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Chemical characterization of sunflower oil oxidized by UV and ozone with different degrees of oxidation and study of their antimicrobial action

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SUMMARY: Oxidation by the action of ozone takes place at high rates and involves the reaction of ozone molecules with fatty acid double bonds followed by the formation of stable oxidation products with biological activity. In the present work, a comparative study on sunflower oil oxidized by ultraviolet (UV) light and ozone was carried out. This study involved the chemical characterization of sunflower oil oxidized by UV irradiation and ozonation, in addition to assessing the germicidal activity of oxidized oils obtained under various conditions. The results indicated that under the conditions studied, the increase in the dose of UV irradiation did not produce significant changes in the level of oxidation of the oil. Ozonation promoted the formation of oxygenated compounds at higher rates, increasing in concentration as the applied dosage of ozone increased. The germicidal activity of the oils behaved similarly, with considerably higher activity found in the ozonized oils.

KEYWORDS: *Oxidation; Ozone; Spectroscopy; Sunflower oil; UV irradiation.*

RESUMEN: ***Caracterización química del aceite de girasol oxidado mediante UV y ozono con diferentes grados de oxidación y estudio de su acción antimicrobiana.*** La oxidación por acción del ozono tiene lugar a tasas muy altas e implica la reacción de las moléculas de ozono con los dobles enlaces de los ácidos grasos, seguida de la formación de productos de oxidación estables con actividad biológica. En el presente trabajo se realizó un estudio comparativo del aceite de girasol oxidado por luz UV y por ozono. Este estudio consistió en la caracterización química del aceite de girasol oxidado por irradiación UV y por ozonización. En segundo lugar, se evaluó la influencia en la actividad germicida potencial del producto final obtenido en varias condiciones de ozonización. Los resultados indicaron que, en las condiciones estudiadas, el aumento de la dosis de irradiación UV no produjo cambios significativos en el nivel de oxidación del aceite. La ozonización promovió la formación de compuestos oxigenados en mayor proporción, aumentando su concentración a medida que aumentaba la dosis de ozono aplicada. La actividad germicida de los aceites se comportó de forma similar, encontrándose una actividad considerablemente mayor en los aceites ozonizados.

PALABRAS CLAVE: *Aceite de girasol; Espectroscopía; Irradiación UV; Oxidación; Ozono.*

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1. INTRODUCTION

Vegetable oils are composed of 95% triacylglycerols (TAG). Depending on their origin, they have different compositions of saturated and unsaturated fatty acids, such as oleic, stearic and palmitic acid. These fatty acids are esterified to the hydroxyl residues of the glycerol molecule (Firestone *et al*., 2013).

Ozonized vegetable oils present a more complex composition than the initial oils (Ledea-Lozano *et al.*, 2001). The interaction of ozone with the unsaturated molecules of vegetable oil generates the formation of a complex mixture of chemical compounds including ozonides, peroxides, and aldehydes (Bailey 1978; Soriano *et al*., 2003; De Almeida *et al*., 2016). Ozone reacts with the double bonds present in unsaturated fatty acids to form 1,2,3-trioxolanes or molozonides, which are unstable. In practice, the validity of Criegge's mechanism has been verified, which establishes that in the presence of water, a carbonyl fragment and a terminal hydroxyl hydroperoxide are produced. The hydroxyl-hydroperoxides (HHPs) have high biological activity and provide hydrogen peroxide through their decomposition into a carbonyl derivative. Vegetable oils easily react with ozone, causing the peroxide index to increase to high values. The amount of ozone that reacts with oils depends on the degree of unsaturation of their fatty acids. Ozonized vegetable oils have applications in dermatology and cosmetics, showing a well-known disinfectant and healing effect (Sechi *et al*. 2001; Skalska *et al*., 2009; Valacchi *et al*., 2013; Anzolin 2020). The study of the antimicrobial activity of ozonized oils has revealed that their germicidal character is due to the formation of oxygenated compounds such as hyperoxides, ozonides, aldehydes, and polyperoxides (Bouzid *et al*., 2021). However, the chemical characterization of ozonized vegetable oils is difficult due to the wide variety of oxygenated species they may contain (Ledea-Lozano *et al*., 2019a). Furthermore, vegetable oils also undergo the autooxidation phenomenon, which is the most widespread alteration of oils and fats. This is a complex process that generates a variety of intermediate and final oxygenated compounds such as organic peracids, alcohols, aldehydes, ketones, carboxylic acids, along with combinations of the above. The most widely accepted mechanism is through the free radical mediated by oxygen. The mechanism of free radical formation from fatty acids or acylglycerols is accelerated in the presence of electromagnetic radiation (light), heat, traces of heavy metals (such as iron and copper), and the presence of other active free radicals. (Tenllado van der Reijden, 2013).

