

Research Article



Oxidative Stability of Edible Oils Improved by *Gaultheria* procumbens L. Essential Oil During Long-term Storage

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The oxidative stability of edible oils is a key determinant of their shelf life and nutritional quality. Growing consumer demand for natural antioxidants has prompted research into plant-based additives capable of preventing lipid oxidation. Gaultheria procumbens L. essential oil (GEO), known for its phenolic-rich composition, may provide protective benefits when added to oil matrices. This study investigates the effect of GEO on the antioxidant capacity of selected edible oils during prolonged storage. This study investigated the effects of GEO supplementation on the total antioxidant capacity (TAC) of rapeseed, olive, and grapeseed oils during 120 days of storage. The results showed that all three oils experienced a gradual decrease in TAC due to oxidative degradation over time. However, the addition of GEO consistently mitigated this decline, with the most significant antioxidant protection observed at 30 and 120 days of storage. In rapeseed oil, GEO significantly increased the TAC by 19.30% at 120 days. Olive oil enriched with GEO retained a significantly higher TAC at 120 days (loss reduced from 19.93 to 7.97%), while grapeseed oil showed the most stable antioxidant profile, including a significant increase in TAC of 13.78% at 120 days. The protective effects of GEO are attributed to its high content of phenolic compounds with radical scavenging properties. Differences in the oils' fatty acid profiles and intrinsic antioxidant levels influenced the extent of GEO's efficacy. The observed benefits of GEO supplementation highlight its promising potential as a natural preservative in food technology. Its ability to enhance oxidative stability without the use of synthetic additives is in line with current trends favouring clean label and plant-based solutions. Furthermore, the efficacy of GEO in different oil matrices suggests broad industrial applicability and opens avenues for future research into its use in emulsions, functional foods, and nutraceutical formulations.

Keywords: Gaultheria procumbens, essential oil, total antioxidant capacity, oxidative stability, storage, natural antioxidants

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Introduction

Edible oils are an important part of the human diet, providing essential fatty acids, fat-soluble vitamins, and energy (Jafari et al., 2022). However, their high unsaturated fatty acid content makes them particularly susceptible to oxidative degradation during storage, especially when exposed to oxygen, light, or elevated temperatures (Zhuang et al., 2022). Oxidation leads to the formation of reactive oxygen species (ROS) and lipid peroxides, which not only degrade the sensory and nutritional quality of oils, but also generate toxic compounds that pose health risks, including increased inflammation and risk of chronic diseases such as cardiovascular disease and cancer (Ayala et al., 2014; Pizzino et al., 2017). As a result, improving the oxidative stability of edible oils has become a major focus of food science and technology (Blasi and Cossignani, 2020; Fadda et al., 2022).

Traditionally, synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have been added to edible oils to retard lipid peroxidation (Mohammadi et al., 2016; Lankanayaka et al., 2025). Despite their effectiveness, concerns about the safety and potential carcinogenicity of synthetic antioxidants have led to a shift towards natural alternatives (Hassanpour and Doroudi, 2023). In this context, essential oils derived from medicinal plants have emerged as promising candidates due to their high content of bioactive phenolic compounds with potent antioxidant properties (Diniz do Nascimento et al., 2020). Among them, Gaultheria procumbens essential oil (GEO) has attracted attention for its anti-inflammatory, antioxidant, and photoprotective activity (Liu et al., 2013; Michel and Olszewska, 2024).

Gaultheria procumbens L., commonly known as American wintergreen or eastern teaberry, is a small shrub native to northeastern North America, traditionally used by indigenous peoples for its anti-inflammatory, analgesic, and antimicrobial properties (Luo et al., 2018; Michel and Olszewska, 2024). Its aerial parts - particularly the leaves and fruits - and the essential oil rich in methyl salicylate (wintergreen oil) have been used to treat rheumatoid arthritis, respiratory infections, and various painful conditions (Liu et al., 2013; Luo et al., 2018). Over time, phytochemical studies have identified more than 70 hydrophilic constituents (e.g. methyl salicylate glycosides, flavonoids, phenolic acids) and numerous lipophilic compounds (e.g. triterpenes, sterols) in the plant, along with more than 130 volatile compounds in the essential oil, predominantly methyl salicylate (Michel and Olszewska, 2024).

