

Oxidative stability of essential oils under thermal and UV stress: potential application as an additive in aquaculture feeds

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Abstract

Oxidative instability represents a critical barrier to the practical application of essential oils as functional additives in aquaculture feeds. This study evaluated the oxidative resistance of three commercially relevant essential oils: cinnamon leaf (*Cinnamomum verum*), peppermint (*Mentha piperita*), and tea tree (*Melaleuca alternifolia*), under conditions simulating feed processing and storage, including UV-C radiation (254 nm) and elevated temperature (60 °C) for up to 48 hours. Chemical composition was determined by GC-MS, and lipid peroxidation was quantified by malondialdehyde (MDA) accumulation using the TBARS method. The results revealed a clear relationship between chemical profile and oxidative stability. Tea tree oil exhibited greater oxidative resistance, with lower MDA levels compared to cinnamon leaf and peppermint oils after 48 hours of thermal and UV stress ($p < 0.05$). These findings indicate that oxidative stability is a criterion for technical and economic feasibility in the selection of essential oils for aquaculture. While tea tree oil shows high potential for direct application, the use of unstable oils depends on the development of technological protection strategies, thereby redirecting research and development efforts toward economically viable formulations.

Keywords: feed quality; lipid peroxidation; malondialdehyde; TBARS.

Resumo - Estabilidade oxidativa de óleos essenciais sob estresse térmico e UV: potencial aplicação como aditivo em rações para aquicultura

A instabilidade oxidativa representa uma barreira crítica para a aplicação prática de óleos essenciais como aditivos funcionais em rações para aquicultura. Este estudo avaliou a resistência oxidativa de três óleos essenciais de relevância comercial: canela-folhas (*Cinnamomum verum*), hortelã-pimenta (*Mentha piperita*) e melaleuca (*Melaleuca alternifolia*), sob condições que simulam o processamento e o armazenamento de rações, incluindo radiação UV-C (254 nm) e temperatura elevada (60 °C) por até 48 horas. A composição química foi determinada por GC-MS, e a peroxidação lipídica foi quantificada pelo acúmulo de malondialdeído (MDA) utilizando o método TBARS. Os resultados revelaram uma relação clara entre o perfil químico e a estabilidade oxidativa. O óleo de melaleuca apresentou maior resistência oxidativa, com menores níveis de MDA em comparação aos óleos de canela-folhas e hortelã-pimenta após 48 horas de estresse térmico e por UV ($p < 0,05$). Esses achados indicam que a estabilidade oxidativa é um critério de viabilidade técnica e econômica na seleção de óleos essenciais para a aquicultura. Enquanto o óleo de melaleuca apresenta alto potencial para aplicação direta, o uso de óleos instáveis depende do desenvolvimento de estratégias tecnológicas de proteção, redirecionando assim os esforços de pesquisa e desenvolvimento para formulações economicamente viáveis.

Palavras-chave: malondialdeído; peroxidação lipídica; qualidade da ração; TBARS.

Resumen - Estabilidad oxidativa de los aceites esenciales bajo estrés térmico y UV: posible aplicación como aditivo en piensos para acuicultura

La inestabilidad oxidativa representa una barrera crítica para la aplicación práctica de los aceites esenciales como aditivos funcionales en los alimentos para la acuicultura. Este estudio evaluó la resistencia oxidativa de tres aceites esenciales de relevancia comercial: hojas de canela (*Cinnamomum verum*), menta piperita (*Mentha piperita*) y tea tree (*Melaleuca alternifolia*), bajo condiciones que simulan el procesamiento y el almacenamiento de alimentos, incluyendo radiación UV-C (254 nm) y temperatura elevada (60 °C) durante hasta 48 horas. La composición química se determinó mediante GC-MS, y la peroxidación lipídica se cuantificó por la acumulación de malondialdehído (MDA) utilizando el método TBARS. Los resultados

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revelaron una relación clara entre el perfil químico y la estabilidad oxidativa. El aceite de tea tree presentó una mayor resistencia oxidativa, con niveles más bajos de MDA en comparación con los aceites de hoja de canela y menta piperita después de 48 horas de estrés térmico y por UV ($p < 0,05$). Estos hallazgos indican que la estabilidad oxidativa es un criterio de viabilidad técnica y económica en la selección de aceites esenciales para la acuicultura. Mientras que el aceite de tea tree muestra un alto potencial para la aplicación directa, el uso de aceites inestables depende del desarrollo de estrategias tecnológicas de protección, redirigiendo así los esfuerzos de investigación y desarrollo hacia formulaciones económicamente viables.

