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Influence of storage conditions on the stability of ozonized vegetable oils

Influência das condições de armazenamento na estabilidade de óleos vegetais ozonizados

Influencia de las condiciones de almacenamiento en la estabilidad de aceites vegetales ozonizados

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Abstract
Ozonation of polyunsaturated vegetable oils imparts antimicrobial, wound healing, and antioxidant properties, expanding their potential applications in pharmaceutical, cosmetic, and food industries. These oils have been increasingly used as natural alternatives in wound treatments due to the formation of reactive oxygen species, such as peroxides, hydroperoxides, and ozonides, during the ozonation process. This study aimed to evaluate the ozonation levels of six polyunsaturated vegetable oils (sunflower, coconut, copaiba, grape seed, olive, and rosehip), using peroxide value as an indicator. Among these, sunflower oil exhibited the highest peroxide value and was selected for detailed monitoring of the ozonation process, including infrared spectroscopy and peroxide value analyses. To assess the effects of storage, the ozonized oil was stored under three conditions: freezer (-18°C), refrigerator (4°C), and room temperature (25°C), with evaluations every 30 days over 180 days. Results demonstrated that room temperature significantly reduced the peroxide value, compromising oxidative stability, while freezer and refrigerator storage better preserved reactive species, such as peroxides, hydroperoxides, and ozonides, with the freezer offering the highest level of conservation. These storage conditions are essential to maintain the antimicrobial and wound-healing potential of ozonized oils, particularly for therapeutic applications. This study highlights the importance of adequate storage strategies to ensure the efficacy and longevity of ozonized oils, contributing to the development of high-value-added products.
Keywords: Ozonized oils; Ozonides; Peroxide value; Infrared; Storage; Oxidative stability.

Resumo
A ozonização de óleos vegetais poli-insaturados confere propriedades antimicrobianas, cicatrizantes e antioxidantes, ampliando o potencial de suas aplicações nas indústrias farmacêutica, cosmética e alimentícia. Esses óleos têm sido cada vez mais utilizados como alternativas naturais em tratamentos de feridas devido à formação de espécies reativas de oxigênio, como peróxidos, hidroperóxidos e ozonídeos, durante o processo de ozonização. Este estudo teve como objetivo avaliar os níveis de ozonização de seis óleos vegetais poli-insaturados (girassol, coco, copaíba, semente de uva, oliva e rosa mosqueta), utilizando o índice de peróxidos como indicador. Entre esses óleos, o óleo de girassol apresentou o maior índice de peróxidos e foi selecionado para o monitoramento detalhado do processo de ozonização, incluindo análises por espectroscopia no infravermelho e índice de peróxidos. Para avaliar os efeitos do armazenamento, o óleo ozonizado foi armazenado em três condições: freezer (-18°C), refrigerador (4°C) e temperatura ambiente (25°C), com avaliações realizadas a cada 30 dias ao longo de 180 dias. Os resultados demonstraram que a temperatura ambiente reduziu significativamente o índice de peróxidos, comprometendo a estabilidade oxidativa, enquanto o armazenamento no freezer e no refrigerador preservou melhor as espécies reativas, como peróxidos, hidroperóxidos e ozonídeos, sendo o freezer a condição que ofereceu o maior nível de conservação. Essas condições de armazenamento são essenciais para manter o potencial antimicrobiano e cicatrizante dos óleos ozonizados, especialmente para aplicações terapêuticas. Este estudo destaca a importância de estratégias adequadas de armazenamento para garantir a eficácia e a longevidade dos óleos ozonizados, contribuindo para o desenvolvimento de produtos de alto valor agregado.
Palavras-chave: Óleos ozonizados; Ozonídeos; Índice de peróxidos; Infravermelho; Armazenamento; Estabilidade oxidativa.

