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Chapter 5

OZONIZED VEGETABLE OILS: PRODUCTION, CHEMICAL CHARACTERIZATION AND THERAPEUTIC POTENTIAL

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ABSTRACT

Ozonated vegetable oils have demonstrated promising results for clinical application, and they have been the focus of great pharmaceutical interest to treat dermatological disorders, such as infections of skin ulcers and chronic wounds. There are reports of these products as effective to heal refractory wounds, where conventional treatments and available medications prove ineffective. In fact, in some European countries, such as Germany, they can be obtained on prescription from pharmacies. Countries such as Cuba have developed commercial ozonated oils, and they have been successfully tested to treat many diseases. Cuba is one of the pioneers in the implementation of this therapy in Public Health Services for over 22 years. Ozone reacts with the double bonds of unsaturated fatty acids of vegetable oils, providing stable ozonation products, mainly ozonides, hydroperoxides and polyperoxides (depending on reaction conditions) with therapeutic potential. Several studies have demonstrated their antimicrobial and antifungal activity, as well as their role as wound healing modulators, showing no cytotoxicity when tested against NIH/3T3 murine fibroblast cells. Simple analytical techniques such as peroxide value, iodine value and viscosity determination have been extensively used for characterization of products, together with spectroscopic techniques of NMR (^{1}H and ^{13}C) and infrared, chromatography and thermal analysis (DSC). This chapter aims to highlight recent contributions to the production, characterization and biological activities of ozonated vegetable oils.

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Keywords: ozonized vegetable oil, ozonides, antimicrobial activity

INTRODUCTION

Oils or vegetable fats are lipids (glycerol esters) extracted from seeds, nuts and native fruits or short crop cycle or long/perennial. They can be extracted by mechanical (molding) or by chemical solvent extraction or a combination of these methods [1].

The oils are products rich in monounsaturated fatty chains and poly-unsaturated acids, antioxidants, fat-soluble vitamins and other components in minor proportions. Vegetable oils have low allergenic capacity, are nontoxic and used as fuel, food, functional product due to the intrinsic therapeutic properties or structural, cosmetic changes, industrial and pharmaceutical uses [2].

The cutaneous application of vegetable oil as a therapeutic practice and dietary coadjuvant has been described mainly in adult patients at risk for essential fatty acid deficiency [3]. Vegetable oils and volatile oils are able to increase the potential penetration of different drugs. Formulations with different vegetable oils for medicinal purposes facilitates transdermal permeation, mainly due to entrainment efficiency and bioavailability [4]. The concentration of vegetable oils in the formulations increases permeation and this is attributed to the fatty acids content as confirmed by studies on release kinetics and transdermal permeation [4, 5].

Modification of unsaturated fatty acids by ozonation at a different time is an option to carry out the synthesis of new drugs and/or preparation of different pharmaceutical forms. The ozonation fatty acids and chemical transformation allow preparation of a single product carrier which is bioactive and at the same time for multiple purposes. Unsaturated vegetable oils can be ionized or produced as oleogel for direct filling. The oleogel has spreadability, adhesiveness and melting temperature close to body temperature. Assays with gram-positive and gram-negative resistant bacteria strains and tumor cells showed a biological activity of vegetable oils and their applicability for wound healing and treatment of chronic wounds. This chapter discusses the synthesis, mechanisms, characterization and biological activity of ozonated vegetable oils for healing chronic wounds.

The chemical synthesis for obtaining ozonized vegetable oils using unsaturated oils, bubbling a gas mixture (O_2/O_3) of a porous ceramic for different periods of time in a reactor. The preparation of oleogel is by its deposition on a water bed (up to 10%) in contact with the porous ceramic to distribute the gas and O_3 reaction with unsaturated vegetable oil fatty acids to form ozonides and lipoperoxides (aldehydes, ketones, peroxides) [6]. Ozone reaction with unsaturated constituents of vegetable oils occurs exclusively with the carbon-carbon double bonds of unsaturated acids and ozonides to produce lipid peroxides. Various oxygen compounds are formed depending on the ozonation time: aldehydes, diperoxides and polyperoxides; which are partly responsible for part of the biological activity of ozonated oils [6, 7].

Despite simplicity of the reaction which normally uses direct ozone bubbling in the vegetable oil, the quality and process reproducibility for obtaining oils patterns depend on the quality and purity from vegetable oils and other process parameters, such as: (i) the type of ozone generators; (ii) the conditions of ozonation, reactor, ozonation time, reactor material

and process scale iii) the presence of water or catalysts; (iv) the efficiency of the ozonator, flow and concentration of O_3 carrier gas/diluent and yet the purity of oxygen used in addition to other parameters [8].

The characterization of the species produced in the ozonation needs to be performed, as well as details of the reaction kinetics at different process times. The physicochemical properties of the products obtained in different ozonation times should also be assigned. Accordingly, the quality of ozonized products must be checked by various spectroscopic and chromatographic techniques and/or classical analytical techniques for the complete characterization and accurate quantification of ozonized products. The most widely used techniques in the ozonation are Fourier Transformed Infrared (FT-IR), $^{1}H/^{13}C$-NMR and analytical methods for the determination of peroxide levels, acidity, viscosity and iodine value [9].

The ozone and ozonized product from the ozone reaction with fatty acids and other substrates have intense biological activity (bactericidal, virucidal, etc.). Ozone disinfectant properties are well revealed in the use of ozonated vegetable oil. Ozonated oil was found to have antiseptic activity. These products stimulate the immune system and act on the healing and tissue repair. The biological activity of the product stability and performance in different formulations allow its use in pre-clinical and clinical testing [10]. Unsaturated fatty acids are essential components of vegetable oils and cellular membranes, therefore, the effects of their ozonation have been widely studied. In the literature, many studies are available using different vegetable oils, especially in topical applications (external use), although there are reports of the oral administration. In these studies, the majority of vegetable oils used for these different purposes are the olive and sunflower oils. However, different vegetable oils or essential oils can be used for producing ozonized products for different cosmetic or therapeutic applications. Ozonized commercial oils are available for a variety of applications. Unsatured oils are best known and available for nutritional purposes [11]. The composition of unsaturated fatty acid are chemical intermediates for ozonation and production of several products for treating of skin (acne, herpes, wrinkle, psoriasis, cracking and peeling), treatment and healing of chronic wounds and other applications. Some examples of such oils are the sunflower, thyme, sesame, soybean, coconut, olive, hemp seed, grape seed, jojoba, sweet almond, avocado, flax seed, and others.

Currently, to treat skin diseases, there are a variety of anti-infective agents available. Some products of these commercial products for topical use have become inefficient due to the emergence of resistance of pathogens. The ozone has strong biological activity, eliminate pathogens from the release of oxygen species, while active fibroblast proliferation, induces the reconstruction of the intercellular matrix, keratinoblasts proliferation, and wound healing. Accordingly, new products have been proposed for the synthesis and its proper use to meet the demands of the pharmaceutical industry.

In this scenario, the use of ozone and vegetable oils allow the production of new assets that are both bioactive and vehicle with excellent antibacterial properties, fungicides and, with a healing property. The ozone is an unstable molecule with intense biological activity by its decomposition into singlet oxygen. This species is highly reactive to pathogens (viruses, bacteria, protozoa and other microorganisms) and can degrade natural or synthetic compounds and their metabolites residues. The produced ozonated derivatives of unsaturated oily substrates allow the production of a multiplicity of commercial products of wide application. The control difficulties of the oxidative process in the production and topical

application, stability, and adequacy of the application form have been carried out by new techniques, pharmaceutical forms and strategies in the ozonation of vegetable oils, in addition to increasing the stability and conservation of characteristics of the obtained products [9].

Numerous diseases can lead to failure of organs, mucous membranes and arteries. This problem has been exacerbated with aging and many skin lesions are subject, ulcers and generalized infections with high socioeconomic cost. In this respect, Zanardi et al. (2013) [12] shows that ozonized oils are a low-cost option compared to conventional antibiotics for topical application to the reduction of the infection, and wound healing. The intense bactericidal and immunostimulant effect of these products accelerates healing. The study also shows that the removal of debris and exudates is a necessary increase in the bactericidal effect of the ozonated condition products [12].

The effect of the ozonization in olive oil, soybean oil, oleic-, linoleic- and linolenic acid was performed by Sadowskaet et al. [13]. The products obtained from different time ozonation were analyzed by different techniques: ^{1}H NMR [14], ^{13}C NMR. The amounts of peroxide and acid contents, viscosity and molecular weight were determined in pure and ozonized oils. Results showed that the chains of fatty acids showed a gradual decrease in the unsaturation level with increasing time of ozonation. The reaction products were identified by Criegee mechanism [15]. The produced ozonides and disappearance of the unsaturations were sequential, with increasing values of the indices peroxide and acid to the oils, the highest increases were observed for the soybean oil. The long time ozonation produced a number of products with different molecular weights were identified as oligomeric ozonides and cross-ozonides respectively [13].

A recent study by Moureu et al. [16] reported that ozonation conditions change the composition of ozonated oils, opening new perspectives for the application of ozonated oil. The study showed that the consumption of double bonds is the same carrying out the ozonation conditions with or without water. However, the emphasis has been pointed out that water increases the formation of peroxides and acid species. This result indicates that the bioactivity of the product and the bactericidal activity is better for ozonized oils in the presence of water and this biological activity is specifically related to the existence of peroxides species [16].

PRODUCTION AND CHEMICAL CHARACTERIZATION OF OZONIZED VEGETABLE OILS

Production of Ozonized Vegetable Oils

The ozone gas used to the reaction is generated from extra-dry oxygen (99.9%). Vegetable oils are ozonated in a semi-batch reactor or Drechsel bottle with a sintered filter at the bottom using ozone generator with the ozone concentration and gas flow defined. [17, 18]. Guerra-Blanco describes the use of ozone analyzer connected to a computer, capable of detecting ozone in gas phase outlet in the reactor. The ozone monitoring is used to measure the ozone consumption and degree of ozonation [17]. Despite there are highly sophisticated ozonolysis equipments, it is possible to build an ozone generator using parts easily obtained on the market and its performance is perfectly suitable for common laboratory work [19].

