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# O-Alkyl derivatives of ferulic and syringic acid as lipophilic antioxidants: effect of the length of the alkyl chain on the improvement of the thermo-oxidative stability of sunflower oil†

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Lipid oxidation is the major cause of the deterioration of fat-containing foods, especially those containing polyunsaturated fatty acids (PUFAs). Antioxidant additives of synthetic origin are added to matrices rich in PUFAs, such as sunflower oil (SO). However, there is controversy regarding their safety, and their low solubility in both water and fat has led to the search for new covalent modifications through lipophilicity. This work presents the synthesis of *O*-alkyl acid derivatives from ferulic and syringic acids and the study of their antioxidant capacity and effect on the thermoxidative degradation of SO. Antioxidant activities were evaluated by employing ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assays in a concentration range of 10–100  $\mu\text{g mL}^{-1}$ . The  $\text{IC}_{50}$  values for DPPH scavenging activity ranged from 15.61–90.43  $\mu\text{g mL}^{-1}$ . The results of the FRAP assay for both *O*-alkyl ferulic (**3a–f**) and syringic (**5a–f**) series revealed a “cut-off” effect on antioxidant activity in carbon five (C5). Thermoxidation study of additives **3b–c** and **5b–c** showed a decrease in the slope of extinction coefficients  $K_{232}$  and  $K_{270}$  in comparison with  $\text{SO}_{\text{control}}$ . Furthermore, **3c** presented higher antioxidant activity than **3b** and **1**, with a power to decrease the thiobarbituric acid reactive species (TBARS) 6 times higher than  $\text{SO}_{\text{control}}$  at 220 °C. Additives **5b–c** exerted a protective effect on the thermoxidation of SO. The results suggest that increasing lipophilic and thermal properties of antioxidants through *O*-alkyl acid derivatization is an effective strategy for accessing lipophilic antioxidant additives with potential use in food matrices.

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## 1. Introduction

Vegetable edible oils are a significant source of fat for human consumption, providing energy and nutritional components,

and the main compounds are triglycerides (~95%), which are composed of fatty acids (saturated or mono-/polyunsaturated). They also include tocopherols, essential fatty acids, phytosterols, vitamins, phenolic compounds, and volatile organic compounds.<sup>1</sup> Chemical and physical refining processes of edible oils lead to the loss of endogenous antioxidants, such as tocopherols, phenolic compounds, and carotenoids, resulting in increased susceptibility to oxidative deterioration.<sup>2</sup> The overall stability and oxidation resistance of oils and fats refer to their ability to resist oxidative rancidity (or deterioration) over processing and storage periods depending on their composition and the conditions to which they are subjected. Generally, the oxidation process begins when the oil is exposed to heat, air, light, *etc.* The degradation process is characterized by a radical mechanism that produces hydroperoxides, which can generate products such as ketones, aldehydes, alcohols, lactones, and acids.<sup>3,4</sup> Partially refined and refined edible oils are usually fortified with synthetic or natural antioxidants to compensate for the loss of endogenous compounds.<sup>5</sup> There are many studies on the antioxidant properties of polyphenolic compounds from vegetable extracts or of synthetic origin and their ability to

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improve the thermoxidative stability of edible oils. In this context, Elhamirad and Zamanipoor<sup>6</sup> reported the inhibition of the thermal oxidation of sheep tallow olein by a series of antioxidants, such as  $\alpha$ -tocopherol, polyphenolic compounds (quercetin and catechin), and simple phenolic acids (caffeic and gallic acids), using the Rancimat method. They reported that the phenolic acids were more effective at a temperature of 120 °C, but at 180 °C, quercetin was the most effective, whereas polyphenolic compounds were more effective than  $\alpha$ -tocopherol at all temperatures used;  $\alpha$ -tocopherol, despite being one of the most important and studied endogenous antioxidants in oils, is thermally unstable and offered limited protection at high temperatures.<sup>7</sup> Owing to the issues and drawbacks of antioxidants obtained from natural extracts, the use of synthetic or semi-synthetic antioxidants has been extensively promoted to improve the thermal-oxidative stability of edible oils. Therefore, synthetic antioxidants, such as butylated hydroxytoluene (BHT), *tert*-butylhydroquinone (TBHQ), and butylated hydroxyanisole (BHA), are commonly added to extend the shelf life of edible oils.<sup>8,9</sup> TBHQ is considered more effective than BHT and BHA in preventing the thermoxidation of frying oils and increasing oxidative stability.<sup>10</sup> Despite the positive effects of preventing the oxidation of edible oils, there is controversy regarding their safety; studies refer to cytotoxic effects and severe damage to the liver and kidney tissues.<sup>11,12</sup> In addition, the low solubility of these compounds in both water and fat has led to the search for new covalent modifications of antioxidants through lipophilization as this is an important field of research aiming at developing antioxidants with improved physicochemical properties.<sup>13</sup>

Lipophilic phenolic compounds (Lipo-PCs), whether derived from natural sources or synthesized, have emerged as a promising option for preventing the oxidation of lipids. The synthesis of Lipo-PCs from natural hydrophilic phenolic compounds (Hydro-PCs) is ongoing *via* chemical or enzymatic acylation/alkylation.<sup>14</sup> In this context, the synthesis of ester derivatives from natural phenolic acids was described with a gradual increase in their alkyl chains. Thus, caffeates of medium alkyl chain-length (butyl, octyl, and dodecyl) added to fish oil-enriched mayonnaise resulted in better oxidative stability than caffeates with shorter (methyl) or longer (octadecyl) alkyl chains,<sup>15</sup> while the addition of the octanoate ester of caffeic acid increased the induction time of sunflower oil (SO),<sup>16</sup> and octyl sinapate enhanced the antioxidant capacity of rapeseed–linseed oil mixture.<sup>17</sup>

Moreover, an assessment was conducted involving ferulic acid, caffeic acid, and their respective esters in fish oil-enriched milk. The results indicated that the most effective antioxidants were short-chain esters (with chain lengths of C1 or C4) for both phenolic compounds.<sup>15,18</sup> Notably, when phenolipids with longer chain lengths were considered, significant distinctions emerged between caffeic acid esters and ferulic acid esters. Medium alkyl ferulates, such as octyl ferulate and dodecyl ferulate, exhibited a notable prooxidative effect at a specific antioxidant concentration, while longer alkyl ferulates (C16 and C20) displayed either weak prooxidant or weak antioxidant properties. In contrast, caffeic acid esters, including medium

and long-chain esters, did not exhibit prooxidative effects; instead, they functioned as weak antioxidants.

Studies of homologous series of antioxidants have shown that antioxidant capacity increases as the alkyl chain length increases until a hydrophobicity threshold (*i.e.*, critical chain length) is reached. If the alkyl chain continues to increase, a collapse of the antioxidant capacity occurs (*e.g.* “cut-off” theory).<sup>19,20</sup>

Moreover, ester derivatives have poor stability at high temperatures. Propyl gallate (PG) is a food additive that is slightly soluble in both water and fat. Nevertheless, PG is inadequate for frying at temperatures higher than 190 °C because of its poor stability at high temperatures.<sup>21,22</sup>

Previous reports highlight the importance of the carboxylic acid group in antioxidant capacity; for example, gallic acid was found to be a DPPH radical scavenger (DPPH<sup>•</sup>) significantly stronger than pyrogallol due to better solvent-based molecular interactions arising from the –COO–electron donor group.<sup>23</sup> In the same way, it has been shown that alkoxy groups also play a crucial role in the antioxidant capacity of phenolic compounds.<sup>24</sup> In phenolic compounds, the antioxidant potency can be promoted by the introduction of different electron-donating/withdrawing groups to specific positions of the phenolic ring.<sup>21</sup> For example, functional groups substituted to the *ortho*- or *para*-positions of phenolic rings are reported to be more effective than those attached to *meta*-position in changing the performance of phenolic antioxidants.<sup>25</sup>

Despite the important antioxidant properties presented by phenolic acid esters, there is great interest in new compounds with better antioxidant capacity/lipophilicity/thermal stability ratios that can become new safe alternatives for use as antioxidant additives on lipids, especially edible oils. For this reason, in the present research, a new approach based on the generation of ethers derived from phenolic acids was investigated. It is known that ethers derived from hydroxytyrosol present good antioxidant capacity despite having the hydroxyl group blocked.<sup>26</sup> Ferulic and vanillic acids have proven effects over the inhibition of the lipoperoxidation process,<sup>27,28</sup> and limited efficiency due to solubility and thermal stability issues.

