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Short communication

# Gas chromatography–mass spectrometry profile of urinary organic acids of Wistar rats orally treated with ozonized unsaturated triglycerides and ozonized sunflower oil

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**Abstract**

The main products in the ozonolysis of unsaturated triglycerides or vegetable oils are peroxides, aldehydes, Criegee ozonides and carboxylic acids. Some of these compounds are present in different concentrations in the biological fluids. The aim of this work is to study, using gas chromatography–mass spectrometry (GC–MS), the organic acid excretion in urine of rats orally treated with ozonized sunflower oil (OSO), ozonized triolein or ozonized trilinolein. Oral administration of OSO to Wistar rats has produced changes in the urinary content of dicarboxylic organic acids. Among others heptanedioic (pimelic acid) and nonanedioic acids (azelaic acid) were the major increased dicarboxylic acids found. The urinary dicarboxylic acid profiles of rats which received ozonized triolein only showed an increase in heptanedioic and nonanedioic acids. However, when ozonized trilinolein is applied, the profile is similar to that obtained when OSO is administered. A biochemical mechanism is proposed to explain the formation of dicarboxylic acids from ozonated unsaturated triglycerides.
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*Keywords:* Ozonized sunflower oil; Dicarboxylic acids; Ozonized triglycerides

## 1. Introduction

Ozonized sunflower oil (OSO) for topical application (OLEOZON®) is a registered drug that is obtained from the reaction between ozone and sunflower oil under appropriate conditions according to a process developed in our center [1]. OLEOZON has shown antimicrobial effects against virus, bacteria and fungi [2–4]. In addition, toxicological studies on OLEOZON have demonstrated that this product is not mutagenic or genotoxic [5,6] and clinical studies have not shown any adverse reactions in patients [2].

On the other hand, since 1994, the use of ozonated sunflower in the treatment of some parasitism has been studied in animal models and in humans by oral administration. For example, the treatment with OSO of *giardia lamblia* have shown very good results [7–9].

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It is known that pharmacokinetic studies are part of the essential requirements for the approval of an oral administration drug. These studies include firstly the determination of the drug and its metabolite concentrations in biological fluids (plasma, urine, etc.), tissues and feces. These pharmacokinetic studies can be carried out with the active principle or with its metabolites [10].

The mechanism of action of ozonated sunflower oil on the biological systems is relatively unknown. Therefore, it is necessary to determine changes in the metabolic profiles of the biological fluids. In such a case, it becomes necessary to analyse substances closely related to the drug active principles or its metabolites.

The metabolic profile is obtained as the result of a multicomponent chromatographic analysis of a certain biological fluid or tissues. These profiles can reveal the differences between normal metabolism and the pathological one. A powerful technique to carry out these chromatographic studies is gas chromatography–mass spectrometry (GC–MS).

The reaction between ozone and unsaturated triglycerides occurs by the well-known Criegee mechanism [11]. Taking into consideration the unsaturated fatty acids composition of sunflower oil, and the ozone–olefin reaction mechanism, during the ozonation of unsaturated triglycerides, it is expected that aldehydes and carboxylic acids with three, six and nine carbon atoms would be obtained. In this reaction, hydroperoxides, ozonides and some other peroxidic or polyperoxidic species can also be obtained [12]. The peroxidic and hydroperoxidic species partially decompose forming aldehydes and carboxylic acids with different numbers of carbon atoms in their structures [13,14].

Some of these compounds could be naturally present in different biological fluids as a result of the lipid oxidation process, one of the most important metabolic pathways in the body. Urine is a biological fluid that has a great content of carboxylic acids and is easy to work with because of its accessibility and clean manipulation.

As carboxylic acids or their precursor are produced during the ozonation of unsaturated compounds and different carboxylic organic acids are the final products of lipid metabolism, changes in its urine concentration could be expected. The aim of this work was the study of the urinary organic acid profiles of Wistar rats, orally treated with ozonized standard triglycerides (Triolein and Trilinolein), and ozonized sunflower oil using a combination of a liquid–liquid extraction method with the GC–MS technique.