The efficiency of ozone reaction with ethyl oleate has been monitored by iodometry. The unreacted ozone emerging from the bubble reactor was trapped in a KI and determined each hour by titration with $Na_2S_2O_3$ (sodium thiosulfate) 0.1 M. Ozone reaction with ethyl oleate is nearly quantitative for several hours, and the unreacted O_3 emerges from the bubble reactor when the molar ratio $O_3/C=C \approx 1$ is reached [18].

The ozonation of different vegetable oils, such as sunflower, olive, grape, flaxseed, baru, coconut, sesame and canola oil under different conditions have been studied. However, the comparison between the results remains difficult due to the large amount of parameters influencing the reaction, such as ozone flow rate and concentration, and use of solvent. Table 1 shows some reactions conditions.

Ozone reacts with carbon–carbon double bonds unsaturated fatty acids (free and as esters in triglycerides), according to the mechanism described by Criegee [15] to form different oxygenated species such as ozonides, hydroperoxides, polymeric peroxides and aldehydes, depending on reaction condictions [16, 18].

Scheme 1 shows the mechanism of ozonolysis reaction described by Criegee in 1975 [15]. The first product of the reaction is called malozonide (1), which is very unstable and decomposes to give a zwitterion (2) and a carbonyl compound (aldehyde or ketone, depending of starting material) (3). In the absence of polar solvent, the zwitterion must react either with itself or with the carbonyl compound. Reaction with the carbonyl compound (3) to form a monomeric ozonide (4) as the major product and ozonides polymeric as minor products. The zwitterion (2) generally dimerizes to form 5 or polymerizes when 3 is a ketone, less susceptible to nucleophilic attack [11]. In the presence of protic solvents, 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.

Spectroscopy

Several techniques have been used to characterize ozonized vegetable oils. Spectroscopic methods are adequate because they make it possible to detect chemical changes that occur from the ozone reaction. Fourier transform infrared (FT-IR) and nuclear magnetic resonance (^{1}H and ^{13}C NMR) are used to study these reactions where the signals corresponding to reactant and product groups are well identified.

In FT-IR spectra of oils before ozonolysis reaction is observed characteristic bands of C=C double bond stretching (1651 - 1654 cm $^-$1) and C=C–H stretching (3009 cm$^-$1). Upon increasing reaction times, these bands diminished and a new band at 1005 - 1106 cm^{-1} appeared, attributed to C–O stretching of ozonides. [17, 23] Kogawa et al. and, other authors report that aldehyde bands were absent from the IR spectra of the ozonized oils [23, 26].

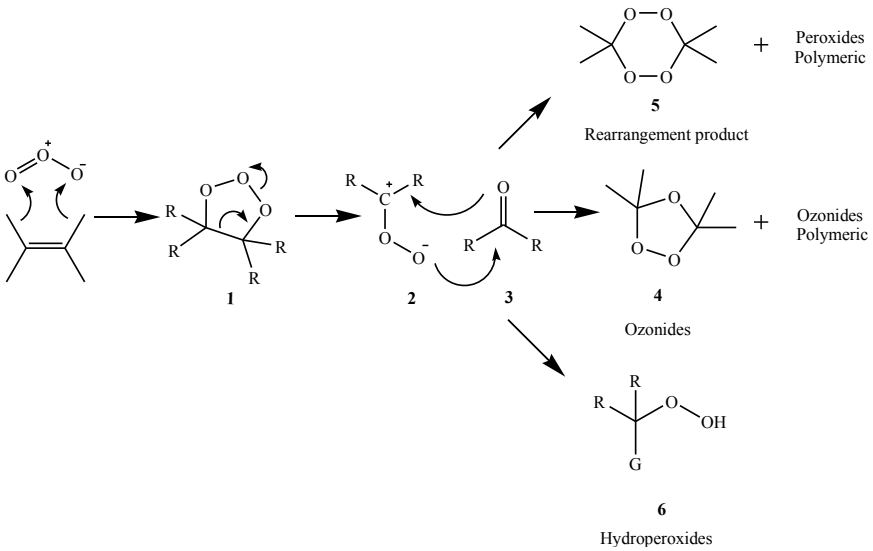
The FT-IR data for sunflower oil and ozonized sunflower oil of 24 hours shows absent bands of C=C–H stretching (3009 cm^{-1}) and appeared of C–O stretching of ozonides at 1106 cm^{-1}. ^{13}C NMR and Iodine Value described above confirmed the disappearance of double bonds [23].

The FT-IR spectrum of ethyl oleate before ozonolysis reaction presents two bands in this region at about 1117 and 1097 cm^{-1} attributable to the C-O stretching of the ester group. The strong ozonide band at about 1110 cm^{-1} gradually overlaps these two bands [18].

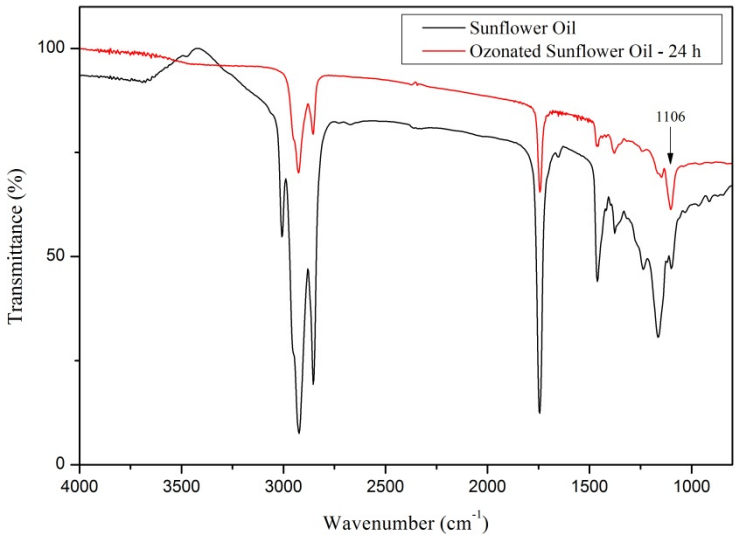
Oils are mainly formed of triglycerides, with different substitution patterns due to the length, degree and kind of unsaturation of the acyl groups, and by minor components such as mono- and di-glycerides. The chemical shifts of the triglycerides at ^{1}H NMR spectra are well known and presents the same standard for different vegetable oils [29].

Table 1. Ozononation reactions conditions

Author	Vegetable Oil	Sample/ solvent	Gas Flow	Ozone Concentration	Time of reaction
Soriano et al., [20]	Sunflower Oil	50 mL oil 50 mL oil/400 mL deionized water	0,5 L/min	33-39 g/m^3	Until Solidification
Díaz et al., [21]	Sunflower Oil	150 g oil	42 L/h	79,5 mg/L	2 h
Díaz et al., [22]	Sunflower Oil	192g oil/20 mL of water	72 L/h	81.6 mg/L	3,5, 7 h
Díaz et al., [7]	Sunflower and Olive Oil	80 mL oil/8 mL water	30 L/h	75,2 mg/L	8,05 h (Sunflower Oil) 5,73 h (Olive Oil)
Moureu et al., [16]	Sunflower Oil	50 g oil 50g oil/5g ultra pure water	30 L/h	65 mg/L	1-7 h
Guerra-Blanco et al., [17]	Sunflower and Grape Oil	9 g each oil	0,5 L/min	30 mg/L	5 h (Until complete reaction)
Kogawa et al., [23]	Sunflower Flaxseed and Baru Oil	200 mL each oil Sunflower Oil/Water 9% (v/v)	1L/min	60 µg/mL	6, 12, 24 and 36 h 24h
Díaz and Gavín, [24]	Methyl Linoleato	1,6 mL/ 0,16 mL water	42 L/h	69 mg/L	7,25 min
Cataldo, [18]	Ethyl Oleato	130 mL	3,5 L/min	0.0260 mol/h	1, 2, 6, 8,10 and 15 h
Díaz et al., [25]	Coconut Oil	150 g 150g/15 mL of water or ethanol	54 L/h	37,5 mg/L	74,4 min
Zanardi et al., [26]	Sesame Oil	40 mL	1,5 L/min	55 mg/L	15, 30, 60, 90 e 120 min
Sega et al., [27]	Sesame Oil	40 mL	1,5 L/min	45 mg/L	15, 30, 45, 60, 75, 90, 105, and 120 min
Omonov et al., [28]	Canola Oil	100g/600 mL ethanol, methanol and ethyl acetate.	6,5 L/min	50 g/m^3	90 min



Scheme 1. Criegee mechanism for the reaction of ozone with carbon-carbon double bonds.



(Source: authors)

Figure 1. IR spectra of sunflower oil and ozonized sunflower oil – 24 hours.

In the ^{1}H NMR spectrum obtained from sunflower oil, the signs of olefinic hydrogens were observed in the region between 5.20 to 5.39 ppm. The signals at 2.00 and 2.74 ppm correspond to the protons of the methylene (-C**H**$_2$-CH=CH-), and the methylene group across the double bonds (-CH=CH-C**H**$_2$-CH=CH-), respectively. The double doublets at 4.10 and 4.26 ppm are assigned to hydrogens of methylene group *sn*-1 and 3 positions of the glycerol moiety (Figure 2), these signals remain in the ozonized oil spectra, indicating that there not changes occur in the glycerol moiety during the ozonolysis reaction [30]. The methyl group terminal presented a difference in the chemical shift depends of acyl groups, for linolenic acyl at 0.9 ppm and other acyl groups at 0.8 ppm [29].