In the present study, the antioxidant capacity and thermo-stability activity of *O*-alkyl functionalized phenolic derivatives were evaluated by applying different methods to assess the physical-chemical characteristics such as hydrophilicity/hydrophobicity and structure–activity relationships responsible for modulating and improving the thermoxidative stability of edible oils, such as SO.

## 2. Experimental

### 2.1. Chemicals and reagents

Ferulic and syringic acids and alkyl bromides (ethyl, propyl, pentyl, hexyl, octyl, and decyl bromides) were purchased from Merck Millipore (Burlington, Massachusetts, USA). Methanol and hexane were obtained from Arquimed (Santiago, Chile), while gallic acid, Folin–Ciocalteu reagent, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Aldrich (St. Louis, MO, USA). All reagents were of analytical grade.



## 2.2. Vegetable oils

The olive and sunflower virgin oils used in this study were obtained from the local market.

## 2.3. General procedure for the synthesis of *O*-alkyl benzoic and hydroxycinnamic acid derivatives

The *O*-alkyl phenolic acid derivatives were synthesized and purified following the methodology reported by Tandel *et al.*, 2014 (ref. 29) with slight modifications. Briefly, commercially available ferulic (**1**) and syringic (**4**) acids (1.44 mmol) were *O*-alkylated using the respective alkyl bromides (1.44 mmol; ethyl (**2a**), propyl (**2b**), pentyl (**2c**), hexyl (**2d**), octyl (**2e**) and decyl (**2f**) bromides) and sodium hydroxide (2.88 mmol) in EtOH/H<sub>2</sub>O (6 mL) 2:1 solution in a 1:1:2 equivalent molar. The reaction mixture was refluxed for 17 h. After completion of the reaction, the mixture was poured over ice water, and 20 mL of HCl (1 M) was added. The precipitate obtained was filtered, washed with water, and dried to result in the corresponding *O*-alkyl phenolic acid derivatives. Structure confirmation was achieved based on spectroscopic analyses (<sup>1</sup>H-NMR and <sup>13</sup>C-NMR) (see ESI: Fig. S1–S18<sup>†</sup>). The synthetic procedure for obtaining the *O*-alkyl phenolic derivative acids **3a–f** and **5a–f** is outlined in Scheme 1.

**2.3.1. Ferulic acid 4-ethyl ether.** (*E*)-3-(4-Ethoxy-3-methoxyphenyl)acrylic acid (**3a**) 59% yield. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ (ppm): 7.48 (d, *J* = 17.3 Hz, 1H), 6.95 (d, *J* = 10.6 Hz, 2H), 6.82–6.62 (m, 1H), 6.14 (dd, *J* = 15.8, 2.9 Hz, 1H), 4.12–3.87 (m, 2H), 3.76 (s, 3H), 1.32 (dt, *J* = 6.9, 3.5 Hz, 2H), 1.11 (d, *J* = 2.7 Hz, 3H) according to the literature data.<sup>30</sup>

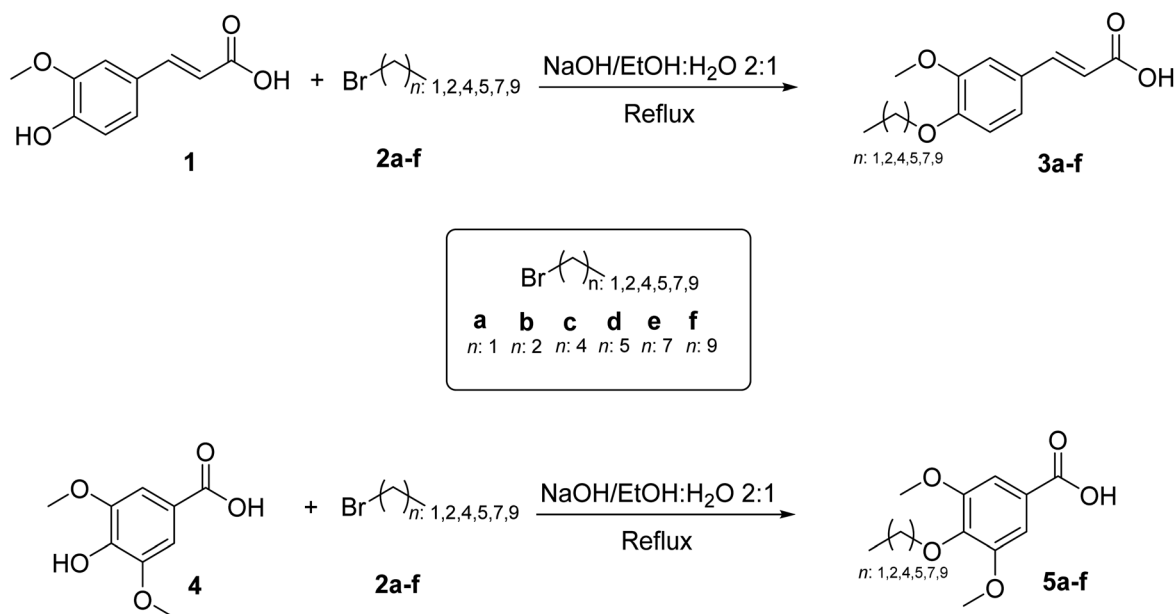
**2.3.2. Ferulic acid 4-propyl ether.** (*E*)-3-(3-Methoxy-4-propoxyphenyl)acrylic acid (**3b**) 47% yield. <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) δ (ppm): 7.49 (d, *J* = 15.9 Hz, 1H), 6.96 (d, *J* = 9.8 Hz, 2H), 6.75 (d, *J* = 8.1 Hz, 1H), 6.16 (d, *J* = 15.9 Hz, 1H), 3.77 (s, 3H), 1.74 (h, *J* = 7.2 Hz, 2H), 0.91 (t, *J* = 7.4 Hz, 3H) according to the literature data.<sup>29,31</sup>

**2.3.3. Ferulic acid 4-pentyl ether.** (*E*)-3-(3-Methoxy-4-(pentyloxy)phenyl)acrylic acid (**3c**) 43% yield. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ (ppm): 7.73 (d, *J* = 15.9 Hz, 1H), 7.11 (d, *J* = 8.3 Hz, 1H), 7.08 (s, 1H), 6.87 (d, *J* = 8.3 Hz, 1H), 6.31 (d, *J* = 15.8 Hz, 1H), 4.05 (t, *J* = 6.9 Hz, 2H), 3.90 (s, 3H), 1.86 (p, *J* = 6.9 Hz, 2H), 1.42 (dtq, *J* = 21.2, 14.1, 7.0 Hz, 4H), 0.93 (t, *J* = 7.0 Hz, 3H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ (ppm): 172.82, 151.38, 149.70, 147.22, 126.96, 123.26, 114.85, 112.44, 110.44, 69.16, 56.15, 28.86, 28.18, 22.57, 14.11.