Resumen
La ozonización de aceites vegetales poliinsaturados confiere propiedades antimicrobianas, cicatrizantes y antioxidantes, ampliando su potencial de aplicación en las industrias farmacéutica, cosmética y alimentaria. Estos

aceites se han utilizado cada vez más como alternativas naturales en tratamientos de heridas debido a la formación de especies reactivas de oxígeno, como peróxidos, hidroperóxidos y ozónidos, durante el proceso de ozonización. Este estudio tuvo como objetivo evaluar los niveles de ozonización de seis aceites vegetales poliinsaturados (girasol, coco, copaiba, semilla de uva, oliva y rosa mosqueta), utilizando el índice de peróxidos como indicador. Entre estos, el aceite de girasol mostró el mayor índice de peróxidos y fue seleccionado para el monitoreo detallado del proceso de ozonización, incluyendo análisis por espectroscopía infrarroja y evaluaciones del índice de peróxidos. Para evaluar los efectos del almacenamiento, el aceite ozonizado se almacenó en tres condiciones: congelador (-18°C), refrigerador (4°C) y temperatura ambiente (25°C), con evaluaciones cada 30 días durante 180 días. Los resultados demostraron que la temperatura ambiente redujo significativamente el índice de peróxidos, comprometiendo la estabilidad oxidativa, mientras que el almacenamiento en congelador y refrigerador preservó mejor las especies reactivas, como peróxidos, hidroperóxidos y ozónidos, siendo el congelador la condición que ofreció el mayor nivel de conservación. Estas condiciones de almacenamiento son esenciales para mantener el potencial antimicrobiano y cicatrizante de los aceites ozonizados, especialmente para aplicaciones terapéuticas. Este estudio resalta la importancia de estrategias de almacenamiento adecuadas para garantizar la eficacia y la longevidad de los aceites ozonizados, contribuyendo al desarrollo de productos de alto valor añadido.
Palabras clave: Aceites ozonizados; Ozónidos; Índice de peróxidos; Infrarrojo; Almacenamiento; Estabilidad oxidativa.

1. Introduction

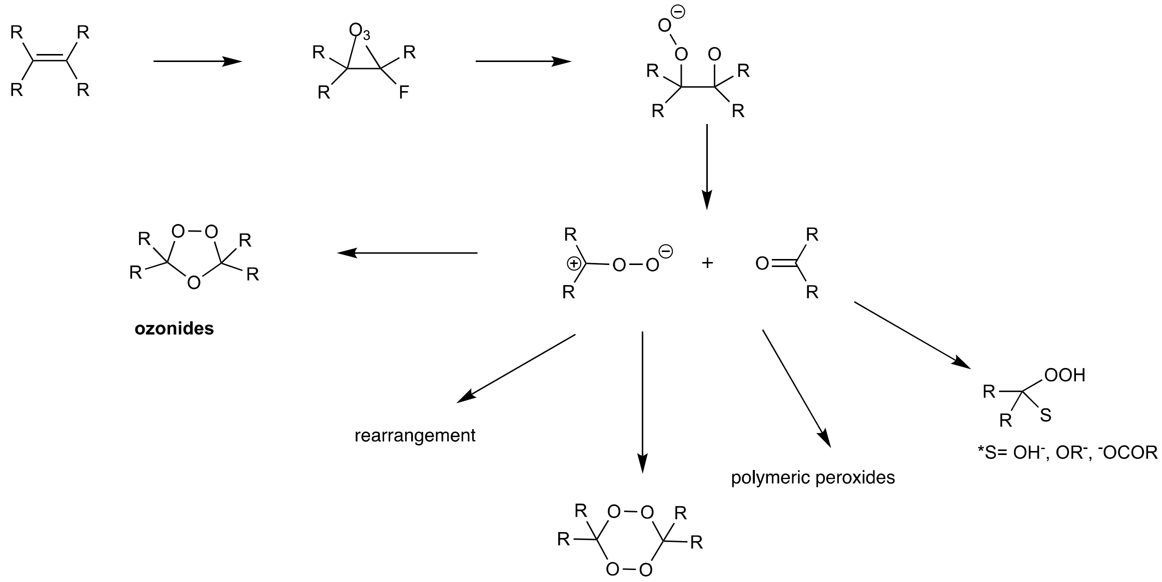
Ozone (O_3) is a molecule composed of three oxygen atoms arranged in an angular structure, a characteristic that grants it high instability and remarkable oxidative capacity (Sciorsci et al., 2020). Due to its mesomeric states, ozone is widely recognized as one of the most powerful oxidants, exhibiting effective antimicrobial activity against a broad spectrum of pathogens, including bacteria, fungi, viruses, and protozoa (Braidy et al., 2018; Azarpazhooh & Limeback, 2008). In the current era of increasing antimicrobial resistance, the search for therapeutic alternatives capable of inactivating pathogens without promoting resistance development has become a priority. In this context, ozone stands out as a promising tool due to its rapid and nonspecific mechanism of action, which minimizes the adaptive capacity of microorganisms. Additionally, growing evidence suggests that ozone has anti-inflammatory and wound-healing properties, with proven efficacy in patients with chronic wounds (Valacchi et al, 2013; Xiao et al, 2017; Lin et al, 2019; Lu et al, 2023).

