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Chemistry

Effect of aqueous extract from leaves of *Ocimum gratissimum* L. (Lamiaceae) on the oxidative stability of grapeseed oil

Efeito do extrato aquoso de folhas de *Ocimum gratissimum* L. (Lamiaceae) na estabilidade oxidativa do óleo de sementes de uva

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ABSTRACT

The growing interest in replacing synthetic antioxidants with natural ones in order to inhibit or delay lipid oxidation in edible vegetable oils has encouraged research on different plant sources, characterization of raw materials, and identification of new antioxidant compounds. *Ocimum gratissimum* L. (Lamiaceae) is popularly known as clove basil and is largely consumed by the population as a spice, condiment, and vegetable. Some authors have reported the use of natural extracts as antioxidants in vegetable oils. This study aimed to assess the effect of the lyophilized aqueous extract from the leaves of *O. gratissimum* on the oxidative stability of grapeseed oil. The oxidative stability of grape seed oil containing 0.1% of the extract was performed according to the accelerated oxidation (aging) test using the Rancimat method. Molecular absorption spectra were recorded in the range of 200-800 nm. The antioxidant potential was evaluated using an *in vitro* method with the DPPH reagent. The Folin-Ciocalteu method was used to quantify the phenolic compound content. The analysis of the grapeseed oil showed that adding the aqueous extract obtained by a decoction of leaves from O. gratissimum increased its oxidative stability.

Keywords: Antioxidant activity; Phenolic compounds; Oxidative stability

RESUMO

O crescente interesse na substituição de antioxidantes sintéticos por naturais afim de inibir ou retardar a oxidação lipídica em óleos vegetais comestíveis tem incentivado a pesquisa de diferentes fontes vegetais, a caracterização de matérias-primas e a identificação de novos compostos antioxidantes. *Ocimum gratissimum* L. (Lamiaceae) é popularmente conhecida como manjericão-cravo e é amplamente

consumida pela população como tempero, condimento e hortaliça. Alguns autores relataram o uso de extratos naturais como antioxidantes em óleos vegetais. Este trabalho teve como objetivo avaliar o efeito do extrato aquoso liofilizado das folhas de *O. gratissimum* sobre a estabilidade oxidativa do óleo de semente de uva. A estabilidade oxidativa do óleo de semente de uva contendo 0.1% do extrato foi realizada de acordo com o teste de oxidação acelerada (envelhecimento) pelo método Rancimat. Os espectros de absorção molecular foram obtidos por espectrofotometria na faixa de 200-800 nm O método *in vitro* utilizando o reagente DPPH avaliou o potencial antioxidante. O teor de substâncias fenólicas foi avaliado com o reagente de Folin-Ciocalteu. A análise do óleo de semente de uva mostrou que a adição do extrato aquoso obtido pela decocção das folhas de *O. gratissimum* aumentou a estabilidade oxidativa do óleo.

Palavras-chave: Atividade antioxidante; Compostos fenólicas; Estabilidade oxidativa

1 INTRODUCTION

The ingestion of vegetable oils from different natural sources is essential in the human diet since they are one of the main raw materials composed of edible lipids (Ying et al., 2018). Grape (*Vitis vinifera* L. (Vitaceae)) is one of the largest fruit crops in the world because of wine production, thus grape pomace and its seeds are residues that must have a correct destination/usage (Maier et al., 2009; Shinagawa et al., 2015). Germany, France, and Italy have produced grapeseed oil (GSO) since the 1930s (Shinagawa et al., 2015). Its applications are diverse, including in the food and cosmetics industries (Luque-Rodríguez et al., 2005). GSO is rich in unsaturated fatty acids (90%), phenolic compounds, and phytosterols (Bail et al., 2008; Garavaglia et al., 2016). The high content of unsaturated fatty acids makes this oil more susceptible to oxidation, which reduces its shelf-life (Berto et al., 2020).