The ^{13}C NMR spectrum shows two signals, at 172 and 173 ppm, refers to carbonyl carbons esters, sp^2 carbons corresponding to the unsaturation of the fatty acids were identified as the signals between 127 and 130 ppm. The signals 62 and 68 ppm refer to the carbon linked to the oxygen of the glycerol moiety, CH_2 and CH respectively, and the signals between 34 and 22 ppm refer to other carbon ($-CH_2-$) present in the structure [30]. The Table 2 shows mainly chemical shifts of ^1H and ^{13}C NMR for vegetable oils.

More specific information is obtained by ^1H and ^{13}C NMR spectroscopy in order to identify the changes in the chemical structure that occur during the reaction. Guerra Blanco et al., [17] observed in the ozonized oil spectral data, four different types of signals. These types are identified as conserved, decreasing, increasing, and increasing/decreasing signals [17].

The conserved signals are those that do not suffer any modification after treatment with ozone. These signals correspond to the α- and β-hydrogens of carbonyl groups, some methylene, and methyl of acyl groups and glycerol moiety. Therefore, protons that are not in proximity to double bonds not change its chemical shifts. Signals corresponding to the hydrogen of the double bonds (sp^2) (C**H**=C**H**), and to vinyl hydrogen (C**H**$_2$–C=C–C**H**$_2$ and C**H**$_2$–C=C–CH_2–C=C–C**H**$_2$), (C=C–C**H**$_2$–C=C), show a decreasing after reaction [17].

New signal in ^1H NMR spectra of ozonized sesame oils at 5.15 ppm are attributed to the hydrogen of ozonide or 1,2,4 -trioxolane. Such evidence was confirmed by the appearance of a signal in ^{13}C NMR spectra at 104.33 ppm [26].

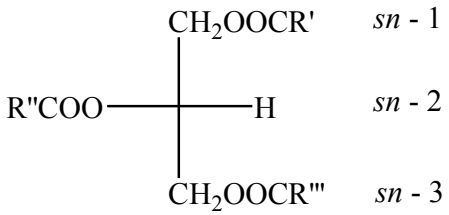
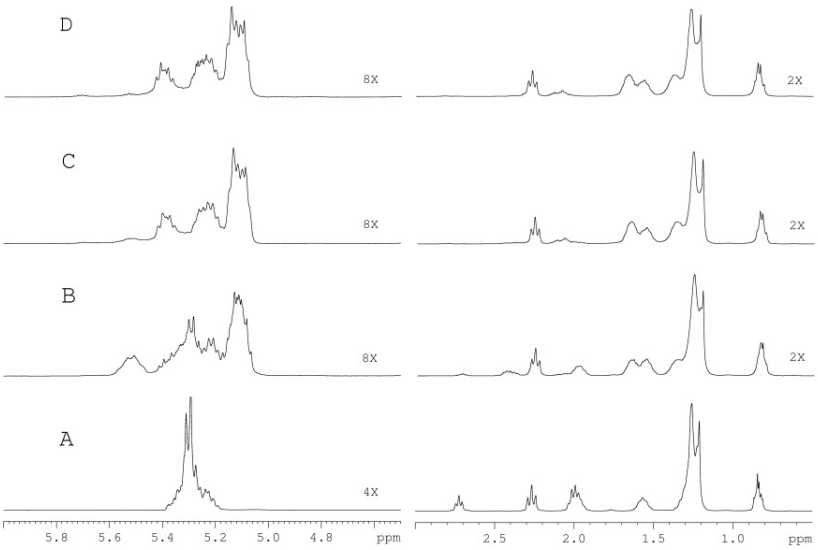


Figure 2. Glycerol moiety.

Table 2. ^1H and ^{13}C NMR data of vegetable oils (untreated) (Almeida et al. [30]; Sega et al. [27]; Guillén and Ruiz [29])

Moiety	^1H δ(ppm)	^{13}C δ(ppm)
$-CH_3$	0.90 – 0.80 (m)	14
$-(CH_2)_n-$	1.40 – 1.15 (m)	22 – 32
$-OCO-CH_2-CH_2-$	1.70–1.50 (bs)	24
$-OCO-CH_2-$	2.35–2.20 (t)	33-34
$-CH_2-CH=CH-$	2.10–1.90 (m)	27
$-CH=CH-CH_2-CH=CH-$	2.80–2.70 (t)	25
$-CH_2OCOR$ (*sn*-1 and 3 glycerol)	4.32 – 4.10 (dd, dd)	62
> CHOCOR (*sn*-2 glycerol)	5.26–5.20 (m)	68
–CH=CH–	5.40–5.26 (m)	127-130
–OCOR	--	172-173

bs: broad signal; m: multiplet; t: triplete, dd; doublet of doublet



(Source: authors)

Figure 3. ^{1}H-NMR spectra expansions from 0 to 3.0 ppm and 4.5 to 6.0 ppm for sunflower oil ozonized for different exposure times: (A) 0 h; (B) 12 h; (C) 24 h; (D) 36 h. 2x, 4x and 8x: larger by factor.

Díaz et al., [21] observed in spectra of sunflower oil additional signals at 9.74 ppm and 9.63 ppm (triplets from aldehydic hydrogens), 5.6 ppm (olefinic protons signal could arise from hydroperoxides), 5.15 ppm (multiplet from ozonides). In ^{13}C NMR, aldehyde carbons resonating from 199 to 203 ppm, methynic carbons corresponding to ozonides and hydroperoxides from 104 to 122 ppm, methylenic carbons belong to ozonides and hydroperoxides from 42 to 44 ppm, and 23 to 24 ppm belong to methylenic carbons vicinal of ozonides [21].

Figure 3 shows the ^{1}H NMR spectra of ozonized sunflower oil at different times of reaction. Multiplet at 2.0 (–C**H$_2$**–CH=CH–), a triplet at 2.7 ppm (–CH=CH–C**H$_2$**–CH=CH–) and multiplet at 5.2 (–C**H**=C**H**–) present prior to reaction initiation, were absent from the spectra obtained after 24 and 36 h of ozonolysis. With the formation of ozonides, new signals appeared at 1.66 and 2.08 ppm, corresponding to methylene groups at the α-position and between ozonides, respectively. The multiplet at 5.5 ppm in the spectrum of ozonized sunflower oil 12h (B, Figure 2) and its absence from other spectra (C and D, Figure 2) indicate the formation of a homoallylic ozonide, with chemical shift of the signal for the remaining double bond hydrogens from 5.3 to 5.5 ppm. Table 3 shows the main ^{1}H and ^{13}C NMR chemical shifts for the vegetable oils [23, 30].

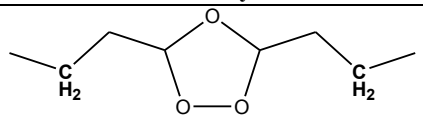
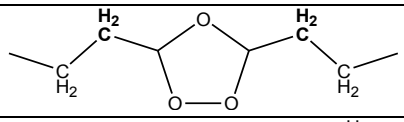
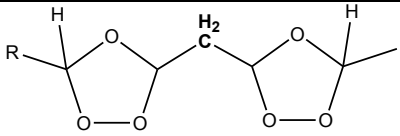
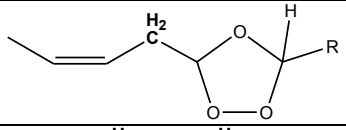
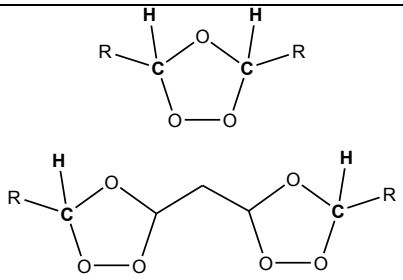
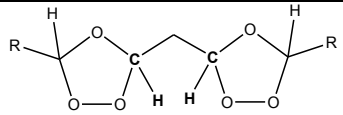
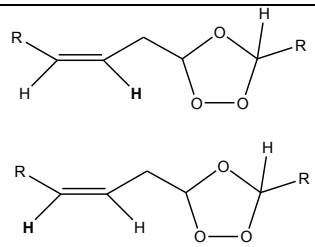
Physicochemical and Analytical Techniques

Some authors have remeasured the viscosity of sunflower [17], grape [17], sesame [26, 27] and coconut oil [25] and correlated viscosity increases with the ozonation degree increase. This observation is commonly explained by a decrease in the flexibility of ester chains because the total number of unsaturations has decreased, and by the formation of chemical species with higher molecular mass, which are assumed to be oligomers [25]. Modification of

the unsaturated acyl chains ozonation kinetic affects the mobility and the reactivity of the species involved in the reaction. This aspect is of particular importance in terms of product characterization and evaluation of the ozonation reaction [26].

The ozonized coconut oil in ethanol showed lower viscosity. This behavior might be due to the solubility of oil with the ethanol. When water is used in the systems the viscosity increases because the emulsion appears due to the poor solubility of oil in water [25].

Table 3. ^{1}H and ^{13}C NMR data of ozonized oils. (Guerra Blanco et al., [17]; Sega et al., [27]; Kogawa et al., [23])

Moiety	^{1}H δ(ppm)	^{13}C δ(ppm)
	1.3 – 1.4 (bs)	23-24
	1.6 – 1.7 (m)	30
	2,0 – 2.1 (m)	35 – 36
	2.3 – 2.5 (m)	41 – 42
	5.0 – 5.2 (m)	103 – 104
	5.2 – 5,3 (m) 5.3 – 5.4 (m)	100
	5.3 – 5,4 (m) 5,5 (m)	120 – 121 133 – 134

bs: broad signal; m: multiplet;

The study of the physicochemical properties of ozonized vegetable oils has a great importance for its characterization. Analytical methods such as peroxide, acidity, and iodine values usually carried out to follow-up the ozonization process and for determining the quality of ozonized vegetable oils [22].

