ISSN 1984-6428
ONLINE
www.orbital.ufms.br

| Vol 4 | | No. 4 | | October-December 2012 |

Review

Ozonized vegetable oils and therapeutic properties: A review

Nathália R. de Almeida[a], Adilson Beatriz[a] , Ana Camila Micheletti[a], and Eduardo J. de Arruda[b]*

[a]Laboratório de Síntese e Transformações de Moléculas Orgânicas – SINTMOL -Centro de Ciências Exatas e Tecnologia, Universidade Federal do Mato Grosso do Sul. Av. Senador Felinto Müller, nº 1555, Cidade Universitária, 79074-460, Campo Grande, MS, Brazil.
[b]Faculdade de Ciências Exatas e Tecnologia – Universidade Federal da Grande Dourados. Rodovia Dourados - Itahum, Km 12. Cidade Universitária, 79804-970, Dourados, MS, Brazil.

Received: 15 December 2012; revised: 02 January 2013; accepted: 10 January 2013. Available online: 15 January 2013.

ABSTRACT: *Ozonized oils represent an interesting pharmaceutical approach to the management of a variety of dermatological pathologies. Ozone reacts with carbon-carbon double bonds of unsaturated fatty acids according to the mechanism described by Criegee, forming ozonides or 1,2,4 trioxolane rings and peroxides as the most important products, responsible for the antimicrobial activity and stimulating tissue repair and regeneration properties. The ozonized vegetable oils can be liquids or semisolids at room temperature and have stability periods that may be adequate for commercial distribuition. Ozonized sunflower oil (Oleozon®), a drug registered nationally and developed in the Ozone Research Center in Cuba has been tested and it was found to have valuable antimicrobial activity against bacteria, fungi and virus. FT-IR and NMR technics are used to confirm the structural changes undergone by oil during the ozonation. For determining the quality of ozonized oils, analytical methods such as peroxide, acidity and iodine values are usually carried out. Products are available for an alternative use of available resources, natural and renewable sources, simple technology, low cost and with extensive biological activity with reduced collateral effects.*

Keywords: *ozonized oil; 1,2,4-trioxolanes; ozonides; ozonetherapy; antimicrobial activity*

Introduction

Although there is no precise data in Brazil, some authors estimate that almost 3%

* Corresponding author. E-mail: nathrodrigues@live.com

of the Brazilian population suffers from any type of skin lesion. Furthermore, approximately four million people present chronic lesions or some form of complication in the cicatricial process [1]. This demands that health professionals have not only a broader knowledge and training to work with the problem, but also implies the need for greater investment in research, both to quantify this population more precisely and to discover new resources and technologies, with lower cost and greater effectiveness, besides being more appropriate and more accessible to the Brazilian population [2].

It has been reported that ozonetherapy and derived products are very useful in treating many pathologies, such as chronic osteomyelitis, pleural emphysema, abscesses with intractable fistulae, infected wounds, bed sores, chronic ulcers and initial gangrene, necrotizing fasciitis, diabetic foot, skin, mouth, vaginal and rectal bacterial, fungus, viral infections and burns [3], especially when in combination with topical therapy by ozonated oils due to permeation and controlled release of active oxygen species into the skin layers.

The skin barrier offers microbiological, physical and biological barrier against external aggressions and has access routes for penetration of cosmetics or active pharmaceuticals [4]. The skin layers are stratum corneum (10-20 μm), viable epidermis (50-100 μm), the dermis (1-2 mm) and subcutaneous tissue. Each layer has specific functions and features [5].

Barata [4] reports that a product will be absorbed by the body following ways of penetration: transdermal (very slow penetration *via*, but of considerable importance); by sudoriparous glands (lines minor penetration), and by pilo-sebaceous apparatus (easier penetration zones). Vegetable oils may alter skin permeation through three different mechanisms namely: increasing occlusion, widening the polar pathway and widening the non-polar pathway [6]. In addition, it has been found that vegetable oils in general produce virtually no skin irritation or sensitization problems [7].

The best absorption and skin penetration enhancers are oils with a high proportion of unsaturated fatty acids such as oleic acid (omega 9), linoleic acid (omega 6) and linolenic acid (omega 3), but mainly oleic and linoleic acids [8]. Absorption and penetration of products into the skin are influenced due to the skin condition and composition of the product to be applied. Gomes [9] described that to improve the absorption and penetration it's important to study the biological, physiological, physicochemical and cosmetologycal features that facilitate or interfere with the application of the product. The effective penetration into the skin aims the best use of the product in the body and the desired effects are the efficiency of the therapeutic properties of active prevention, balance and maintenance of healthy skin [9].

In recent years there has been a growing interest in modificating technology of

oils and fats. It can be attributed to the fact that these materials are obtained from natural sources and used as important raw materials for the chemical industries, pharmaceutical, cosmetic and food [10].

Ozone reacts with the carbon-carbon double bonds of unsaturated fatty acids from vegetable oils giving rise to the formation of chemical species, such as ozonides and peroxides that are responsible for the germicidal action, as well as the properties of stimulating tissue repair and regeneration [11, 12]. The antimicrobial properties of ozonized oils represent an interesting pharmaceutical approach to the management of a variety of dermatological pathologies [12, 13].