Ocimum gratissimum L. (Lamiaceae) an herbaceous plant known as clove basil, is widely distributed in tropical and temperate regions of the globe (Alabi et al., 2018). The species is extensively and globally used in folk medicine to treat pneumonia, cough, high fever, epilepsy, flu, abdominal pains, and conjunctivitis, among others (Priyanka et al., 2018). *O. gratissimum* is one of the most used medicinal plants for its strong antioxidant and antibacterial properties (Priyanka et al., 2018). It is cytoprotective (Chiu et al., 2013), breast cancer preventive (Nangia-Makker et al., 2007), anesthetic (Silva et

al., 2012), larvicidal (Okigbo et al., 2010), antifungal (Koba et al., 2009), antileishmanial (Ueda-Nakamura et al., 2006), and hypoglycemic (Aguiyi et al., 2000).

To increase the oxidative stability, some studies have added plant extracts into vegetable oils, such as the addition of catnip, hyssop, lemon balm, oregano, sage, and thyme extracts in sunflower oil and the methanolic extract of *Agaricus blazei* mushroom into soybean oil (Abdalla & Roozen, 1999; da Silva et al., 2009).

No previous study evaluated the performance of the aqueous extract of the leaves of *O. gratissimum* in improving the oxidative stability of vegetable oils. Also, plant extracts have not yet been used to increase the oxidative stability of grapeseed oil.

Considering the aforementioned, the present study shows the evaluation of the oxidative stability of grapeseed oil with the addition of 0.1% of the aqueous extract obtained by decoction of leaves from *O. gratissimum*.

2 MATERIALS AND METHODS

2.1 Chemicals

Isopropanol was purchased from Merck (Darmstadt, HE, Germany), and Folin-Ciocalteau reagent and 1,1-diphenyl-2-picrylhydrazil from Sigma-Aldrich (St. Louis, USA). Ethanol 95% was from Pershy Chemicals (São Gonçalo, RJ, Brazil). The purified water was obtained using a Gehaka MS 2000 ultra-purifier (São Paulo, SP, Brazil). The grapeseed oil (GSO) was purchased from Bela Vida Natural (São Paulo, Brazil). Its label contained the following description: obtained by cold pressing, followed by filtration and refining, without the incorporation of antioxidants.

2.2 Plant material and extract preparation

Leaves from *O. gratissimum* were collected at the Horto de Plantas Medicinais of the Federal University of Grande Dourados, Brazil (UFGD), and a voucher was deposited at the herbarium of UFGD under the number #5429.

Fresh leaves of *O. gratissimum* were powdered, and the extract was obtained by heating for 10 minutes at 100 ºC (vegetable mass and volume of distilled water at 1:10 (m/v)). The mixture remained in contact for more 30 minutes until reaching 25 °C. The extract was filtered and frozen for subsequent lyophilization (Christ, Alpha 1-2 LD Plus, Osterode, Germany). The process was performed in triplicate.

2.3 Forced stability test

To obtain the samples for the forced stability test, the GSO extract was used for the preparation of the GSOE. The GSOE sample was prepared by incorporating 0.1% (m/v) of the lyophilized aqueous extract of *O. gratissimum* in GSO. GSO was used as a control for the evaluations. After preparation (time zero), GSO and GSEO were submitted to the analyses (molecular absorption spectrum, antioxidant activity, total phenolic content, and oxidative stability index) in triplicate, at different times (after preparation (time zero), on the fifth and tenth day).

Samples used in the forced stability test on the fifth and tenth day were maintained at a constant temperature of 50 °C and submitted to the same analyses carried out with the samples at time zero.

2.4 Molecular absorption spectrum

Samples were analyzed directly, without dilution, on a spectrophotometer (FEMTO 700 PLUS, São Paulo, Brazil), between the wavelengths 200 and 800 nm, with 1 nm of intervals.

2.5 Antioxidant activity

Analyses were performed employing the reagent DPPH, according to Kumaran & Joel Karunakaran (2006). Samples were analyzed by spectrophotometry (FEMTO 700 PLUS, São Paulo, Brazil). The solvent for the DPPH solution (0.004%) was isopropanol, and samples were prepared at concentrations ranging from 7 to 70 μg mL^{-1}. A curve

(percentage of inhibition versus sample concentration) was prepared to obtain the IC_{50} value. Analyses were done in triplicate, and isopropanol was used as the blank.