**2.3.4. Ferulic acid 4-hexyl ether.** (*E*)-3-(4-(Hexyloxy)-3-methoxyphenyl)acrylic acid (**3d**) 41% yield. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ (ppm): 7.73 (d, *J* = 15.8 Hz, 1H), 7.11 (d, *J* = 8.3 Hz, 1H), 7.07 (s, 1H), 6.87 (d, *J* = 8.3 Hz, 1H), 6.31 (d, *J* = 15.8 Hz, 1H), 4.05 (t, *J* = 6.8 Hz, 2H), 3.90 (s, 3H), 1.85 (p, *J* = 7.0 Hz, 2H), 1.45 (q, *J* = 6.9 Hz, 2H), 1.40–1.25 (m, 4H), 0.90 (t, *J* = 6.8 Hz, 3H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ (ppm): 172.68, 151.26, 149.59, 147.10, 126.85, 123.14, 114.74, 112.34, 110.33, 69.07, 56.03, 31.57, 29.00, 25.61, 22.58, 14.02.

**2.3.5. Ferulic acid 4-octyl ether.** (*E*)-3-(3-Methoxy-4-(octyloxy)phenyl)acrylic acid (**3e**) 40% yield. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ (ppm): 7.73 (d, *J* = 15.8 Hz, 1H), 7.11 (d, *J* = 8.3 Hz, 1H), 7.08 (s, 1H), 6.87 (d, *J* = 8.3 Hz, 1H), 6.31 (d, *J* = 15.9 Hz, 1H), 4.05 (t, *J* = 6.9 Hz, 2H), 3.90 (s, 3H), 1.86 (p, *J* = 7.0 Hz, 2H), 1.54–1.40 (m, 2H), 1.39–1.19 (m, 8H), 0.88 (t, *J* = 6.7 Hz, 3H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ (ppm): 172.25, 151.07, 149.40, 146.89, 126.65, 122.94, 114.50, 112.14, 110.12, 68.89, 55.84, 31.61, 29.15, 29.02, 28.83, 25.74, 22.46, 13.90.

**2.3.6. Ferulic acid 4-decyl ether.** (*E*)-3-(4-(Decyloxy)-3-methoxyphenyl)acrylic acid (**3f**) 20% yield. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ (ppm): 7.73 (d, *J* = 15.9 Hz, 1H), 7.11 (d, *J* = 8.3 Hz, 1H), 7.07 (s, 1H), 6.87 (d, *J* = 8.3 Hz, 1H), 6.31 (d, *J* = 15.9 Hz, 1H), 4.05 (t, *J* = 6.9 Hz, 2H), 3.90 (s, 3H), 1.86 (p, *J* = 6.9 Hz, 2H), 1.38–1.16 (m, 14H), 0.88 (t, *J* = 6.7 Hz, 3H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ (ppm): 172.18, 151.39, 149.73, 147.21, 131.06, 128.99,



Scheme 1 Synthesis of *O*-alkyl ferulic (**3a–f**) and syringic (**5a–f**) acid derivatives.

126.97, 123.25, 114.72, 112.46, 110.44, 69.21, 65.72, 56.17, 32.03, 29.68, 29.45, 29.16, 26.06, 22.82, 19.33, 14.25.

**2.3.7. Syringic acid 4-ethyl ether.** 4-Ethoxy-3,5-dimethoxybenzoic acid (**5a**) 30% yield.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 7.37 (s, 2H), 4.15 (q,  $J = 7.0$  Hz, 2H), 3.91 (s, 6H), 1.38 (t,  $J = 7.0$  Hz, 3H) according to the literature data.<sup>32</sup>

**2.3.8. Syringic acid 4-propyl ether.** 3,5-Dimethoxy-4-propoxybenzoic acid (**5b**) 38% yield.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 7.37 (s, 2H), 4.03 (t,  $J = 6.8$  Hz, 2H), 3.91 (s, 6H), 1.79 (h,  $J = 7.2$  Hz, 2H), 1.02 (t,  $J = 7.4$  Hz, 3H) according to the literature data.<sup>32</sup>

**2.3.9. Syringic acid 4-pentyl ether.** 3,5-Dimethoxy-4-(pentyloxy)benzoic acid (**5c**) 42% yield.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 7.37 (s, 2H), 4.06 (t,  $J = 6.8$  Hz, 2H), 3.90 (s, 6H), 1.77 (p,  $J = 6.9$  Hz, 2H), 1.41 (ddq,  $J = 27.6, 14.6, 7.3, 6.8$  Hz, 4H), 0.92 (t,  $J = 7.1$  Hz, 3H) according to the literature data.<sup>32</sup>

**2.3.10. Syringic acid 4-hexyl ether.** 4-(Hexyloxy)-3,5-dimethoxybenzoic acid (**5d**) 38% yield.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 7.37 (s, 2H), 4.06 (t,  $J = 6.8$  Hz, 2H), 3.90 (s, 6H), 1.76 (p,  $J = 6.9$  Hz, 2H), 1.46 (p,  $J = 7.6, 6.8$  Hz, 2H), 1.38–1.21 (m, 4H), 0.90 (t,  $J = 6.6$  Hz, 3H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 171.97, 153.40, 142.63, 124.00, 107.65, 73.83, 56.37, 31.74, 30.21, 25.59, 22.77, 14.18.

**2.3.11. Syringic acid 4-octyl ether.** 3,5-Dimethoxy-4-(octyloxy)benzoic acid (**5e**) 36% yield.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 7.37 (s, 2H), 3.90 (s, 6H), 1.76 (p,  $J = 6.9$  Hz, 2H), 1.45 (p,  $J = 8.1, 6.7$  Hz, 2H), 1.37–1.17 (m, 8H), 0.88 (t,  $J = 5.7$  Hz, 3H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 171.80, 153.41, 142.63, 124.00, 107.66, 73.83, 56.38, 31.98, 30.25, 29.49, 29.42, 25.93, 22.80, 14.23.

**2.3.12. Syringic acid 4-decyl ether.** 4-(Decyloxy)-3,5-dimethoxybenzoic acid (**5f**) 18% yield.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 7.37 (s, 1H), 4.06 (t,  $J = 6.8$  Hz, 1H), 3.90 (s, 3H), 1.76 (s, 1H), 1.45 (p,  $J = 6.9$  Hz, 2H), 1.29 (d,  $J = 12.5$  Hz, 10H), 0.88 (t,  $J = 6.5$  Hz, 2H) according to the literature data.<sup>32</sup>

## 2.4. Thermal analysis

The study aimed to assess the thermal stability of the treated oils by subjecting them to thermal analysis using a TGA-Q500 thermogravimetric analyzer (TAI Instruments, USA) at a constant heating rate of  $5\text{ }^\circ\text{C min}^{-1}$ . The analysis involved heating the oils in air, as the reactive gas, from ambient temperature to  $700\text{ }^\circ\text{C}$ . A mass flow of  $60\text{ mL min}^{-1}$  was maintained for the reactive gas, while  $40\text{ mL}$  per minute of  $\text{N}_2$  was utilized as a protective gas in the electronic balance. Each analysis involved placing approximately  $10\text{ mg}$  of the oil into a Pt crucible. After the analysis of TG/DTG curves, specific temperatures were chosen to monitor the oil degradation process. These temperatures included room temperature ( $25\text{ }^\circ\text{C}$ ), an intermediate temperature between room temperature and the initial degradation point ( $100\text{ }^\circ\text{C}$ ), the frying temperature ( $180\text{ }^\circ\text{C}$ ), and the onset of degradation temperature ( $220\text{ }^\circ\text{C}$ ).

## 2.5. Heating of oils

A  $1\text{ mL}$  sample of sunflower oil (SO) and olive oil (OO) was introduced into a  $5\text{ mL}$  beaker; then, this beaker was

subsequently positioned on a hot plate equipped with a thermometer for temperature regulation. The samples were exposed to various heating temperatures ( $25, 100, 180$ , and  $220\text{ }^\circ\text{C}$ ) for 30 minutes. The determinations of extinction coefficients ( $K_{232}$  and  $K_{270}$ ) and lipoperoxidation assay (TBARS assay) were performed in triplicate for each selected temperature.

