



Ciencia Latina
Internacional

Ciencia Latina Revista Científica Multidisciplinar, Ciudad de México, México.
ISSN 2707-2207 / ISSN 2707-2215 (en línea), julio-agosto 2024,
Volumen 8, Número 4.

https://doi.org/10.37811/cl_rcm.v8i4

DETERMINATION OF THE OXIDATIVE STABILITY OF OMEGA-3 OIL USING ELECTROCHEMICAL IMPEDANCE SPECTROSCOPY

**DETERMINACIÓN DE LA ESTABILIDAD OXIDATIVA
DEL ACEITE OMEGA-3 UTILIZANDO ESPECTROSCOPIA
DE IMPEDANCIA ELECTROQUÍMICA**

Brenda Patricia Rodríguez Villanueva

Universidad de Puerto Rico, Puerto Rico

Sandra Milena Rodríguez Villanueva

Universidad de Puerto Rico, Puerto Rico

Roberto Del Cristo Martínez Mejía

Universidad de Puerto Rico, Puerto Rico

Henry Nuñez Coavas

Universidad del Atlántico, Colombia

DOI: https://doi.org/10.37811/cl_rcm.v8i4.12993

Determination of the Oxidative Stability of Omega-3 Oil Using Electrochemical Impedance Spectroscopy

Brenda Patricia Rodríguez Villanueva¹brenda.rodriguez1@upr.edu<https://orcid.org/0000-0001-7117-1681>

Universidad de Puerto Rico

Puerto Rico

Sandra Milena Rodríguez Villanuevasandra.rodriguez2@upr.edu<https://orcid.org/0000-0001-9512-1873>

Universidad de Puerto Rico

Puerto Rico

Roberto Del Cristo Martínez Mejíaroberto.martinez6@upr.edu<https://orcid.org/0000-0003-1998-8728>

Universidad de Puerto Rico

Puerto Rico

Henry Nuñez Coavashenrynunez@mail.uniatlantico.edu.co<https://orcid.org/0000-0002-7319-1640>

Universidad del Atlántico

Colombia

ABSTRACT

One of the main challenges in the supplements and food industry is maintaining the oxidative stability of Omega-3 rich oils, which are highly susceptible to oxidation. The oxidation of these oils not only affects their quality and flavor but can also diminish their health benefits, such as reducing inflammation and supporting brain function. This study evaluates the oxidative stability of Omega-3 oil using electrochemical impedance spectroscopy (EIS), a technique that offers a faster and non-destructive alternative compared to traditional chemical methods. Oil samples were subjected to autoxidation at room temperature and analyzed over a frequency range of 3 kHz to 100 kHz, while peroxide levels were measured using traditional bromatological techniques for comparison. The results revealed a strong linear correlation ($R^2 = 0.99$) between peroxide levels and imaginary dielectric permittivity, highlighting the potential of EIS to effectively characterize the oxidative stability of Omega-3 oil. This finding is significant because EIS allows for faster and non-destructive evaluation, facilitating real-time monitoring of oxidation in Omega-3 oils, which could be particularly beneficial for manufacturers seeking to ensure the quality of their products more efficiently. Additionally, the use of EIS can reduce costs and analysis times, improving the efficiency of quality control processes. However, possible disadvantages include the need for specialized equipment and precise calibration of the method for different types of oils. Future research should focus on further optimizing and validating this method under different storage and processing conditions, as well as exploring its applicability to other types of edible oils and Omega-3 derived products to ensure their quality and safety. Moreover, it would be beneficial to investigate the integration of EIS into automated production lines for continuous monitoring, which could revolutionize the way Omega-3 oil quality is assured in the industry.

Keywords: omega-3, oxidative stability, electrochemical impedance spectroscopy

¹ Autor principal.