2.6 Total phenolic content

It was done using the Folin–Ciocalteu reagent based on the procedure from Djeridane et al. (2007). The oils were diluted on isopropanol at 1 mg mL^{-1}, and the solutions were analyzed by spectrophotometry (FEMTO 700 PLUS, São Paulo, Brazil) at 760 nm. A standard curve was prepared with gallic acid (5 to 1000 µg mL^{-1}). The results were expressed as mg of gallic acid equivalent (GAE) per g of sample and they were done in triplicate.

2.7 Oxidative Stability Index (OSI)

The determination of OSI was performed using equipment for the analysis of oxidative stability (893 Professional Biodiesel Rancimat, Metrohm, Herisau, Switzerland). This method was adapted from the AOCS Cd 12b-92 standard, in which 3.0 g of each sample was placed in a sealed reaction tube at a temperature of 100 °C and airflow rate of 20 L h^{-1}. The OSI data are calculated automatically through plots of the water conductivity against time by the software StabNet provided by the equipment manufacturer (AOCS Cd 12b-92, 1997). Analyses were performed in triplicate.

2.8 Statistical analyzes

The analysis was performed in RStudio (R Core Team, 2022), considering a significance of 5% in all statistical tests. The Levene test was performed to evaluate the homogeneity of the samples with *car* package (Fox & Weisberg, 2019), and the normality was tested on the Shapiro-Wilk. All samples presented homogeneity and normality, so the results were expressed as mean and standard deviation. Sequentially, an analysis of variance (ANOVA) was performed. After this, a Tukey test was performed, with the *multcompView* package for the visualization of formed groups (Graves et al., 2024).

To analyze the similarity between the samples, the Euclidean distance coefficients were determined and the cophenetic correlation was calculated using the *Vegan* package (Oksanen et al., 2022). Principal component analysis (PCA) was performed with the *FactoMineR* package (Le et al., 2008) and *factoextra* (Kassambara et al., 2022).

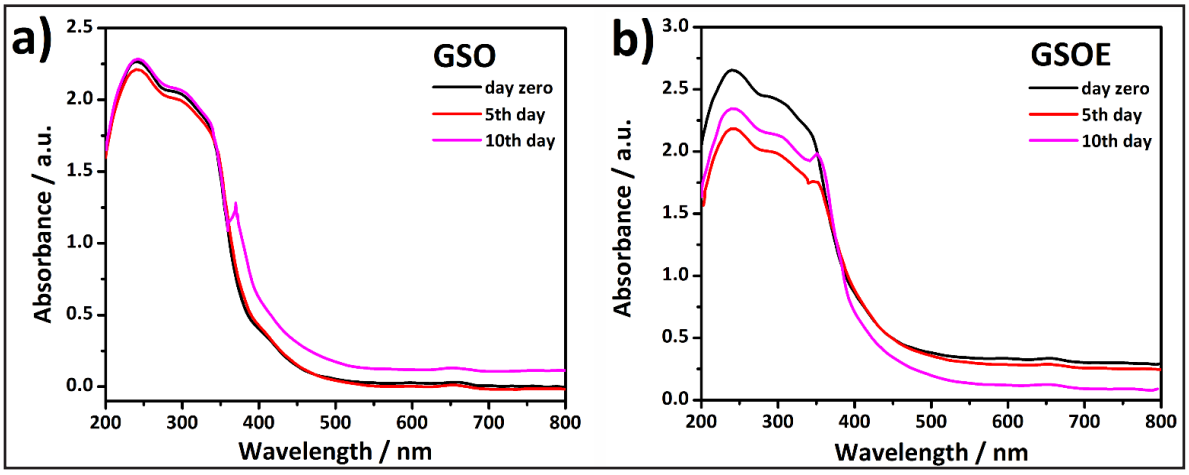
3 RESULTS AND DISCUSSION

The stability of vegetable oils depends on oxidation resistance during processing and storage, and the balance between chemical composition and antioxidants. A forced stability test shows how much a sample can endure in drastic conditions, and absorption spectra can accompany modifications in the composition of the samples over time.