## 2. Experimental

### 2.1. Solvents and reagents

Diethyl ether, ethyl acetate, methanol, anhydrous sodium sulfate, sodium chloride, potassium hydroxide, and hydrochloric acid of analytical grade were obtained from BDH (Poole, UK). *N*-Nitro-*N*-methyl-*p*-toluenesulfonamide, trilinolein (99%), and triolein (99%) were purchased from Sigma (St Louis, MO, USA). The edible quality sunflower oil was supplied by Agustin Roig (Tarragona, Spain). All the reagents were used without previous purification.

### 2.2. Ozonation process

All the substrates (sunflower oil and unsaturated triglycerides) were ozonized under the same conditions: 50 ml of the appropriate substrate was placed in a 100 ml bubbling reactor, with an oxygen flow of 10 l/h. The reactor was immersed in a water bath at $25 \pm 0.1$ °C. An OZOMED-400 ozone generator (Ciudad de la Habana, Cuba) was used with an ozone production of 1 g/h. The reaction was finished after 4 h, stopping the ozone (gas) generation. The ozone concentration was determined by measuring the absorbance at 256 nm, in an Ultraspect III spectrophotometer (Pharmacia LKB, Uppsala, Sweden).

### 2.3. Animals

Two animal experiments were carried out. A detailed description is given below.

#### 2.3.1. Study of oral administration of ozonated sunflower oil

Twenty-four female Wistar rats weighing from 180 to 200 g were placed in metabolic cages (Tecniplast, Buguggiate, Varese, Italy) under con-

trolled conditions of temperature and humidity, water ad libitum and appropriate standard feeding. Two control groups were established. The first group, Control 1, included 12 animals without any treatment and the second group, Control 2, included six animals that were orally treated with a unique dose of 0.3 ml of sunflower oil per kg animal weight. The last group of six animals was orally treated with the same unique dose of ozonated sunflower oil.

All the compounds assayed were given to the animals through an intragastric cannula. The urine samples were collected during 24 h post-treatment and immediately subjected to the extraction of the urinary acids.

#### *2.3.2. Study of oral administration of ozonated unsaturated triglycerides*

Twenty-four female Wistar rats weighing from 180 to 220 g were placed in metabolic cages (Tecniplast, Buguggiate, Varese, Italy) with controlled conditions of temperature and humidity, water ad libitum and appropriate standard feeding. A group of six animals were orally treated with a unique dose of 0.3 ml of ozonated triolein per kg animal weight. The other six animals were treated with the same dose of ozonated trilinolein. Two control groups of six animals each were used with the same doses of triolein and trilinolein, respectively. The urine of each animal was collected during 24 h and kept at −10 °C until they were analyzed.

### *2.4. Creatinine determination*

The Jaffé method was employed for creatinine determination. One milliliter of urine was diluted with 49 ml of redistilled water. A small fraction (0.2 ml) of this sample was mixed in a glass cuvette with 2 ml of a solution containing picric acid (35 m*M*) and sodium hydroxide (0.32 mol/l), followed by the absorbance measurement at 490 nm versus air [15].

### *2.5. Liquid–liquid extraction of urinary organic acids and methyl ester formation*

Urine samples (5 ml), containing appropriate amounts of internal standard (*n*-heptadecanoic acid) and sodium chloride, were acidified with hydrochloric acid (pH 1) and extracted twice, first with ethyl ether and later with ethyl acetate; an equal volume of both solvents was used (5 ml). The organic phases were mixed and dried with anhydrous sodium sulfate. The mixture was filtered and the solvents were removed under nitrogen flow at room temperature. One millilitre of ethyl ether was used to dissolve the extracted residue, and an excess of diazomethane was bubbled to obtain the methyl esters of the dicarboxylic acids present. Nitrogen was used for evaporation until dryness, and 50 μl of ethyl acetate were used to redissolve the final products. The samples were kept at −20 °C until the GC–MS analysis.