Correspondencia: brenda.rodriguez1@upr.edu

Determinación de la Estabilidad Oxidativa del aceite Omega-3 Utilizando Espectroscopia de Impedancia Electroquímica

RESUMEN

Uno de los principales desafíos en la industria de los suplementos y alimentos es mantener la estabilidad oxidativa de los aceites ricos en Omega-3, que son altamente susceptibles a la oxidación. La oxidación de estos aceites no solo afecta su calidad y sabor, sino que también puede disminuir sus beneficios para la salud, como la reducción de la inflamación y el apoyo a la función cerebral. Este estudio evalúa la estabilidad oxidativa del aceite Omega-3 utilizando espectroscopia de impedancia electroquímica (EIE), una técnica que ofrece una alternativa más rápida y no destructiva en comparación con los métodos químicos tradicionales. Se expusieron muestras de aceite a autooxidación a temperatura ambiente y se analizaron en un rango de frecuencias de 3 kHz a 100 kHz, mientras que los niveles de peróxido se midieron mediante técnicas bromatológicas tradicionales para proporcionar una base de comparación. Los resultados revelaron una fuerte correlación lineal ($R^2 = 0.99$) entre los niveles de peróxido y la permitividad dieléctrica imaginaria, destacando el potencial de la EIE para caracterizar eficazmente la estabilidad oxidativa del aceite Omega-3. Este hallazgo es significativo porque la EIE permite una evaluación más rápida y no destructiva, facilitando el monitoreo en tiempo real de la oxidación en los aceites Omega-3, lo que podría ser particularmente beneficioso para los fabricantes que buscan asegurar la calidad de sus productos de manera más eficiente. Además, el uso de EIE puede reducir costos y tiempos de análisis, mejorando la eficiencia de los procesos de control de calidad. Sin embargo, se mencionan posibles desventajas, como la necesidad de equipos especializados y la calibración precisa del método para diferentes tipos de aceites. Futuras investigaciones deberían centrarse en optimizar y validar aún más este método en diferentes condiciones de almacenamiento y procesamiento, así como explorar su aplicabilidad en otros tipos de aceites comestibles y productos derivados de Omega-3, para garantizar su calidad y seguridad. Asimismo, sería beneficioso investigar la integración de EIE en líneas de producción automatizadas para un monitoreo continuo, lo que podría revolucionar la forma en que se asegura la calidad de los aceites Omega-3 en la industria.

Palabras clave: omega-3, estabilidad oxidativa, espectroscopia de impedancia electroquímica

*Artículo recibido 28 julio 2024
Aceptado para publicación: 15 agosto 2024*



INTRODUCTION

Omega-3 oil is widely used in various pharmaceutical supplements due to its health benefits, including reducing inflammation and supporting brain function (Montgomery, Conway & Spector, 1992). However, the high susceptibility of Omega-3 fatty acids to oxidation can degrade the product, affecting its quality and safety (Ortega-Nieblas, Robles-Burgueño & Vázquez-Moreno, 2001). To ensure it is suitable for consumption, these oils must meet strict regulations, such as the peroxide limit established by the United States Pharmacopeia Convention, which must not exceed 5 milliequivalents of peroxide per kilogram of oil (5 mEq/kg) (Ortega-Nieblas, Robles-Burgueño & Vázquez-Moreno, 2001).

The conventional method for assessing the oxidative stability of Omega-3 oils is the peroxide index, which, although accurate, is laborious and time-consuming. The need for more efficient methods has been highlighted in several studies. For example, Shahidi & Zhong (2010) emphasize the importance of developing rapid and reliable techniques to measure oxidation levels due to the increasing demand for Omega-3 supplements. Similarly, Frankel (2005) discusses the limitations of traditional methods and the potential of advanced techniques such as electrochemical impedance spectroscopy (EIS) to provide real-time monitoring of oxidation.

EIS, already successfully used in other fields such as evaluating the stability of biodiesel (Biernat et al., 2019) and gasoline (Bocian et al., 2021), offers a rapid and non-destructive methodology. Additionally, Corugedo et al. (2014) have demonstrated the effectiveness of EIS in diagnosing transformer oils, suggesting that this technique could be adapted to assess edible oils like Omega-3.

Recent studies have investigated oxidative stability in vegetable oils using innovative techniques. For example, Rodríguez et al. (2015) evaluated the oxidative stability and shelf life of *sacha inchi* oil (*Plukenetia volubilis* L.) using traditional methods. Although this study does not employ EIS, the methodology applied and the results obtained are relevant to evaluating oxidative stability in similar oils. Applying EIS in these cases could improve the accuracy and efficiency of assessments.