Figure 1 presents the UV-Vis profiles of GSO (a) and GSOE (b) at times zero, 5, and 10 days of storage at 50 °C. Analyzing these molecular absorption spectra, there was a difference in the composition of GSO and GSOE over time, with the appearance of a band between 330 and 380 nm, with maximum absorptions at 370 (GSO) and 352 nm (GSOE). The change in the spectrum profile shows an alteration in composition during the storage period, beginning at 5 days for GSOE and 10 days for GSO (Figure 1). These changes in composition are probably because of oxidation reactions of the fatty acids in the oil.

Other tests performed during the storage period can also show the changes in oil composition, as seen in Table 1. A significant difference was observed between the antioxidant potential of GSO and GSOE only at time zero, while the antioxidant potential of GSOE was higher at time 0 and after 10 days. Furthermore, GSO had a significant increase ($p > 0.05$) in the minimum inhibitory concentration of DPPH (IC50), indicating a reduction in antioxidant potential, while only time zero of GSOE differed significantly ($p < 0.05$). It is possible that phenolic compounds were consumed during the analysis by inhibiting oil degradation (Zeb, 2020), thus preserving the antioxidant potential of grape oil.

Figure 1 – Molecular absorption spectra of samples during the forced stability study of 10 days a) GSO at the first, fifth, and tenth days of storage b) GSOE at the first, fifth, and tenth days of storage



Source: Authors' private collection (February 2023)

Table 1– Values of antioxidant concentration (IC_{50}), total phenolic content, and Oxidative Stability Index (OSI) during the forced stability test for GSO and GSOE

Sample (time)	IC_{50} (μg mL^{-1})	Phenolic content	OSI (h)
GSO (time zero)	14.62 ± 0.14b	203.50 ± 9.33a	11.04 ± 1.09a
GSOE (time zero)	10.11 ± 0.29a	228.50 ± 12.63b	10.90 ± 0.94a
GSO (five days)	15.89 ± 1.63bc	201.35 ± 13.58a	5.54 ± 1.29b
GSOE (five days)	13.16 ± 1.02b	219.78 ± 20.17ab	6.84 ± 1.21b
GSO (ten days)	19.96 ± 2.64c	200.84 ± 11.21a	3.91 ± 0.36d
GSOE (ten days)	13.85 ± 1.97b	215.61 ± 18.13ab	4.76 ± 0.51c

Different letters indicate significative differences in column ($p < 0.05$) on the Tukey test. Source: Authorship (2023)

The results showed a significant reduction in OSI over time for both samples ($p > 0.05$), indicating a clear effect of time on the stability of grapeseed oil. Regarding the effect of the extract, there was a significant difference between the samples only after

10 days ($p < 0.05$). This effect after 10 days may be associated with the antioxidant action mechanism of phenolic compounds, which is associated with reactions of breakdown of chains in the oxidative process that causes this long-term effect (Zeb, 2020).

Other authors have already studied the stability of grapeseed oil (Table 2). These studies explore the stability of oils obtained by cold pressing in the laboratory (Lutterodt et al., 2022; Heshmati et al., 2022; Pardo et al., 2009); only the work by Shinagawa et al. (2018) explored the stability of commercial oil.