## 2.6. Determination of antioxidant capacity

**2.6.1. DPPH $^\bullet$  scavenging activity.** To evaluate the scavenging activity of *O*-alkyl benzoic and cinnamic acid derivatives, we employed the DPPH $^\bullet$  free radical as a model, following a method adapted from Brand-Williams *et al.*<sup>33</sup> and Molyneux.<sup>34</sup> In a nutshell,  $75\text{ }\mu\text{L}$  of different compounds and a control solution (80% methanol) were mixed with  $150\text{ }\mu\text{L}$  of DPPH. The resulting mixture was vigorously shaken, allowed to stand at room temperature for 30 minutes, and protected from light. Subsequently, the mixture was measured spectrophotometrically at  $515\text{ nm}$ . The free radical scavenging activity was determined as the percentage of DPPH decolorization using the following formula: % scavenging DPPH free radical =  $100 \times (1 - \text{AE}/\text{AD})$ , where AE represents the absorbance of the solution after the addition of the analyzed compound and AD is the absorbance of the DPPH solution (blank). Ferulic and syringic acids were used as reference compounds. Each measurement was performed in triplicate.

**2.6.2. Determination of ferric antioxidant power (FRAP).** The determinations were carried out following Benzie and Strain's method<sup>35</sup> with some modifications.<sup>36</sup> The FRAP reagent was prepared by mixing  $300\text{ mM}$  acetate buffer (pH 3.6),  $10\text{ mM}$  2,4,6-tripyridyl-*s*-triazine solution, and  $20\text{ mM}$   $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  in a  $10:1:1$  (v/v/v) ratio and then heated to  $37\text{ }^\circ\text{C}$  in a water bath. Subsequently,  $1.5\text{ mL}$  of the FRAP reagent was added to a test tube, and an initial reading was taken at  $593\text{ nm}$ . To this mixture,  $50\text{ }\mu\text{L}$  of additional antioxidant compounds and  $150\text{ }\mu\text{L}$  of distilled water were introduced into the cuvette. After adding the sample to the FRAP reagent, a second reading was taken at  $593\text{ nm}$  following a 90 minute incubation at  $37\text{ }^\circ\text{C}$ . Changes in absorbance 90 minutes after the initial blank reading correlated with a standard curve. Known standards of  $\text{Fe}^{2+}$  concentrations were analyzed, encompassing concentrations ranging from  $0.1$  to  $1\text{ mM}$ , and a standard curve was generated by plotting the FRAP values of each standard against its concentrations. The outcome was expressed as the concentration of antioxidants with ferric reducing capacity in  $1\text{ gram}$  of the sample ( $\mu\text{mol Fe}^{2+}\text{ g}^{-1}$ ). Ferulic and syringic acids were employed as reference compounds, and each measurement was conducted in triplicate.

## 2.7. Determination of oxidation parameters

**2.7.1. Lipoperoxidation assay.** To determine lipoperoxidation, we employed a thiobarbituric acid reactive substance (TBARS) assay. In this assay,  $0.5\text{ mL}$  of the different oils were mixed with  $2\text{ mL}$  of a solution containing thiobarbituric acid, trichloroacetic acid, and hydrochloric acid (TBA/TCA/HCl). The mixture was immediately vortexed upon combination. Subsequently, the samples were centrifuged at





3500 rpm for 5 minutes, and 2 mL of the supernatant was then boiled at 90 °C for 15 minutes. The absorbance at 535 nm was measured using a spectrophotometer and compared to a concentration curve of malondialdehyde (MDA) for quantification. The results were expressed as nmol of MDA per mL of oil, following the method by Maqsood and Benjakul (2010).<sup>37</sup> Each measurement was conducted in triplicate.

**2.7.2.  $K_{232}$  and  $K_{270}$  extinction coefficients.** The extinction coefficients ( $K_{232}$  and  $K_{270}$ ) for the oil samples were determined by analyzing the absorption values at 232 and 270 nm, respectively. The UV analysis of the oil was conducted using a scanning spectrophotometer (ATI UNICAM Helios, Alpha, UK) with 1 cm pathlength quartz cuvettes and hexane as the reference. The calculations followed the following formula:  $K\lambda = D\lambda/C$ , where  $K\lambda$  represents the specific extinction coefficient for each wavelength,  $D\lambda$  is the absorption, and  $C$  is the oil concentration in g per 100 mL. Each measurement was replicated three times.

**2.7.3. Determination of oxidative stability.** The aging process of SO and OO supplemented with the additives with the best antioxidant performance was studied using the AOCS oven storage test for the accelerated aging of oils, Cg5-97, also known as the Schaal Oven Test.<sup>38</sup> This stability assay is employed to simulate the real-time aging of oils, assuming that one day at 60 °C is equivalent to a month at 20 °C. In this method, the oil is stored at temperatures of  $60 \pm 5$  °C; the samples are collected periodically, and the oxidation parameters are measured.<sup>39</sup> In this context, the Schaal Oven Test was carried out for 12 days to simulate one year of storage of these supplemented oils. The samples were taken every 3 days, and the  $K_{232}$  extinction coefficient and TBARS values were measured.

## 2.8. Cytotoxicity

**2.8.1. Cell cultures.** Vero and Caco-2 cells (American Type Culture Collection, ATCC-CCL-81 and ATCC-HTB-37) were grown in monolayer culture in Dulbecco's modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS) (Gibco, NY, USA) and antibiotic-antimycotic (Gibco, NY, USA) at 37 °C in a humidified 5% CO<sub>2</sub> incubator. HUVEC cells (ATCC-CRL-1730) were grown in monolayer culture in DMEM-F12 medium with 10% fetal bovine serum (FBS) (Gibco, NY, USA) and antibiotic-antimycotic (Gibco, NY, USA) at 37 °C in a humidified 5% CO<sub>2</sub> incubator.

**2.8.2. Cell viability assays.** For the cell viability assays, each cell type was seeded in 96-well plates at a density of 5.000 cells per well 24 hours before the start of the experiment and before adding the stimulus compounds. Dimethyl sulfoxide (DMSO) at 10% was used as a positive control for cell death, and cells incubated with only DMEM or DMEM-F12 1% in FBS served as a negative control. The dilutions of the stimulus compounds were prepared in DMEM with 1% FBS for Caco-2 and Vero cells, and in DMEM-F12 with 1% FBS for HUVEC cells, from a stock solution of 100 mM in DMSO (Merck, Germany) in six concentrations (1, 10, 30, 50, 100, and 150  $\mu$ M). After 24, 48, and 72 hours, the effect on cell viability from the treatments was quantified using the resazurin fluorometric assay. Resazurin salt, the blue-colored N-oxide of the fluorescent dye resorufin,

can detect reductive activity in cells and is suitable for measuring proliferation and mitochondrial metabolic activity. 20  $\mu$ L of a 0.5 mg mL<sup>-1</sup> resazurin solution in phosphate-buffered saline (PBS) was added per well and incubated for 4 hours under standard conditions. Then, the plates were read for fluorescence at an excitation wavelength of 560 nm and emission of 590 nm using a 96-well microplate reader TECAN, Infinite 200Pro (Tecan, Männedorf, Switzerland). The IC<sub>50</sub> values were determined using the statistical software GraphPad Prism version 10 (GraphPad Software Inc., California, USA).<sup>40</sup>

## 2.9. Prediction of physicochemical properties and pharmacokinetic properties

The physical and chemical characteristics, along with the pharmacokinetic parameters, such as absorption, distribution, metabolism, excretion, and toxicity (ADMET), for all the compounds were forecasted using the ADMETLab 2.0 platform.<sup>41</sup>

## 3. Statistical analysis

The data are presented as the mean  $\pm$  standard deviation (SD). Statistical analysis ANOVA was carried out using the software Statistical Product and Service Solutions (SPSS) version 15.0 (SPSS Inc., Chicago, IL, USA). The significance level for statistical tests was defined as  $p < 0.05$ .