Modern pharmacological research has shown that G. procumbens has significant anti-inflammatory, antioxidant, and photoprotective activity, supported by in vitro and ex vivo studies (Liu et al., 2013; Michel and Olszewska, 2024; Michel and Olszewska, 2024; Michel et al., 2024). While the essential oil shows moderate antimicrobial and insecticidal potential, the hydrophilic compounds - particularly the glycoside gaultherin - are mainly responsible for the plant's therapeutic effects, with some in vivo efficacy also confirmed (Hammer et al., 1999; Mullen et al., 2014; Kiran and Prakash, 2015; Verdi et al., 2022). Despite its long-standing traditional use and promising bioactivity, the lack of clinical trials and comprehensive toxicological evaluations highlights the need for further research to determine safe dosages, mechanisms of action, and suitable pharmaceutical formulations (Michel and Olszewska, 2024).

The oxidative stability of edible oils varies considerably depending on their fatty acid composition (Maszewska et al., 2018). Rapeseed oil is rich in monounsaturated fatty acids (MUFA), especially oleic acid, which is relatively stable against oxidation (Abrante-Pascual et al., 2024). Olive oil is also rich in MUFAs and intrinsic antioxidants such as tocopherols and polyphenols (Jimenez-Lopez et al., 2020). In contrast, grapeseed oil is particularly rich in polyunsaturated fatty acids (PUFAs), especially linoleic acid, which makes it highly susceptible to oxidative degradation (Fruehwirth et al., 2020). This variability requires targeted antioxidant strategies tailored to the specific oxidative susceptibilities of each oil type.

The present study investigated the effect of GEO supplementation on the total antioxidant capacity (TAC) of rapeseed, olive, and grapeseed oils during extended storage periods of up to 120 days. By measuring TAC at different time points, this work aimed to elucidate both the immediate and long-term effects of GEO on oxidative stability. A comparative approach between different oil types provided insights into the interaction between exogenous antioxidants and oil composition. In particular, this study addressed whether GEO can effectively mitigate oxidative losses in oils with different fatty acid profiles and whether its protective effect is consistent over time or dependent on the degree of native antioxidant depletion.

Materials and Methodology

Gaultheria Essential Oil

The commercial GEO was provided by Polish essential oil manufacturers (Bamer, Włocławek, Poland). Gaultheria oil is obtained by steam distillation of the leaves of *Gaultheria* spp. The oil obtained from this plant has an intense and slightly minty fragrance. Samples were stored in re-sealable vials at 5 °C in the dark, but allowed to equilibrate to room temperature before testing. Geographical origin was excluded, as this information was mostly not available.

Rapeseed, Olive, and Grapeseed Oils

The rapeseed, olive, and grapeseed oils were purchased from a local shop. Rapeseed oil (Wyborny, Poland) is refined rapeseed oil. The energy value of 100 ml is 3,464 kJ (828 kcal), fat 92 g, including 6.4 g saturated fatty acids, 58 g monounsaturated fatty acids, and 28 g polyunsaturated fatty acids.

Olive oil (Casa de Azeite, Italy) is a high-quality extra virgin olive oil. The energy value of 100 ml is 3,374 kJ (821 kcal), fat 91 g, including 13 g of saturated fatty acids, 72 g of monounsaturated fatty acids, and 6.3 g of polyunsaturated fatty acids.

Grapeseed oil (Monini, Italy) is a product containing polyunsaturated fatty acids. The energy value of 100 ml is 3404 kJ (828 kcal), fat 92 g, including 11 g of saturated fatty acids, 24 g of monounsaturated fatty acids, and 57 g of polyunsaturated fatty acids.

The rapeseed, olive, and grape seed oil samples (5 ml) were incubated with 0.1 ml GEO (final concentration $20 \ \mu g.mL^{-1}$) at 25 °C for 240 days. This reaction mixture was gently shaken at fixed intervals during incubation at 25 °C. Samples were collected for analysis after 0, 8, 15, 30, 60, and 120 days of storage. The rapeseed, olive, and grape seed oil samples without additives were used as control samples.

Measurement of Total Antioxidant Capacity (TAC)

The level of TAC in the samples was estimated by measuring the level of 2-thiobarbituric acid reactive substances (TBARS) after oxidation of Tween-80. This level was determined spectrophotometrically at 532 nm (Opryshko et al., 2021). The sample inhibits the Fe²⁺/ ascorbate-induced oxidation of Tween 80, resulting in a decrease in the TBARS level. The amount of TAC in the sample (%) was calculated from the absorbance of the blank.