Palabras clave: calidad del alimento; malondialdehido; peroxidación lipídica; TBARS.

Introduction

Modern aquaculture faces the dual challenge of intensifying production while reducing reliance on conventional antibiotics, driven by concerns related to microbial resistance and food safety (Bondad-Reantaso et al., 2023). In this context, essential oils have emerged as promising alternatives, exhibiting antimicrobial, antioxidant, and immunostimulatory activities in aquaculture species (Kalaiselvan et al., 2024; Pudota et al., 2025; Terrazas-Pineda et al., 2025). However, a critical barrier limits their large-scale application is their chemical instability during feed processing and storage (Onomu and Okuthe, 2024).

Essential oils represent complex biochemical systems composed of a wide variety of plant secondary metabolites (Dhifi et al., 2016). Their structural diversity, predominantly consisting of monoterpenes, sesquiterpenes, and phenols, confers the desired bioactive properties but also makes them susceptible to oxidative degradation (Tisserand and Young, 2014). The presence of reactive sites such as conjugated double bonds, phenolic hydroxyl groups, and α,β -unsaturated systems favors reactions with reactive oxygen species, promoting the oxidation of volatile constituents (Miguel, 2010).

This degradation is particularly critical in aquaculture, where essential oils are incorporated into feeds that often undergo thermal processing by extrusion and are subsequently stored for prolonged periods under variable environmental conditions (Glencross, 2020). Oxidation may compromise not only the biological efficacy of essential oils but also generate compounds that negatively affect feed palatability, growth, and the aquatic animal health (Laohabanjong et al., 2009). Malondialdehyde (MDA), a final product of lipid peroxidation, serves as a reliable marker of this degradative process (Ayala et al., 2014).

The oxidative stability of essential oils does not result from a simple sum of the activities of their components; rather, it is strongly influenced by synergistic and antagonistic interactions among their constituents (Turek and Stintzing, 2013). This complexity is intensified by the high chemical diversity of commercially available essential oils (Baser and Buchbauer, 2015). Nevertheless, despite the recognized potential of essential oils as feed additives in aquaculture (Kolygas et al., 2025), one of the major limitations for their industrial application is the lack of specific data on critical degradation parameters for different chemical classes under conditions relevant to the feed industry, such as oxidative resistance under thermal and light-induced stress.

Considering that the economic and technical feasibility of using essential oils in aquaculture depends directly on their ability to maintain chemical stability throughout the feed's shelf life, this study aimed to evaluate the oxidative resistance of three commercially relevant essential oils: cinnamon leaf (*Cinnamomum verum*), peppermint (*Mentha piperita*), and tea tree (*Melaleuca alternifolia*), under conditions simulating the stresses of feed processing and storage. The objective was to establish a relationship between the chemical profile of each essential and its oxidative stability, providing insights for the rational use of natural additives in aquaculture production.