### *2.6. Gas chromatography–mass spectrometry analysis*

An Automass GC–MS system (UNICAM, Cambridge, UK) was used. A FFAP Supelco capillary column (30 m×0.32 mm I.D., 0.25 μm film thickness) was employed for the separation of dicarboxylic acid methyl esters. The temperature programming column conditions employed were: 80 °C initial temperature (2 min), 8 °C/min to 220 °C, and held at 220 °C for 10 min. An ionization voltage of 70 eV over the mass range of 30–400 a.m.u. was used to fragment the components. The interface and the ion source temperature were set at 240 and 250 °C, respectively. Injector temperature was set at 280 °C. The helium gas carrier was maintained at a linear flow-rate of 1 ml/min and the injection volume was 0.1 μl. An automated mass spectra library was used for identifying the compound [16]. An internal standard method (heptadecanoic acid) was used to quantify the detected compounds, taking into consideration the respective response factors.

## 3. Results and discussion

The chromatogram corresponding to the methylated urinary organic acids of the Wistar rats without any treatment (Control 1) was quite complex and to analyze it, a coupled system GC–MS was necessary (Fig. 1a). The components were identified with the help of an automated database and other mass spectra previously reported for rats or human

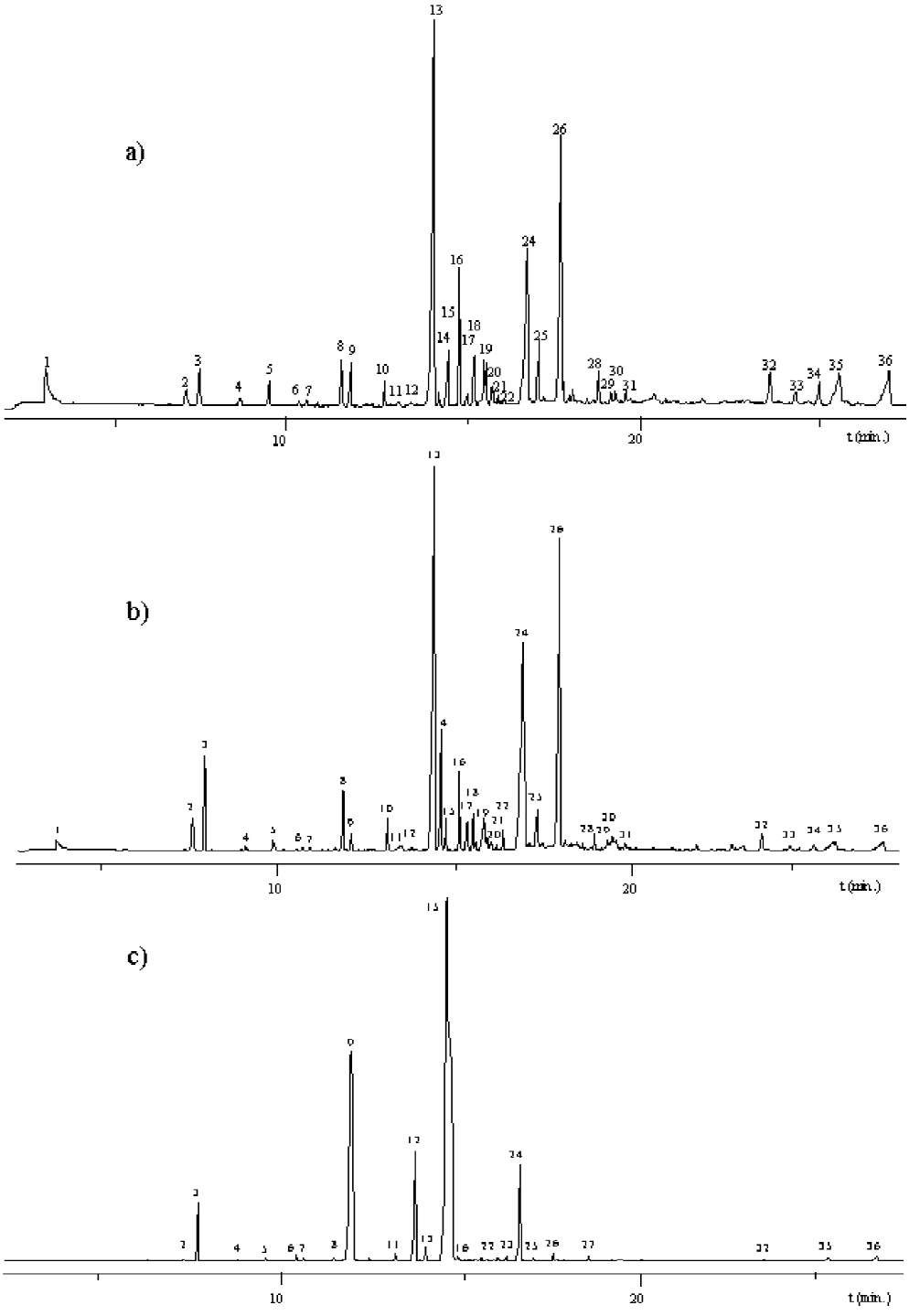



Fig. 1. Chromatographic profile of methylated urinary organic acid of Wistar rats: (a) without any treatment (Control 1), (b) orally treated with sunflower oil (Control 2), (c) orally treated with ozonated sunflower oil.

Table 1
Compounds present in the different urinary chromatographic profiles of Wistar rats

| Peak | Name | Peak | Name |
|---|---|---|---|
| 1 | Lactic acid | 19 | Methylaconitic acid isomer |
| 2 | Butanedioic acid | 20 | Decanedioic acid |
| 3 | Benzoic acid | 21 | α-Hydroxybenzenepropanoic acid |
| 4 | Pentanedioic acid | 22 | Unidentified |
| 5 | Benzeneacetic acid | 23 | Decenedioic acid |
| 6 | Hexanedioic acid | 24 | Heptadecanoic acid (internal standard) |