## 4. Results and discussion

The *O*-alkyl ferulic (**3a-f**) and syringic (**5a-f**) derivatives were obtained with overall yields of 20–59% and 18–38%, respectively. Some of the synthesized compounds have been reported in previous works.<sup>29,42–45</sup> The <sup>1</sup>H- and <sup>13</sup>C-NMR spectra and HPLC analysis are displayed in ESI: Fig. S1–S18.†

To evaluate the *in vitro* antioxidant effects of *O*-alkyl phenolic derivatives **3a-f** and **5a-f**, DPPH and FRAP assays were applied. The values for the DPPH and FRAP assays of the *O*-alkyl phenolic derivative **3a-f**–**5a-f** are shown in Table 1 and Fig. 2, respectively. The antioxidant capacities for both methods were evaluated in a concentration range of 10–100  $\mu$ g mL<sup>-1</sup>, and the IC<sub>50</sub> range of values for DPPH scavenging activity was  $4.53 \pm 0.19$ – $90.43 \pm 1.63$   $\mu$ g mL<sup>-1</sup>.

These results attest that the radical scavenging activities of the *O*-alkyl ferulic acid **3a-b** (IC<sub>50</sub> =  $56.50 \pm 2.38$   $\mu$ g mL<sup>-1</sup> (**3a**);

Table 1 Antioxidant capacity (DPPH) of the different compounds is expressed as the average inhibitory concentration (IC<sub>50</sub>)

| Compound  | IC <sub>50</sub> ( $\mu$ g mL <sup>-1</sup> ) | Compound  | IC <sub>50</sub> ( $\mu$ g mL <sup>-1</sup> ) |
|-----------|---|-----------|---|
| <b>1</b>  | $15.61 \pm 1.82$                              | <b>4</b>  | $4.53 \pm 0.19$                               |
| <b>3a</b> | $56.50 \pm 2.38$                              | <b>5a</b> | $9.20 \pm 0.91$                               |
| <b>3b</b> | $90.43 \pm 1.63$                              | <b>5b</b> | $11.47 \pm 0.58$                              |
| <b>3c</b> | >100  | <b>5c</b> | $34.98 \pm 2.23$                              |
| <b>3d</b> | >100  | <b>5d</b> | >100  |
| <b>3e</b> | >100  | <b>5e</b> | >100  |
| <b>3f</b> | >100  | <b>5f</b> | >100  |

$90.43 \pm 1.63 \mu\text{g mL}^{-1}$  (**3b**)) and *O*-alkyl syringic acid **5a–c** ( $\text{IC}_{50} = 9.20 \pm 0.91 \mu\text{g mL}^{-1}$  (**5a**);  $11.47 \pm 0.58 \mu\text{g mL}^{-1}$  (**5b**);  $34.98 \pm 2.23 \mu\text{g mL}^{-1}$  (**5c**)) were higher in comparison with *O*-alkyl ferulic (**3c–f**) ( $\text{IC}_{50} > 100 \mu\text{g mL}^{-1}$ ) and *O*-alkyl syringic (**5d–f**) ( $\text{IC}_{50} > 100 \mu\text{g mL}^{-1}$ ) acids, respectively.

It is well-accepted that phenolic compounds scavenge radicals by proton donation.<sup>21</sup> Furthermore, it is a well-established fact that the number and position of the hydroxyl group on the ring in the *ortho*-position increases the radical scavenging activity.<sup>46</sup>

In the *O*-alkyl phenolic compounds reported in the present study, the hydroxyl group was blocked, highlighting that the antioxidant capacity depends on the number of *O*-alkyl groups present in the molecule, *e.g.* methoxy, which leads to an increase in the radical scavenging activity of the antioxidant.<sup>47</sup> As an example, the *O*-alkyl syringic acid **5a** ( $\text{IC}_{50} = 9.20 \pm 0.91 \mu\text{g mL}^{-1}$ ) is almost six times more powerful as an antioxidant than the *O*-alkyl ferulic acid **3a** ( $\text{IC}_{50} = 56.50 \pm 2.38 \mu\text{g mL}^{-1}$ ). In this same line of comparison, **5b** is approximately eight times more powerful than **3b**. Moreover, it is evident that the antioxidant capacity decreases as the *O*-alkyl phenolic derivative length increases. Thus, the antioxidant capacity in decreasing order was **3a** > **3b** > **3c** and **5a** > **5b** > **5c** for ferulic and syringic *O*-alkyl phenolic acid derivatives, respectively. Furthermore, it was observed that the influence of  $-\text{CO}_2\text{H}$  and  $-\text{CH}=\text{CH}-\text{CO}_2\text{H}$  groups was not explicit.

The different radical-scavenging effects observed can be attributed to the different abilities of the individual phenolic acids to react with DPPH $\cdot$ , giving a stable non-radical product. A proposed chemical mechanism for the reaction between DPPH $\cdot$  and rosmarinic acid was presented by Brand-Williams *et al.*, 1995.<sup>33</sup>

Additionally, Karamac *et al.*<sup>48</sup> reported that the hydroxybenzoic acid, syringic acid (**4**), was more active than its hydroxycinnamic acid counterpart, sinapic acid. This trend of higher antioxidant capacity of hydroxybenzoic acids over hydroxycinnamic acids was also observed in the present study when the antioxidant capacity of syringic acid **4** ( $\text{IC}_{50} = 4.53 \pm 0.19 \mu\text{g mL}^{-1}$ ) was compared to that of ferulic acid **1** ( $\text{IC}_{50} = 15.61 \pm 1.82 \mu\text{g mL}^{-1}$ ). This tendency was maintained

throughout the *O*-alkyl series of syringic acid (**5a–c**) over the *O*-alkyl series of ferulic acid (**3a–c**).

However, the *O*-alkyl ferulic (**3a–f**) and syringic (**5a–f**) acid series were selected for the FRAP scavenging activity study (Fig. 1). The FRAP values in increasing order for the *O*-alkyl ferulic and syringic acid series were **3a** > **3b** > **3c** and **5a** > **5b** > **5c**, respectively. The decrease in ferric reducing/antioxidant power when the *O*-alkyl length increases is in agreement with the values of DPPH scavenging activity. The comparison of antioxidant capacity values between **3a–b** and **3c–f** showed a significant difference ( $p < 0.001$ ). In the same way, the comparison between **5a–c** and **5d–f** showed a significant difference ( $p < 0.001$ ) in the FRAP values.

To evaluate and verify the influence of the structure and composition on the thermal degradation of OO and SO oils, thermal analysis of the samples was undertaken in a temperature range of 25–700 °C. The mass loss (TG) and derivative (DTG) curves are presented in Fig. 2 and 3, respectively. A plateau was observed in both oils, indicating thermal stability of the materials of up to 200 °C. The thermal oxidative

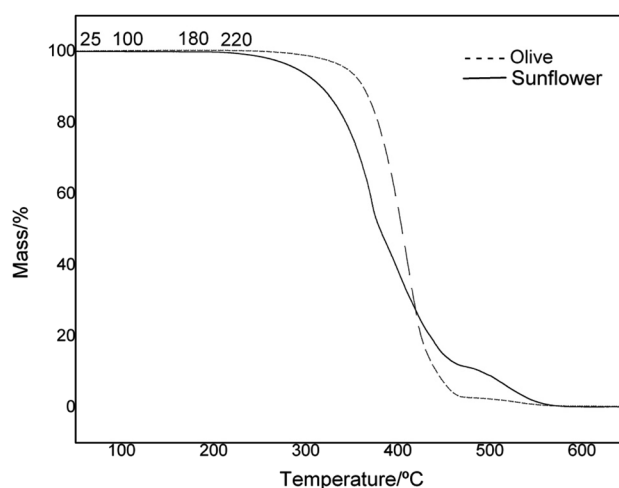


Fig. 2 Comparison of TG curves for sunflower and olive oils at the selected temperatures.