Materials and Methods

Ethical Note

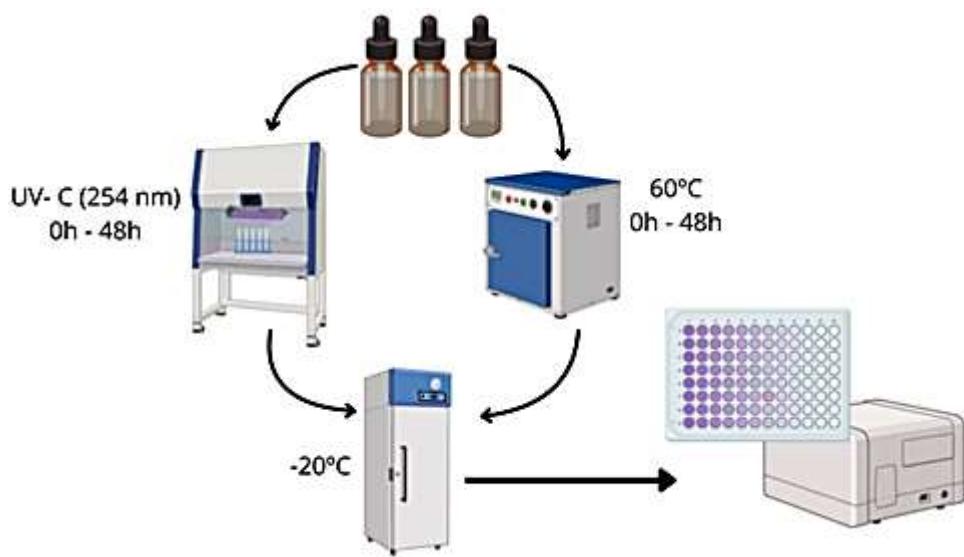
The authors declare that there are no conflicts of interest in the execution or dissemination of this research. Commercial brands of the essential oils were not disclosed in order to maintain scientific neutrality.

General Conditions

Three commercial essential oils were selected: cinnamon leaf (*C. verum*), peppermint (*M. piperita*), and tea tree (*M. alternifolia*). The selection was based on their relevance as potentially beneficial additives for aquaculture, as well as their contrasting chemical profiles, which allow for a comprehensive evaluation of the influence of composition on oxidative stability. The essential oils, which had purity and quality certification

from the Biodynamic Institute (IBD), were purchased and stored at -20 °C for up to two weeks before the beginning of the assays to preserve their original characteristics. Figure 1 illustrates the experimental scheme, from storage to the lipid peroxidation analyses.

Figure 1. Scheme for storage, photo-exposure assay, thermal assays, and analyses of the essential oils used in the present study.



Chemical Profile Analysis of Essential Oils by Gas Chromatography-Mass Spectrometry (GC-MS)

The chromatographic report of the essential oils was provided directly by the company responsible for their commercialization, and no chemical profile analysis was performed by the authors for this result. The company provides the chemical profile of the essential oils as well as the methodology used for compound quantification. The chemical profile of the essential oils was determined through gas chromatography coupled to mass spectrometry (GC-MS), performed by the supplier's laboratory.

UV Radiation Oxidation

To simulate photo-oxidative degradation that may occur during feed storage, samples of 1 g of each essential oil were evenly distributed in penicillin tubes and exposed to UV-C radiation (254 nm) in a controlled chamber ($25 \pm 2^\circ\text{C}$, Pachane® PA400). The chamber contained 15 W UV-C lamps positioned 40 cm from the samples. The estimated irradiance at the sample plane was approximately $500 \mu\text{W}/\text{cm}^2$, based on measurements reported by Mahesh and Siewersen (2020), a value similar to that of typical low-pressure lamps (Chang et al., 2007). The samples, in triplicate, were exposed for periods of 0, 12, 24, and 48 hours. After each interval, the samples were collected under reduced ambient light and immediately stored at -20°C for subsequent lipid peroxidation analysis.

Thermal Oxidation

To evaluate degradation by thermal oxidation, analogous to the stress experienced during feed extrusion processing, samples of 1 g of each essential oil were placed in glass tubes (2 mL) with PTFE caps to prevent contamination. The tubes were kept protected from light in a temperature-controlled oven (Marconi®, MA033/1I300) set to $60 \pm 1^\circ\text{C}$ for periods of 0, 12, 24, and 48 hours. At the end of each interval, the oxidative reactions were halted by immediately cooling the samples in an ice bath (4 °C), after which they were stored at -20 °C until analysis. The assay was conducted in triplicate.

