OLIVE OIL OZONIDE AND ITS FUNGICIDAL QUALITY.*

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Received February 19th, 1934. Published April 28th, 1934.

Ozone has been much used recently not only in the determination of the structure of unsaturated organic compounds, but commercially for sterilization of drinking water in the reservoirs of large cities such as Paris and Petrograd, and in the paint and varnish industries.

The present investigation is concerned with a therapeutic agent for the infectious fungus skin disease, known as ringworm. For this purpose the following conditions, at least, should be satisfied.

First, while the chemically combined ozone must be stable at room temperature, contact with the heat of the human body should cause it to give off sufficient nascent oxygen to destroy the pathogenic micro-organisms.

Second, the compound and its decomposition products as a whole must not have any irritating or harmful effects on a delicate skin or tissue.

Third, the compound must have a moderate oxidizing power combined with a strong penetrating power upon the skin or tissue in which it should remain for some time.

On the basis of these considerations various ozonides of unsaturated fatty acids or their glycerides were roughly studied. However, these substances did not meet fully with the above requirements. The ozonide of olive oil, however, gave a fairly satisfactory result. The effect of ozone upon olive oil was studied by Fenaroli,(1) and Dover and Appleby.(2)

The compositions of olive oils from different sources are given in Table 1. The linoleic acid glyceride content varies from 0.5 to 7%, sometimes being as high as 17%. The presence, in the oil, of a large amount of linoleic acid or its glyceride is undesirable, since the ozonides of these compounds might decompose easily producing various aldehydes or acids of shorter carbon chains and giving relatively high acidity or low *p*H to the product as a whole. The acidity thus produced might be irritating or harmful to a delicate skin or tissue. In the present experiment the effective substance in olive oil is the glyceride of oleic acid or triolein which has the following structural formula :

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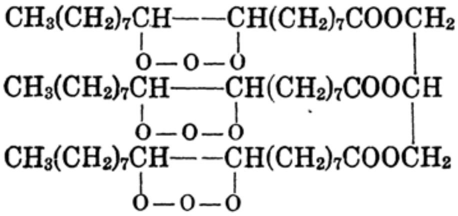
* Patent pending.

(1) *Gazz. Chim. Italiana*, **36**, ii (1906), 292.

(2) *Ind, Eng. Chem.*, **18** (1923), 63.

$$\begin{array}{l} CH_3(CH_2)_7CH = CH(CH_2)_7COOCH_2 \\ CH_3(CH_2)_7CH = CH(CH_2)_7COOCH \\ CH_3(CH_2)_7CH = CH(CH_2)_7COOCH_2 \end{array}$$

This combines with three molecules of ozone when brought in contact with it. The double bonds or ethylene linkages of the three oleic acid groups of triolein, take up three molecules of ozone and a definite saturated chemical compound, triolein ozonide, is produced. The structural formula may be represented as follows:



When this ozonide comes in contact with heat it decomposes at the ozone linkages and produces nascent oxygen slowly or rapidly depending upon the degree of heat which is added.

Table 1.

Analysis of Olive Oil.

	California (J. & B.)[1]	Italian (J. & B.)[1]	Spanish (J.)[2]		Spanish (T. & S.)[3]
Acid value	1.5	—	1.8	Acid value	0.62
Iodine value	85.1	—	83.7	Iodine value	82.7
Sapon. No.	190.0	—	192.4	Sapon. No.	192.6
Olein	84.4	83.1	80.5	Total as oleic acid	83.94
Linolein	4.6	3.9	6.9	,, Linoleic	0.51
Myristin	trace	trace	0.2	,, Glycerol (Calc.)	4.44
Palmitin	6.9	9.2	9.4	,, Palmitic	7.55
Stearin	2.3	2.0	1.4	,, Stearic	2.27
Arachidin	0.1	0.2	0.2	Unsapon. m. and triolein and α-palmitodiolein	0.79
Unsapon. m.	1.0	1.1	0.8		

(1) G. S. Jamieson and F. W. Banghman, *J. Oil and Fat Ind.*, **2** (1925), 40, 110.
(2) G. S. Jamieson, *J. Oil and Fat Ind.*, **4** (1927), 426.
(3) K. Taufel and J. Sarria, *Chem. Abs.*, **20** (1926), 1723.

To ozonize olive oil, a series of three glass aerating vessels was used, the principal reaction occcurring in the first, and the other two serving as auxiliaries to catch excessive ozone. They were half filled with pure olive oil and a current of ozone, washed by passing through a dilute soda solution to remove oxides of nitrogen, was drawn through by means of a sufficiently high vacuum suction. The distinct yellow colour of the oil in the first vessel became pale yellow or nearly colourless at the end of the process. This aeration served not only to remove the volatile aldehydes, or acids which were formed as by-products in the ozonization of the oil, but to maintain the temperature of the whole not higher than the room temperature throughout the operation.

To ozonize, for example, one gallon of olive oil to the saturation point it was necessary to treat the oil far more than 200 hours with a current of about 1.5 grams ozone per hour. It was found that further treatment was a great disadvantage, for the oil became viscous and a white foam resulted. To clear up the foam a few days of standing at room temperature were required.

Table 2.

Changes of Physical and Chemical Constants of Olive Oil by Ozone Treatment.