This paper presents a bibliographic review on ozonized oils, synthesis of ozonides, physico-chemical characterization, therapeutic properties and antimicrobial activity. Generally, in the literature there is little information about the chemical composition of ozonized oils, due to the complexity of the mixture of compounds formed during the reaction. This aspect is a major constraint to the registration of these products as drugs.

Ozonetherapy

Ozonetherapy is a term of medicine that describes a number of different practices in which oxygen, ozone or hydrogen peroxides are administered *via* gas, water or oil to kill microorganisms, improve cellular function and promote the healing of damage tissues [14].

The history of ozone began in 1839, through the German chemist Christian Friedrich Schonbein, who initially identified its characteristic odor and began to investigate it. Ozone occurs naturally in the atmosphere, 6 to 30 miles above the earth's surface at a concentration of approximately 10 ppm (parts per million). This ozone layer helps protect the earth's surface from harmful ultraviolet radiation and prevents heat loss from the surface [15].

Ozone, the triatomic form of oxygen, is a colorless gas of pungent odor. It is a very strong oxidizer and can oxidize organic substances, destroying microorganisms, such as bacteria, sterilizing the air, and destroying odors [16].

On the 19th century, ozone had been already identified as a potent bactericidal gas and it was used during World War I for treating German soldiers affected by gaseous gangrene due to *Clostridium* anaerobic infections. In two pioneristic studies, Stroke reported the first 21 medical cases successfully treat with ozone at the Queen Alexandria Military Hospital [17, 18].

Ozone has been used therapeutically in several countries such as Cuba, Germany, Italy, Switzerland, Austria, Spain, Russia, Japan, Chile, Peru, Argentina, United States, among others. Cuba is one of the pioneers in the implementation of this therapy in Public

Health Services for over 22 years [19]. OLEOZON® is a therapeutical drug obtained from the reaction of ozone with sunflower oil. The process was developed at the Ozone Research Center – National Center for Scientific Research in Cuba [20]. Topical ozonetherapy have been reported to activate local microcirculation, improve cellular oxygen up-take, stimulate oxidative defensive enzymatic systems and to improve granulation and tissue growth [21].

The ozone gas quickly becomes unstable in the atmosphere so it must be generated practically before use. Ozonization of vegetable oils seems to enhance its stability for clinical uses, keeping activity for a period of up to 2-3 years [22, 23]. Ozonated materials, in which the ozone molecule is stabilized as an ozonide, have the capacity to deliver nascent oxygen deep into the treated area without causing irritation [24].

As a natural preparation, ozonized oil is available in several countries [25]. Ozonized sunflower oil (Oleozon®) has been tested and it found to have valuable antimicrobial activity against bacteria, fungi and virus [26-28].

At the University Hospital pharmacy of Siena, they make their own preparation by bubbling ozone in pure olive oil for at least 30 minutes in a cooled bath. In other countries the pure olive oil is ozonized for two days until it solidifies [29].

There are many other commercially produced ozonated vegetable oils on the market like Cocozone – made from coconut oil in Great Britain, OOO (Ozonized Olive Oil) – made in Canada, O2-Zap – made from olive oil in USA [30].

There are numbers of pharmaceuticals and cosmetics on the market that use ozonized oils as active principles, including Oxaktiv® (Pharmoxid Arznei GmbH&Co.KG, German) [31], Oleoforte® (NaturOzone, Spain) [32] and Ozonia 10® (Innovares, Italy) [33].

Ozonized vegetable oils

The vegetable oils are formed by 97-98% of triglycerides. Depending on their origin and nature they have a variable composition of saturated and unsaturated fatty acids bonded to the glycerol backbone [34].

Industrial exploitation of oils and fats, both for food and oleochemical products, is based on chemical modification of both the carboxyl and alkene groups present in fatty acids, especially *via* oxidation process. The unsaturated triglycerides give the oil many favorable properties. Oxidation of double bonds is used to cleave the alkyl chain or to introduce additional functionality along the chain [35].

An important example described in literature involves the oxidative cleavage of

double bonds using ozonolysis reaction. Ozonolysis is a convenient and highly effective method owing to the complete reaction of ozone with the starting material [36].

The reaction of the ozone with unsaturated fatty acids from vegetable oils generates ozonides, peroxides and aldehydes [37]. The peroxides are the most important products formed. This group includes ozonides, hydroperoxides, polymeric peroxides and other organic peroxides [38, 39] and, probably, is responsible for the wide biological activity of described ozonized vegetable oils [40].