Lipid Peroxidation

Lipid peroxidation was quantified by malondialdehyde (MDA) levels using the TBARS method (Buege and Aust, 1978). Analysis was performed in triplicate. Samples (200 μL) of the essential oils subjected to UV and temperature stress were homogenized with 400 μL of TBARS reagent (15% trichloroacetic acid, 0.375% thiobarbituric acid, and 0.25 mol/mL HCl). The mixture was incubated at 90°C for 40 minutes in a water bath. After cooling, 600 μL of n-butanol was added, followed by centrifugation at 9500 rpm for 5 minutes. The absorbance of the supernatant (200 μL) was measured 535 nm using a microplate spectrophotometer. MDA concentration was determined using a standard curve of 1,1,3,3-tetramethoxypropane, and results were expressed as μmol of MDA per mL of essential oil.

Statistical Analysis

Data normality and homogeneity of variance were verified using the Shapiro-Wilk and Bartlett tests, respectively. A one-way analysis of variances (ANOVA) was applied, and upon detection of significant differences ($p < 0.05$), Tukey's test was used for multiple comparisons. All analyses were conducted in the RStudio environment (Version 2025.09.0), adopting a significance level of 5% ($\alpha = 0.05$).

Results

Chemical Profile of the Essential Oils

The results revealed distinct chemical profiles among the three essential oils (Table 1). This allows the relationship between their composition and oxidative stability, and consequently their potential application as feed additives in aquaculture, to be established. The cinnamon leaf oil (*C. verum*) showed a marked predominance of phenolic compounds, with eugenol as the major constituent (71.55%), followed by eugenyl acetate (8.99%) and trans-cinnamaldehyde (1.06%). Sesquiterpenes such as β -caryophyllene (2.60%) and cinnamyl acetate (2.01%) were also identified. The peppermint oil (*Mentha piperita*) exhibited a composition dominated by monoterpenols, with menthol (44.07%) and isomenthone (24.45%) as the main constituents. Menthyl acetate (9.28%) and 1,8-cineole (6.01%) were the most significant secondary components. The presence of pulegone (0.64%) was detected, while minor compounds accounted for 1.19% of the total composition. Tea tree oil (*M. alternifolia*) demonstrated the most complex and balanced profile, with terpinen-4-ol (35.13%) as the major constituent, followed by γ -terpinene (17.84%) and α -terpinene (9.90%). Monoterpenes, including α -terpinolene (4.01%) and 1,8-cineole (2.63%) were also found. Sesquiterpenes such as ledene (2.18%) and δ -cadinene (1.55%) completed the composition.

Table 1. Chemical profile of the essential oils of cinnamon leaf (*C. verum*), peppermint (*M. piperita*), and tea tree (*M. alternifolia*) determined by gas chromatography-mass spectrometry (GC-MS).