On the other hand, Sanaeifar and Jafari (2019) used an integrated system of dielectric spectroscopy and computer vision to determine the oxidative stability of olive oil. This combination of advanced techniques provides a detailed and accurate evaluation of oxidation, which could be adapted for Omega-3 oils, offering an advanced tool for real-time monitoring and improving product quality.



Moreover, Gashi et al. (2024) evaluated the oxidative thermal stability of fish oil with the addition of pumpkin seed oil or rosemary extract, demonstrating how natural antioxidants can enhance oxidative stability. These findings complement studies using EIS to evaluate these effects in Omega-3 oils, providing a basis for improving the formulation and storage of these oils.

The use of advanced techniques like EIS is not limited to edible oils. Flynn et al. (2018) conducted an EIS study on the interaction of supported lipid bilayers with free docosahexaenoic acid, demonstrating the applicability of this technique in various lipid matrices. Similarly, Bocian et al. (2021) have used EIS to study the oxidative stability of gasoline, developing a chemical stability index that could be adapted for Omega-3 oils.

Additionally, Rodríguez, Escobar, and Ortiz (2010) developed an oxidative stability test for fish oil, comparing traditional bromatological techniques with differential scanning calorimetry. Their findings highlight the potential of advanced analytical techniques in improving the understanding and assessment of fish oil oxidation, further supporting the relevance of exploring EIS for this purpose.

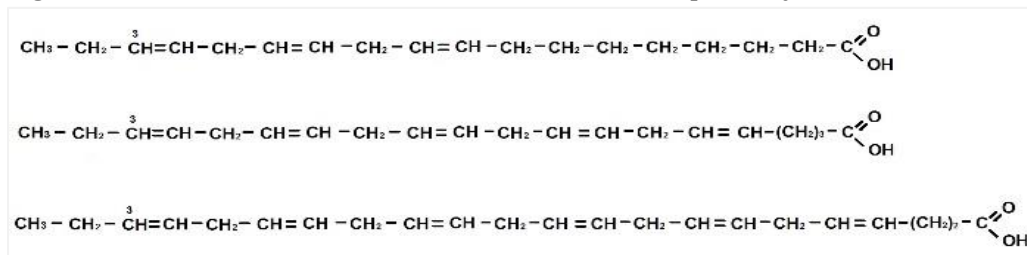
Finally, Lázaro Mora (2023) has demonstrated how image analysis techniques can monitor the oxidation of marine oils, suggesting that a combination of EIS and image analysis could offer an even more robust methodology for evaluating oxidative stability.

The primary objective of this research is to explore the use of EIS as a novel and efficient method for assessing the oxidative stability of Omega-3 oils. This study investigates the relationship between peroxide levels and the imaginary dielectric permittivity of Omega-3 oils, validates the accuracy and reliability of EIS compared to conventional bromatological methods, and demonstrates the potential of EIS for real-time, non-destructive monitoring of oxidation in Omega-3 oils.

Composition of Omega-3 Fatty Acids

Omega-3 oils are polyunsaturated fatty acids that contain more than one carbon-carbon double bond. The omega-3 family oils are composed of alpha-linolenic acid (ALA) found in plants, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) both found in fish. These acids have 3, 5, and 6 carbon-carbon double bonds in a chain of 18, 20, and 22 carbon atoms respectively, shown in fig. 1.

Figure 1. Chemical structures of ALA, EPA and DHA respectively

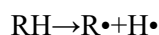


Autoxidation Process

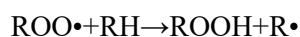
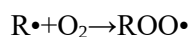
The autoxidation of Omega-3 oils is a significant factor affecting their stability and shelf life. This process, driven by the interaction of unsaturated fatty acids with oxygen, involves a series of complex chemical reactions that ultimately lead to the deterioration of the oil's quality and nutritional value (Shahidi & Zhong, 2010).

Stages of Autoxidation

1. **Initiation** The initiation stage involves the formation of free radicals, highly reactive species that can start chain reactions. In Omega-3 oils, this can occur due to exposure to light, heat, or metal catalysts (Frankel, 2005). The presence of these factors causes the homolytic cleavage of C-H bonds in the fatty acid molecules, leading to the formation of lipid radicals ($R\cdot$).