Ozone treatment in hours	Increased viscosity at 25°C.	Iodine value	Refractive index at 28°C.	Acid value in KOH mg.	Bleaching time in mins. 10 c.c. of 0.(02% indigo carmine solution	Available oxygen after 3 months standing at 25° to 32°C. in %
0	0	85.86	1.4656	1.0	—	—
24	27	78.46	1.4653	4.5	140	0.11
48	73	68.27	1.4653	6.0	37	0.19
72	127	60.83	1.4652	8.7	24	0.23
96	200	55.79	1.4651	13.5	18	—
111	245	53.18	1.4651	13.8	15	—
114	254	53.05	1.4650	13.4	14	—
117	264	53.43	1.4650	13.7	12	—
120	273	53.81	1.4650	13.5	12	0.37
135	318	47.19	1.4650	13.4	11	—
144	345	48.52	1.4652	14.0	9	—
159	382	44.15	1.4653	13.6	9	0.61
164	468	43.40	1.4653	13.9	9	0.64
180	600	38.66	1.4655	17.6	8	0.71
203	855	34.19	1.4659	19.1	13	0.86

Physical and chemical constants of this ozonized oil are related to the time of treatment under the conditions described. As shown in Table 2 the refractive index of the olive oil gradually dropped from 1.4656 to 2.4650 and then rose again to 1.4659 by the passing of ozone for 120 to 203 hours, while its iodine value (this test is not reliable for ozonized oil except as a general index) dropped steadily from 85 to 34. The viscosity and the available oxygen content of the oil increased markedly and in proportion to the time of ozone treatment. The acid value of the oil rapidly increased from 1.0 to 13.5 with 96 hours and then remained constant up to 164 hours of treatment. The acidity was probably due to the presence of the decomposition products of the ozonide of trilinolein. A further increase of acidity by further treatment was probably due to the decomposition products of triolein ozonide.

For the purposes and under the conditions which are described above, it was found sufficient to treat the oil for only about 120 hours. The colour of the product or ozonide thus obtained was pale yellow, but on standing for some days it gradually became colourless. The available oxygen content of this product was found to be about 0.45% of its own weight. This strength is taken as its standard for commercial purposes by the author. The method for the determination of the available oxygen content or of the oxidizing power will be reported in a separate paper.

The product has a slightly bitter taste and an agreeable odour. It is fairly stable at room temperature. For *Staphylo Cocci* the phenol coefficient of the ozonized oil containing 0.37% available oxygen was about 0.05. When 1 c.c. of the product was shaken with 10 c.c. of water, the *p*H of the aqueous solution was found to be not less than 3.0 at 37°C. The oil itself does not give any irritation even to a delicate skin or tissue.

When the ozonide was brought in contact with human skin or tissue at 37°C. or body temperature it gave off nascent oxygen immediately but moderately. It had strong penetrating but no irritant qualities even for delicate tissue. At the same time it remained for an indefinite period in the oxidizing condition on or in the skin or tissue. Therefore the product mignt destroy easily pathogenic micro-organisms especially ringworm fungi in skin lesions.

The experimental data on the fungicidal qualities for the fungi, *Trichophyton interdigitale*, *Trichophyton violaceum*, *Epidermophyton inguinale*, and *Aspergillus oryzae* which is abundantly cultured for the diastatic enzyme preparation,[3] are represented in Table 4, including a comparison with the common oxidizing agents, namely hydrogen peroxide, and sodium hypochlorite which is sold under the name Zonite.

(3) T. Harada, *Ind. Eng. Chem.*, **23** (1931), 1434.

Table 3.

Stability of Olive Oil Ozonide.

Time in hours	At 25 to 32°C gas formed in c.c. by decomposition in the presence of light*	Available oxygen in per cent.	At 8–10°C gas formed in c.c. by decomposition*	Available oxygen in per cent.
0	0	0.48	0	0.48
3	5	0.44	0	—
12	21	0.42	0	0.48
21	32	0.40	?	—
35	41	0.40	—	0.48

* The top lip of a burette was suspended so that it touched the bottom of a beaker containing the ozonide and the burette was filled with 60 c.c. of the ozonide. The figures are empirical values which do not represent the gas formed by decomposition of 60 c.c. of the ozonide, for as the gas is formed and rises in the burette, an equal volume of the oil flows out into the beaker.

Table 4.

Fungicides and Fungi.

Oxidizing agents	Available oxygen in per cent.	*T. interdigitale*	*T. violaceum*	*E. inguinale*	*A. oryzae*
H_2O_2	1.04	1:71 – 36	—	1:24 – 15	1:8 – 5.6
NaOCl	0.28	1:24 – 18	—	1:19 – 15	1:8 – 5.6
Olive oil ozonide	0.37	1:360–180	1:360–263	1·180–121	1:28–15

The figures in Table 4 are ratios of the agent concentrations to the amounts of the medium. The figures at the left indicate the concentrations at which the fungi grew while the figures at the right indicate the concentrations too high for specific fungus growth. The technique of the experiments is reported in a previous paper.

It is suggested that olive oil ozonide has not only fungicidal, antiseptic, and germicidal qualities, but a remarkable property as the healing and soothing remedy for the eczema caused by ringworm infection and the like.* Moreover at the same time, it restores poisoned and oxygen-starved cells to a normal condition by its production of nascent oxygen.

* This clinical problem is being carried on in co-operation with Dr. D. Stetson of Roosevelt Hospital, New York, N.Y.

Summary.

The requirements which a pathogenic fungicide must satisfy for use on human skin or tissue are pointed out. The ozonide of olive oil was prepared and examined in these respects with fairly satisfactory results. Changes in physical and chemical constants produced by ozone treatment of olive oil are given.

Certain qualities of the ozonide with regard to its fungicidal effect for ringworm are discussed.

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