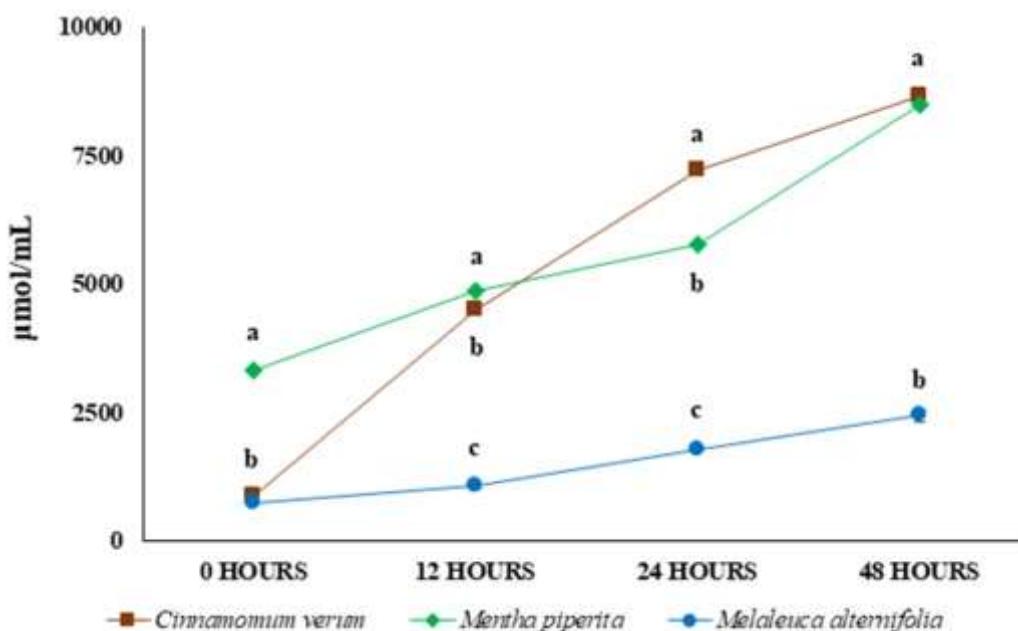
Cinnamon Leaf (<i>Cinnamomum verum</i>)		Peppermint (<i>Mentha piperita</i>)		Tea Tree (<i>Melaleuca alternifolia</i>)	
Compounds	(%)	Compounds	(%)	Compounds	(%)
α -Pinene	0.37	α -Pinene	0.41	β -Thujene	0.86
Camphene	0.11	β -Pinene	0.37	α -Pinene	3.20
p-Cymene	0.45	p-Cymene	0.33	Sabinene	0.50
D-Limonene	0.26	D-Limonene	1.25	β -Pinene	0.69
Eucalyptol	0.10	Eucalyptol	6.01	Myrcene	0.58
Linalool	1.98	Isopulegol	0.28	α -Phellandrene	0.46
Terpinen-4-ol	0.20	Iso-Menthone	24.45	α -Terpinene	9.90
α -Terpineol	0.39	neo-Menthol	7.32	p-Cymene	4.80
trans-Cinnamaldehyde	1.06	Iso-Menthol	2.59	D-Limonene	2.13
Safrole	1.06	Menthol	44.07	Eucalyptol	2.63
Eugenol	71.55	neoiso-Menthol	0.31	γ -Terpinene	17.84
Copaene	0.91	α -Terpineol	0.26	α -Terpinolene	4.01
Caryophyllene	2.60	Pulegone	0.64	cis- ρ -Menth-2-en-1-ol	0.30
trans-cinnamyl acetate	2.01	Piperitone	0.25	Terpinen-4-ol	35.13
α -Humulene	0.65	Menthyl acetate	9.28	α -Terpineol	4.64
δ -Cadinene	0.20	Caryophyllene oxide	0.55	α -Gurjunene	0.52
Eugenyl acetate	8.99	Minor (< 0.26%)	1.19	Caryophyllene	0.34
Caryophyllene oxide	1.41	Unidentified	0.45	Aromandendrene	1.96
Humulene 1,2-epoxide	0.17	-	-	Alloaromadendrene	0.54
Benzyl benzoate	4.50	-	-	Ledene	2.18
Minor (< 0.10%)	0.76	-	-	δ -Cadinene	1.55
Unidentified	0.28	-	-	Minor (< 0.30%)	3.75
-	-	-	-	Unidentified	1.50

Peroxidation of Essential Oils Under UV Radiation

Exposure to UV-C radiation (254 nm) promoted a significant increase in MDA levels in all essential oils, revealing distinct susceptibilities to photo-oxidation (Figure 2). Peppermint oil exhibited the greatest instability, with a sharp increase within the first 12 hours (4888.89 $\mu\text{mol}/\text{mL}$) and reaching its maximum value at 48 hours (8504.33 $\mu\text{mol}/\text{mL}$). Cinnamon leaf oil also showed pronounced degradation, increasing from an initial value 870.08 $\mu\text{mol}/\text{mL}$ to 8667.66 $\mu\text{mol}/\text{mL}$ at 48 hours. In contrast, tea tree oil displayed greater resistance, maintaining significantly lower MDA levels at 12, 24, and 48 hours of exposure.

Figure 2.