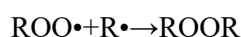
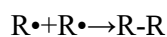


2. **Propagation** During the propagation stage, the lipid radicals react with oxygen to form peroxy radicals ($ROO\cdot$). These peroxy radicals are highly reactive and can further react with other lipid molecules, continuing the chain reaction and producing lipid hydroperoxides ($ROOH$) (McClements & Decker, 2000).



The lipid hydroperoxides can decompose into secondary products such as aldehydes, ketones, and alcohols, which contribute to off-flavors and odors in the oil (Choe & Min, 2006).

3. **Termination** The termination stage involves the reaction of free radicals with each other, forming non-radical products that do not propagate the chain reaction. This can occur through various pathways, such as the combination of two peroxy radicals or the reaction of a peroxy radical with a lipid radical (Frankel, 2005).



The termination stage effectively halts the autoxidation process, but by this point, significant oxidative damage may have already occurred (McClements & Decker, 2000).

Factors Influencing Autoxidation

Degree of Unsaturation Omega-3 fatty acids, being polyunsaturated, have multiple double bonds, making them more susceptible to oxidation compared to monounsaturated or saturated fats. The higher the degree of unsaturation, the greater the susceptibility to autoxidation (Shahidi & Zhong, 2010).

Presence of Antioxidants Natural antioxidants, such as vitamin E (tocopherols), can inhibit the autoxidation process by donating hydrogen atoms to free radicals, stabilizing them and preventing further propagation. Synthetic antioxidants are also used in the food industry to extend the shelf life of oils (McClements & Decker, 2000).

Environmental Conditions Exposure to light, heat, and oxygen accelerates the autoxidation process. Proper storage conditions, such as keeping oils in dark, cool, and airtight containers, can significantly reduce the rate of oxidation (Choe & Min, 2006).

Measurement of Oxidative Stability

The oxidative stability of Omega-3 oils can be measured using various methods, including: Peroxide Value (PV) This method quantifies the primary oxidation products (hydroperoxides) formed during the initial stages of autoxidation. The peroxide value is commonly used as an indicator of the oil's freshness and the extent of oxidation (Shahidi & Zhong, 2010) y Electrochemical Impedance Spectroscopy (EIS) EIS is an advanced technique that measures the dielectric properties of oils. By analyzing the imaginary dielectric permittivity, researchers can infer the extent of oxidation and the presence of oxidative degradation products (McClements & Decker, 2000).

The standard technique used in the pharmaceutical industry for oxidative characterization of omega-3 oil is the chemical bromatological for peroxide index. This technique consists of the determination of the peroxide value of the sample measuring the volume needed to titrate a solution of the omega-3 oil (Rodríguez, Escobar, & Ortiz, 2010 & Spellman, 2009). The titration procedure consists of mixing 5g of the omega-3 oil with 30mL of a solution of acetic acid – chloroform (3:2). After swirl the solution



until is completely dissolved 0.5mL of saturated potassium iodide must be added and then swirl for one minute. Immediately 30mL of distilled water must be added and shaken vigorously. Now the solution is titrated with 0.1N sodium thiosulfate and mixed until obtain a light yellow color. Then 5mL of starch is added as indicator, the solution will take a blue gray color. Finally titrate until the blue color disappears. It is necessary to make a blank determination of the reagents. Then the peroxide value is given by:

$$\text{Peroxide Value} = \frac{1000(V_s - V_b)N_{\text{thiosulfate}}}{W}$$

Where V_s is the titration volume of the sample, V_b is the titration volume of the blank and W is the weight of the sample in grams. The bromatological peroxide index technique requires a wide use of reagents and longtime of measurement making it inefficient for a high number of samples. Rodriguez et al. (2010) has shown the use of the differential scanning calorimetry as a substitute for the chemical bromatological technique to measure the oxidative stability of omega-3 oil. Herby this research aims to find an alternative more efficient method of peroxide characterization using the imaginary dielectric spectrum to measure the electric properties of omega-3 oil (Barsoukov & Macdonald, 2005).