However, its practical application of ozone faces significant challenges. Its ephemeral nature and short lifespan in the gaseous state limit its use, necessitating on-site production to ensure effectiveness. A promising approach to overcoming these limitations is the incorporation of ozone into stable matrices, such as vegetable oils, through ozonation processes. This technique, which exploits the reactivity of ozone with unsaturated fatty acids, has shown great potential in the development of products for the pharmaceutical, cosmetic, and food industries (Soriano et al, 2003; Sadowska et al, 2008).

Ozonized vegetable oils, generally liquid or semi-solid at room temperature, possess sufficient stability for storage and commercial use. Oils such as olive, sunflower, sesame, grape seed, and coconut have been widely investigated due to their distinct fatty acid compositions, which directly influence the chemical properties and stability of the final products (Díaz et al., 2005, 2006b; Díaz-Gómez et al., 2009; Valacchi et al., 2011). The quality of ozonized oils, however, depends on various technical factors, including the type and quality of the ozone generator, ozone concentration and gas flow, and ozonation conditions such as reaction time, type of material, and presence of water or catalysts.

Ozonation involves complex reactions described by the Criegee mechanism (Figure 1), in which ozone reacts with unsaturated double bonds to form unstable intermediates known as primary ozonides. These intermediates rapidly decompose, generating zwitterions and carbonyl fragments that can recombine under anhydrous conditions to form cyclic trioxolanes (ozonides) or, in the presence of water, produce aldehydes and peroxides (Criegee, 1975; Jardines et al., 2003). Although ozonides are relatively stable, their degradation can be accelerated by exposure to light, elevated temperatures, or humidity, resulting in secondary products such as aldehydes, which contribute to unpleasant odors (rancidity).

Figure 1 - Mechanism proposed by Criegee in 1953.



Source: Authors.

With the growing demand for sustainable methods and natural products, interest in the ozonation of vegetable oils has increased substantially, as evidenced by the rising number of scientific studies and patent registrations on their industrial applications (Justia Patents, 2022; US Patent Office, 2006; European Patent Office, 2009; Russian Patent Office, 2017; WIPO, 2008).

This study aims to evaluate the durability of ozonized vegetable oils under different storage conditions: freezer (8°C), refrigerator (4°C), and room temperature (25°C). Critical parameters such as peroxide value, acidity, and oxidative stability will be monitored over time to determine the quality and integrity of these products. Understanding these variables is essential to ensuring the efficacy and safety of ozonized oils, as well as providing practical guidelines for preserving their active properties and maximizing their shelf life. Thus, this study seeks to contribute to the development of high-quality products that meet growing industrial demands, offering consumers safe and durable alternatives.

2. Methodology

This study was conducted in an experimental, laboratory-based, and quantitative manner, aiming to ensure methodological rigor and reproducibility of the results. The approach allowed careful control of the variables involved in the ozonation process and the storage of vegetable oils. The methodology was developed based on previously established procedures and adapted to meet the specific objectives of the research, as described by Pereira et al. (2018).

2.1 Materials and Reagents

The reagents used in the chemical analyses were all analytical grade, including glacial acetic acid, chloroform, and indicators for peroxide determination. Refined vegetable oils were used, including sunflower seed oil **(***Helianthus annuus***)**, coconut oil **(**Cocos nucifera**)**, olive oil **(**Olea europaea**)**, grape seed oil **(***Vitis vinifera***)**, copaiba oil **(***Copaifera* spp.), and rosehip oil (*Rosa rubiginosa*), purchased from local stores and pharmacies. The ozone generator used for the oil synthesis was a Wier® brand (Brazil), with a capacity of 10 g/h.

2.2 Ozonation Process

The vegetable oils sunflower, olive, coconut, grape seed, rosehip, and copaiba were subjected to the ozonation process. An ozone generator (Wier, 10 g/h capacity) was used, calibrated to a flow rate of 0.5 L/min to ensure the optimal ozone concentration for an effective reaction. Each sample was prepared with 50 mL of vegetable oil, to which 0.5 mL of distilled water was added to enhance ozone dispersion in the reaction medium. The ozonation process was conducted in a well-ventilated fume hood using a closed reactor to prevent ozone gas release. The reaction parameters, such as temperature and time (20 minutes), were strictly controlled to promote efficient ozonide formation. After ozonation, the oils were stored in amber glass bottles at -18°C to prevent degradation caused by light and temperature.