| 7 | 4-Methoxy-phenol | 25 | Methylaconitic acid isomer |
| 8 | 2,6-Ditherbutyl-3-methylphenol | 26 | Citric acid |
| 9 | Heptanedioic acid | 27 | Dodecenedioic acid |
| 10 | Unidentified | 28 | 3,5-Bis (1,1-dimethylethyl)-4-hydroxybenzoic acid |
| 11 | Octanedioic acid | 29 | 1-Hydroxy 1,2,3-propanetricarboxylic acid |
| 12 | Octenedioic acid | 30 | 2-(Formilamine) benzoic acid |
| 13 | 2-Methoxy-2-pentenedioic acid | 31 | 3,4,5 Trimethoxybenzoic acid |
| 14 | Unidentified | 32 | 4-Hydroxybenceneacetic acid |
| 15 | Nonanedioic acid | 33 | 3-(3,4-Dimethoxyphenyl)-2-propenoic acid |
| 16 | *P*-Cresol | 34 | 4-Hydroxybenzenepropanoic acid |
| 17 | Unidentified | 35 | Hippuric acid |
| 18 | Methylaconitic acid | 36 | *N*-(Phenylacetyl)-glycine |

urine metabolic profiles [17]. These compounds are numbered consecutively (Table 1).

The chromatographic urinary acids' profile was characterized by the presence of aromatic acids as a consequence of the metabolism of aromatic amino-acids and short or medium chain dicarboxylic acids (DCA) mainly formed from fatty acid oxidation. Other characteristics were the presence of lactic and citric acid and some artifacts reported previously in the specialized literature (benzoic and methylaconitic acids) [18,19].

The chromatographic profile of the Control 2 group (rats treated with sunflower oil) showed similar characteristics to those of Control 1 (Fig. 1b). In the chromatographic urinary organic acids profile (Fig. 1c) of Wistar rats that received ozonized sunflower oil, obvious changes are obtained with respect to the control groups 1 and 2. The compounds that had significant changes were the acids: heptanedioic (pimelic acid, peak 9), octenedioic (peak 12), and nonanedioic (azelaic acid, peak 15). All these acids are endogenous compounds. In addition, two other dicarboxylic acids were observed that were not detected in the control profile: the decenedioic acid (peak 23) and the dodecenedioic acid (Peak 27). These results are summarized in Table 2.