The peroxide value (PV) represents the quantity of peroxide in the sample (meq $kg^{-}1$); acid value (AV) represents the present free acids (mg of KOH $g^{-}1$); and iodine value (IV) is a measure of total number of double bonds in the sample (g of iodide (100 g)$^{-}1$ of sample). All these values are well described according to the European pharmacopoeia [31, 32] and Official Methods of Analysis of the Association of Official Analytical Chemists [33].

The literature describes an increase of acid and peroxide value and a decrease of iodine according increase of time of ozonation [16, 23, 7, 26, 27]. Acid value increases with the reaction time, indicating that acids can be formed through decomposition of ozonides or directly during the reaction. From the investigated samples, oleogel exhibited the highest acidity value, which can be explained by the formation of other peroxidic compounds in the presence of water, such as hydroxy-hydroperoxides [23]. According to Moureu et al., the increase seems to be slower for the samples ozonized without water, which is significantly higher for the oils ozonized with water [16].

It is well-known that iodine value is a measure of the total number of double bonds present in an oil sample [32]. The results also showed a decrease in iodine values with longer reaction times. The ozonized baru oil presented iodine value of 0.71 g (100 g)$^{-}1$) after 24 hours of reaction. Nonetheless, flaxseed oil showed an iodine value of 44.61 g (100 g)$^{-}1$ after 24 hours, indicating the presence of double bonds, as observed by the signals at 136–120 ppm in the ^{13}C NMR spectra [23]. Zanardi et al., reported a decrease of iodine value of 113.65 to 13.39 with the greater ozone dose used (9,900 mg) in Sesame oil [26].

The determination of the amount of peroxide present in oils ozonized is of fundamental importance because ozonides are responsible for the biological activity of these substances [7]. The peroxide value is expressed in milliequivalents (mEq) of active oxygen the quantity of peroxide present in 1000 g of sample, and commonly determined by iodometrics techniques, due to the ability of these compounds to oxidize iodide to iodine. Then, iodine is titrated with sodium thiosulfate solution [30].

According to the literature, for samples with high concentration of peroxides as ozonized oils, the experimental procedure described by American Oil Chemists' Society (A.O.C.S.) [33] becomes inadequate. The one-minute time, after addition iodide is insufficient for the peroxides present in the sample oxidize iodide to iodine. The dialkylperoxides and peroxides high molecular weight react slowly with iodide. Therefore, changes such as temperature and reaction time may be performed to obtain better results [34, 35].

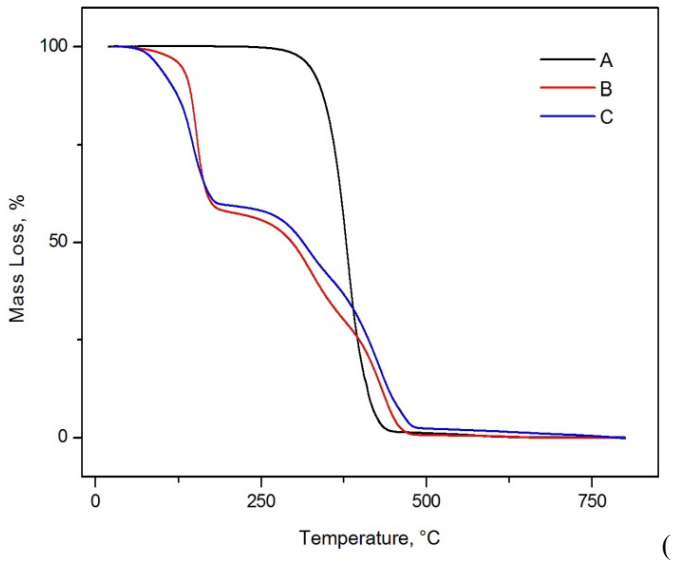
Furthermore, the iodometrics methods used have some limitations. The two main sources of error in iodometrics tests are: (i) iodine reaction with the unsaturation of fatty acids and (ii) release of iodine from potassium iodide by oxygen in the solution to be titrated [36]. The determination of the amount of peroxide present in the ozonized oils is of great significance and new methodologies to quantify the peroxide must be developed.

Thermal Behavior (TG/DTG - DSC)

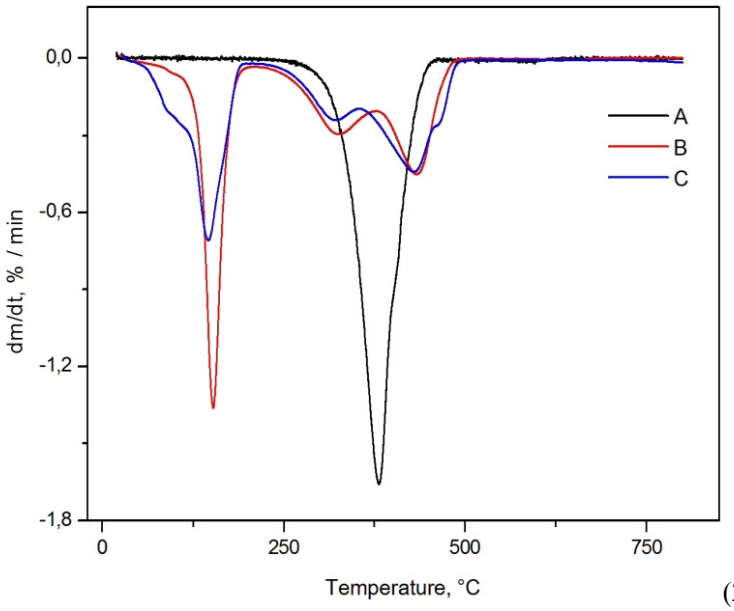
The TG curves (Figure 4) show mass losses for sunflower oil and ozonized oils. Sunflower oil (A) presented mass loss in one step in temperature between 300 and 450ºC and ozonated sunflower oils shows mass losses at three and four main steps, respectively. For oleogel (C), the first mass loss started at 70°C and for ozonized oil by 24 h (B) mass loss started at about 93ºC, revealing higher thermal stability of the latter.

Several samples of oils and ozonized oils were subjected to Differential Scanning Calorimetry (DSC) analysis. The study shows DSC curves corresponding by heating, cooling and degradation. DSC heat curve for sunflower oils (Figure 5) shows endothermic transitions corresponding to triglyceride (TAG) melting. Samples of sunflower oils show more than one endothermic transition to melting, suggesting polycrystalline state and the ozonized oil samples underwent endothermic transitions at higher temperatures than oils without ozonolysis. For the oleogel ozonized sunflower oil in presence of water was observed a Glass transition at about ⁻56ºC, which is a feature of polymeric materials or substances that undergo non-crystalline solidification. Glass transition was followed by an endothermic peak, corresponding to melting temperatures from ⁻30 to 12ºC and a fusion heat (ΔH) of 19.78 J g^{-1} [23].

Exothermic transitions observed in the cooling curve for DSC (Figure 6), corresponding to crystallization of fatty acids, sunflower oil exhibited transition characteristic of unsaturated acids, at ⁻20.80ºC. The ozonolysis leads to a decrease of unsaturated fatty acid decrease due to ozone reaction with the double bonds, leading the ozonized oils to present a similar thermal behavior to that of saturated fatty acids [23]. Melting and crystallization profiles from oil samples with high degrees of saturation involved higher temperature than for oils with high degrees of unsaturation [37].



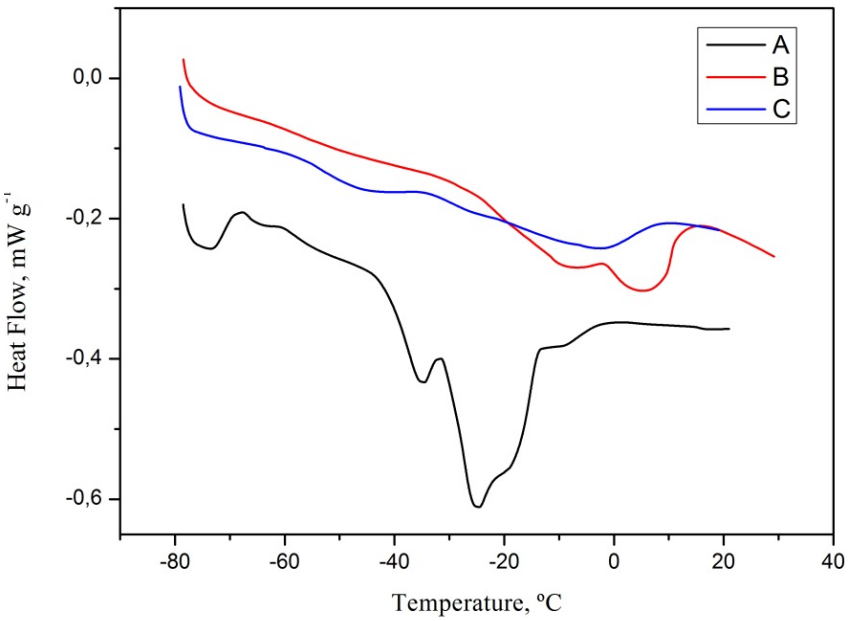
(1)



(2)

(Source: authors)

Figure 4. (1) TG (2) DTG curves for sunflower oils in a N_2 atmosphere (A) 0 h; (B) 24 h; (C) oleogel 24h.



(Source: authors)

Figure 5. Heat DSC curves for sunflower oil: (A) 0 h; (B) 24 h; (C) oleogel 24h.