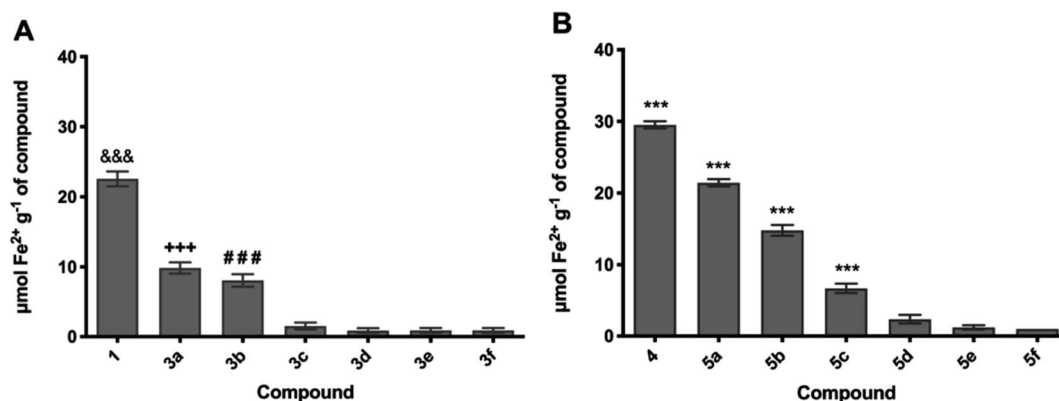


Fig. 1 Ferric reducing/antioxidant power (FRAP) of the evaluated compounds. (A) Ferulic acid and *O*-alkyl derivatives (**3a–f**), and (B) syringic acid and *O*-alkyl derivatives (**5a–f**). &&&  $p < 0.001$  against (**3a–f**), \*\*\*  $p < 0.001$  against all compounds.



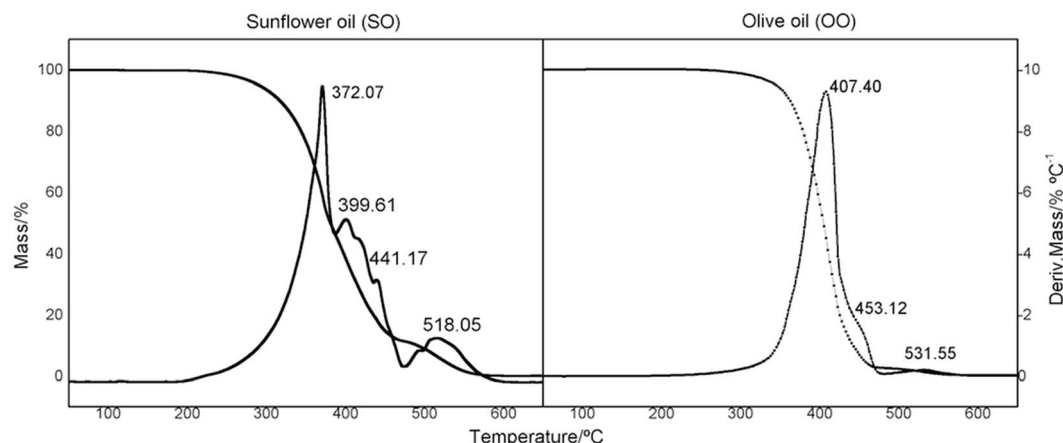


Fig. 3 Comparison of TG/DTG curves for sunflower and olive oils.

decomposition processes occurred in the temperature range of 200–600 °C for OO and SO. OO decomposed in three steps, with a total mass loss of 95%, while SO decomposed in four steps, with a total mass loss of 98%. The maximum degradation temperature for OO was 407 °C, while that for SO was 327 °C. This high thermal stability of OO can be largely attributed to the endogenous presence of polyphenolic compounds, such as hydroxytyrosol and oleuropein.<sup>49,50</sup>

As the thermal behaviour was similar in both oils (OO and SO), four temperatures (25, 100, 180, and 220 °C) were chosen from the TG curve for the study of improvement of the thermal stability of SO with additives **3b**, **3c**, **5b**, and **5c**.

A concentration of 200 ppm of additives **3b**, **3c**, **5b**, and **5c** was added to the samples of SO based on the existing food additive regulation of the U.S. Food and Drug Administration (FDA). This regulation allows the use of BHA and BHT individually or in combination at a maximum level of 0.02% or 200 ppm, including their use in edible animal fats and vegetable oils, based on the lipid content of food products, as specified by the Code of Federal Regulations (CFR).<sup>51,52</sup>

To analyze the thermal stability of SO supplemented with **3b**, **3c**, **5b**, and **5c** at 200 ppm concentration, thermal analyses were

performed in an interval of 20–700 °C. The TG curves are shown in Fig. 4 (from 20 to 600 °C).

The TG plateaus for SO + **3b**, **3c**, **5b**, and **5c** showed differences in the onset temperature of mass loss ( $T_{\text{onset}}/^{\circ}\text{C}$ ) (Table 2) in comparison with SO. The additive **3b–c** had an increase of approx. 63 °C, while the additive **5b** and **5c** increased by 24 and 37 °C, respectively.

The SO degradation temperatures associated with the loss of mass of 5, 10, and 50% obtained were 341, 365, and 419 °C, respectively. Additionally, the treatments with additives **3b–c** and **5b–c** showed an increase in these values. In this regard, additive **3b** showed the highest values with differences of 35, 25, and 4 °C in comparison to SO at 5, 10, and 50% mass loss, respectively. In addition, a small difference was observed between the same series of additives; for example, the  $T_{\text{onset}}/^{\circ}\text{C}$  of **3b** (C3) in comparison with **3c** (C5) was only 1 °C, with the trend being observed throughout 5, 10, and 50% of the mass loss. However, in the **5b–c** series, the difference in the  $T_{\text{onset}}/^{\circ}\text{C}$  of **5b** (C3) in comparison to **5c** (C5) was 13 °C, and the temperatures corresponding to the 5, 10, and 50% of the mass loss are similar with a variation of 4–6 °C.

The homogenized samples were heated to 25, 100, 180, and 220 °C and kept at these temperatures for 30 min with a heating

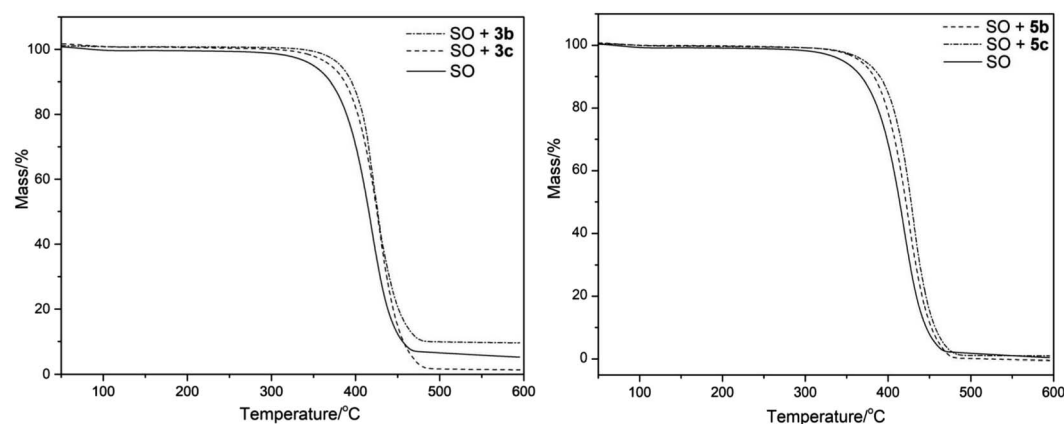


Fig. 4 TG curves of sunflower oil (SO) and SO supplemented with the *O*-alkyl derivatives **3b–c** and **5b–c**.