For the stability studies during storage, 150 mL of sunflower oil, with 1% distilled water added, were ozonized at a higher flow rate of 1.0 L/h for 2 hours. The ozonized oil was then divided into three 50 mL aliquots, which were stored under distinct conditions to evaluate their stability over time.

2.3 Peroxide Value

The vegetable oils sunflower, olive, coconut, grape seed, copaiba, and rosehip were analyzed using the peroxide value according to the AOCS Cd 8-53 standard method, as well as the stability studies conducted on sunflower oil.

2.4 Infrared Analysis

Aliquots of ozonized sunflower oil with increasing concentrations were collected throughout the ozonation process and analyzed using an Agilent FT-IR spectrometer.

2.5 Storage Conditions

Ozonized sunflower seed oil (50 mL) with a peroxide value of 380 mEq O_2/Kg was stored under three different conditions: freezer (-18°C), refrigerator (4°C), and room temperature (25°C). Stability evaluations were conducted at intervals of 30, 60, 90, 120, and 180 days to monitor changes in quality parameters and determine the oil's durability under different storage conditions.

2.6 Statistical analysis

The data obtained were analyzed using appropriate statistical tests, including ANOVA and Tukey's test, to compare the storage conditions and identify significant differences in the stability parameters of the oils.

3. Results and Discussion

3.1 Degree of Ozonation in Vegetable Oils

In this study, six vegetable oils (sunflower, coconut, copaiba, grape seed, olive, and rosehip) were subjected to ozonation to evaluate their oxidative reactivity, measured by peroxide value. The detailed results of these indices are presented in Table 1, providing a comparative overview of the analyzed oils.

Table 1 - Peroxide value of vegetable oils after ozonation.

Vegetable Oil	Peroxide Value (mEq O_2/Kg)
Copaiba	76,2
Rosehip	110
Olive	200
Grapeseed	116
Coconut	88
Sunflower	230

Source: Author's data.

Sunflower oil exhibited the highest peroxide value (230 mEq O_2/Kg), reflecting the greatest degree of ozonation among the tested oils, which justifies its selection for stability and storage studies. This result can be attributed to the high concentration of polyunsaturated fatty acids in its composition, such as linoleic acid, which are highly susceptible to reaction with ozone. In contrast, copaiba oil showed the lowest peroxide value (76.2 mEq O_2/Kg), possibly due to its terpene-rich composition. These compounds have chemical structures that react differently with ozone, resulting in lower peroxide formation compared to unsaturated fatty acids.

Similarly, coconut oil (88 mEq O_2/Kg) exhibited low reactivity, which can be explained by its predominant composition of medium-chain saturated fatty acids, such as lauric acid. These fatty acids are less prone to oxidation by ozone due to the absence of double bonds in their carbon chains. On the other hand, olive oil (200 mEq O_2/Kg) and grape seed oil (116 mEq O_2/Kg) showed intermediate peroxide values, suggesting a moderate capacity to form ozonides. In the case of olive oil, the presence of monounsaturated fatty acids, such as oleic acid, confers higher reactivity to ozone compared to coconut oil, although still lower than sunflower oil, due to its lower amount of polyunsaturated fatty acids. Meanwhile, grape seed oil, with a higher proportion of linoleic acid, would theoretically exhibit a reactivity profile closer to sunflower oil. However, it showed a peroxide value of 116 mEq O_2/Kg, lower than expected, which may be attributed to the presence of natural antioxidant compounds in its composition. These antioxidants may act as modulators, reducing the oil’s susceptibility to oxidation even in the presence of a high concentration of polyunsaturated fatty acids.