The mechanism of ozonolysis (Figure 1) was described by Criegee in 1975 [41]. First step is a 1,3 dipolar cycloaddition of ozone to the olefin leading to the malozonide (1) (Criegee intermediate), which is very unstable and decomposes to give a zwitterion (2) and a carbonyl compound (3) In the presence of reactive solvent, such as water or alcohol, the zwitterion interact with the solvent to give hydroperoxides (4) in high yield, since the concentration of the solvent far exceeds that of any other substances with which the zwitterion may react. Ozonides (5), dimeric (6) or polymeric peroxides may be by-products. When the solvent is inert, the zwitterion must react either with itself or with carbonyl compound. Reaction with carbonyl compound (3) to form a monomeric ozonide (5) as the major product and polymeric ozonides as minor products usually predominates if (3) is an aldehyde. The zwitterion (2) generally dimerizes to form 6 or polymerizes when 3 is a ketone, less susceptible to nucleophilic attack [11].

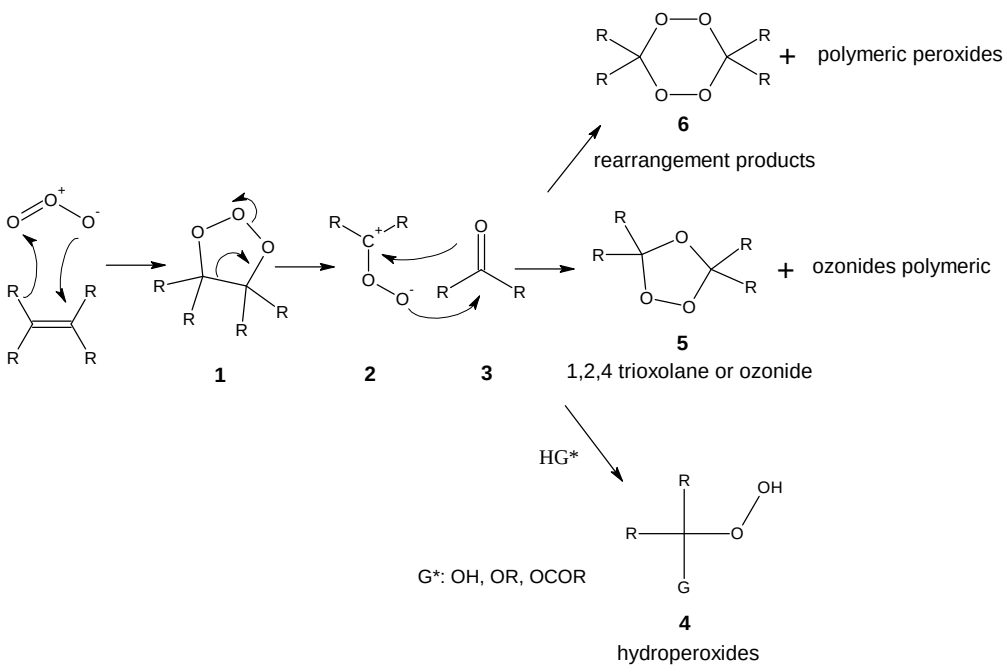

Figure 1: Mechanism of ozonolysis proposed by Criegee [11].

The chemical reactions of ozone when bubbled into oil are very complex. The

analyses of these reactions provide information on the functional group change during ozonation as well as the identification of the products without use of prior separation techniques [41].

The yield of products depends on reaction conditions, such as temperature, time, ozone generator, reactor type and ozone concentration [12]. Referring to ozonolysis of vegetable oils, many oils such as olive [28], canola [37], sunflower [28, 40, 42, 43], sesame [12, 44] and coconuts [45] have been investigated.

Analyses FT-IR, NMR ^{1}H and ^{13}C confirm structural changes undergone by oil during the ozonation. In IR spectra, the bands corresponding to both C=C (1654 cm^{-1}), =C-H (3009 cm^{-1}) stretching and to ozonide C-O stretching (1105 cm^{-1}) are the most important. The intensity of the bands corresponding to the double bonds (C=C) decrease and the band that identify the formation of ozonides increase with respect to the time reaction [12, 46, 47].

The main reaction is the formation of 1,2,4-trioxolane rings, that can be identified by signals observed at ^{1}H NMR spectra with characteristic chemical shifts, like 5.17-5.08 ppm (protons on oxolane ring carbons), at 1,63 (methylene protons α to oxolane ring) and at 1.35 ppm (methylene protons β to ring carbons) [44, 48, 59, 50]. In ^{13}C NMR spectra, oxolane ring carbons have chemical shift at about 103-104 ppm.

Almeida et al. [43] perfomed the ozonolysis reaction in sunflower oil under different conditions and the product is in the process of patent. The IR and ^{1}H and ^{13}C NMR of ozonized oils confirm the formation of 1,2,4-trioxolane ring according to the mechanism proposed by Criegee [43].

Díaz & Gavín [51] studied the products of ozonated methyl linoleate (7) using ^{1}H, ^{13}C and 2DCOSY RMN spectroscopy. Figure 2 shows oxygenated compounds along with their molecular weight that could possibly be obtained in the reaction. All functional groups of the products were well characterized as ozonides (8), hydroperoxides (9) and aldehydes (10) present in ozonized methyl linoleate [51].

In the absence of any participating solvent, the cyclic intermediate (1) called malozonide (Figure 1) leads to the formation of 1,2,4-trioxolanos and peroxide oligomer [47].