The results demonstrated the changes taking place

Table 2
Urinary dicarboxylic acid concentration (mg acid/mg creatinine) in the different experimental groups

| Metabolites (Dicarboxylic acids) | Control I ($N=12$) | Control II ($N=6$) | Ozonated sunflower oil ($N=6$) |
|---|---|---|---|
| Heptanedioic acid (pimelic acid) | 0.06±0.01 | 0.07±0.02 | 9.6±0.8 |
| Octanedioic acid (suberic acid) | 0.019±0.004 | 0.021±0.005 | 0.22±0.02 |
| Octenedioic acid | 0.016±0.002 | 0.018±0.004 | 1.6±0.2 |
| Nonanedioic acid (azelaic acid) | 0.07±0.02 | 0.07±0.02 | 69±4 |
| Decenedioic acid | n.d | n.d. | 0.047±0.005 |
| Dodecenedioic acid | n.d. | n.d. | 0.043±0.005 |

n.d., not detected.

in the Wistar rats after oral administration of ozonated sunflower oil. Nonanedioic acid (azelaic acid) was the most incremented dicarboxylic acid even regarding their absolute concentration (69 mg/mg creatinine) followed by heptanedioic acid (pimelic acid) with 9.6 mg/mg creatinine. Another acid that showed a significant increment is octenedioic acid (Table 2).

It is well known that the fatty acids (FA) metabolism is carried out by the β- or ω-enzymatic oxidation process [20,21]. Dicarboxylic acids (DCA) are formed as a consequence of the monocarboxylic acids ω-oxidation process under normal conditions. This oxidation pathway is the least favored one. Only between 4 and 5% of fatty acids are oxidized in this way [20]. The DCA are usually completely excreted without being catabolized [22]. Therefore, DCA were present in the metabolic profiles of the control groups 1 and 2.

A significant increment of the dicarboxylic acid concentration in urine has been used for diagnosis of defects in the β-oxidation of fatty acids in the mitochondria. For example, the dicarboxylic aciduria that is a congenital metabolic illness in neonatal children can be detected by a high concentration of these acids with carbon atom numbers 4, 6, 8 and 10 in the urine [17,23–26].

The carboxylic acids detected in urine of rats orally treated with OSO have two origins: one is the result of ozonation process where ozonides and substances with structures of nine and 12 carbon atoms holding functional groups like carbonylic, carboxylic and peroxidic could be obtained; and the other is the normal metabolic process of free fatty acids (see Fig. 2, pathways I and II).

Once the lipids are absorbed by the intestine, the lipases react in the blood flow, being species with two carboxylic functional groups [9,27,28]. The aldehydes and the hydroperoxides can be oxidized to acids by enzymes, like aldehyde dehydrogenase and peroxidase. The ozonides are reduced to their respective aldehydes with the participation of the glutathione-*S*-transferase and the reduced glutathione. Later both are oxidized, as previously described [29]. As a result of these biochemical processes, dicarboxylic acids could be formed. These compounds are the azelaic and the dodecenedioic acids.

Azelaic acid could be later β-oxidated and pimelic acid is obtained [17,25,30]. On the other hand, decenedioic and octenedioic acids are formed by two β-oxidation processes from dodecenedioic acid. Finally, the suberic acid is formed starting from the decenedioic acid by the unsaturated acid β-oxidation mechanism [20].