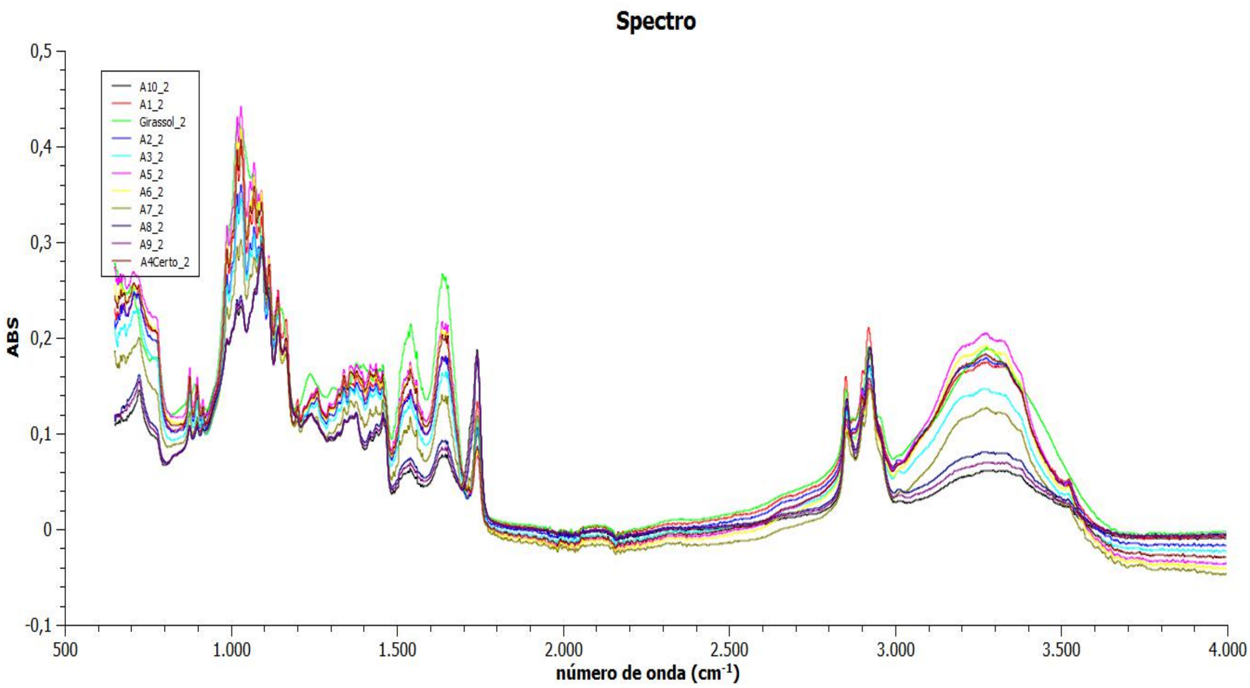
These results, presented in Table 1, highlight the variability in the formation of reactive species, such as peroxides, hydroperoxides, and ozonides, among the different analyzed oils. This variability is intrinsically linked to the chemical profiles of each oil, particularly the concentrations of unsaturated fatty acids and the presence of other bioactive components, such as terpenes and antioxidants.

Sunflower oil, with its high concentration of polyunsaturated fatty acids, showed the highest levels of oxidation, while copaiba and coconut oils demonstrated lower susceptibility due to their specific structural characteristics. These differences underscore the importance of considering the chemical composition of oils when evaluating their applicability in processes involving ozonation. Moreover, the findings provide a foundation for selecting appropriate oils for different applications, particularly in contexts where oxidative stability or the formation of bioactive compounds is a determining factor.

3.2 Characterization of Ozonized Sunflower Oil by FT-IR

The main bands of interest in the infrared spectrum of ozonized sunflower oil were observed in the regions of 1000–1800 cm^{-1} and 2800–3500 cm^{-1}, which are associated with the formation of oxygenated compounds such as ozonides, peroxides, and hydroperoxides. These regions are critical for understanding the chemical changes resulting from the ozonation process, as they reflect the introduction of new functional groups into the oil matrix. Figure 2 illustrates the infrared spectrum of sunflower oil prior to ozonation and following the reaction at varying concentrations, highlighting the chemical transformations induced by the process. In the 1650 cm^{-1} region, a progressive reduction in the intensity of the band corresponding to C=C double bonds was observed, indicating the saturation of these bonds by ozone.

Figure 2 - Infrared spectra of sunflower oil before ozonation and at increasing ozonation concentrations.



Source: Author's data.