The autooxidation mechanism of oils consists of three stages. The first is induction, in which the homolytic breakage of a carbon-oxygen bond occurs, generating a free radical. The second stage is propagation, in which by a reaction between molecular oxygen and the previously formed radical, a peroxide radical is formed. This radical can abstract hydrogen atoms from the carbon chains to form hydroperoxides, generating new free radicals that feed and expand the reaction. The hydroperoxides formed can also decompose, contributing to the propagation of the reaction. Termination reactions occur when free radicals react with each other forming new bonds, stopping to participate in the propagation of the reaction (Villanueva *et al*., 2017).

As already mentioned, edible oil oxidation can be activated by ultraviolet and visible light (Luna *et al*., 2006), which accelerates the formation of free-radicals from fatty acids or acylglycerols. The energy required for hydrogen abstraction depends on its position and proximity to the unsaturated molecules. The hydrogen atoms adjacent to the double bond, especially the one attached to the carbon between the two double bonds, require less energy for abstraction (Choe *et al*., 2006).

This work characterizes sunflower oil oxidized by two routes, the first by photoxidation with UV light and the second by ozone, establishing the main differences in terms of the compounds formed at different applied doses of the oxidizing agent, as well as the germicidal activity they acquire when they are oxidized by one of the selected pathways.

2. MATERIALS AND METHODS

2.1. Sunflower oil

The sunflower oil used in this work was of edible quality and supplied by the Borges Company, Spain.

2.2. UV irradiation of sunflower oil

The irradiation of sunflower oil samples was carried out in a stainless-steel photochemical reactor, equipped with a Philips TUV mercury vapor lamp, 254 nm and 11 w power. The samples were stirred at

200 rpm, the contact time was 2 and 4 h, G UV 2h and G UV 4h.

2.3. Ozonation of sunflower oil

A volume of 40 mL of sunflower oil was ozonized in a bubbling reactor at a controlled temperature of 25.0 ± 0.1 °C. The ozone gas flow rate was 30 L/h and the total ozonation time was 30 min. Ozone was generated through AQOZO equipment (CNIC, Cuba), with an initial ozone concentration of 70 mg/L, measured on an Ultrospec III spectrophotometer (Pharmacia LKB, Sweden). The reaction was carried out in the absence of solvents. The applied ozone doses were 4.3 and 25.8 g /L, for G10 and G30 ozonized oils respectively.

2.4. Determination of the peroxide value (PV)

The sample weight was 0.5 g, which was mixed with a solution of glacial acetic acid:chloroform 3:2 v/v. A volume of 0.5 mL of a potassium iodide saturated solution was added. The mixture was left in the dark for two minutes, then 30 mL of distilled water were added and the mixture was titrated with a sodium thiosulfate solution of 0.1 mol/L. The result is expressed as milligrams of active oxygen per kilogram of sample. The peroxide index value was calculated using the expression:

$$PV = [(M \cdot V)/Pm] \cdot 1000$$

where:
M: Concentration of sodium thiosulfate (0.1 mol/L).
V: Volume of sodium thiosulfate consumed in the titration (mL).
Pm: Weight of the sample (mg)

2.5. Determination of TAG composition

TAG analysis was carried out by gas chromatography on an Agilent 6890 gas chromatograph (Palo Alto, CA) using a Quadrex Aluminium-Clad 400-65HT capillary column (30 m length, 0.25 mm inner diameter, 0.1 μm film thickness: Woodbridge, CT, USA) and a flame ionization detector. The working temperatures were 360 °C in the injector and 370 °C in the detector. The injection split ratio was 1:80, with hydrogen as the carrier gas at a rate of 50 cm/s. The method used was isothermal at 335 °C, with a pressure gradient of 100 to 180 kPa. The peaks were identified by their retention times, which were compared to those of the database obtained from the analyses of oils of known composition.

2.6. Fatty acid determination

Fatty acids were determined as their fatty acid methyl esters (FAMEs) by GC. For this purpose, sunflower oil was transesterified to its corresponding methyl esters by treating 5 mg of sample with 1.5 mL of methanol/toluene/sulfuric acid (88/10/2; v/v/v) for 1 hour at 80 ºC. This determination was made on an Agilent 6890 gas chromatograph (Palo Alto, CA) with a flame ionization detector (FID operating at 200 ºC). The silica gel capillary column used was SP-2380 (30 m x 250 μm x 0,20 μm, Bellefonte, PA). Hydrogen was used as a carrier gas at a rate of 28 cm/s. The injection volume was 1 μL.

2.7. Separation of non-polar and polar triacylglycerol fractions

The separation of the non-polar and polar lipid compounds of the oxidized samples was carried out by adsorption chromatography on a silica gel column, according to the method established by Waltking and Wessels (1981).