Soriano & collaborators [47] performed the decomposition of linoleate and oleoate in neat sunflower oil and in presence of water. In the IR spectrum of ozonated oil in presence of water showed a band at 3471 cm^{-1}, due to the presence of OH groups. According to the Criegee mechanism, water reacts the intermediate carbonyl oxides to give hydroxyl-alkyl-hydroperoxides, thereby preventing the formation of 1,2,4-trioxolane.

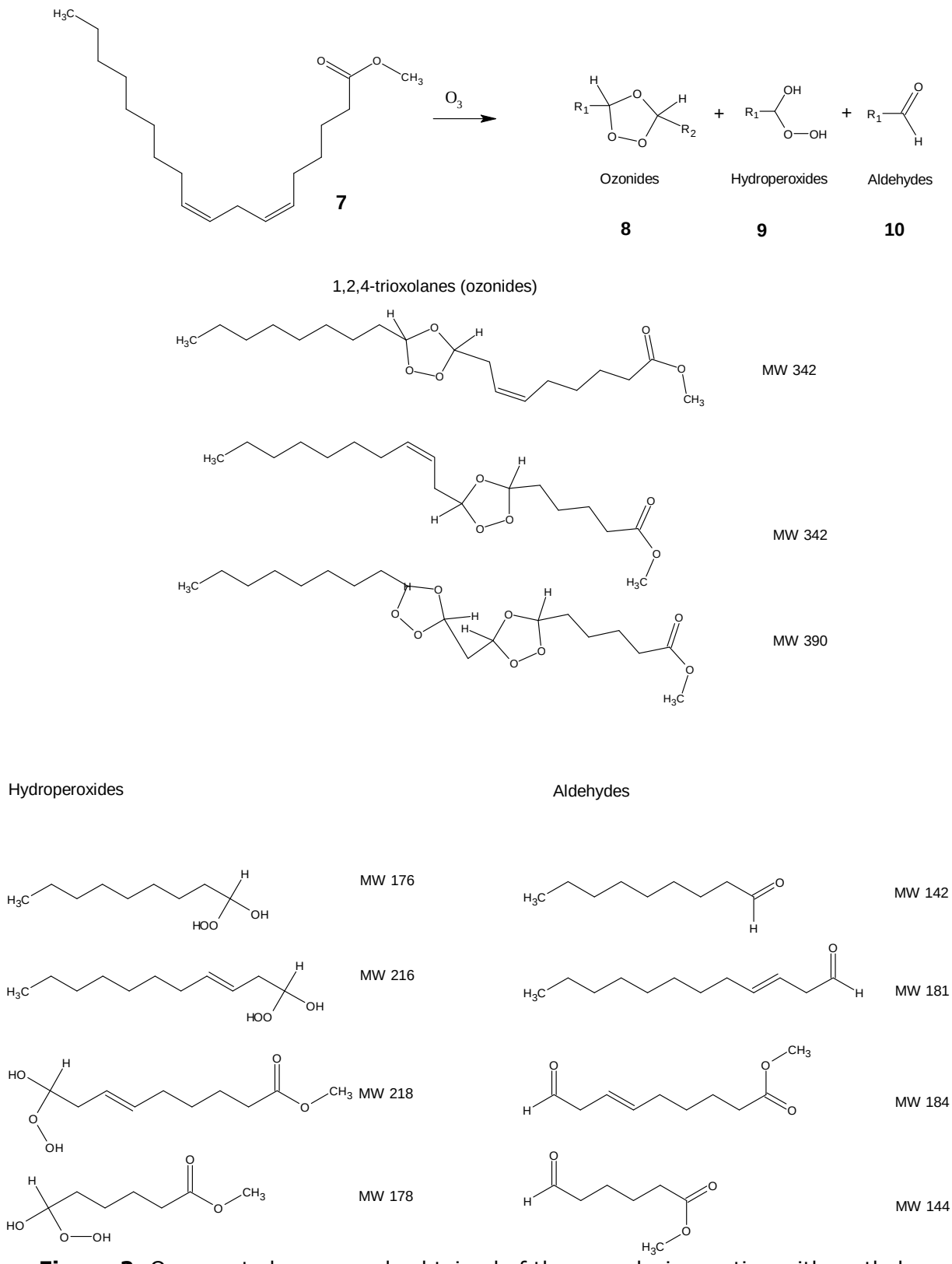

Figure 2: Oxygenated compounds obtained of the ozonolysis reaction with methyl linoleate [51].

The presence of α-hydroxyl-alkyl-hydroperoxides would be evidenced in ^{1}H NMR spectrum, with signals for protons that was expected to resonate between 4.9 and 5.1

ppm [52]. What was not observed by Soriano et al. [46]. The hydroxihydroperoxides may lose hydrogen peroxide to give aldehyde or rearrange to carboxylic acids.

Acording to Diaz et al. [45], the ozonization of coconut oil in presence of water leads to higher ozonides formation. When ethanol is added to the reaction of coconut oil with ozone, higher peroxide decomposition occurs and this favors formation of acids and aldehyde [45].