This change directly indicates the reactivity of the double bonds in unsaturated fatty acids such as oleic and linoleic acids, which predominate in sunflower oil. The decrease in this band not only confirms the conversion of unsaturated fatty acids into oxygenated products but also highlights the efficiency of ozone as an oxidizing agent in the context of ozonation. Another relevant aspect was the increased intensity of the bands between 1700–1740 cm^{-1}, attributed to C=O stretching of aldehydes and ketones. These compounds are expected products of the decomposition of ozonides and peroxides formed during the ozonolysis reaction. The presence of these bands reinforces the idea that, in addition to primary products (ozonides), there is significant conversion to secondary compounds, especially aldehydes, which may influence the chemical and functional properties of ozonized oil. In the 3400 cm^{-1} region, an increase in the width and intensity of the bands associated with hydroxyl groups (-OH) was observed. These changes indicate the formation of hydroperoxides, compounds frequently found as intermediates during the oxidation of unsaturated fatty acids. The presence of these hydroxyl groups is relevant to the antimicrobial properties of ozonized oil, as hydroperoxides and peroxides are known for their ability to release reactive oxygen species.

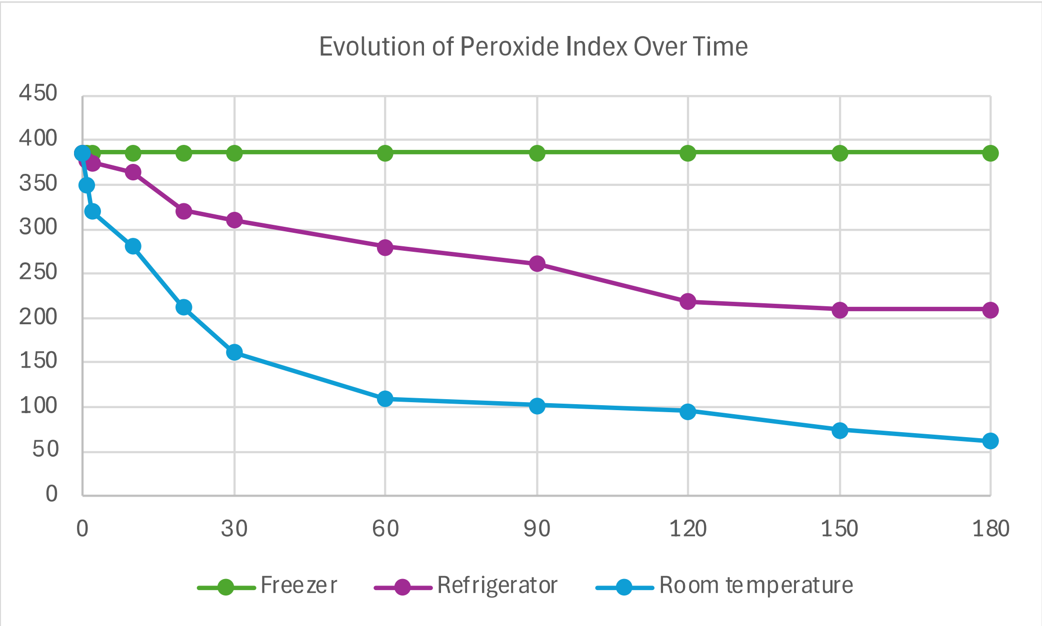
The spectral data suggest that ozonation of sunflower oil promotes significant modifications in its chemical composition, transforming unsaturated fatty acids into a complex mixture of oxygenated compounds. These structural changes have direct implications for the biological and industrial properties of the oil, such as its antimicrobial activity, therapeutic potential, and chemical stability. The reduction in the C=C band reflects the extent of the ozonation process, while the more pronounced C=O and -OH peaks highlight the generated products, such as aldehydes, ketones, and hydroperoxides.

Furthermore, the spectroscopic analysis evidences the role of unsaturated fatty acids in determining the chemical behavior of the oil during ozonation. For example, oleic acid, with a single double bond, and linoleic acid, with two double bonds, react differently to ozone, resulting in varying proportions of ozonides, aldehydes, and hydroperoxides. These factors are crucial for understanding the reactivity of sunflower oil compared to other vegetable oils, such as coconut or olive, whose fatty acid compositions vary significantly.

3.3 Effects of Storage Conditions on Ozonized Oils

Ozonized sunflower oil, with a peroxide value of 385 mEq O_2/Kg, was stored under three distinct conditions (freezer at -18°C, refrigerator at 4°C, and room temperature at 25°C) and evaluated over a 180-day period. Peroxide value were used as a parameter to monitor the oxidative stability of the oils. As illustrated in the graph (Figure 3), oils stored at room temperature exhibited a sharp decline in peroxide value over time, reaching minimum values after 180 days of storage. In contrast, oils stored in the refrigerator showed a slower and more moderate reduction, while oils stored in the freezer remained stable throughout the evaluation period, with no evidence of degradation of oxidation-associated active compounds.