Table 2 – Studies concerning the thermal stability of grapeseed oil

Sample	Amount	Temperature (°C)	Flow (L h^{-1})	OSI (h)	Reference
Muscadine	4 mL	80	7	19.7	Lutterodt et al., (2022)
Chardonnay				26.9	
Concord				40.0	
Ruby red				23.4	
Siahe	3 g	120	20	7.53	Heshmati et al., (2022)
-	-	120	20	1.37 – 3.09*	Shinagawa et al., (2018)
Monastrell	3.5 g	98.7	10	9.36	Pardo et al., (2009)
Monastrell				6.38	
Garnacha Tintorera				8.14	
Petit Verdot				8.07	
Syrah				7.90	

Source: Authorship (2023) *Commercial samples collected in different cities - = Uninformed

Based on the literature, it is possible to verify that the OSI of grapeseed oil varies according to the variety and parameters used in the analysis (Table 2). The stability of the oil analyzed in the present study was superior to that obtained in

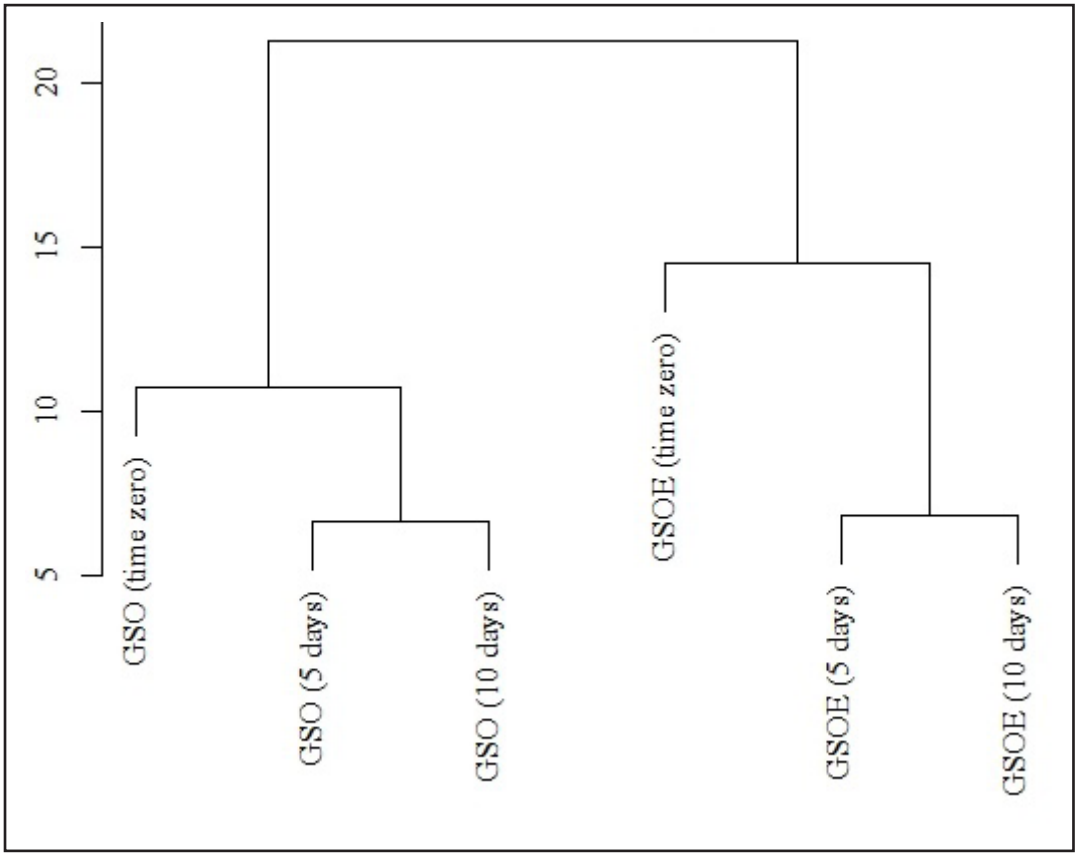
the works of Heshmati et al. (2022), Pardo et al. (2009), and Shinagawa et al. (2018). The most significant difference between OSI was observed when comparing the results obtained with those of Lutterodt et al. (2022), possibly due to the milder analysis conditions used by these authors.

Ying et al. (2018) determined the OSI for sixteen cold-pressed edible oils at 110 °C and 20 L h^{-1}; and only four of them (black cumin, argan, milk thistle, and macadamia oils) had OSI at the same order of magnitude as ours at time zero, and these are the samples with the higher stability performances. Hence, both GSO and GSOE exhibit oxidative stabilities comparable to many other vegetable oils.