The study of the physico-chemical properties of ozonated vegetable oils has great importance for their characterization and identification. For determining the quality of ozonized products, analytical methods such as peroxide, acidy and iodine values are usually carried out [12, 38, 53].

The peroxide value (PV) represents the quantity of peroxide in the sample; acid value (AV) represents the present free acids; and iodine value (IV) is a measure of total number of double bonds in the sample. All values are well described according to the European pharmacopoeia [54, 55, 56] and Official Methods of Analysis of the Association of Official Analytical Chemists [57, 58].

The peroxide value represents the quantity of peroxide expressed in milliequivalents of active oxygen contained in a 1000 g sample. In the case of materials characterizated by a high peroxide content, some authors determined the PV introducing changes into the method described in the official monograph due the slow iodide reactivity with diallylperoxides [12, 59]. In accordance with the official methods of analysis, after addition of potassium iodide, the sample is allowed to stand for 1 minute so that the peroxide oxidizes iodide to iodine. During the ozonolysis of sunflower oil, polymeric peroxides and other organic peroxides have been formed [60], and due the high concentration of peroxides a long reaction time is required for these compounds the oxidize iodide to iodine [39]. Some methods include increased reaction time and reflux until 60 ºC. Peroxide content of ozonated sunflower oil using iodometric assay achieved the maximum values at 24 hours of reaction time [39].

Other difficult found in the iodometric assay is susceptible to interference by molecular oxygen as well as the reaction of liberated iodine with other components in the systems [61].

According to a Cuban patent (U.S. Pat No. PI 0309256-1 A), published in 2005, the ozonolysis reaction was continued until obtaining peroxide value between 600-800 units and acid number less than 15 mg/g for the sunflower oil; peroxide value between 1000-1200 units and acid value below 30 mg/g for cacao oil, used in the preparation of therapeutic and cosmetic creams formulations [62].

The acid value of ozonized oils does not directly indicate the oil quality or process

of rancidity [12]. An increase in acid value was observed in several works as increasing the reaction time [12, 44, 63], that may be due to acid formation during the ozonation and due to peroxide decomposition.

For the ozonated oils, the iodine value showed a decrease in relation to applied ozone dose. Ozone reaction with the unsaturated fatty acids led to rapid decrease of iodine values [12, 63].

Antimicrobial property of ozonated oils

Ozone germicidal action was widely proved on a broad group of microorganisms, including Gram-positive and Gram-negative bacteria as well as fungi spores and vegetative cells [64].

Telles Silveira et al. [65] compared antimicrobial activities of ozone and sodium hypochlorite on *Enterococcus sp* (Gram positive bacteria), which are often found in hospital sewage. Ozone was more effective than sodium hypochlorite against these microorganisms, even on Vancomycin-resistant Enterococcus.

Inactivation of bacteria by ozone is a complex process once ozone attacks numerous cellular constituents, including proteins, unsaturated lipids and respiratory enzymes in cell membranes, peptidoglycans in cell wall, enzymes and nucleic acids in the cytoplasm, and proteins and peptidoglycan in spore coats and virus capsids. Some authors concluded that molecular ozone is the main inactivator of microorganisms, while others emphasize the antimicrobial activity of the reactive by-products of ozone decomposition such as OH^-, O_2^-, and $OH^\cdot$ [66-68].

Chlorine acts specifically by diffusion through the cell wall, acting on the vital elements within the cell, such as enzimes, protein, DNA and RNA. Unlike chlorine, the ozone may oxidize various components of cell envelope including polyunsaturated fatty acids, membrane-bound enzymes, glycoproteins and glycolipids, leading to leakage of cell contents and eventually causing lysis [69]. It acts directs on the cell wall, causing rupture and death in a short contact time, preventing the recovery of microorganisms of the attack [70].

Bactericidal, fungicidal and virucidal properties of ozone are attributed to its ability to destroy many of the enzymatic structures. Naturally each microorganism has specific sensitivity to ozone. Bacteria are more sensitive than yeast and fungi. As a result of differences in structure of the cells walls, Gram positive bacteria are more sensitive to ozone than Gram negative ones [20, 64, 71].

Probably, when stable ozonide comes into contact with the warn exudate of the wound, it slowly decomposes in different peroxides, that can explain the prolonged antimicrobial and stimulatory activity of tissue repair [24].

Ozonized sunflower oil has a wide antimicrobial spectrum showing inhibition and lethal activity on gram positive and gram negative bacteria resistant to antibiotics, as *Mycobacterium* species, yeast of the gender Candida and some protozoa like *Giardia lamblia* [25, 27, 72, 73].

The ozonized olive and sunflower oils show activity against *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 10536, *Bacillus subtilis* ATCC 6633 and *Pseudomonas aeruginosa* ATCC 27853. The olive and sunflower ozonized oil with peroxide value of 2439 and 2506 mmol-equiv. Kg^{-1} respectively, showed Minimum Inhibitory Concentrations (MICs) of 0.95 mg Ml^{-1} for all tested bacteria. Diaz et al. [28] research indicates that at higher peroxide value, higher antimicrobial activity of ozonized sunflower oil.