Table 2 Thermal decomposition temperatures of SO supplements with **3b**–**c** and **5b**–**c** additives at 200 ppm

| Treatment | Temperature (°C)         |             |                |                |
|-----------|--------------------------|-------------|----------------|----------------|
|           | $T_{\text{onset}}^a$ /°C | $T_5^b$ /°C | $T_{10}^c$ /°C | $T_{50}^d$ /°C |
| SO        | 281                      | 341         | 365            | 419            |
| <b>3b</b> | 344                      | 376         | 390            | 423            |
| <b>3c</b> | 343                      | 370         | 386            | 424            |
| <b>5b</b> | 305                      | 364         | 382            | 420            |
| <b>5c</b> | 318                      | 368         | 388            | 426            |

<sup>a</sup> Decomposition temperature (°C): onset temperature. <sup>b</sup> 5% mass loss.

<sup>c</sup> 10% mass loss. <sup>d</sup> 50% mass loss.

ramp of 2 °C min<sup>−1</sup>. Extinction coefficients  $K_{232}$  and  $K_{270}$  and TBARS values were measured through heating as SO oxidation parameters. In Fig. 5 and 6, the evolution in  $K_{232}$  and  $K_{270}$  values is presented, respectively. As expected, both  $K_{232}$  (primary oxidation) and  $K_{270}$  (secondary oxidation) indexes increased in a temperature-dependent manner, which indicated the formation of conjugated dienes and trienes over time.

The  $K_{232}$  (3.32) and  $K_{270}$  (1.68) values obtained at 25 °C for SO resemble those reported by Kalantzakis and coworkers.<sup>53</sup> The values of  $K_{232}$  and  $K_{270}$  showed a lower increase in the range of 25–100 °C, not observing differences in the treatments with compounds **3b**–**c** and **5b**–**c**. Simultaneously, at frying temperature (180 °C), it was observed that the slopes of the  $K_{232}$  and  $K_{270}$  curves were low compared with the corresponding slopes at 220 °C. Thus, in Fig. 5 and 6, these trends are observed at 220 °C, which are much more evident in the *O*-alkyl phenolic acid derivatives **3c** and **5c**.

The TBARS assay (Fig. 7 and 8) is the most widely used method for the measurement of secondary oxidation products. In all treatments with the compounds, a continuous and temperature-dependent increase in TBARS values was observed. The SO<sub>control</sub> exhibited an increase in the TBARS values from 4.30 to 13.80 nmol L<sup>−1</sup>, while OO<sub>control</sub> TBARS values declined

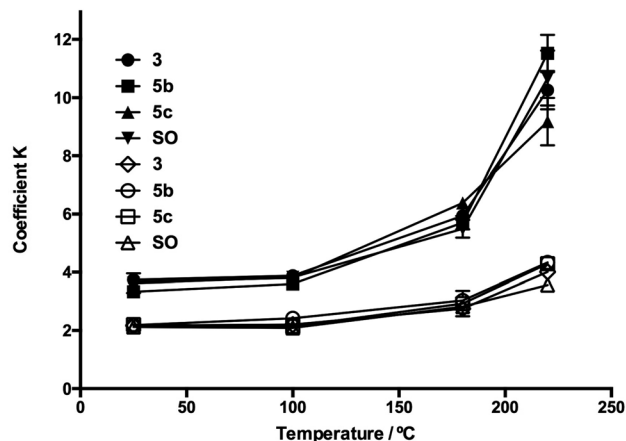


Fig. 6 Evolution of extinction coefficients  $K_{232}$  and  $K_{270}$  through the heating process for oil additives **5b** and **5c**. SO: sunflower oil without additive,  $K_{232}$ : symbol in black, and  $K_{270}$ : symbol in white.

through heating from 10.47 to 8.46 nmol L<sup>−1</sup> in the temperature range of 25–220 °C. The high polyunsaturated fatty acid (PUFA) content, such as linoleic acid content (61.37%), and the absence of endogenous antioxidants are the main factors responsible for the poor resistance of SO to thermoxidation. Although OO contains a low PUFA content (8.576%),<sup>54</sup> the presence of different endogenous antioxidants, such as oleuropein, pinorresinol, and tyrosol,<sup>55,56</sup> makes it more resistant to thermoxidation.

For the evaluation of the *O*-alkyl phenolic acid derivatives as oil additives, **3b**–**c** and **5b**–**c** were selected, and these compounds showed a slow rate of TBARS formation at 25–100 °C range. With the temperature increase (180 °C), TBARS levels were increased without a significant difference between SO<sub>control</sub> and the additives, while significant differences were observed when the temperature was increased to 220 °C. In this sense, the *O*-alkyl syringic acid additives **3b** and **3c** showed TBARS values of 8.88 and 7.68 nmol mL<sup>−1</sup> respectively. The reduction in secondary oxidation to 5 (**3b**) and 6 (**3c**) times in comparison with SO<sub>control</sub> (13.80 nmol L<sup>−1</sup>) was also observed, with a significant difference ( $p < 0.05$ ) of SO concerning **3c**. Meanwhile, the *O*-alkyl ferulic acids **5b** and **5c** reduce the TBARS levels 5 times more than those of SO<sub>control</sub>. There is no significant difference between **5b** and **5c**, but there is a considerable difference compared to the SO<sub>control</sub> ( $p < 0.05$ ).

In line with our findings, Zhang *et al.*<sup>57</sup> reported the use of carnosic acid (CA) (phenolic diterpene compound) found in the leaves of rosemary as an oil additive to improve the oxidative stability of SO during accelerated storage (60 °C). CA exhibited a stronger antioxidant capacity in SO than in BHT and BHA. However, vegetable extracts have been evaluated as edible oil additives. Recently, our research group investigated the antioxidant potential of maqui (*Aristotelia chilensis* (Mol.) Stuntz) leaf methanolic extract, which acted as an excellent additive in the thermoxidative process of avocado oil, reduced its oxidation parameters  $K_{232}$ ,  $K_{270}$ , and its content in polar compounds.<sup>58</sup> Additionally, an increase in the  $T_{\text{onset}}$ /°C temperature of mass

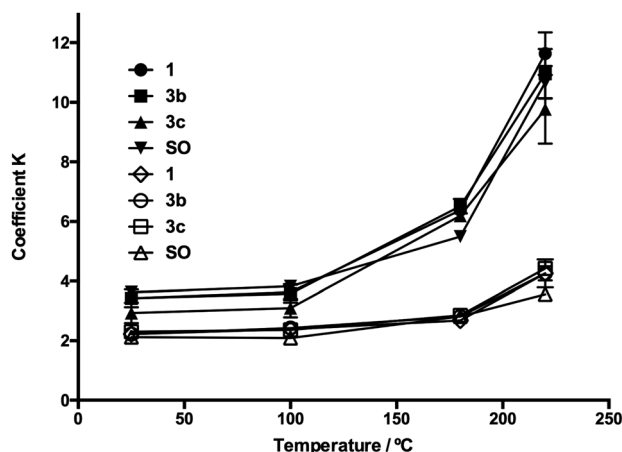


Fig. 5 Evolution of extinction coefficients  $K_{232}$  and  $K_{270}$  through the heating process for oil additives **3b** and **3c**. SO: sunflower oil without additive,  $K_{232}$ : symbol in black, and  $K_{270}$ : symbol in white.