Electrochemical impedance spectroscopy is a method widely used to characterize the dielectric properties of a system (Barsoukov & Macdonald, 2005). This technique requires no sample preparation and the relatively simple electrical measurements makes it ideal for automated characterization evaluating a high volume of samples in a short time. The permittivity spectra is obtained from the measurement of an AC current from the sample as result of an AC voltage input. The ratio between the voltage and current as function of the applied frequency is given by the impedance ($z(\omega)$).

$$\frac{V(\omega)}{I(\omega)} = z(\omega) = |z|e^{-i\omega t}$$

In general the impedance is a complex number and ω is the angular frequency of the applied potential ($\omega=2\pi f$). Experimentally impedance measurements are taken using a capacitance cell in which the sample is placed and its relative permittivity can be calculated by:

$$\epsilon_r(\omega) = \frac{C_x}{C_0}$$

$$C_x = \frac{1}{i\omega z(\omega)}$$

$$C_0 = \epsilon_0 \frac{A}{d}$$

Where ϵ_0 is the vacuum permittivity, C_x is the capacitance of the cell with the sample and C_0 is the capacitance of the empty cell (A = cell area, d = cell separation). Relative permittivity is a complex number $\epsilon_r = \epsilon'_r - i \epsilon''_r$ and the complex impedance as function of the applied frequency is represented in a Blode plot where the frequency is in logarithmic scale. A system can be classified according to the ratio of the imaginary part of the permittivity to the real part.

$$\frac{\epsilon''_r}{\epsilon'_r} = \sigma$$

In general the higher this ratio more conductive is the sample. A perfect conductor will have a ratio of $\sigma = \infty$ while a perfect dielectric (has no conductivity) will have a ratio of $\sigma = 0$.

Understanding the autoxidation process is crucial for both the food and pharmaceutical industries. In the food industry, oxidative stability determines the shelf life and sensory qualities of Omega-3-rich products. In pharmaceuticals, maintaining the integrity of Omega-3 oils in supplements is essential for ensuring their therapeutic efficacy and safety (Shahidi & Zhong, 2010; McClements & Decker, 2000).

METHODOLOGY

The study utilized Omega-3 oil samples from the same lot, provided by PROCAPS S.A. The samples were subjected to autoxidation at room temperature with exposure times of 1, 3, and 5 days to simulate different levels of oxidative degradation. The specifications of the oil samples, including their initial peroxide levels and fatty acid composition (samples extracted on May 14, 2009, and analyzed on May 24, 2009), are detailed in Table 1.

Table 1. Omega-3 oil analysis performed

Specifications	Method	Results
Free fatty acid	Iso 660 1996	0.42%
Moisture	Iso662b 1998	0.02%
Iodine index	Aoac 920.158 2005	193 hanus
Peroxide	Aocs cd 18-90 1998	3 meq/kg
EPA	Aocs ce1b-89 1998	20.0%
DHA		12.4%

The bromatological technique used for peroxide index determination is the one established by United States Pharmacopeial Convention and Food Ingredient Expert Committee (2009) for fats and oils. Samples mass were weighed to 5 ± 0.0001 g using a Mettler Toledo model XP205DR electronic scale. High purity reagents and solvents were used. This procedure was performed by PROCAPS S.A. (Barranquilla- Colombia).

The primary variables considered in this study were peroxide levels and imaginary dielectric permittivity. Peroxide levels were determined using the chemical bromatological method, providing a measure of the oxidative degradation of the Omega-3 oil samples. Imaginary dielectric permittivity was measured using electrochemical impedance spectroscopy (EIS), which offered insights into the electrical properties of the oil and their correlation with oxidative stability.

To analyze the relationship between these variables, a linear correlation analysis was performed. This statistical approach helped to establish the connection between peroxide levels and imaginary dielectric permittivity. The strength and reliability of this correlation were assessed using the RMS value of R^2 , which quantified the degree of linearity between the variables and confirmed the potential of EIS as an effective method for evaluating the oxidative stability of Omega-3 oils.