Statistical Analysis

Results are expressed as means. All variables were tested for normal distribution using the Kolmogorov-Smirnov test (p >0.05). The significance of differences in TAC levels between samples (significance level p <0.05) was tested using the Mann-Whitney test according to Zar (1999). All statistical calculations were performed on separate data from each sample using STATISTICA 13.3 software (TIBCO Inc., USA).

Results and Discussion

The effect of GEO addition and storage time on the TAC of rapeseed, olive, and grapeseed oils at 7, 15, 30, 60 and 120 days of storage is shown in Figs 1–3.

The total antioxidant capacity (TAC) of rapeseed oil after 7, 15, 30, 60 and 120 days of storage decreased by 13.35% (p > 0.05), 15.74% (p > 0.05), 51.15% (p < 0.05), 45.94% (p < 0.05) and 19.36% (p > 0.05), respectively, compared to the initial value (0 day). In rapeseed oil enriched with GEO, the TAC was reduced by 13.28% (p >0.05), 20.41% (p >0.05), 49.66% (p <0.05), 41.91% (p <0.05) and 5.32% (p >0.05), respectively, over the same storage periods. The addition of GEO to rapeseed oil increased the TAC at 30 and 120 days of storage by 4.79% (p >0.05) and 19.30% (p <0.05), respectively, compared to the control samples. At 7 and 60 days, a slight, non-significant increase in TAC levels was also observed in the GEO-enriched samples compared to the control, by 1.75% (p > 0.05) and 9.28%(p >0.05), respectively (Figure 1).

The total antioxidant capacity of olive oil after the addition of GEO at 7, 15, 30, 60, and 120 days of storage is shown in Figure 2.

The TAC of olive oil after 7, 15, 30, 60 and 120 days of storage decreased by 14.39% (p >0.05), 25.23% (p <0.05), 32.93% (p <0.05), 32.52% (p <0.05) and 19.93% (p >0.05), respectively, compared to the initial value (0 day). In olive oil enriched with GEO, the TAC was reduced by 8.66% (p >0.05), 19.59% (p >0.05), 15.91% (p >0.05), 32.96% (p <0.05) and only 7.97% (p > 0.05), respectively, over the same storage periods. The addition of GEO to olive oil increased the TAC at 30 and 120 days of storage by 11.07% (p >0.05) and 19.22% (p <0.05), respectively, compared to the control samples. At 7 and 15 days, non-significant decreases in TAC of 5.49% (p >0.05) and 4.71% (p > 0.05), respectively, were also observed in the GEOenriched samples compared to the control. After 60 days, a significant decrease of 11.97% (p < 0.05) was observed compared to the control (Figure 2).



Figure 1 Effect of GEO addition and storage time on the total antioxidant capacity (TAC, %) of rapeseed oil after 7, 15, 30, 60, and 120 days of storage

* Changes are significantly different between control and GEO addition (p < 0.05, n = 6)



Figure 2 Effect of GEO addition and storage time on the total antioxidant capacity (TAC, %) of olive oil after 7, 15, 30, 60, and 120 days of storage

* Changes are significantly different between control and GEO addition (p < 0.05, n = 6)

The total antioxidant capacity of grapeseed oil after the addition of GEO at 7, 15, 30, 60, and 120 days of storage is shown in Figure 3.

The TAC of grapeseed oil after 7, 15, 30, 60 and 120 days of storage decreased by 6.22% (p >0.05), 12.89% (p >0.05), 19.38% (p <0.05), 22.36% (p <0.05) and 14.25% (p >0.05), respectively, compared to the initial value (0 day). In grapeseed oil enriched with *Gaultheria* essential oil (GEO), the TAC changed by +5.25% (p >0.05), -1.36% (p >0.05), -14.03% (p >0.05), -24.27% (p <0.05) and +2.73% (p >0.05), respectively, over the same storage periods. The addition of GEO to grapeseed oil increased the TAC by 6.57% (p >0.05), 7.53% (p >0.05), 1.33% (p >0.05), and 13.78% (p <0.05) compared to control samples at 7, 15, 30, and 120 days, respectively. At 60 days, a decrease in TAC of 7.33% (p >0.05) was observed in the GEO-enriched samples compared to the control (Figure 3).