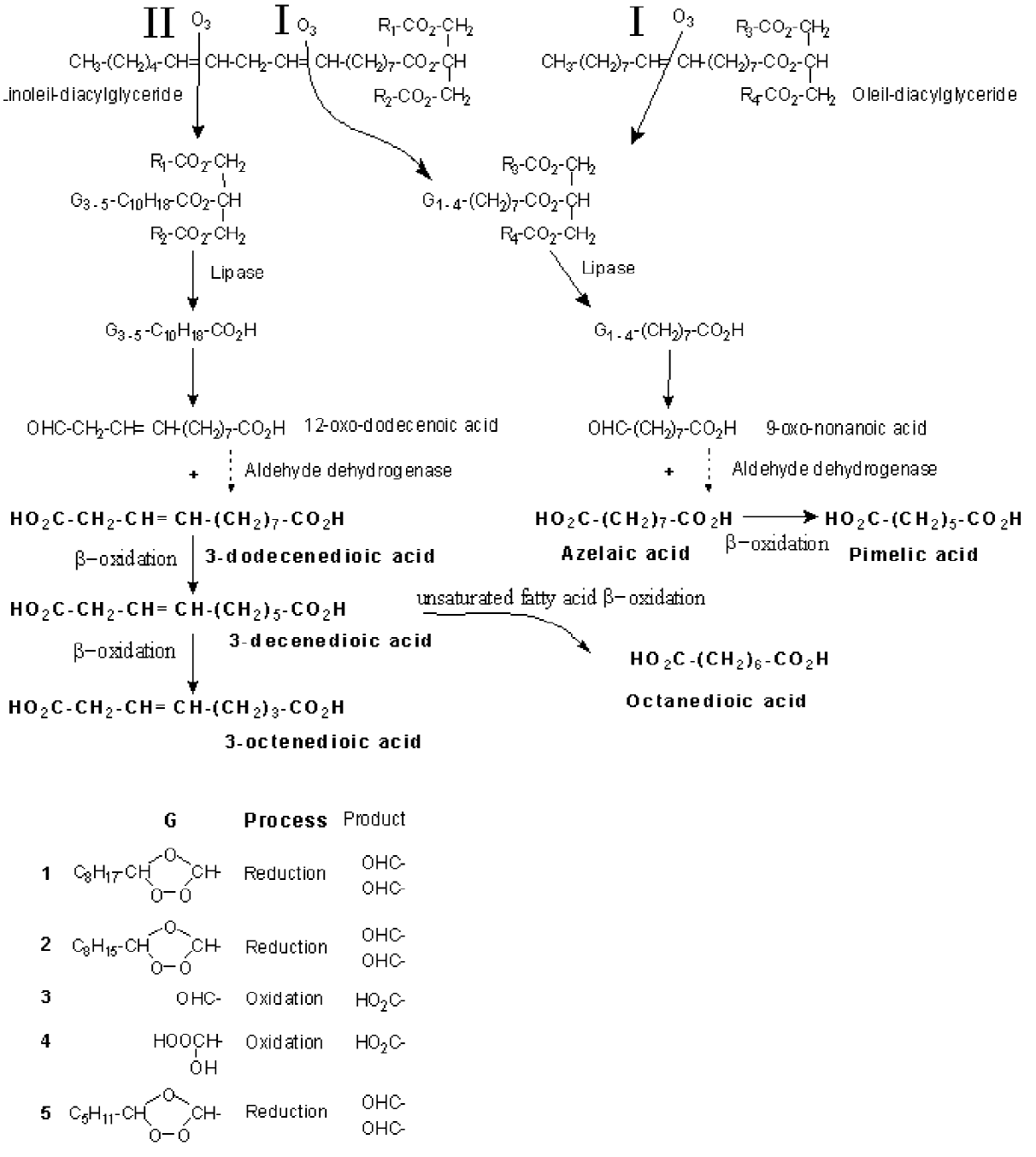
In the sunflower oil, the double bond in the $C_9$ position is in a greater proportion than in the $C_{12}$ position. It is because the double bond in $C_9$ is present in linoleic and oleic acids, fatty acids that are more abundant in sunflower oil triglycerides [31,32]. The $C_{12}$ double bond is only present in linoleic acid. Therefore, the highest probability of the ozone reaction is with the double bond in the $C_9$ position, explaining the enormous increase in azelaic acid.

Azelaic acid does not show chronic toxic properties [22,33]. On the contrary, it has anti-comedogenic, anti-viral, and anti-fibrinolitic properties [34–37]. Many of these properties explain its topical use, e.g. in the treatment of acne [30,33,37]. It is possible that this acid would be one of the active principles of the OSO. It would be necessary, therefore, to carry out additional investigations to prove this hypothesis, whereby the azelaic acid could be an appropriate compound for pharmacokinetic studies using OSO; it is highly abundant in the samples. In addition, this compound is easily detectable without any interference in the chromatograms (see peak 15 in Fig. 1).

The proposed mechanism forming the profiles of urinary organic acids cause by ingestion of OSO can be explained by the results concerning the DCA of Table 2.

The study of the urinary organic acids profile of Wistar rats orally treated with ozonized model triglycerides (triolein and trilinolein) was carried out to support this mechanism and the hypothesis of the origin of the dicarboxylic acids starting from oleic and linoleic acids.