Malondialdehyde (MDA) levels in essential oils exposed to UV-C radiation (254 nm) for different durations. Different lowercase letters indicate significant differences among oils within the same exposure time ($p < 0.05$).

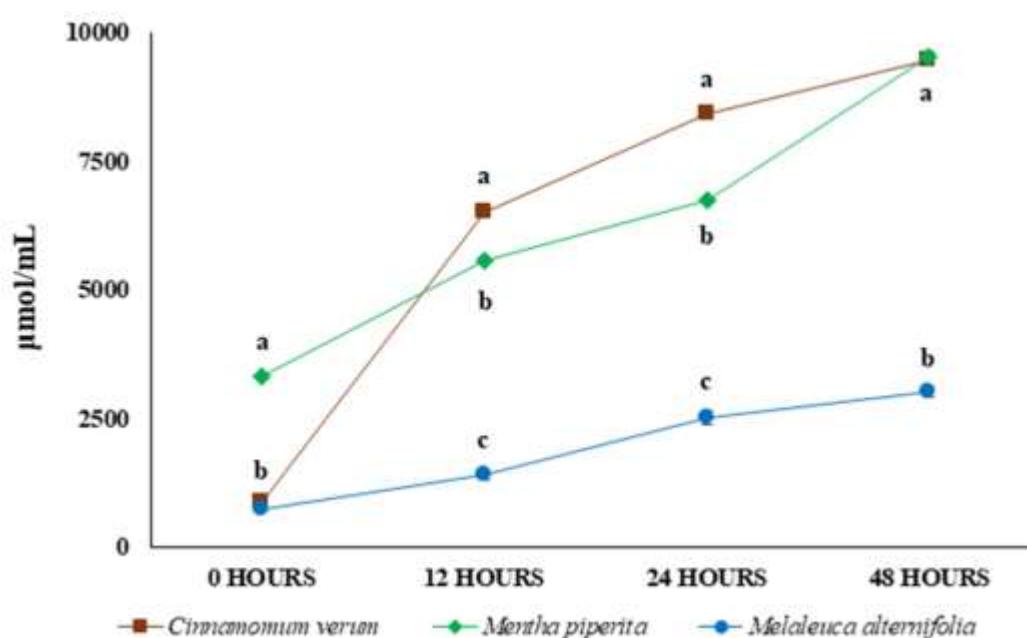


Peroxidation of Essential Oils Under Temperature Stress

The essential oils exhibited distinct degradation profiles when subjected to thermal stress at 60°C (Figure 3). Peppermint oil showed high susceptibility, reaching 5584.26 $\mu\text{mol}/\text{mL}$ of MDA at 12 hours and 9551.10 $\mu\text{mol}/\text{mL}$ at the end of the assay. Cinnamon leaf oil showed an increase in MDA levels from 870.08 $\mu\text{mol}/\text{mL}$ (0 hours) to 9486.76 $\mu\text{mol}/\text{mL}$ (48 hours). In contrast, tea tree oil exhibited the highest resistance to thermal oxidation, maintaining the lowest MDA levels across all intervals, with a final value of 3035.39 $\mu\text{mol}/\text{mL}$ after 48 hours of exposure.

Figure 3.

Malondialdehyde (MDA) levels in essential oils subjected to thermal stress (60°C) for different durations. Different lowercase letters indicate significant differences among oils within the same exposure time ($p < 0.05$).



Discussion

The results of this study provide insights into the oxidative stability of essential oils with potential application in aquaculture, revealing distinct patterns directly associated with their chemical signatures. The comparative analysis among the essential oils from cinnamon leaf (*C. verum*), peppermint (*M. piperita*) and tea tree (*M. alternifolia*) demonstrates how the chemical profile determines resistance to oxidative processes, a crucial factor for their viability as functional additives in aquaculture feeds.