Experimental Procedure

The bromatological technique for peroxide index determination followed the United States Pharmacopoeia Convention (USPC, 2009) standards for fats and oils. Five grams of each sample were weighed with a precision of ± 0.0001 g using a Mettler Toledo model XP205DR electronic scale. High purity reagents and solvents were used, and the procedure was carried out by PROCAPS S.A. in Barranquilla, Colombia. The results were recorded and analyzed to establish baseline peroxide levels for each sample.

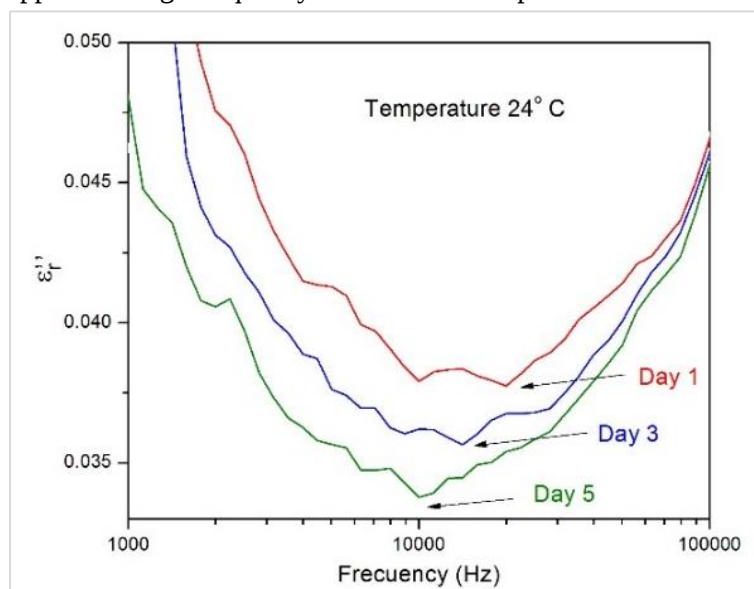
Imaginary impedance spectra were obtained using a YSI Inc. electrochemical cell model 3402 with a platinum electrode configuration (M/sample/M). A Solartron signal analyzer model 1260 was used, covering a frequency range from 3 kHz to 100 kHz. The measurements were performed at a constant room temperature of 24°C. The impedance data were collected and processed to obtain the imaginary component of the dielectric permittivity for each sample.

RESULTS AND DISCUSSION

The results of the bromatological peroxide index analysis for the Omega-3 oil samples show a clear proportional increase in the peroxide index with the duration of the oil's exposure. This progressive increase, as shown in Table 2, indicates a continuous oxidation process occurring in the oil samples over time.

The imaginary component of the complex relative permittivity of Omega-3 oil as a function of the applied voltage frequency at a constant temperature of 24°C. The spectra for each oxidation time begin to converge at frequencies above 50 kHz, eventually overlapping at approximately 100 kHz, as shown in Figure 3. This convergence suggests that EIS is sensitive to changes in the oxidative state of the oil, with the imaginary permittivity serving as a reliable indicator of peroxide levels.

Figure 3. Imaginary component of the complex relative permittivity of Omega-3 oil as function of the applied voltage frequency at a constant temperature of 24°C



It was observed that for frequencies above 50 kHz, the imaginary permittivity spectra for each oxidation time begin to converge until they overlap around 100 kHz. In this frequency region, the electric response of the Omega-3 oil is independent of the peroxide index. However, between 1 kHz and 50 kHz, a considerable difference among the spectra is observed. The imaginary permittivity of Omega-3 oil decreases as the peroxide index increases. The minimum values of the imaginary component of the complex permittivity obtained were 38×10^{-3} , 36×10^{-3} , and 34×10^{-3} for the first, third, and fifth days, respectively.