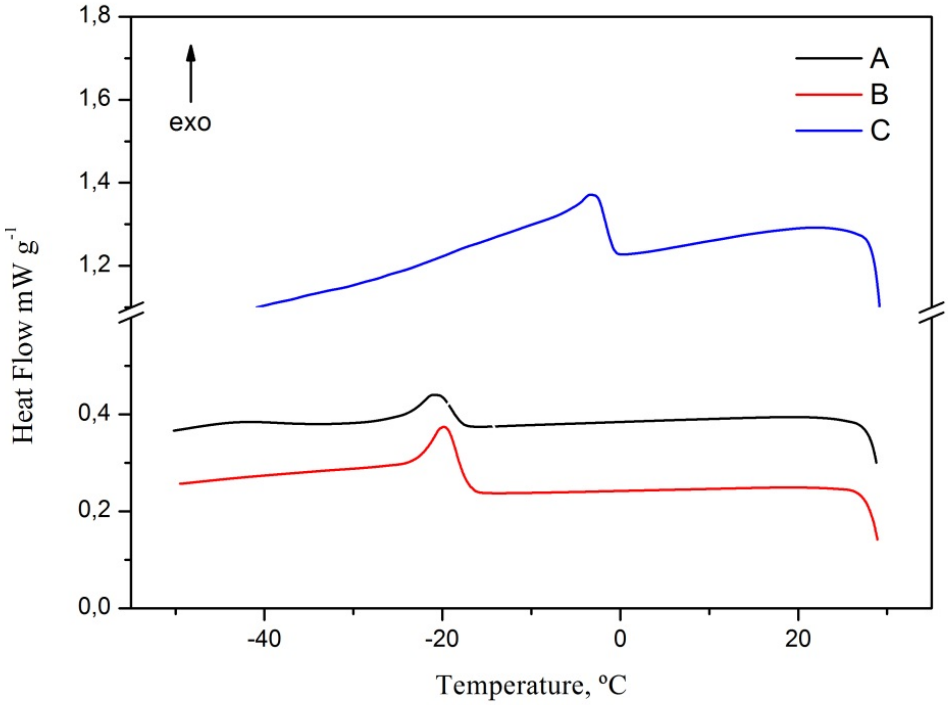


Figure 6. Cooling DSC curves for sunflower oil: (A) 0 h; (B) 12 h; (C) 24h.

Cataldo and Kogawa et al. carried out a study of thermal behavior by Differential scanning calorimetry (DSC) [18, 23]. The ozonized vegetable oils, which seem completely stable at room temperature, should not be heated above 100 °C. The thermal analysis shows that the ozonides of ethyl oleate decompose showing a peak at about 155◦C and a broad exotherm. The accepted mechanism of the ozonide thermal decomposition involves the homolytic cleavage of O-O bond which is also considered to be the rate-determining step leading to aldehydes, carboxylic acid and other minor products [18]. Previously, Soriano et al. reported that ozonized sunflower oil decomposes at 152°C with a decomposition enthalpy of 878.7 J/g [38].

Kogawa et al. showed that thermal degradation by DSC and ozonized sunflower oil presented exothermic peaks attributed to degradation followed by oxidative decomposition of ozonides at about 150°C [23]. Oxidative decomposition was probably caused by oxygen released from ozonide degradation. The thermal analysis for oleogel exhibited exothermic peaks related to decomposition at similar ozonized sunflower oil of 24 h. Figure 7 shows thermal degradation DSC curves for sunflower oils

DSC analysis ethyl oleate ozonized and other ozonized vegetable oils may be used to monitor the degree of ozonization achieved [18]. Therefore, the thermal decomposition enthalpy is proportional to the degree of ozonization achieved and the increase of the amount of ozonized oils is in ozonides is manifested by an increase in the amount of energy released (ΔH Decomposition) during thermal decomposition [23].

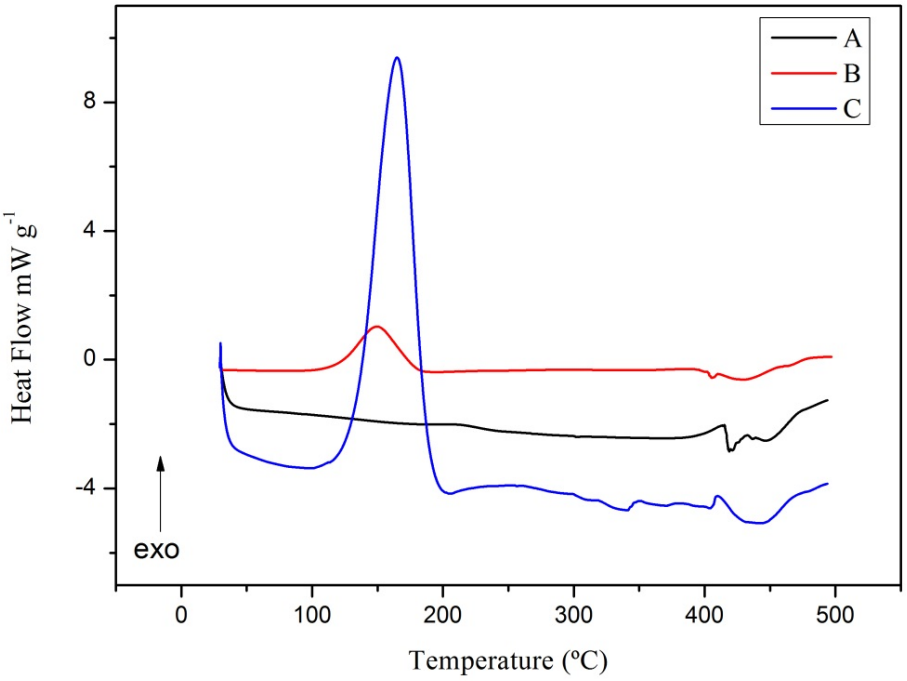


Figure 7. Thermal degradation DSC curves for sunflower oils. (A) 0 h; (B) 12 h; (C) 24h.

BIOLOGICAL ACTIVITY

There is plenty of scientific literature dealing with the biological activities of ozonized vegetable oils, especially with respect to antimicrobial activity (antibiotic, antifungal and antiparasitic), as well as wound healing and antiulcer activities.

Pharmaceutical products based on ozonized vegetable oil are commercially available, mainly for dermatological treatments such as stimulants of wound healing and/or as disinfectants. Oleozon® (ozonized sunflower oil) is manufactured in Cuba; Cocozone in Britain by coconut oil ozonolysis; OOO and O2-ZAP are manufactured in Canada and the USA, respectively, from olive oil [39].

The commercial product Oleozon® was evaluated for its antimicrobial activity against resistant strains of methicillin-sensitive *Staphylococcus aureus* and *Staphylococcus epidermidis* [40], with MIC_{90} of 9.5 mg/mL at a time of action of 60 to 180 minutes. The ozonized sunflower oil was evaluated by Sechi et al. (2001) [41] as an antibiotic agent front various bacterial, sensitive and resistant strains of *Staphylococcus aureus*, *Enterococcus faecalis*, *Enterococcus faecium*, *Streptococcus pyogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, and various species of *Mycobacterium*. The results were very satisfactory, with MICs of 2.37 to 9.95 mg/mL for the species of mycobacteria, and 1.18 to 9.5 mg/ml for all other bacteria. At first, MIC values on this scale seem too big, but vegetable oils are complex

mixtures of antioxidants and high-molecular-weight triacylglycerols, acting as a matrix capable of releasing active oxygen from ozonides, which have antimicrobial activity [41].

Table 2. Ozonized vegetable oils and antimicrobial activity

Author	Product	Bacterial strain	MIC (mg/mL)/ inhibition zone (mm)*/other information
Rodrigues et al. [42]	Bioperoxoil® (sunflower ozonized oil)	*S. aureus*, *E. coli*, *P. aeruginosa*, *Candida albicans* and *Salmonella typhimurium*	2.0-3.5 mg/mL
Díaz et al. [25]	Coconut ozonized oil (with or without protic solvent)	*S. aureus* and *P. aeruginosa*	17- 28.5 mm
Díaz et al. [22]	Sunflower ozonized oil (aqueous media)	*S. aureus*, *E. coli*, *P. aeruginosa*, *Bacillus subtilis*	9.5-38 mg/mL
Díaz et al. [7]	Sunflower and olive ozonized oils (aqueous media)	*S. aureus*, *E. coli*, *P. aeruginosa*, *B. subtilis*	0.95-14.25 mg/mL
Torres et al. [43]	Ozonized theobroma oil	*C. albicans*	5.78-93.75 mg/mL (depending on exposure time)
Gomez et al. [44]	Ozonized theobroma oil	*C. albicans*	2 – 15 mg/mL
Skalska et al. [39]	Sunflower ozonized oil	*E. coli*, *C. albicans*, *B. subtilis*	200-250 mg O_3/ g oil**
Daud et al. [45]	Bioperoxoil® (sunflower ozonized oil)	*Microsporum canis*	According to the authors, there is statistical indication that the ozonized oil acts against *M. canis* and there is clinical evidence of its action over this dermatophyte.
Menéndez et al. [46]	Oleozon® (sunflower ozonized oil)	phase III simple-blind study on onychomycosis patients	better therapeutic effect than topical ketoconazole
Gerrer et al. [47]	Bioperoxoil® (sunflower ozonized oil)	*C. albicans*, *Candida parapsiloses*, *Candida guilliermondii*, *Candida tropicalis*, *Tichosporum asaii*	11 – 19 mm
Ozyildis et al. [48]	Microcapsules containing ozonized red pepper seed oil	*E. coli*, *P. aeruginosa*, methicillin resistant *S. aureus*, vancomycin resistant *E. faecium*, *C albicans*	17 – 22.5 mm
Díaz et al. [49]	Sunflower ozonized oil (different sources for ozone formation)	*S. aureus*, *E. coli*, *P. aeruginosa*	4.75 – 28.5 mg/mL
Montececchi et al. [50]	Novox® (olive ozonized oil)	*S. aureus*, *Porphyromona gingivalis*	19.0 – 30.7 mm (0 – 1:128 dilutions)
Moureu et al. [16]	"Classical" and a "high oleic" sunflower oils ozonized with or without water	*S. aureus*, *E. coli*, *Streptococcus uberis*	5 - 40 mg/mL
Kogawa et al. [23]	Sunflower, flaxseed and baru ozonized oils (with or without water)	*S. aureus*, *E. coli*, *P. aeruginosa*, *E. faecalis*, oxacillin resistant *S. aureus*, vancomycin resistant *E. faecalis*	3 – 10 mg/mL

* MIC= minimal inhibitory concentration, using dilution methods; inhibition zone = using agar diffusion method. ** 100% of growth inhibition using the dose of 200 or 250 O_3 mg/g of oil during ozonolysis reaction.