The behavior of peppermint essential oil, which showed the highest lipid peroxidation indices in both assays, raises significant concerns for its application in aquaculture. Its composition, rich in menthol (44.07%) and isomenthone (24.45%), represents a particularly vulnerable system, considering that monoterpenols are known for their susceptibility to oxidation, and this inherent instability is enhanced by the fact that approximately 85% of the components are monoterpenoids, a class highly reactive under thermal and light stress (Turek e Stintzing, 2013). The significant MDA formation observed suggests that the potential of this oil as an immunostimulant in aquaculture may be severely compromised during extrusion processing, which involves high temperatures, and during feed storage (Ganosi et al., 2023). The accelerated degradation may not only reduce feed palatability, but may produce toxic compounds to aquatic organisms (Yun et al., 2013).

The cinnamon leaf essential oil presents particular relevance for aquaculture applications and, despite its major component, eugenol, having recognized antioxidant, antimicrobial, and antiparasitic activities (Habiba et al., 2021; Bandeira-Junior et al., 2022), the significant peroxidation values observed indicate vulnerabilities that may compromise its effectiveness against pathogens in aquaculture. This apparent contradiction may be explained by several factors, among them the pro-oxidant behavior of trans-cinnamaldehyde at low concentrations (Raveendran et al., 1993; Yu et al., 2020) and the potential of eugenol, the major component, to act as an electron donor (Prakash et al., 2025). These factors suggest that protective formulations would be necessary to preserve the original characteristics of this oil in aquaculture feeds.

In contrast, the stability of tea tree essential oil under conditions of thermal and UV stress positions it as the most promising candidate among the oils evaluated in the present study for application in aquaculture feeds. Its major component, terpinen-4-ol, possesses a hydroxyl group that acts as an efficient electron donor for free radicals, interrupting the propagation chains of peroxidation (Kim et al., 2004; Zhang et al., 2018). Additionally, the synergy among the various monoterpenes present, such as γ -terpinene, α -terpinene and α -terpinolene, creates a cascade protective system, where less stable compounds tend to protect more valuable ones in a process known as the “umbrella effect” (Frankel, 2005). This antioxidant protection network, which resulted in lower MDA levels than the other oils after 48 hours of exposure, suggests that tea tree oil has greater potential to resist feed manufacturing processes and to maintain the activity of its bioactive compounds of interest in aquaculture feeds (Souza et al., 2019; Reis et al., 2021; Yasin et al., 2021).

The practical implications of these findings for the aquaculture industry are significant. For unstable oils such as cinnamon leaf and peppermint, protection strategies such as encapsulation (Lim et al., 2023) or nanoemulsion (Singh e Pulikkal, 2022) become indispensable. For tea tree oil, whose stability has been demonstrated, more economical storage solutions, such as UV-filter packaging under controlled environmental conditions (20°C to 30°C), appear suitable. This recommendation is particularly relevant given that the thermal resistance of this oil allows for greater logistical flexibility in the feed production chain. The implementation of these protocols in aquaculture requires special attention to critical points in the logistic chain. The direct relationship established between major components and oxidative stability offers a valuable predictive model for the selection of essential oils and the development of feed formulations, allowing maximization of bioactivity retention while minimizing economic losses. This study reinforces that general solutions for diverse oils are inadequate for chemically diverse systems, highlighting the need for specific protocols based on the chemical signature of each essential oil for aquaculture applications.

Conclusion

Oxidative stability is a fundamental and discriminating criterion for selecting essential oils as functional additives in aquaculture feeds. The results confirm that the chemical profile determines resistance to degradation under conditions that resemble feed processing and storage. Tea tree oil (*M. alternifolia*) proved to be the most viable option due to its thermal and photo-oxidative stability, attributed to its chemical profile. In contrast, cinnamon leaf (*C. verum*) and peppermint (*M. piperita*) oils exhibited high susceptibility to oxidation, indicating that their practical use is conditioned upon the development of technological protection strategies to preserve their original characteristics. Thus, it is concluded that the technical and economic feasibility of incorporating essential oils into aquaculture feeds depends directly on their oxidative resistance.

The findings provide an evidence-based model to guide decision-making in the feed industry, promoting the more effective and safer use of natural additives in sustainable aquaculture production.

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