The polar lipid content was determined using the following formula:

$$CP\ (\%) = [pM\text{-}pF1]/pM \cdot 100$$

where:
CP- content of polar compounds,
pM- weight of the sample
pF1-weight of the non-polar lipid fraction.

2.8. Molecular exclusion HPLC analysis

The sample preparation consisted of dissolving 10 mg of sample into 1 mL of tetrahydrofuran, which was injected into the HPLC system for molecular exclusion analysis. The HPLC system consisted of a Waters 2695 separation module equipped with a 2420 ELS detector. Two stainless steel columns of 30 cm in length and 7.7 mm internal diameter, packed with a styrene-divinylbenzene copolymer with a particle diameter of 5 μm and different pore

sizes were used. One had a pore size of 10 nm and the other of 50 nm (Waters Associates, USA). The columns were connected in series starting with the 50 nm column. Tetrahydrofuran was used as the mobile phase at a flow rate of 1 mL/min. Stearic acid monoglyceride was used as an internal standard for quantification (1 mg/mL).

2.9. Minimal inhibitory concentration (MIC) of ozonized oils

Antimicrobial activity was determined by the agar dilution method according to CLSI (2012) guidelines; the final inoculum was 103 cfu/mL. Petri plates were prepared with Mueller Hinton agar containing oxidized sunflower oil samples at concentrations ranging from 5 mg/mL to 100 mg/mL. The MIC was defined as the lowest concentration of oils inhibiting visible bacterial growth after incubation for 20 h at 37 °C. Staphylococcus aureus ATCC 25923, Pseudomonas aeruginosa ATCC 27853, Escherichia coli ATCC 25922 and yeast Candida albicans ATCC10231 were used.

2.10. Statistical analysis

The mean and standard deviation of the three determinations performed in each analysis were given.

3. RESULTS AND DISCUSSION

3.1. Photooxidation of sunflower oil with UV light

The irradiated sunflower oil showed an initial PV of 19.8. Irradiation produced a rapid increase in PV in the first 2 h, reaching values of 95.5 (Table 1). However, this rapid production of peroxides declined at longer exposure times, dropping to a PV of 59.0 after 4 h of exposure to UV radiation. This result shows a double effect of radiation, which induced the formation of peroxides but also destroyed them. Thus, irradiation would cause the decomposition of hydroperoxides resulting in secondary degradation products at long exposure times, producing compounds such as carboxylic acids and aldehydes, which do not contribute to PV value, which tended to decrease, as observed in Table 1.

Table 1. Oxidation parameters and unsaturated fatty acid content in sunflower oils oxidized by UV radiation. Results are the average of three determinations.

			Content (%)	
	PV	**Polar fraction**	**18:1**	**18:2**
Initial oil	19.8	n.d.	52.30 ± 0.01	38.40 ± 0.02
UV2h	95.5	15.3	52.85 ± 0.07	35.75 ± 0.07
UV4h	59.0	11.4	53.00 ± 0.20	35.80 ± 0.42

3.2. Determination of the content of polar lipid compounds in irradiated samples

Fractionation according to the polarity of compounds is one of the techniques used for the characterization of oxidized samples (Ledea-Lozano *et al*., 2005). Its main objective is to quantify the amount of modified triacylglycerols (polar lipid fraction) and those that have not been modified (non-polar lipid fraction). In this study, the results showed that at two hours of irradiation the polar lipid fraction was 15.3%; while at four hours it was 11.4% (Table 1). This result shows that there was degradation of the oxidation products at longer exposure times, in good correlation with the results of PV. Furthermore, photooxidation was mainly affected by linoleic acid. Thus, the results in Table 1 showed that the only fatty acid that showed a decrease in its proportion was that fatty acid with a decrease of approximately 2.6% in the oxidized samples. This result agrees with the higher rates of autoxidation expected for all polyunsaturated fatty acids. The level of linoleic acid in the oils oxidized at 2 and 4 h was the same, indicating that there was no advance in the propagation of the reaction.