When analyzing the dendrogram by Euclidean distance of the OSI, total phenolic content, and antioxidant activity of the samples (Figure 2), it is possible to verify the formation of two distinct groups, separating the samples into GSO and GSOE. It is verified the greater similarity between the samples stored after 5 and 10 days in both main groups. The dendrogram showed a cophenetic correlation of 0.77, indicating the reliability of the analysis. Two main groups were formed, one from the GSO and the other from the GSOE (Figure 2).

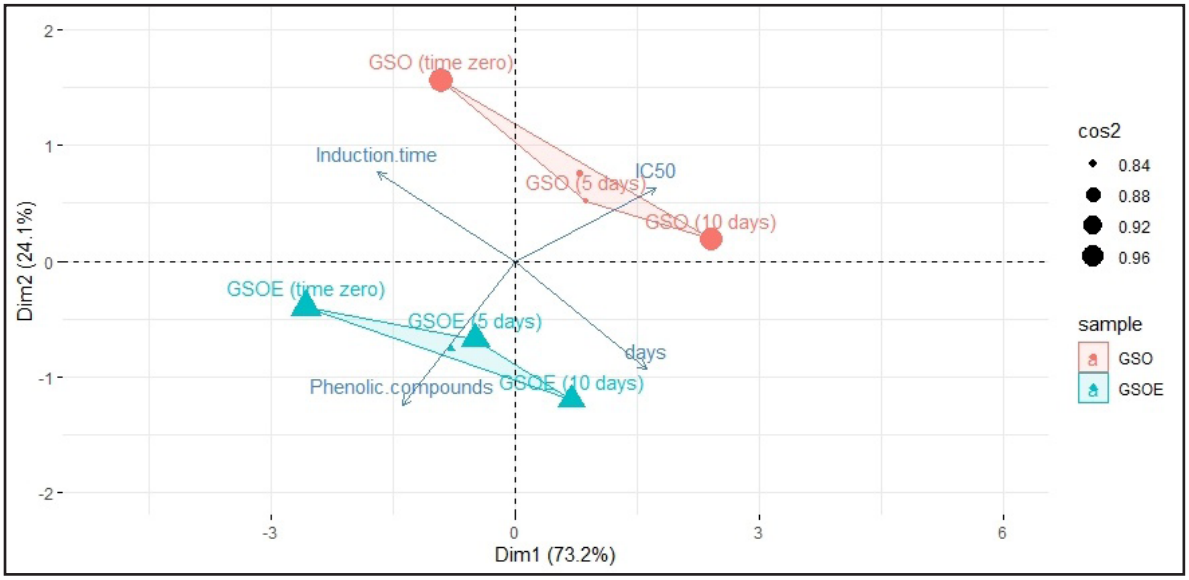
The results of the dendrogram (Figure 2) confirm the variation in composition observed in the molecular absorption spectra (Figure 1), antioxidant potential, and total phenolic content (Table 1). Similar to the dendrogram, PCA analysis shows a split between the samples according to the presence of *O. gratissimum* aqueous extract. In this sense, it was noticed that the increase in storage days culminates in the reduction of OSI, total phenolic content, and antioxidant potential in GSO and GSOE (Figure 3).

Figure 2 – Similarity of grapeseed oil (GSO) and grapeseed oil with aqueous extract of leaves from *O. gratissimum* (GSOE) at different storage times



Source: Authors' private collection (February 2023)

Figure 3 – Principal components analysis of grapeseed oil (GSO) and grapeseed oil with aqueous extract of *O. gratissimum* (GSOE) samples at different storage times



Source: Authors' private collection (February 2023)

4 CONCLUSIONS

The results presented in this study make it possible to infer that the lyophilized aqueous extract from the leaves of *O. gratissimum* improves the oxidative stability of grapeseed oil, being an interesting substitute for synthetic antioxidants in this vegetable oil.

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