Because of the antimicrobial properties of ozonized vegetable oils, there are many patents describing their use for the treatment of infectious diseases such as dermatitis, acne, ulcers, sores, burns and other skin lesions [74-77], treatment of asthma [78], use as a laxative and for treating intestinal infections, where they act against pathogenic intestine microorganisms [79], therapy for gastroduodenal ulcers [78] and to treat *Giardia lamblia* infections [80]. Recently their use has been described for the treatment of infections caused by pinworms, genital herpes simplex, human papilloma virus (HPV), and fungi, such as microorganisms of the genus *Candida* [38].

Conclusion

Ozonetherapy, ozonized vegetable oils are an interesting alternative for investigation. The availability of vegetable oils and fatty materials in Brazil and the ease of obtaining these products, in addition to low operating costs and high cost-benefit ratio allow the use of ozone and related products in the treatment of various diseases, especially chronic wounds. Ozonized oils, containing 1,2,4-trioxolane rings formed in unsaturated fatty acid chain, can be considered as an active principle and a vehicle at the same time, enhancing absorption and skin penetration. Using and/or synthesizing natural molecules or modified bioinspired products are rational strategies for achieving adhesiveness, biological and positive immunological activities, to control drugs' release and to promote easier penetration into normal skin. The technology for oils and fats modifications considers traditional knowledge for the synthesis of low cost and innovative products, with new applications due to biological activity resulting from generation of radicals and oxidizing species. Possessing extensive biological activity with reduced collateral effects these products are an alternative to use of available resources, natural and renewable sources, using simple low cost technology.

Acknowledgments

The authors would like to thank Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul (FUNDECT), Coordenação de Aperfeiçoamento de Pessoal de Nivel Superior (CAPES) and CNPq (Brazil) for their scholarships and financial support.

References and Notes

[1] Mandelbaum, S. H.; Di Santis, E. P.; Mandelbaum, M. H. S. *An. Bras. Dermatol.* **2003**, *78*, 525. [Link]

[2] Mandelbaum, S. H.; Di Santis, E. P.; Mandelbaum, M. H. S. *An. Bras. Dermatol* **2003**, *78*, 393. [Link]

[3] Bocci, V. *Arch. Med. Res.* **2006**, *37*, 425. [CrossRef]

[4] Barata, E. A. F. A Cosmetologia: princípios básicos. São Paulo: Tecnopress, 2003.

[5] Baumann, L. Dermatologia Cosmética: princípios e prática. Rio de Janeiro: Revinter, 2004.

[6] Robinson, J. R.; Lee, V. H. Transdermal Therapeutic Systems. 2nd ed. 29, 1987, 182.

[7] Patel, H. R.; Patel, R. B.; Patel, G. N.; Patel. M. M. *East Cent. Afr. J. Pharm. Sci*. **2010**, *13*, 19. [Link]

[8] Leonardi, G. R. Cosmetologia Aplicada. 2ª ed. São Paulo: Livraria e Editora Santa Isabel, 2008.

[9] Gomes, R. K. Cosmetologia: descomplicando os princípios ativos. 3ª. ed. São Paulo: Livraria Médica Paulista, 2009.

[10] Castro, H. F.; Mendes, A. A.; Santos, J. C.; Aguiar, C. L. *Quim. Nova* **2004**, *27*, 146. [CrossRef]

[11] Bailey, P. S.; Ozonation in Organic Chemistry, Volume 1 Olefinic Compounds. New York: Academic Press, 1978.

[12] Zanardi, I.; Travagli, V.; Gabbrielli, A.; Chiasserine, L.; Bocci, V. *Lipids* **2008**, *43*, 877. [CrossRef]

[13] Valacchi, G.; Fortino, V.; Bocci, V. *Br. J. Dermatol.* **2005**, 153,1096. [CrossRef]

[14] Giunta, R.; Coppola, A.; Luongo, C.; Sammartino, A.; Guastafierro, S.; Grassia, A.; Giunta, L.; Mascolo, L.; Tirelli, A.; Coppola, L. *Ann. Hematol*. **2001**, *80*, 745. [CrossRef]

[15] Franken, L. The application of ozone technology for public health and industry. *Food Safety & Security at Kansas State University*. 2005

[16] Kunz, A.; Freire, R. S.; Rowedler, J. J. R.; Mansilla, H.; Rodriguez, J.; Duran, N.; *Quim. Nova* **1999**, *22*, 425. [CrossRef]

[17] Stroker, G. *Lancet* **1916**, *188*, 712. [CrossRef]

[18] Stroker, G. *Lancet* **1917**, *189*, 797. [CrossRef]

[19] Oliveira, J. T. C.; Revisão sistemática de literatura sobre o uso terapêutico do ozônio em feridas. [Master's thesis.] São Paulo, Brazil: Escola de Enfermagem, Universidade de São Paulo, 2007.