3.3. Influence of irradiation on TAG aggregation

The study of the impact of photooxidation on sunflower oil was completed by looking into the composition of the polar lipid fractions by molecular exclusion HPLC. This technique separated glycerolipids of different molecular weights and thus gives information on the formation of dimers and oligomers. These compounds are formed by the condensation of TAGs carrying peroxide derivatives, and their presence alters the oil's physical properties and increases parameters such as oil viscosity. The results of the TAG aggregation of the polar fractions in the irradiated oils are shown in Figure 1. It can be observed that as a result of irradiation, there is no formation of oligomers, but only dimers, and the concentration of dimers was higher (2.41%) at 2 h of irradiation than at 4 h. No significant differences were observed in the

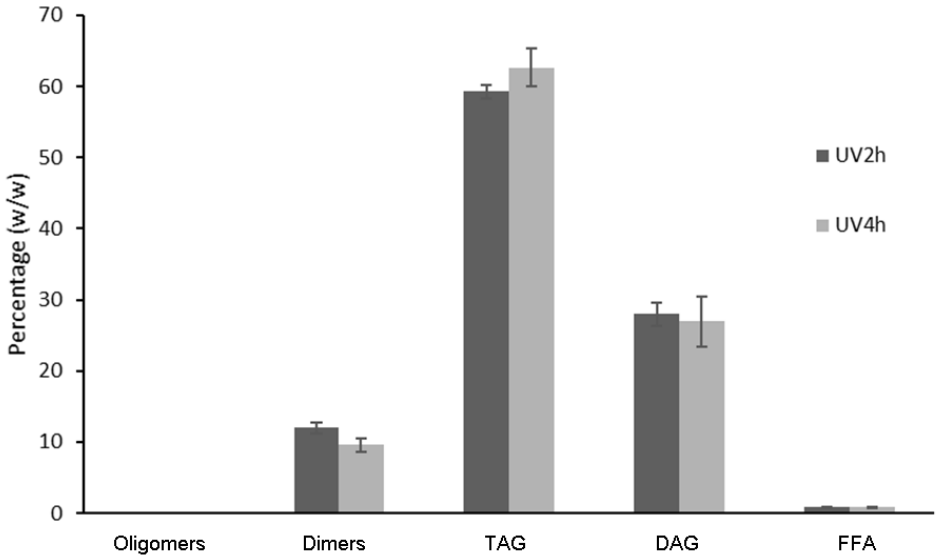

Figure 1. Composition of polar lipid fraction of UV light-irradiated sunflower oil. Data correspond to the average, plus or minus SD, of 3 independent experiments. TAG: altered triacylglycerols; FFA: free fatty acids