Previous works were followed by many researchers using other microorganisms. Additional parameters were analyzed starting from different vegetable oils. Table 1 summarizes this information.

It is interesting to note that many authors could relate the antimicrobial activity results to the peroxide index (PI) values showing that, to some extent, the higher the oil peroxide value, the greater the activity. After a certain point, an increase in PI does not influence the activity [16, 22, 25, 39, 43, 44]. This relationship suggests that the antimicrobial mechanism of action of ozonized vegetable oils is related to the action of oxidizing species, which was reported by Diaz et al. [49].

Studies show the influence of ozonation conditions and of the initial fatty acid composition of ozonized sunflower oils on their iodine index, peroxide index, acidity value and, antibacterial activity. The results indicated that fatty acid composition of the oils has no significant effect on the antimicrobial activity. However, the addition of water has a direct impact on the increase in peroxide index and so better antibacterial activity of oils ozonized with water [16, 23]. Recent results of our research group (not yet published) indicate that the neem oil ozonized in the presence of water also showed better antimicrobial activity. These results reveal opportunities for further studies and applications of ozonized oils.

There are also reports in the literature of antiparasitic activity of ozonized vegetable oils. Hernandez et al. evaluated the cytotoxic effect of Oleozon® on *Giardia duodenalis* trophozoites. The total cytotoxic effect on $15x10^4$ cells was obtained with 30µL (28.6 mg) of Oleozon® [51]. Direct cytotoxic-oxidant effect on the parasite *in vitro* may be one of the mechanisms of action for a parasitocidal effect of this product. Rajabi et al. studied the action of ozonized olive oil on *Leishmania major* promastigotes, the parasite that causes cutaneous leishmaniasis [52]. The authors found that IC_{50} were 120 mg/mL and 165 mg/mL for Glucantim and almost 2 and 3.5 µg/mL for ozonized olive oil, considering mean alive promastigotes determined by MTS method and light microscope [52].

Pai et al. described the potential of ozonized sesame oil to augment wound healing in rats. The animals were treated with two doses of ozonized sesame oil (peroxide values 500 and 700 mEq/1000 g, respectively), and framycetin, an antibacterial drug commonly used for treating wounds, was used as positive control [53]. Ozonized oil treated wounds had significantly higher tensile strength, collagen content and superoxide dismutase activity than that of the control treated wounds, which means that area treated with ozonized oil revealed better healing activity.

Some authors have reported other activities related to increasing on antioxidant enzyme activity. Rodriguez et al. [54] and Zamora et al. [55] reported the antiulcer activity of ozonized sunflower oil in rats. Rodriguez et al. evaluated the protective effect of ozonized oil on damage to digestive mucosa caused by ethanol action. Results demonstrate that OSO pretreatment exerts protective effects in ethanol-induced gastric ulcers in rats and these protective effects are mediated, at least partially, by stimulation of some important antioxidant enzymes such as SOD (superoxide dismutase) and GSH-Px (glutathione peroxidase), which are scavengers of ROS (reactive oxygen species) and therefore, prevent gastric injury induced by them [54]. Zamora et al. investigated the potential cytoprotective

effects of ozonized sunflower oil in the damage of rat gastric mucosa induced by indomethacin. They also observed the cytoprotective effects of OSO in rat gastric mucosa. It was concluded that these cytoprotective effects are mediated somewhat by upregulation of the antioxidant system and mainly SOD [55].

Abu-Gharbieh et al. [56], similarly, evaluated the potential protective effect of ozonized olive oil in 2,4-dinitrobenzene sulfuric acid (DNBS) induced colitis in rats. Their results demonstrated that pretreatment with ozonized oil exerts protective effects in DNBS induced colitis in rats and provided evidence that protective the effects are mediated by stimulation of some antioxidant enzymes, once CAT (catalase), GSH-Px, and SOD activities were significantly increased in the distal colon of inflamed animals pretreated with olive ozonized oil with respect to control group dose dependently.

Sanchez et al. tested the antioxidant activity of a cosmetic formulation made with ozonized theobroma oil on rat skin irradiated with ultraviolet light. Again, the ozonized vegetable oil was able to stimulate the activity of antioxidant enzymes SOD and GSH-Px, which prevent skin injury induced by ultraviolet radiation [57].

Although ozonized oils have been used in many countries in the treatment of wounds and skin ulcers, reports on their cytotoxicity are scarce. Kogawa et al. [23] investigated sunflower and flaxseed ozonized oils (different ozonolysis times and conditions) for cytotoxicity against NIH/3T3 fibroblasts, but proved non-toxic when compared with doxorubicin, which is a desirable trait for the safe use of ozonized oils in patients. Also, authors evaluated the antitumor potential of the products against cancer cell lines: 786-0 (ATCC-CRL-1932, renal adenocarcinoma) HT-29 (ATCC-HTB-38, colon adenocarcinoma), MFC-7 (ATCC-HTB-22, breast adenocarcinoma), PC-3 (ATCC-CRL-1435, prostate adenocarcinoma), and B16-F10 (ATCC-CRL-6322, murine melanoma), and concluded that the oils were potentially active against neoplastic cell lines [23].

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[56] Abu-Gharbieh, E., Bayoumi, F. A. & Ahmed, N. G. (2014). Alleviation of antioxidant defense system by ozonized olive oil in dnbs-induced colitis in rat. *Mediators Inflammation*, 6 pages.

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BIOGRAPHICAL SKETCH

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Affiliation: Institute of Chemistry of Federal University of Mato Grosso do Sul

Education:

- Ph.D student at Federal University of Mato Grosso do Sul (2013 to present)
- Master, degree in Chemistry, field of Organic Chemistry, Federal University of Mato Grosso do Sul (2013)
- Bachelor's degree in Pharmacy at Universidade Católica Dom Bosco (2008)

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Research and Professional Experience: Organic Chemistry/Organic Synthesis

Professional Appointments:

- Junior Associate Editor of Orbital - The Electronic Journal of Chemistry (2014 to present)

Honors:

- **2012**: Finalist Project – SANTANDER Entrepreneurship - Prize of Biotechnology and Health.

Publications Last 3 Years:

1. Kogawa, N. R. A.; De Arruda, E. J.; Micheletti, A. C.; Matos, M. F. C.; De Oliveira, L. C. S.; De Lima, D. P.; Carvalho, N. C. P.; Oliveira, P. D.; Cunha, M. C.; Ojeda, M.; Beatriz, A. Synthesis, characterization, thermal behavior and biological activity of ozonides from vegetable oils. *RSC Advances: an international journal to further the chemical science*s, 2015, 5, 65427.

2. Almeida, N. R.; Beatriz, A.; Micheletti, A. C.; Arruda, E. J. Ozonized vegetable oils and therapeutic properties: A review. *Orbital: Electron. J. Chem*, 2013, 4, 313.

Name: Adilson Beatriz

Affiliation: Institute of Chemistry of Federal University of Mato Grosso do Sul (Brazil)

Education:

- Ph.D. degree in Science, field of Organic Chemistry from University of São Paulo – USP (2001).
- Bachelor's degree in Technological Chemistry by the Federal University of Mato Grosso do Sul (1995).

Address: Av. Senador Filinto Müller, 1555, CEP 79074-460 – Campo Grande, MS, Brazil

Research and Professional Experience: Organic Chemistry/Organic Synthesis

Experienced in Organic Synthesis, mainly in the following subjects: i) synthesis of resorcinolic lipids; ii) bioactive cage-like polycyclic compounds (development of new pharmaceuticals and new pesticides) and iii) Synthesis of polymers, surfactants etc. from biomass; iv) Chemical transformations of natural products; iv) Preparative biotransformations: whole cell and isolated enzymes in Organic Synthesis.

Professional Appointments:

- Post-Doctoral fellow, University of São Paulo (2001- 2002).
- Post-Doctoral fellow, University of Santiago de Compostela, USC, Spain (2009-2010).
- Research Associate Professor at Federal University of Mato Grosso do Sul (2006 to present).
- Research advisor in the Master and Doctor Programs in Chemistry (2006 to present).
- Head, Department of Chemistry, The Federal University of Mato Grosso do Sul (2009 to 2011).
- Editor-in-Chief of Orbital - The Electronic Journal of Chemistry (2009 to present).

Honors:

- **2015**: Finalist of the 7th Prize of Innovation Medical Services - Category Tropical Medicine, SANOFI
- **2014**: First Prize – 6th Prize of Innovative Medical Services – New Ways in Public Health, SANOFI and Portal Medical Services.

- **2012**: Finalist Project – SANTANDER Entrepreneurship - Prize of Biotechnology and Health.
- **2008**: First Prize - SANTANDER Regional Prize of Science and Innovation – Category: Biotechnology.