[20] Skalska, K.; Ledakwicz, S.; Perkowski, J.; Sencio, B. *Ozone: Science & Engineering*

2009, *31*, 232. [CrossRef]

[21] Bocci, V. Ozonoterapia Comprensione dei meccanismi di azione e possibilitá terapeutiche. 1st edn. Casa Editrice Ambrosiana, Milan, 2000.

[22] Cardoso, C. C.; Macêdo, S. B.; Carvalho, J. C. T. *Farm. Ter. (Int. J. Drug Ther.)* **2002**, *19, 56.*

[23] Sánchez, G. M.; Re, L.; Perez-Davison, G.; Delaporte, R. H. *Revista Espanola de Ozonoterapia* **2012**, *2*, 121. [Link]

[24] Travagli, V.; Zanardi, I.; Valacchi, G.; Bocci, V. *Mediators of Inflammation* **2010**, Article ID 610418. [Link]

[25] Lescano, I.; Nuñez, N.; Gutierrez, M.; Molerio, J.; Rigüeifeiros, M. G.; Diaz, W. *Rev. CENIC, Cienc. Biol.* **1996**, *27*, 46.

[26] Lescano, I; Nuñez, N.; Espino, M.; Gomez, M. *Ozone Sci. Eng*. **2000**, *22*, 207. [CrossRef]

[27] Sechi, L. A.; Lezcano, I.; Nuñez, N.; Espim. M.; Dupre, I.; Pinna, A. *J. Appl. Microbiol*. **2001**, *90*, 279. [CrossRef]

[28] Díaz, M.F.; Hernández, R.; Martínez, G.; Vital, G.; Gómez, M.; Fernández, H.; Garcés, R. *J. Braz. Chem. Soc.* **2006**, *17*, 403. [CrossRef]

[29] Bocci, V.; Oxygen Ozone Therapy: A critical Evaluation. Kluwer Academia Publischers: Dordrecht, The Netherlands, 2002.

[30] Available from: http://www.ozonatedoils.com/index.htm. Access November, 2012.

[31] Available from: (http://www.ozonosan.de/ozonidcreme_23.htm, Access October, 2012.

[32] Available from: http://www.naturozone.com/. Access October, 2012.

[33] Available from: http://www.innovares.com/product/ozonia-10/. Access October, 2012.

[34] Firestone, D. Physical and Chemical Characteristics of Oils, Fats and Waxes, 2th. AOCS, 2006.

[35] Scrimgeour, C. Chemistry of Fatty Acids. In: Bailey's Industrial Oil and Fat Products. 6 ed. vol 1. John Wiley & Sons, Inc. 2005.

[36] Omonov, T. S.; Kharraz, E.; Curtis, J. M. *J. Am. Oil Chem. Soc*. **2001**, *88*, 689. [CrossRef]

[37] Bailey, P. S. Ozonation in Organic Chemistry. Volume 1, Olefinic Compounds. New York: Academic Press, 1978, p220.

[38] Mirabal, J. M.; Cespero, S. A. M.; Rubi, V. F. D.; Garcia, L. A. F.; Lozano, O. E. L.; Gomes, M. F. D.; Lastre, I. D. L. WO 03/085072 A1

[39] Tellez, G. M.; Lozano, L. O.; Gomes, M. F. D. *Ozone Sci. Eng*. **2006**, *28*, 181. [CrossRef]

[40] Díaz, M. F.; Gavín, J. A.; Gómez, M.; Curtielles, V.; Hérnandez, F. *Ozone Sci. Eng*. **2006**, *28*, 59. [CrossRef]

[41] Criegee, R. *Angew. Chem. Int. Ed*. **1975**, *14*, 745. [CrossRef]

[42] Díaz, M. F.; Sazartonil, J. A. G; Ledea, O.; Hérnandez, F.; Alaiz, M.; Garces, R. *Ozone Sci. Eng*. **2005**, *27*, 247. [CrossRef]

[43] Almeida, N. R.; Oliveira, P. D. ; Arruda, E. J.; Lima, D. P.; Beatriz, A. In: Abstract

of IV Workshop Norte, Nordeste e Centro Oeste de Síntese Orgânica, Bonito, Brasil. *Orbital Elec. J. Chem.* **2012**, *4 (Suppl. 1),* 33. [Link]

[44] Sega, A.; Zanardi, I.; Chiasserini, L.; Gabbrielli, A.; Bocci, V.; Travagli, V. *Chem. Phys. Lipids*. **2010**, 163, 148. [CrossRef]

[45] Díaz, M. F.; Núñez, N.; Quincose, D.; Díaz, W.; Hernández, F. *Ozone Sci. Eng*. **2005**, *27*,153. [CrossRef]

[46] Soriano Jr., N. U.; Migo, V. P.; Matsumura, M. *J. Am. Oil Chem. Soc.* **2003**, *80*, 997. [CrossRef]

[47] Soriano Jr., N. U.; Migo, V. P.; Matsumura, M. *J. Chem. Phys. Lipids*. **2003**, *126*, 133. [CrossRef]

[48] Díaz, M.; Hernandez, F.; Álvarez, I.; Vélez, H.; Ledea, O.; Molerio, J. *Rev. CENIC C. Quim.* **1998**, *29*, 89.