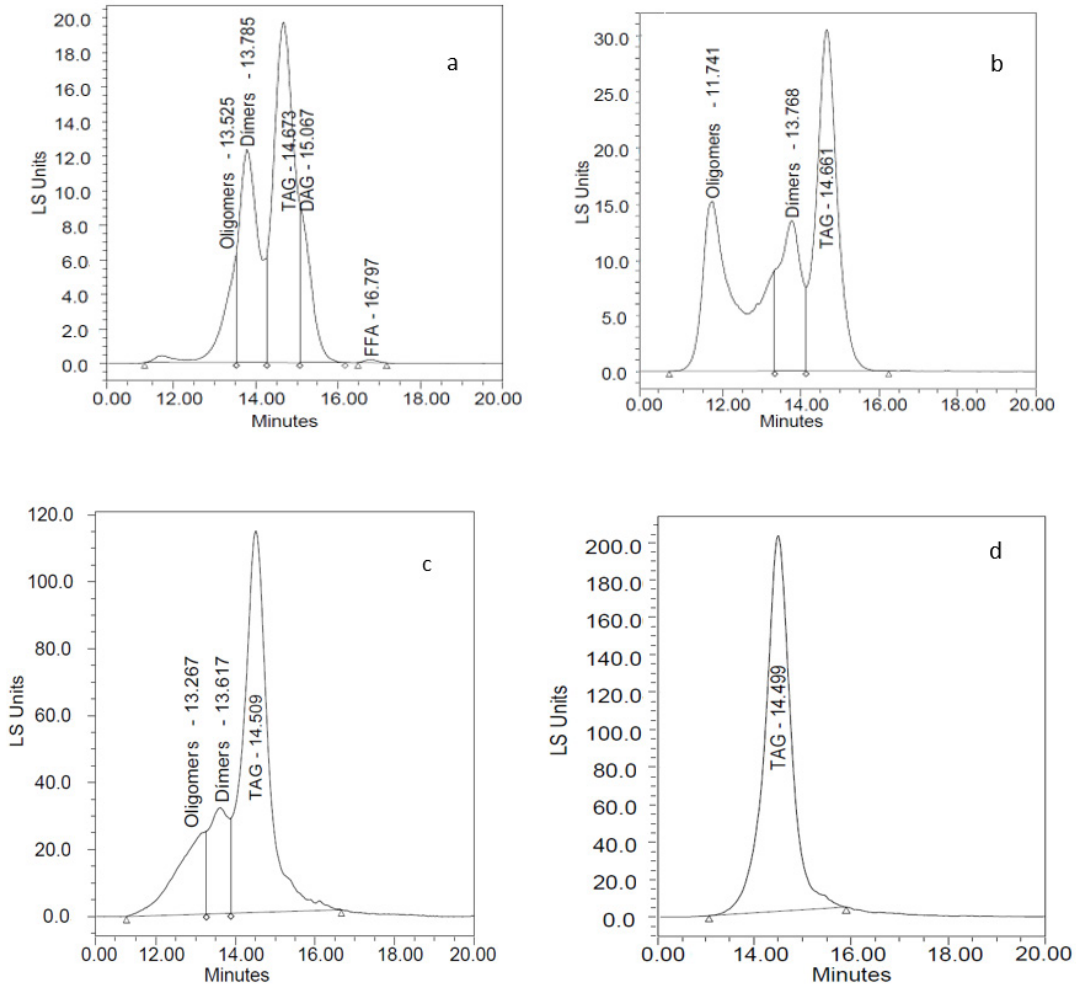

Figure 2. Molecular exclusion HPLC chromatograms from: a) Sunflower oil ozonized with 10% polar lipid compounds; b) Sunflower oil ozonized with 30% polar lipid compounds; c) Sunflower oil ozonized OLEOZON; d) Initial sunflower oil.

other compounds, which were altered TAGs, DAGs, and free fatty acids. The decrease observed in dimer concentration due to the effect of prolonged irradiation was in good agreement with the decrease in peroxides observed in these samples, indicating that at 2 h the termination reactions predominate over those of propagation and no more peroxides with potential biological activity were formed.

3.4. Oxidation parameters of different ozonized sunflower oils

In the present work, 3 ozonized sunflower oils were studied, differing in the dose of ozone applied to them. Therefore, the oils named G10 and G30 are partially ozonized oils, receiving doses of 4.3 and 25.8 g/L ozone. The sample named OLEOZON was the final commercial sample, which received ozone levels of 43 g/L ozone. The oxidative alterations of the oils after reacting with the amounts of ozone are shown in Table 2. When the amount of ozone applied increased, the PV of the oils increased too, with a substantial rise from G10 to G30 (from 49.6 to 159.0). The polar lipid fraction increased accordingly, which showed that ozonation is not subjected to the limitations of photooxidation and is able to increase the amount of peroxides without any apparent limitation. These high levels of oxidation affected the composition of the fatty acids. Therefore, the presence of nonanoic acid in considerable amounts (9:0) in the ozonized samples was remarkable. This fatty acid is not present in the initial oil but accumulated during the ozonation process due to lysis of ozonides formed from oleic acid, and its presence is consistent with previously published results. (Ledea-Lozano *et al*., 2005; Ledea-Lozano *et al.,* 2019). Unsaturated fatty acids decreased in very appreciable amounts. In this case, both oleic and linoleic acid showed substantial decreases in all ozonized oils, although linoleic acid was the one that decreased faster (Table 2). This pattern of decrease in unsaturated fatty acids was expected due to ozone attacks on all the double bonds present in the oil, but there was no preference for those in polyunsaturated fatty acids. In the case of linoleic acid, the possibility of reacting with the added ozone was double, which explains its faster decline. This contrasts with the photooxidation of this oil, which was almost exclusively affected by linoleic acid and in a much lower proportion (Table 1). Changes in the TAG composition observed in the neutral lipid fraction corresponding to ozonized oil samples indicated that those carrying a higher number of double bonds were those that decayed faster throughout ozonation, with important decreases in the most unsaturated LLL and OLL (Table 3). In contrast, the TAGs which displayed a lower level of unsaturation (POO and StOO) experimented an increase in its proportion after the process of ozonation.

Table 2. Oxidation parameters and unsaturated fatty acid content in sunflower oils oxidized by UV radiation. Results are the average of three determinations.

		Content (%)			
	PV	**Polar fraction**	**9:0**	**18:1**	**18:2**
Initial oil	19.8	n.d.	0.00 ± 0.00	52.3 ± 0.04	38.4 ± 0.08
G10	49.6	13.3	0.25 ± 0.07	50.3 ± 0.70	36.3 ± 0.71
G30	159.0	32.2	1.75 ± 0.21	46.3 ± 0.00	29.00 ± 0.21
OLEOZON			3.00 ± 0.14	42.6 ± 0.35	21.5 ± 0.42

Table 3. Triacylglycerol composition of the neutral lipid fraction of the initial sunflower oil and ozonized oils used in this work. TAGs with proportions lower than 2 % were excluded.

	Content (w/w %)								
Sample	**POO**	**POL**	**PLL**	**StOO**	**OOO**	**OOL**	**StLL**	**OLL**	**LLL**
Initial oil	6.2	4.5	5.5	4.3	36.2	8.3	3.3	15.2	11.1
G10	6.6	4.6	5.5	4.5	36.5	8.2	3.3	14.6	10.2
G30	7.8	4.8	5.1	5.4	36.1	7.6	3.3	12.5	7.8

Triacylglycerols were named with 3-letter coding for the fatty acids esterified. P, palmitic, St, stearic, O oleic and L linoleic. Data are the average of 3 determinations. The SDs were under 5% in all cases.