The results of this study indicate that the TAC of all three edible oils – rapeseed, olive, and grapeseed – decreased with time during storage, which is consistent with the well-documented oxidative degradation of unsaturated lipids during prolonged storage (Sun et al., 2020). However, the extent of this decline, as well as the protective effect of GEO, varied with oil type and storage time. In rapeseed oil, TAC decreased significantly after 30 and 60 days of storage (p <0.05), with reductions of more than 45%, reflecting considerable oxidative degradation. In particular, the addition of GEO conferred a protective effect, especially at 30 and 120 days of storage, where increases in TAC of 4.79% and 19.30%, respectively, were observed compared to the control. These results suggest a delayed but significant antioxidant effect of GEO on rapeseed oil. While earlier time points (7 and 15 days) did not show significant improvements in antioxidant stability, a non-significant positive trend was observed (Figure 1).

Similar patterns were observed for olive oil. Although samples fortified with GEO showed lower TAC levels than the control at 7 and 15 days, significant protection was observed at later stages. A significant improvement (p < 0.05) was observed at 120 days, where GEO supplementation reduced the loss in TAC from 19.93% to only 7.97%. The sharp contrast between control and GEO-enriched samples at 30 days (TAC decrease of 32.93% vs. 15.91%) further underlines the efficacy of GEO in stabilising olive oil against oxidative deterioration (Figure 2).

Grapeseed oil showed the most stable TAC profile among the three oils during storage, especially when enriched with GEO. Interestingly, a non-significant increase in TAC was observed at 7 days (+5.25%), followed by slight losses at 15 and 30 days and a moderate decrease at 60 days. Importantly, after 120 days of storage, GEO supplementation resulted



Figure 3 Effect of GEO addition and storage time on total antioxidant capacity (TAC, %) in grapeseed oil after 7, 15, 30, 60, and 120 days of storage

* Changes are significantly different between control and GEO addition (p <0.05, n = 6)

in a significant (p < 0.05) increase in TAC of 13.78% compared to the control, suggesting that GEO is particularly effective in improving the oxidative stability of grapeseed oil during long-term storage (Figure 3).

Taken together, these results confirm the antioxidant potential of GEO as a natural preservative. Its ability to preserve TAC is attributed to its high concentration of phenolic compounds known for their radical scavenging activities (Platzer et al., 2022). The variation in efficacy between oils may be due to differences in fatty acid composition, intrinsic antioxidant content, and susceptibility to lipid peroxidation (Abrante-Pascual et al., 2024). GEO appeared to be particularly effective in oils with lower oxidative stability, such as grapeseed oil, which has a higher proportion of polyunsaturated fatty acids (Fruehwirth et al., 2020).

The observed differences in the efficacy of GEO among the oils tested can be attributed not only to their different fatty acid profiles but also to their intrinsic antioxidant compositions. Rapeseed oil, rich in monounsaturated fatty acids (mainly oleic acid) and natural antioxidants such as tocopherols, has a relatively high initial oxidative stability (Dordevic et al., 2023). In contrast, grapeseed oil contains a higher proportion of polyunsaturated fatty acids (linoleic acid), which are more susceptible to lipid peroxidation (Maszewska et al., 2018; Khakbaz Heshmati et al., 2022). Therefore, the pronounced effect of GEO in grapeseed oil may reflect its greater need for exogenous antioxidant boosting.

Studies have shown that *G. procumbens* leaf and stem extracts possess potent antioxidant properties as confirmed by various in vitro assays, including DPPH, ABTS, FRAP, and linolenic acid oxidation inhibition and peroxide radical scavenging tests. These extracts showed superior activity compared to standard antioxidants such as quercetin and ascorbic acid, particularly in neutralizing radicals such as $\bullet 0_2^-$, OH \bullet , and H₂O₂ (Michel et al., 2014, 2019, 2022). In contrast, fruit extracts showed moderate antioxidant capacity due to the predominance of less reactive salicylic glycosides (Michel et al., 2020). Ex vivo experiments showed that *G. procumbens* extracts (25–150 µg.mL⁻¹) significantly reduced ROS levels in fMLP-stimulated human neutrophils, with leaf and stem extracts showing the strongest effects, attributed to their rich flavonoid and procyanidin content (Michel et al., 2019, 2022). Interestingly, fruit extracts also reduced ROS levels, despite weak direct activity in cell-free assays, probably due to methyl salicylate glycosides affecting pro-inflammatory pathways (Zhang et al., 2012, 2015).

Further cellular studies confirmed the antioxidant potential of ethanolic leaf tinctures, which reduced malondialdehyde levels in H_2O_2 -stimulated Caco-2 cells (Voicu et al., 2019).