With the administration of the ozonized triolein, the profile of urinary organic acids changed as expected. The only dicarboxylic acids which increased were heptanedioic and nonanedioic acid. These DCA are the only ones that should appear within pathway I (Fig. 2). By administration of ozonized trilinolein, the profile of urinary acid was similar to that of OSO. This result supports the priority of pathway II of the proposed mechanism.




| | G | Process | Product |
|---|---|---|---|
| 1 | $C_8H_{17}$-CH(O-O)-CH | Reduction | OHC-<br>OHC- |
| 2 | $C_8H_{15}$-CH(O-O)-CH | Reduction | OHC-<br>OHC- |
| 3 | OHC- | Oxidation | $HO_2C$- |
| 4 | HOOCH-<br>OH | Oxidation | $HO_2C$- |
| 5 | $C_5H_{11}$-CH(O-O)-CH | Reduction | OHC-<br>OHC- |

Fig. 2. Proposed mechanism for urinary dicarboxylic acid formation in Wistar rats after oral administration of ozonated sunflower oil.

The concentrations for pimelic and azelaic acid in the urine dicarboxylic acids profile of rats orally treated with ozonized triglycerides were superior to the corresponding ones after oral administration of the OSO (Table 3). It is well known that the ozonolysis reaction is not selective for a specific

Table 3
Metabolite concentration (mg/mg creatinine) in the ozonized model compounds and the OSO

| Metabolite | Ozonized triolein ($n$=6) | Ozonized trilinolein ($n$=6) | OSO ($n$=6) |
|---|---|---|---|
| Pimelic acid | 32.6±1 | 23.4±0.9 | 9.6±0.8 |
| Suberic acid | n.d. | 0.27±0.05 | 0.22±0.02 |
| Octenedioic acid | n.d. | 1.9±0.1 | 1.6±0.2 |
| Azelaic acid | 101±5 | 79±4 | 69±4 |
| Decenedioic acid | n.d. | 0.040±0.007 | 0.047±0.005 |
| Dodecenodioic acid | n.d. | 0.020±0.005 | 0.043±0.005 |

n.d., not detected.

double bond position. When the triolein is ozonized, all the administered ozone reacts with the only possible double bond $C_9$ position. However, in the trilinolein, two double bonds ($C_9$ and $C_{12}$) can react with ozone. Therefore, other substances are formed, in addition to those produced from triolein ozonation. The composition of FA in OSO regarding $C_9$ and $C_{12}$ is different in comparison to the two triglycerides used as model substances. Therefore, another composition of DCA resulted (Table 3).

## 4. Conclusions

The concentrations of urinary dicarboxylic acids in Wistar rats increase after oral administration of ozonized sunflower oil. Nonanedioic acid (azelaic acid), heptanedioic acid (pimelic acid) and octenedioic acid were found as the major increased dicarboxylic acids. The study of the urinary dicarboxylic acid profile of Wistar rats orally treated with ozonized unsaturated triglycerides revealed the origin of the increment of these acids. This helped to explain the same observation when ozonated sunflower oil was ingested. The results supported the proposed mechanism for ozonized sunflower oil metabolism in rats. In addition, it was demonstrated that the urinary dicarboxylic acid profiles depend on the composition of the ozonized substances orally administered.