Figure 3 - Evolution of peroxide value over time for sunflower oil under different storage conditions. The y-axis represents the peroxide value, while the x-axis corresponds to the storage duration in days.

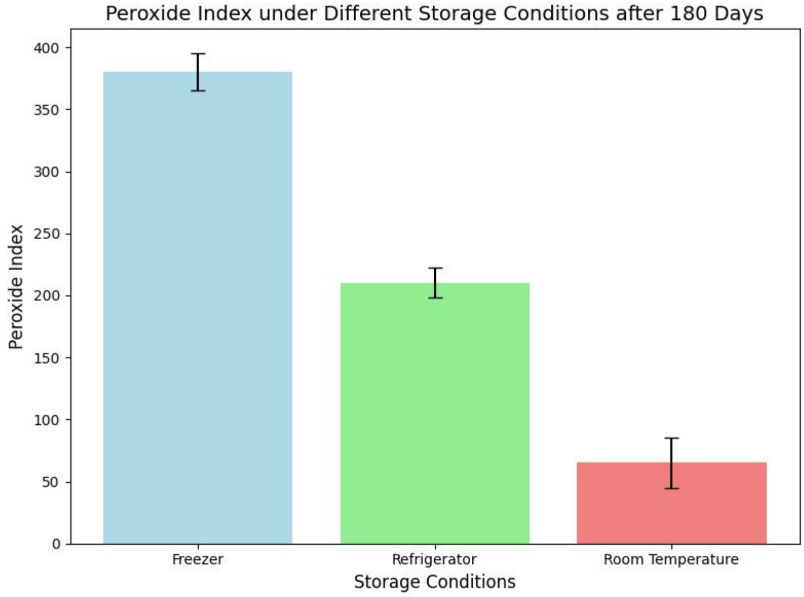


Source: Author's data.

The analysis of variance (ANOVA) revealed statistically significant differences among the evaluated storage conditions ($F = 14.87$, $p < 0.001$), indicating that the type of storage directly influences the stability of ozonized oils. To further investigate these differences, Tukey's multiple comparisons test was applied. The test results confirmed that oils stored at room temperature exhibited significantly lower peroxide values compared to those stored in the freezer and refrigerator. This decline in peroxide values at room temperature reflects the accelerated degradation of reactive species, such as peroxides, hydroperoxides, and ozonides, due to exposure to higher temperatures. In contrast, storage conditions in the freezer and refrigerator proved to be more effective in preserving these reactive species, emphasizing the importance of controlled conditions to maintain the oxidative stability of ozonized oils, particularly for therapeutic and industrial applications.

No significant differences were observed between oils stored in the freezer and refrigerator, as shown in Figure 4, suggesting that both environments are effective in preserving the oxidative stability of the oils. However, freezer storage demonstrated the highest efficiency, maintaining the active compounds unchanged over the entire 180-day period.

Figure 4 - Peroxide value of sunflower oil under different storage conditions. Error bars represent the standard deviation of the data, and statistical differences were analyzed using Tukey's test.



Source: Author's data.

These data confirm the importance of controlled storage conditions to prolong the shelf life of ozonized oils. The results underscore the need for practices that minimize oxidative degradation, particularly in applications where oxidative stability is essential to preserve the functional and therapeutic properties of the products.

4. Conclusion

This study demonstrated that sunflower oil is the most suitable for the ozonation process, showing the highest degree of ozonation among the oils evaluated. Infrared spectroscopy analysis confirmed the expected chemical transformations, highlighting the formation of oxygenated compounds such as peroxides and hydroperoxides, which are essential for the therapeutic properties of the oil. The storage analysis revealed that both freezer (-18°C) and refrigerator (4°C) conditions effectively preserved the oxidative stability of ozonized oil over 180 days, ensuring the maintenance of these active compounds. In contrast, room temperature accelerated the degradation of oxygenated compounds, compromising the oil's chemical stability and therapeutic potential.

These findings provide relevant guidelines for the safe and prolonged storage of ozonized vegetable oils, allowing the maximization of their functional and therapeutic properties. Furthermore, the results underscore the importance of controlled storage conditions as a strategy to ensure the quality and efficacy of these products in industrial and pharmaceutical applications.

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