3.5. Characterization of ozonized oil by molecular exclusion HPLC

Previous studies on ozonized oils indicated that the high level of peroxidation induced by this reaction significantly altered the aggregation of TAGs, causing the presence of TAGs oligomers in considerable proportion, which altered the physical properties of the oil, increasing its viscosity (Ledea-Lozano *et al*., 2019b). This effect of ozonation was also observed in this work. Therefore, the initial sunflower oil showed a single peak corresponding to TAGs in the HPLC exclusion system used in this work (Figure 2a), and the samples with different ozone loads, G10 and G30, considerably increased their proportion of TAG dimers and smaller amounts of oligomers began to appear in the oil (Figure 2b and 2c). Finally, OLEOZON oils, which received the highest ozone load, showed a remarkable amount of polymerized TAGs (Figure 2d). The results of the composition of the different ozonized oils are shown in Figure 3, where the formation of considerable amounts of polymeric compounds can be seen, which increased the viscosity of the product. This result is consistent with the previous ones obtained for ozonized oils (Menéndez *et al*., 2008; Uzun *et al*., 2018; Ledea-Lozano *et al*., 2019a). In the case of OLEOZON, it was the oil that received the highest ozone load and contained a very notable proportion of dimers and oligomers, which reached 30% (Figure 3). It is important to note that the proportion of polymerized TAGs corresponds to