The DC conductivity is directly proportional to the imaginary permittivity, as expressed by the equation:

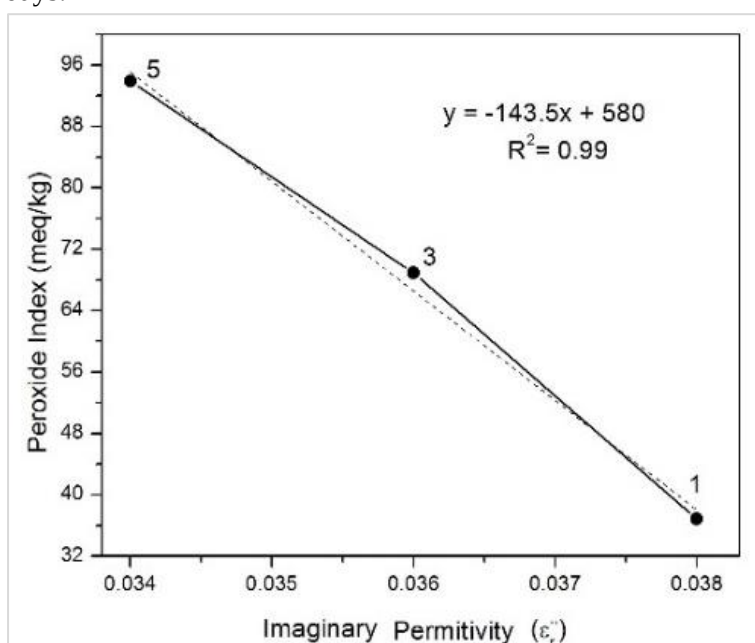
$$\sigma_{DC} = \omega \epsilon_0 \epsilon'' = \omega \epsilon_0^2 \epsilon_r''$$

Therefore, the DC conductivity of Omega-3 oil decreases with higher peroxide index values.

Correlation

The analysis evaluated the linear correlation between peroxide levels and imaginary dielectric permittivity, revealing a strong linear relationship with a correlation coefficient of $R^2 = 0.99$. As shown in Figure 4, this high correlation confirms the potential of EIS as a sensitive and reliable alternative method for assessing oxidative stability in Omega-3 oils.

Figure 4. Peroxide index as function of the Omega-3 oil imaginary permittivity for different exposure days.



CONCLUSIONS

This study has demonstrated that electrochemical impedance spectroscopy (EIS) can effectively characterize the oxidative stability of Omega-3 oils. The strong linear correlation between peroxide levels and the imaginary dielectric permittivity highlights the sensitivity and reliability of EIS in detecting oxidative changes. This technique offers a faster, non-destructive alternative to traditional chemical methods, providing significant advantages for quality control in both the food and pharmaceutical industries.

In the food industry, the ability to monitor oxidative stability in real-time can enhance product quality

and extend shelf life. EIS can facilitate more precise interventions to prevent rancidity and maintain sensory attributes, ultimately improving consumer satisfaction and reducing waste (Choe & Min, 2006). The work of Rodríguez et al. (2015) on the oxidative stability of sachu inchi oil underscores the importance of efficient monitoring methods. While traditional methods were used in their study, the integration of EIS could provide a more efficient and precise approach.

For the pharmaceutical industry, maintaining the integrity of Omega-3 supplements is crucial for ensuring their therapeutic efficacy and safety. EIS offers a reliable method for rapid quality assessment, helping manufacturers comply with stringent regulatory standards and deliver high-quality products to the market (Shahidi & Zhong, 2010). Studies such as those by Rodríguez, Escobar, and Ortiz (2010), which explored the oxidative stability of fish oil using bromatological techniques and differential scanning calorimetry, highlight the potential for EIS to provide similar insights more efficiently.

Future research should focus on further validating the EIS technique for various types of Omega-3 oils and under different storage and processing conditions. Additionally, exploring the integration of EIS with other analytical methods, as demonstrated by Sanaeifar and Jafari (2019) with dielectric spectroscopy and computer vision for olive oil, could enhance the comprehensiveness of oxidative stability assessments. Investigating the potential application of EIS in monitoring other types of lipids and nutritional compounds could also broaden its utility.

Despite its advantages, the implementation of EIS in routine quality control faces challenges, including the initial setup costs and the need for specialized knowledge to interpret the data. Addressing these challenges through the development of user-friendly software and cost-effective solutions will be essential for widespread adoption (Frankel, 2005). The advancements made by Flynn et al. (2018) in applying EIS to lipid bilayers and by Bocian et al. (2021) in creating a chemical stability index for gasoline demonstrate the ongoing evolution and potential for EIS across various applications, emphasizing the need for continued innovation and adaptation in this field.

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