Publications Last 3 Years:

1. Schneider, B. U. C.; De Souza, A. M.; Beatriz, A.; Carvalho, P. C.; Mauro, M. O.; Karaziack, C. B.; De Lima, D. P.; Oliveira, R. J. Cardanol: toxicogenetic assessment and its effects when combined with cyclophosphamide. *Genetics and Molecular Biology*. In Press 2016.
2. Micheletti, A. C.; Honda, N. K.; Carvalho, N. C. P.; De Lima, D. P.; Beatriz, A. Design, Synthesis and *in vitro* Antimicrobial Activity Evaluation of Novel Hybrids of Lichexantone-THC Derivatives. *Orbital: Electron. J. Chem*. 2015, 7, 301.
3. Kogawa, N. R. A.; De Arruda, E. J.; Micheletti, A. C.; Matos, M. F. C.; De Oliveira, L. C. S.; De Lima, D. P.; Carvalho, N. C. P.; Oliveira, P. D.; Cunha, M. C.; Ojeda, M.; Beatriz, A. Synthesis, characterization, thermal behavior and biological activity of ozonides from vegetable oils. *RSC Advances: an international journal to further the chemical science*s, 2015, 5, 65427.
4. Meza, A.; dos Santos, E. dos A.; Gomes, R. S.; De Lima, D. P.; Beatriz, A. Cytosporones: Isolation, Synthesis and Biological Activities of a Promising Class of Phenolic Lipids. *Current Organic Synthesis*, 2015, 12, 618.
5. Oliveira, R. J.; Navarro, S. D.; De Lima, D. P.; Meza, A.; Pesarini, J. R.; Gomes, R. S.; Karaziack, C. B.; Mauro, M. O.; Cunha-Laura, A. L.; Monreal, A. C. D.; Romão, W.; Lacerda Júnior, V.; Beatriz, A. A novel cytosporone 3-Heptyl-4,6-dihydroxy-3H-isobenzofuran-1-one: synthesis; toxicological, apoptotic and immunomodulatory properties; and potentiation of mutagenic damage. *BMC Cancer*, 2015, 561, 1.
6. Araújo, Y. J. K.; Avvari, N. P.; Paiva, D. R.; De Lima, D. P.; Beatriz, A. Synthesis and enzymatic resolution of racemic 2,3-epoxy propyl esters obtained from glycerol. *Tetrahedron Letters*, 2015, 56, 1696.
7. Beatriz, A.; Gomes, R. S.; Constantino, M. G.; da Silva, G. V. J.; de OLIVEIRA, K. T. Synthesis of the Bicyclo[6.2.1] undecane Ring System by a Solvent-free Diels-Alder Reaction. *Tetrahedron Letters*, 2014, 55, 679.
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9. Rizzo, P. V. S.; Boarin, L. A.; Freitas, I. O. M.; Gomes, R. S.; Beatriz, A.; Rinaldi, A. W.; Domingues, N. L. C. The study of biocatalyzed thio-Michael reaction: a greener and multi-gram protocol. *Tetrahedron Letters*, 2014, 55, 430.
10. Polonini, H. C.; Lopes, R. S.; Beatriz, A.; Gomes, R. S.; Silva, A. O.; Lima, R. V.; Nunes, G. A.; Brandão, M. A. F.; Raposo, N. R. B.; De Lima, D. P. Synthesis and evaluation of octocrylene-inspired compounds for UV-filter activity. *Química Nova*, 2014, 37,1004.
11. Naujorks, A. A. S.; da Silva, A. O.; Lopes, R. S.; de Albuquerque, S.; Beatriz, A.; Marques, M. R.; de Lima, D. P. Novel naphthoquinone derivatives and evaluation of their trypanocidal and leishmanicidal activities. *Organic & Biomolecular Chemistry*, 2014, 13, 428.

12. Santos, E. A.; Prado, P. C.; Beatriz, A.; de Lima, D. P. Synthesis and biological activity of sulfur compounds showing structural analogy with combretastatin A-4. Química Nova, v. 36, p. 279-283, 2013.
13. da Silva, A. O.; LOPES, R. S.; de Lima, R, V.; Tozatti, C. S. S.; Marques, M. R.; de Albuquerque, S.; Beatriz, A.; de Lima, D. P. Synthesis and biological activity against Trypanosoma cruzi of substituted 1,4-naphthoquinones. *European Journal of Medicinal Chemistry*, v. 60, p. 51-56, 2013.
14. Micheletti, A. C.; Honda, N. K.; Pavan, F. R.; Leite, C. Q. F.; Matos, M. F. C.; Perdomo, R. T.; Bogo, D.; Alcântara, G. B.; Beatriz, A. Increment of Antimycobacterial Activity on Lichexanthone Derivatives. *Medicinal Chemistry (Hilversum)*, v. 9, p. 904-910, 2013.
15. Magalhães, H. I. F.; Wilke, D. V.; Bezerra, D. P.; Cavalcanti, B. C.; Rotta, R.; de Lima, D. P.; Beatriz, A.; de Moraes, M. O.; Diniz Filho, J.; Pessoa, C. O. (4-Methoxyphenyl) (3,4,5-trimethoxyphenyl) methanone inhibits tubulin polymerization, induces G2/M arrest, and triggers apoptosis in human leukemia HL-60 cells. *Toxicology and Applied Pharmacology*, v. 272, p. 117-126, 2013.

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Research and Professional Experience:

After PhD work on natural product, polymers, metal complexes, sustainable development, use of modified natural product or not, biomass and development of new products and technologies for medical applications and in health. Participated in university and classist boards from 2008 to 2014. It is a referee of national and international journals. The research group with researchers from UFGD, UFMS and UNICAMP has received recognition in the last seven years with the proposed use natural or modified products. Presently, his research focuses on the design and implementation of (bio) natural products, hybrid scaffolds and new strategies, metal-insecticides (metallo-insecticides) and photosensitizers for vector control of tropical diseases.

Professional Appointments:

- Full Professor at Universidade Católica Dom Bosco, Campo Grande, MS (1985 to 2008).
- Post-doctoral UNESP (2009/2010), FEQ / UNICAMP (2014) and UP - University of Porto - PT / INEB/i3S - Institute of Biomedical Engineering (2015).

- Adjunct Professor at School of Chemistry, Federal University of Grande Dourados (2008 to present).

Honors:

- **2014**: First Prize – 6th Prize of Innovative Medical Services – New Ways in Public Health, SANOFI and Portal Medical Services.

Publications Last 3 Years:

1. Silva, E. L.; Arruda, E. J.; Andrade, C. F. S.; Fernandes, M. F.; Teixeira, T. Z.; Scudeler, C. G. S.; Cabrini, I. Avaliação da Susceptibilidade de Populações de Aedes aegypti (Linnaeus) (Diptera: Culicidae) ao Inseticida Temephos nos Municípios de Maracaju e Naviraí, MS. *BioAssay (Piracicaba)*, v. 10, p. 1-5, 2015.
2. Catelan, T. B. S.; Arruda, E. J.; Oliveira, L. C. S.; Raminelli, C.; Cabrini, I.; Gaban, C. R. G.; Arakaki, A. H.; Nova, P. C. C. V. Evaluation of Toxicity of Phenolic Compounds Using Aedes aegypti (Diptera: Culicidae) and Artemia salina. *Advances in Infectious Diseases*, v. 05, p. 48-56, 2015.
3. Lescano, C. H.; Sanjinez-Argandoña, E. J.; Arruda, E. J.; Oliveira, I. P.; Baldivia, D. S.; Silva, L. R. Nutrients content, characterization and oil extraction from Acrocomia aculeata (Jacq.) Lodd. fruits. *African Journal of Food Science*, v. 9, p. 113-119, 2015.
4. Silva, A. K.; Arruda, E. J.; Fonseca, G. G.; Carvalho, C. T.; Silva, C. M.; Nova, P. C. C. V.; Gaban, C. R. G.; Cabrini, I. Evaluation of toxicity of Bordeaux Mixture in Aedes aegypti larvae (L. 1672) (Diptera: Culicidae) and Gram-negative and Gram-positive bacteria. *Journal of Mosquito Research*, p. 1-8, 2015.
5. Finoto, S.; Machulek Junior, A.; Caires, A. R. L.; Arruda, E. J.; Casagrande, G. A.; Raminelli, C.; Andrade, L. H. C.; Lima, S.M. New metalorgano-chalcogenide compounds based on polymeric frameworks constructed by Se-Hg intermolecular interactions: Preparation, structural characterization, and Raman evaluation. *Polyhedron*, v. 99, p. 96-102, 2015.
6. Kogawa, N. R. A.; De Arruda, E. J.; Micheletti, A. C.; Matos, M. F. C.; De Oliveira, L. C. S.; De Lima, D. P.; Carvalho, N. C. P.; Oliveira, P. D.; Cunha, M. C.; Ojeda, M.; Beatriz, A. Synthesis, characterization, thermal behavior and biological activity of ozonides from vegetable oils. *RSC Advances: an international journal to further the chemical science*s, 2015, 5, 65427.
7. Carbonaro, E. S.; Arruda, E. J.; Oliveira, L. C. S.; Nova, P. C. C. V.; Arakaki, A. H.; Machulek Junior, A. A síntese de novos ativos larvicidas para o controle populacional do Aedes aegypti (Diptera: Culicidae). *Revista Brasileira de Inovação Tecnologica em Saúde*, v. 5, p. 10-26, 2015.
8. Scudeler, C. G. S.; Silva, T. G.; Fernandes, M. F.; Teixeira, T. Z.; Andrade, C. F. S.; Silva, I.; Arruda, E. J. Larval Susceptibility of Two Culex quinquefasciatus Populations (Diptera: Culicidae) Temephos® in the City of Naviraí, MS, Brazil. *Orbital: the Electronic Journal of Chemistry*, v. 7, p. 370-374, 2015.
9. Lima, A. R.; Arruda, E. J.; Cabrini, I.; Carvalho, C. T.; Fernandes, M. F.; Kato, M. F. H.; Andrade, C. F. S.; Silva, C. M. Insecticidal activity of Cu(II)-NTA in Aedes

aegypti larvae (Diptera: Culicidae). *Orbital: the Electronic Journal of Chemistry*, v. 7, p. 369-375, 2015.