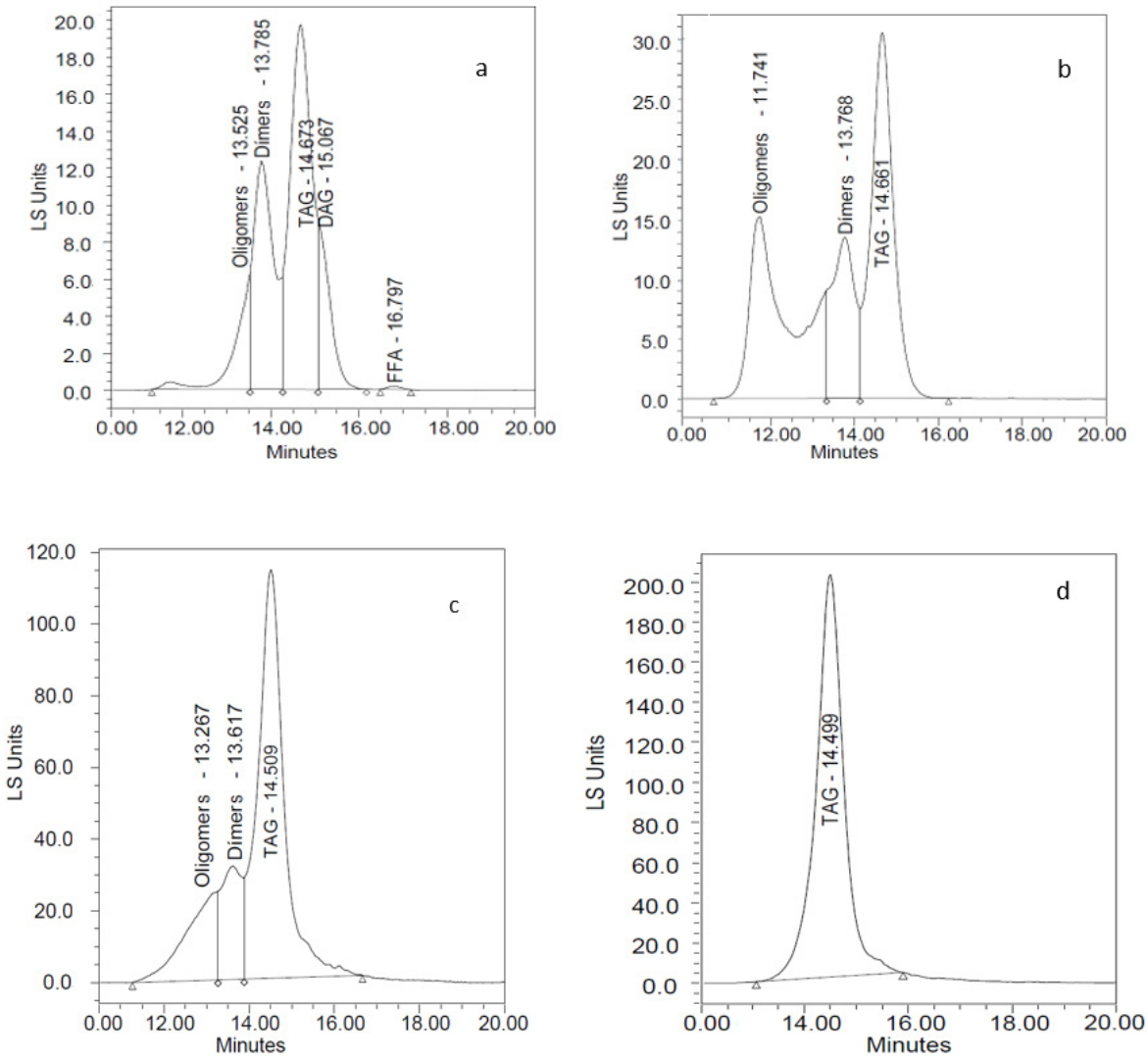

FIGURE 2. Molecular exclusion HPLC chromatograms from: a) Sunflower oil ozonized with 10% polar lipid compounds; b) Sunflower oil ozonized with 30% polar lipid compounds; c) Sunflower oil ozonized OLEOZON; d) Initial sunflower oil.

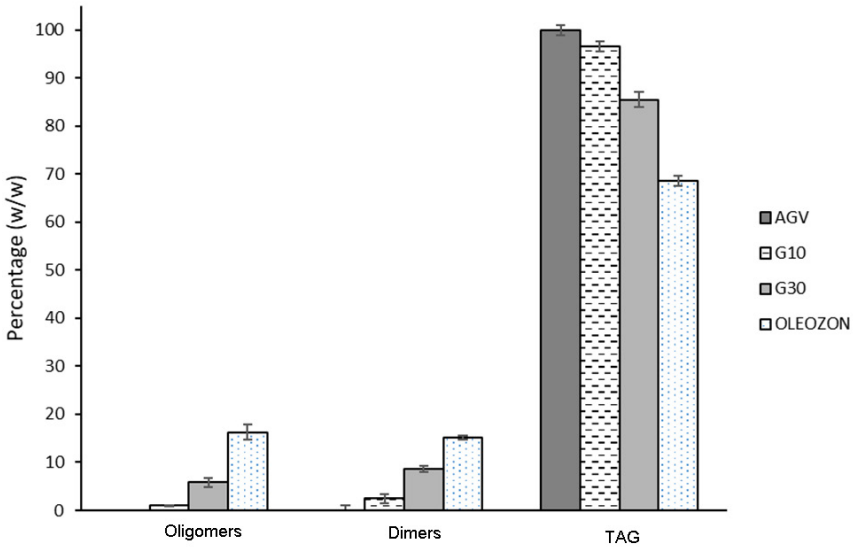

FIGURE 3. Chromatogram of the molecular exclusion HPLC analysis of virgin sunflower oil, partially ozonized sunflower oil and OLEOZON. Data correspond to the average, plus or minus SD, of 3 independent experiments. TAG: triacylglycerols; FFA: free fatty acids

the dose of ozone applied in any case. This result was also reported by other researchers (Guerra-Blanco *et al*., 2021).

3.6. Analysis of the behavior of polymers formed in aged ozonized sunflower oil

A molecular exclusion HPLC analysis of ozonized sunflower oil samples stored at 5 ºC for one and three months (Figure 4) was also carried out. The initial ozonized oil contained 68% non-polymerized TAG and amounts of dimers and oligomers at around 15%. The aged sample showed a significant increase in TAGs, with levels close to 80% at the expense of a notable decrease in the polymerized ones, which remained at around 10% for both groups (dimers and oligomers, Figure 4). This means that during storage, the polymerized TAGs tend to decompose, probably due to the breakage of the peroxide bonds that exist between them. This should involve a loss in viscosity values, which has been previously reported in previous storage trials (Menéndez *et al.*, 2008). Peroxidic species formed during the ozonization of sunflowers are metastable species that tend to decay with storage time. This decay involves loss