[49] Ledea, O.; Molerio, J.; Díaz, M.; Jardines, D.; Rosada, A.; Correa, T. *Rev. CENIC C. Quim.* **1998**, *29*, 75.

[50] Wu, M.; Church, D. F.; Mahier, T. J.; Barker, S. A.; Pryor, W. A. *Lipids* **1992**, *27*,129. [CrossRef]

[51] Díaz, M. F.; Gavín, J. A.; *J. Braz. Chem. Soc.* **2007**, *18*, 513. [CrossRef]

[52] Rebrovic, L. *J. Am. Oil Chem. Soc.* **1992**, *69*, 159. [CrossRef]

[53] Sadowska, J.; Johansson, B.; Johannessen, E.; Friman, R.; Braniarz-Press, L.; Kosenholm, J. B. *Chem. Phys. Lipids.* **2008**, *151*, 85. [CrossRef]

[54] European pharmacopoeia. Acid Value. Concil of Europe, 5th edn. Strasbourg Cedex, France, pp 127.

[55] European pharmacopoeia. Iodine Value. Concil of Europe, 5th edn. Strasbourg Cedex, France, pp 127-128.

[56] European pharmacopoeia. Peroxide Value. Concil of Europe, 5th edn. Strasbourg Cedex, France, pp 128-129.

[57] AOAC. Official Methods of Analysis of the Association of Official Analytical Chemists. 40th ed. Washington, **1984**.

[58] AOCS. Official Methods and Recommended Practices of the American Oil Chemists Society. 3th ed. Champaign, v. 1-2, **1995.**

[59] Richaud, E.; Farcas, F.; Fayolle, B.; Andouin, L. *Verdu. J. Polym. Test*. **2006**, *25*, 829. [CrossRef]

[60] Ledea, O. Estio de la composicion química del aceite de girassol ozonizado OLEOZON registrado. PhD. Thesis. National Center for Scientific Research, Havana City, 2003.

[61] Nourooz-Zadeh, J.; Tajaddin-Sarmadi, I.; Brilorez-Arazon; Wolff, S. *J. Agri. Food Chem.* **1995**, *43*, 17. [CrossRef]

[62] Mirabal, J. M.; Menéndez, S. A. C.; Ledea, O. E. L.; Gómez, M. F. D.; Rubi, W. F. D.; Garcia, L. A. F.; Lastre, I. L. M. L. BR 0309246-1A, 2005.

[63] Díaz, M. F.; Hérnández, R.; Martinez, G.; Vidal, G.; Gomez, M.; Fernandez, H.; Garces, R. *J. Braz. Chem. Soc.* **2006**, *17*, 403. [CrossRef]

[64] Guzel-Seydim, Z. B.; Greene, A. K.; Seydim, A. C. *Food Science and Technology* **2004**, *37*, 453. [Link]

[65] Silveira, C. I. T.; Monteggia, L. O.; Pires, M.; Barth, A. Ozone desinfection of multiressistent bacteria isolated from hospital effluent. IOA 17th Ozone World Congress – Strausbourg, **2005**.
[66] Khadre, M. A.; Yousef, A. E.; Kim, J. G. *J.Food Sci.* **2001**, *66*, 9. [CrossRef]
[67] Chang, S. L. *J. Sanitation Eng. Div.* **1971**, *97*, 689.
[68] Harakeh, K.; Butler, M. *Ozone Sci. Eng*. **1985**, *6*, 235. [CrossRef]
[69] Scott, D. B. M.; Lesher, E. C. *J. Bacteriol.* **1963**, *85*, 367.
[70] Snatural Tecnologias Ambientais LTDA. Ozônio. Available from: http://www.snatural.com.br/Ozonio.htm. Access: 24 October 2012.
[71] Pascoal, A.; Llorca, I.; Canut, A. *Trend Food Sci. Technol*. **2007**, *18*, S29. [CrossRef]
[72] Lescano, I.; Molerio, J.; Rigüeifeiros, M. G.; Contreras, R.; Roura, G. Diaz, W. *Rev. CENIC, Cienc. Biol.* **1998**, *29*, 209.
[73] Díaz, M.; Lescano, I.; Molerio, J.; Hernandez, F. *Ozone Sci Eng*. **2001**, *23*, 35. [CrossRef]
[74] DeVillez, R. L. US 4451480 A, 1984.
[75] DeVillez, R. L. US 4591602 A, 1986.
[76] Twombly, A. H. US 984722 A, 1911.
[77] Molareda, M. A. G.; Dall'Aglio, R.; Melegari, P. WO 0137829 A1, 2001.
[78] Neel, W. D. US 925590 A, 1909.
[79] Knox, W. J. US 1210949 A, 1917.
[80] Mirabal, J. M.; Cepero, S. A. M.; Menéndez, L. E.; Rubi, W. F. D.; Garcia, L. A. F.; Regueiferos, M. C. G. CU 22749 A1, 2002.