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## References

[1] J. Molerio, W. Díaz, I. Lezcano, S. Menéndez, O. Ledea, Registro Médico del Aceite de Girasol ozonizado (OLEOZON®) para el tratamiento de la epidermofitosis. Certificado de inscripción, ERM 783/1999, Republica de Cuba.
[2] T. Cajigas, U. Bastard, S. Menéndez, M. Gómez, L. Eng, Revista CENIC Ciencias Biológicas 20 (1990) 3.
[3] I. Lezcano, N. Nuñez, M. Gómez, S. Espino, Antibacterial activity of Ozonized Sunflower Oil (OLEOZON®) against Staphylococcus aureus and Staphylococcus epidermidis, Ozone Sci. Eng. 22 (2000) 207.
[4] L.A. Sechi, I. Lezcano, N. Nunez, M. Espino, I. Dupre, A. Pinna, P. Molicotti, G. Fadda, S. Zanetti, Antibacterial activity of ozonized sunflower oil (OLEOZON), J. Appl. Microbiol. 90 (2001) 279.
[5] G. Martinez, O.S. León, C. Rodríguez, Revista CENIC Ciencias Biológicas 26 (1995) 2.
[6] A. Remigio, Y. González, Z. Zamora, J. Molerio, Revista CENIC Ciencias Biológicas 28 (1998) 3.
[7] S. Menéndez, M.E. González, E. Blanco, Proceedings of the XII Ozone World Congress, Lille, France, Vol. 3 (1995) pp. 297–300.
[8] D. Hernández, Estudio de los efectos del aceite de girasol ozonizado sobre la giardiasis. Tesis de Especialidad, Instituto Superior de Ciencias Médicas de la Habana, Centro de Investigaciones del Ozono, 1999.
[9] J. Molerio et al., Patente 20-96 Oficina Nacional de Invenciones, Información Técnica y Marcas, Cuba, 1996.
[10] L.S. Goodman, A. Gilman, The Pharmacological Basis of Therapeutics, International ed., London, 1996.
[11] P.S. Bailey, Ozonation in Organic Chemistry: Olefinic Compounds, Vol. I, Academic Press, New York, 1978.
[12] M. Díaz, J. Gavin, F. Hernández, O. Ledea, J. Moleiro, Study of $^1$H NMR methyl linoleate ozonation, Ozone Sci. Eng. 2002 (in press).

[13] O. Ledea, T. Correa, J. Molerio, D. Jardines, M. Escobar, A. Rosado, C. Hernández, Ozone Sci. Eng. 23 (2001) 1.
[14] J.C. Allen, R.J. Hamilton, Rancidity in Foods, Blackie, London, 2000.
[15] The Jaffé method for creatinine determination, Boehringer-Mannheim, Germany, 1979.
[16] National Institute of Standards and Technology Mass Spectra Library, Automated Data Base of 55000 entries, NIST (1990).
[17] S. Lindsdt et al., Clin. Chem. 22 (1976) 1330.
[18] F. Streichsbier et al., Fette Seifen Anstrichmittel 84, Jhrg. No. 8304-308, 1982.
[19] M. Spiteller, G. Spiteller, J. Chromatogr. 164 (1979) 253.
[20] F.D. Gunstone, Structure and Modified Lipids, Marcel Dekker, New York, 2001.
[21] S. Eaton, K. Bartlett, M. Pourfarzan, Biochem. J. 320 (1996) 345.
[22] A.V. Grego, G. Mingrone, Clin. Nutr. 14 (1995) 143.
[23] D. Sontheirmer et al., Monatsschr. Kinderheilkd. 143 (1995) 250.
[24] N. Gregersen, Scand. J. Clin. Lab. Invest. 45(Suppl. 174) (1985).
[25] T. Tserng Kou, R.L. Griffin, D.S. Kerr, Metabolism 45 (1996) 162.
[26] M. Duran, S.K. Wadman, Enzyme 38 (1987) 115.
[27] O. Ledea, T. Correa, J. Molerio, D. Jardines, A. Rosado, Revista CENIC Ciencias Químicas 29 (1998) 3.
[28] N. Poklukar, M. Mittelbach, Monatsh. Chem. 122 (1991) 719.
[29] R.A. Hempenius, J. de Vries, I. Riejtjens, Eur. J. Pharmacol. Environ. Toxicol. Pharmacol. Sect. 228 (1992) 207.
[30] Q.H. Nguyen, T.P. Bui, Int. J. Dermatol. 34 (1995) 75.
[31] T. Rezanka, P. Mares, J. Chromatogr. 542 (1991) 145.
[32] D. Firestone, Physical and Chemical Characteristics of Oils, Fats and Waxes, AOCS Press, USA, 1999.
[33] V. Ferioli et al., Farmaco 49 (1994) 421.
[34] Y.U. Taniguchi et al., Oncol. Res. 7 (1995) 183.
[35] K. Boadu Addo et al., Cancer Lett. 103 (1996) 125.
[36] L. Lemic-Stojcevic, A.H.W. Nias, A.S. Breathnach, Exp. Dermatol. 4 (1995) 79.
[37] H.P.M. Gollnick, Dermatol. Monatsschr. 178 (1992) 143.