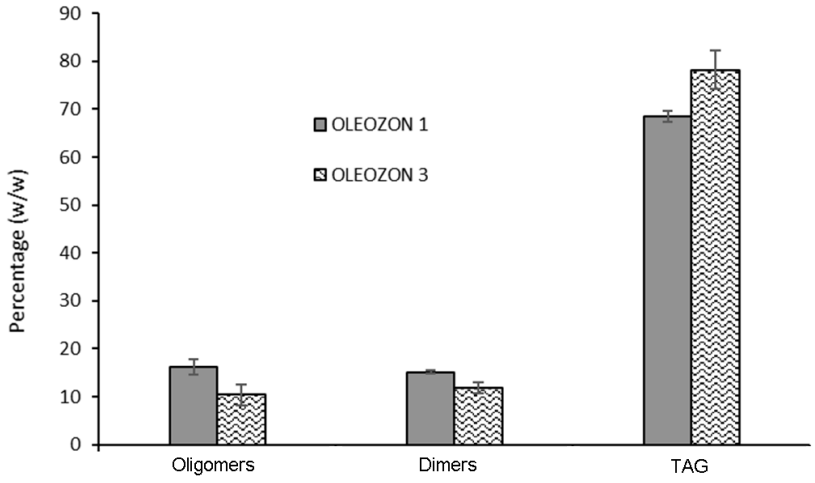

FIGURE 4. Variations in the compositions of TAGs, dimers and oligomers in OLEOZON with one (OLEOZON 1) and three months (OLEOZON 3) of storage from 2 to 8 °C. Data correspond to the average, plus or minus SD, of 3 independent samples. TAG: triacylglycerols; FFA: free fatty acids

in viscosity and, most likely, loss in antimicrobial activity, so it is important to store this product under proper storage conditions in order to avoid these peroxide decompositions, with low temperature storage, and the absence of humidity.

3.7. Determination of the antimicrobial activity of oxidized sunflower oil

The samples irradiated with UV light did not show germicidal activity within the minimum inhibitory concentration (MIC) range established in this work (Table 4), whose upper limit was 100 mg of oxidized oil per mL of sample. MIC values higher than that are not of interest because of the high amount of oil is necessary to obtain any effect. In general, in the case of experiments with UV light, the results indicate that as the irradiation time increases, the peroxidic species formed disappear, so the total amounts of active peroxides accumulated are too low to achieve the necessary inhibitory activity.

Table 4 shows the minimum inhibitory concentration values obtained for ozonized sunflower oil against one Gram-positive bacterium (Staphylococcus aureus ATCC 25923), two Gram-negative (Escherichia coli ATCC 25922 and Pseudomona aeruginosa ATCC 27853) and one yeast (Candida albicans ATCC 10231). The results show that as the applied dose of ozone and the content of polar compounds increased in the ozonized oil, the MIC value decreased, which means that the oil displayed higher antimicrobial activity. This indicated that the peroxides formed during that process are responsible for the biocidal effect and that a longer ozonation times involves the presence of higher amounts of compounds with biocidal activity. This result is consistent with the criterion that the mixture of peroxy compounds formed by ozonation constitutes the active pharmaceutical ingredient in these oils and is in good agreement with previous results (Poznyak *et al*., 2018). The MIC values obtained for each microorganism are related to their morphological and physiological characteristics. Growth inhibition was observed in smaller amounts of ozonized sunflower oil in the case of Gram-positive bacteria and yeast. A greater resistance was observed for the two Gram-negative bacteria. Similar results have been shown for bacteria and yeast treated with ozonized oils (Sechi *et al*., 2001; Ugazio *et al*., 2020; Higa *et al*., 2022). This evaluation shows that even at low doses, ozonized sunflower oil has a remarkable germicidal character that is not present in autoxidized oils.

CONCLUSIONS

Two oxidation methods were applied to sunflower oil, irradiation with UV light and ozonation. The results showed that ozonation supplied a greater formation of peroxidic compounds as the applied dose increased. Irradiation at a higher dose resulted in the decomposition of the peroxidic compounds formed. The study of the polar lipid fraction confirmed this result. The oxidation processes studied induced the polymerization of the triacylglycerols present in sunflower oil, from which dimers, and oligomers were formed. The stored OLEOZON samples showed a decrease in dimers and oligomers and an increase in triacylglycerols between one and three months of storage. The oils irradiated with UV showed no germicidal activity. In the case of ozonized samples, they were able to inhibit the bacteria Pseudomonas

Table 4. Minimum inhibitory concentration values for partially ozonized sunflower and OLEOZON obtained against the studied microorganisms.

	MIC (mg/mL)			
	Staphylococcus aureues ATCC 25923	*Pseudomona aeruginosa* ATCC 27853	Escherichia coli ATCC 25922	*Candida albicans* ATCC 10231
UV2h	nd	nd	nd	nd
UV4h	nd	nd	nd	Nd
G10	44.5	89	89	44.5
G30	22.26	44.5	44.5	11.13
OLEOZON	2.78	5.76	5.76	2.78

nd: inhibition not detected below 100 mg/mL.

Results are the average of 3 determinations. Standard deviation remained below 10% for these values.

aeruginosa, Escherichia coli and Staphylococcus aureus and the yeast Candida albicans, and showed a lower MIC value as the ozone dose applied to the sunflower oil increased.

ACKNOWLEDGMENTS

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DATA AVAILABILITY

The data in this article is available on reasonable demand

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