10. Gaban, C. R. G.; Dourado, D. M.; Silva, L. M. G. E.; Nova, P. C. C. V.; Cabrini, I.; Arruda, E. J. Morphological Changes in the Digestive System of Aedes aegypti L. Induced by [Cu(EDTA)]2- Complex Ions. *Journal of Mosquito Research*, v. 5, p. 1-9, 2015.
11. Andrade, R. C.; Almeida, C. F.; Suegama, P. H.; Arruda, E.J.; Arroyo, P. A.; Carvalho, C. T. Buriti palm stem as a potential renewable source for activated carbon production. *Environmental Technology & Innovation*, v. 3, p. 15-22, 2015.
12. Teixeira, T. Z.; Arruda, E. J.; Andrade, C. F. S.; Crispim, B. A.; Fernandes, M. F.; Silva, E. P.; Nakamura, A. K. S. Suscetibilidade de Larvas de Simulídeos ao Larvicida Temephos em Caarapó, MS. *BioAssay (Piracicaba*), v. 9, p. 1-6, 2014.
13. Nardeli, J. V.; Arruda, E. J.; Carvalho, C. T.; Nova, P. C. C. V.; Cabrini, I.; Arakaki, A. H. Sintese, caracterização e atividade biológica do acetato de Cu(II) para larvas de Aedes aegypti (Diptera: Culicidae) e bactérias Escherichia coli, Staphylococcus aureus, Salmonella typhimurium e Lysteria monocytogenes. *Orbital: the Electronic Journal of Chemistry*, v. 6, p. 122-129, 2014.
14. Lescano, C. H.; Sanjinez-Argandoña, E. J.; Arruda, E. J.; Kassuya, C. A. L.; Moraes, I. C. F. Acrocomia aculeata(Jacq.) Lodd. Oil Microencapsulation by Complex Coacervation: Preservation of Bioactive Compounds. *Journal of Encapsulation and Adsorption Sciences*, v. 04, p. 105-113, 2014.
15. Jacobowski, A. C.; Zobiole, N. N.; Padilha, P. M.; Moreno, S. E.; Arruda, E. J. Efeito Mutagênico do Edetato de Cobre ([Cu(EDTA)]-2) Livre e Nanoencapsulado em Camundongos e Peixes. *Journal of Brazilian Society of Ecotoxicology*, v. 8, p. 13-19, 2013.
16. Santos, G.; Arruda, E. J.; Oliveira, L. C. S.; Nova, P. C. C. V.; Ferreira, V. S. Desenvolvimento de metodologia eletroanalítica para determinação do antioxidante terc-butilhidroquinona (TBHQ) em amostras de biodiesel de soja. *Biofar: Revista de Biologia e Farmácia*, v. 9, p. 79-90, 2013.

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- Master in Organic Chemistry (2007), from Federal University of Mato Grosso do Sul (2007).
- Bachelor's degree in Chemistry by the Federal University of Mato Grosso do Sul (2004).

Address: Av. Senador Filinto Müller, 1555, CEP 79074-460 – Campo Grande, MS, Brazil

Research and Professional Experience: working mainly in: chemistry of lichens and structural modification of natural products, organic synthesis, synthesis of bioactive molecules.

Professional Appointments:

- Professor at Federal University of Mato Grosso do Sul (2011 to present).
- Research advisor in the Master and Doctor Programs in Chemistry (2011 to present).

Honors:
Publications Last 3 Years:

1. Micheletti, A. C.; Honda, N. K.; Carvalho, N. C. P.; De Lima, D. P.; Beatriz, A. Design, Synthesis and *in vitro* Antimicrobial Activity Evaluation of Novel Hybrids of Lichexantone-THC Derivatives. *Orbital: Electron. J. Chem*. 2015, 7, 301.
2. Kogawa, N. R. A.; De Arruda, E. J.; Micheletti, A. C.; Matos, M. F. C.; De Oliveira, L. C. S.; De Lima, D. P.; Carvalho, N. C. P.; Oliveira, P. D.; Cunha, M. C.; Ojeda, M.; Beatriz, A. Synthesis, characterization, thermal behavior and biological activity of ozonides from vegetable oils. *RSC Advances: an international journal to further the chemical science*s, 2015, 5, 65427.
3. Micheletti, A. C.; Honda, N. K.; Pavan, F. R.; Leite, C. Q. F.; Matos, M. F. C.; Perdomo, R. T.; Bogo, D.; Alcântara, G. B.; Beatriz, A. Increment of Antimycobacterial Activity on Lichexanthone Derivatives. *Medicinal Chemistry (Hilversum)*, v. 9, p. 904-910, 2013.
4. Almeida, N. R.; Beatriz, A.; Micheletti, A. C.; Arruda, E. J. Ozonized vegetable oils and therapeutic properties: A review. *Orbital: Electron. J. Chem*, 2013, 4, 313.

Name: Dênis Pires de Lima
Affiliation: Institute of Chemistry of Federal University of Mato Grosso do Sul (Brazil)
Education:

- Bachelor's in Pharmacy from Universidade Federal de Minas Gerais (1986)
- Doctorate at Chemistry from Universidade Federal de Minas Gerais (1994).

Address: Av. Senador Filinto Müller, 1555, CEP 79074-460 – Campo Grande, MS, Brazil.

Research and Professional Experience: Organic Chemistry/Organic Synthesis/Medicinal Chemistry.

Experienced in the area of the molecular transformation of organic natural compounds, synthesis of phenolic compounds comprising naphthoquinones derivatives, analogues of resveratrol, combretastatin, and phenolic lipids as those isolated from cashew nut shell liquid (CNSL). Currently, biotransformation and bioremediation are exploited employing fungi isolated from many sources of the Brazilian biome.

Professional Appointments:

Part of Ph.D. program (1991-1992) was carried out at the UNIVERSITY OF ALBERTA (Edmonton-CANADA) working on the organic synthesis of antihypertensive agent, under supervision of the Professor Derrick L. J. Clive. The postdoctoral fellowship was taken at UNIVERSITY OF LIVERPOOL (Liverpool-UK) as a researcher of the group headed by Professor Stanley M. Roberts in the field of biotransformation.

Honors:

- **2015**: Finalist of the 7th Prize of Innovation Medical Services - Category Tropical Medicine, SANOFI
- **2014**: First Prize – 6th Prize of Innovative Medical Services – New Ways in Public Health, SANOFI and Portal Medical Services
- **2013**: Semifinalists Project – SANTANDER Prize of Science and Innovation
- **2005**: Best work in the 5th International Congress of Pharmaceutical Sciences, International Congress of Pharmaceutical sciences - Ribeirão Preto – SP - Brazil
- **2005**: Honored Mention, in the 5th International Congress of Pharmaceutical Sciences, International Congress of Pharmaceutical sciences - Ribeirão Preto – SP – Brazil

Publications Last 3 Years:

1. Oliveira, R. J.; Navarro, S. D.; De Lima, D. P.; Meza, A.; Pesarini, J. R.; Gomes, R. S.; Karaziack, C. B.; Mauro, M. O.; Cunha-Laura, A. L.; Monreal, A. C. D.; Romão, W.; Lacerda Júnior, V.; Beatriz, A. A novel cytosporone 3-Heptyl-4,6-dihydroxy-3H-isobenzofuran-1-one: synthesis; toxicological, apoptotic and immunomodulatory properties; and potentiation of mutagenic damage. *BMC Cancer*, 2015, 561, 1.
2. Meza, A.; dos Santos, E. dos A.; Gomes, R. S.; De Lima, D. P.; Beatriz, A. Cytosporones: Isolation, Synthesis and Biological Activities of a Promising Class of Phenolic Lipids. *Current Organic Synthesis*, 2015, 12, 618.
3. Micheletti, A. C.; Honda, N. K.; Carvalho, N. C. P.; De Lima, D. P.; Beatriz, A. Design, Synthesis and *in vitro* Antimicrobial Activity Evaluation of Novel Hybrids of Lichexantone-THC Derivatives. *Orbital: Electron. J. Chem*. 2015, 7, 301.
4. Carvalho, P.; Santos, E. A.; Catelan, B. U.; Matuo, R.; Pesarini, J.; Laura, A.; Monreal, A.; de Lima, D. P.; Oliveira, R. J.; Brochado, A. C. M.; Silva, A. Diaryl sulfide analogs of combretastatin A-4: toxicogenetic, immunomodulatory and apoptotic evaluations and prospects for use as a new chemotherapeutic drug. *Environmental Toxicology and Pharmacology*, 2015, 40, 715.
5. Naujorks, A. A. S.; da Silva, A. O.; Lopes, R. S.; de Albuquerque, S.; Beatriz, A.; Marques, M. R.; de Lima, D. P. Novel naphthoquinone derivatives and evaluation of their trypanocidal and leishmanicidal activities. *Organic & Biomolecular Chemistry*, 2014, 13, 428.
6. Araújo, Y. J. K.; Avvari, N. P.; Paiva, D. R.; De Lima, D. P.; Beatriz, A. Synthesis and enzymatic resolution of racemic 2,3-epoxy propyl esters obtained from glycerol. *Tetrahedron Letters*, 2015, 56, 1696.
7. Kogawa, N. R. A.; De Arruda, E. J.; Micheletti, A. C.; Matos, M. F. C.; De Oliveira, L. C. S.; De Lima, D. P.; Carvalho, N. C. P.; Oliveira, P. D.; Cunha, M. C.; Ojeda, M.; Beatriz, A. Synthesis, characterization, thermal behavior and biological activity of ozonides from vegetable oils. *RSC Advances: an international journal to further the chemical science*s, 2015, 5, 65427.
8. Polonini, H. C.; Lopes, R. S.; Beatriz, A.; Gomes, R. S.; Silva, A. O.; Lima, R. V.; Nunes, G. A.; Brandão, M. A. F.; Raposo, N. R. B.; De Lima, D. P. Synthesis and

evaluation of octocrylene-inspired compounds for UV-filter activity. *Química Nova*, 2014, 37, 1004.

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