Further evidence of antioxidant effects was demonstrated in UVA-irradiated Hs68 dermal fibroblasts. *G. procumbens* extracts (5–25 µg.mL⁻¹) significantly reduced ROS formation and restored the activity of key antioxidant enzymes such as superoxide dismutase and glutathione S-transferase (Michel et al., 2024). These findings suggest a protective role for G. procumbens against UV-induced oxidative stress and premature skin ageing (Rinnerthaler et al., 2015). Wintergreen oil from G. procumbens, which is rich in methyl salicylate, generally showed weaker antioxidant effects in cell-free assays compared to plant extracts or standard antioxidants such as vitamin C or gallic acid (Singh and Ali, 2017; Jintanasirinurak et al., 2023). However, some studies reported moderate activity in other models, such as OH• radical inhibition in the Fenton reaction and increased antioxidant capacity in neuronal cells (Eldurini et al., 2021). Despite its limited direct radical scavenging ability, wintergreen oil may exert indirect antioxidant effects by modulating redox-sensitive signalling pathways, including NF- κ B and TNF- α expression (Zhang et al., 2012, 2015). These mechanisms are likely to be responsible for the delayed oxidative degradation observed in GEO-enriched samples, particularly after 30 and 120 days of storage.

It is important to note that although GEO showed statistically significant protective effects at later storage intervals, its efficacy was limited or non-significant at the early stages (7 and 15 days). This delay may be related to the initial abundance of endogenous antioxidants in the oils, which may mask or overlap the effect of GEO. It is only when these native compounds are depleted that the contribution of GEO becomes more evident. This suggests a potential synergistic or complementary role for GEO used with an intrinsic antioxidant combination, rather than as a stand-alone solution during early storage.

Previous studies have also highlighted the effect of essential oil concentration and oxidative stress intensity on their efficacy. It is possible that a higher concentration of GEO could provide greater protection, particularly during the early stages of storage. However, the sensory characteristics of the oils (flavour, aroma) may limit the practical application of higher doses (Burt, 2004). Therefore, an optimal balance between oxidative protection and organoleptic acceptability needs to be determined for industrial applications.

From a technological and consumer health perspective, the incorporation of natural antioxidants such as GEO is in line with current trends to reduce synthetic additives in food products (Lourenço et al., 2019). The growing consumer demand for clean-label products underlines the relevance of this study. In addition, maintaining high antioxidant capacity during storage is essential not only to preserve nutritional quality but also to prevent the formation of harmful lipid oxidation products, which are implicated in several chronic diseases (Bellucci et al., 2022; Petcu et al., 2023). Therefore, the use of GEO may have both technological and functional roles in edible oil preservation strategies.

Thus, incorporation of GEO significantly improved the total antioxidant capacity of rapeseed, olive, and grapeseed oils during storage, particularly at later stages. The protective effect of GEO was most pronounced in grapeseed oil and least effective in rapeseed oil at early storage intervals. These results support the use of GEO as a natural antioxidant additive to extend the shelf life and maintain the nutritional quality of edible oils. However, the efficacy of GEO was dependent on oil type and storage time, highlighting the need for tailored antioxidant strategies in oil preservation.

Conclusions

This study evaluated the effect of *Gaultheria* essential oil (GEO) supplementation on the total antioxidant capacity (TAC) of rapeseed, olive, and grapeseed oils during storage for up to 120 days. All oils showed a progressive decrease in TAC over time. However, GEO-enriched oils consistently showed improved antioxidant stability, with the most significant effects observed at 30 and 120 days of storage. Grapeseed oil showed the greatest improvement in TAC with GEO addition, whereas rapeseed oil showed moderate improvements. These results highlight the potential of GEO as a natural antioxidant to mitigate oxidative degradation in edible oils. This research is also in line with the growing demand for clean-label foods, free from synthetic additives. The incorporation of plant-derived antioxidants such as GEO into edible oils could provide a dual benefit of extending shelf-life and preserving nutritional integrity, while responding to consumer preferences for natural, health-promoting food ingredients (Lourenço et al., 2019; Datsenka et al., 2019). With global trends towards sustainable and

health-conscious food production, this study provides timely and practical implications for the use of GEO as a natural antioxidant in edible oil preservation.

Conflicts of Interest

The authors have no competing interests to declare.

Ethical Statement

This article does not include any studies that would require an ethical statement.

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