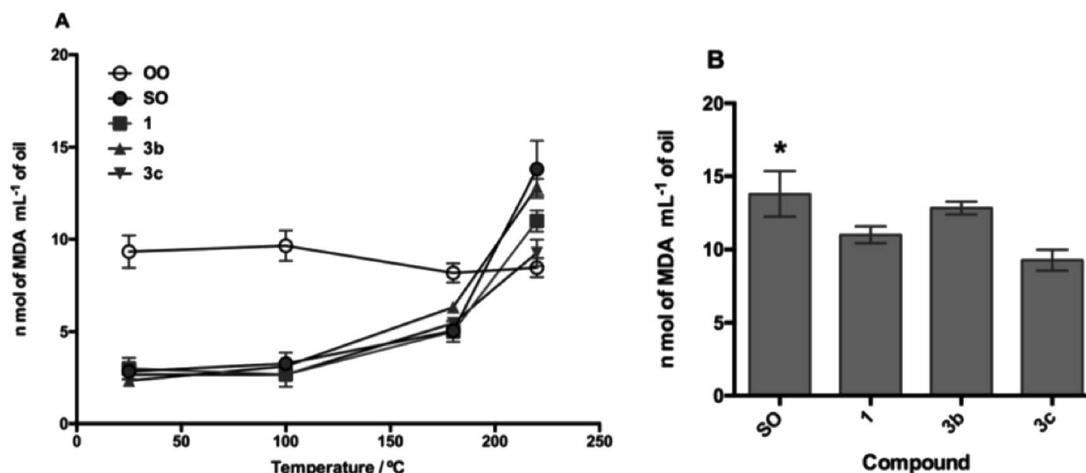


Fig. 7 Lipid peroxidation process in the presence of compound 1 and its derivatives 3b and 3c. (A) Lipid oxidation process as temperature increases and (B) statistical analysis of the oxidation process at 220 °C. SO: sunflower oil. \* $p < 0.05$  with respect to 3c.

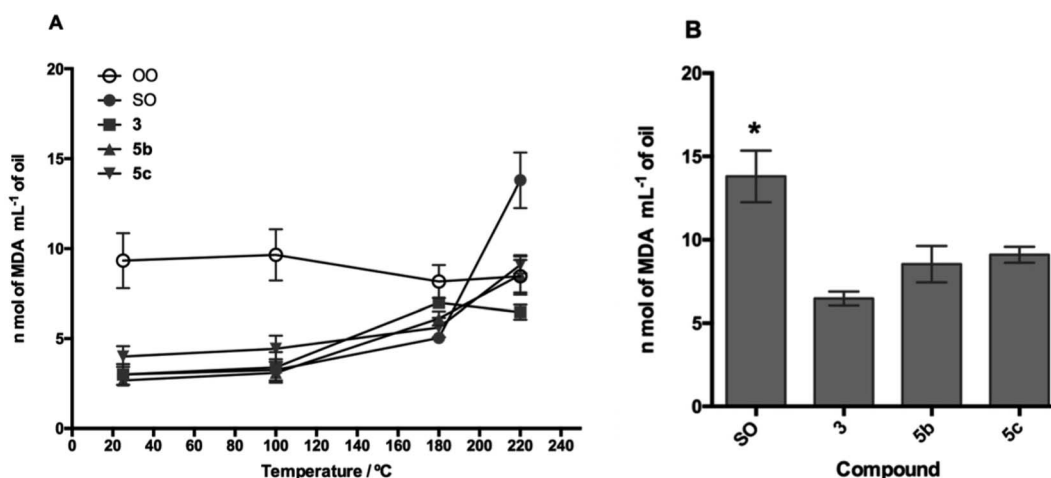


Fig. 8 Lipid peroxidation process in the presence of compound 3 and its derivatives 5b and 5c. (A) Lipid oxidation process as temperature increases and (B) statistical analysis of the oxidation process at 220 °C. SO: sunflower oil. \* $p < 0.05$  for compounds 3, 5b, and 5c.

loss was observed in the TG-DTG curves of avocado oil fortified with maqui leaf methanolic extract.

Moreover, Kamkar *et al.*<sup>59</sup> demonstrated that aqueous and methanolic extracts and essential oils from pennyroyal (*Mentha pulegium* L.) act as antioxidants and their use as oil additives showed content-dependent inhibitory effects on retarding lipid oxidation of edible oils under study storage time of 8 days, resulting in low levels of TBARS compared with BHT through the examined range of concentrations (200–1000 ppm).<sup>59</sup>

It should be noted that these oil additives reported in the present study managed to efficiently reduce the levels of SO oxidation (7.68 nmol L<sup>-1</sup>; 3c) even below the values for OO (8.46 nmol L<sup>-1</sup>) at 220 °C.

The aging process of SO and OO supplemented with the best performance additives (5c and 3c) was carried out for 12 days to simulate one year of storage. The values of the  $K_{232}$  extinction coefficient and TBARS are shown in Fig. 9 and 10, respectively.

The  $K_{232}$  extinction coefficient reveals the presence of conjugated dienes, which are formed from hydroperoxides derived from the unsaturated fatty acids present in the oils. Thus, as illustrated in Fig. 9, a sustained increase in  $K_{232}$  over time for OO and SO was observed. However, the oils supplemented with additives 5c and 3c showed different behaviors. In the case of OO, 5c showed lower values than the OO<sub>control</sub>, with  $\Delta K$  values of 0.04 for days 3 and 9. Moreover, 3c exhibited  $K_{232}$  values higher than the OO<sub>control</sub>. However, SO showed a behavior similar to that presented by OO. In the case of SO, both additives (5c and 3c) decreased the  $K_{232}$  values as a function of time, with additive 3c standing out, which presented  $\Delta K$  values of 0.04 and 0.09 for days 9 and 12, respectively.

All oil supplements with additives 5c and 3c showed a continuous increase in TBARS values (Fig. 10). The OO<sub>control</sub> increases the TBARS value from 6 to 15 nmol L<sup>-1</sup>, while the SO<sub>control</sub> increases the TBARS value from 20 to 120 nmol L<sup>-1</sup> in

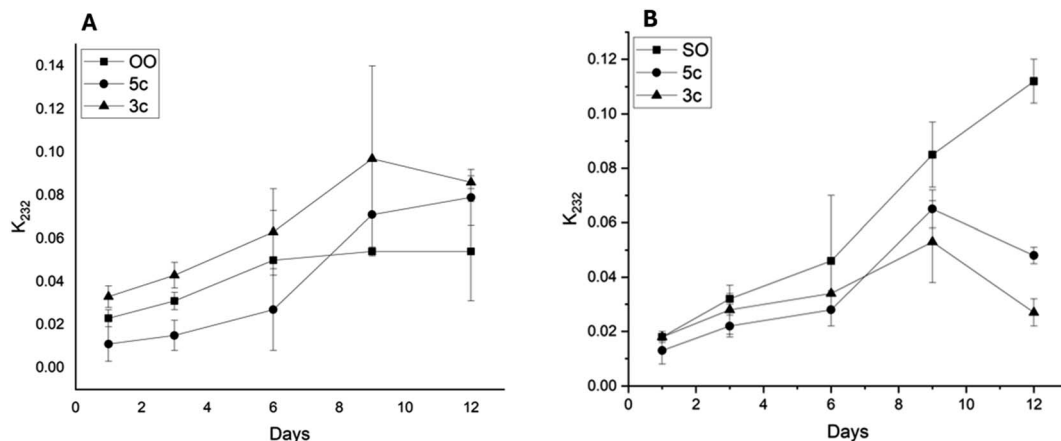


Fig. 9 Evolution of extinction coefficients  $K_{232}$  over time of olive oil (A) and sunflower oil (B) supplemented with additives 5c and 3c at 60 °C in the dark. Data points represent mean ( $n = 3$ )  $\pm$  standard error of the mean.

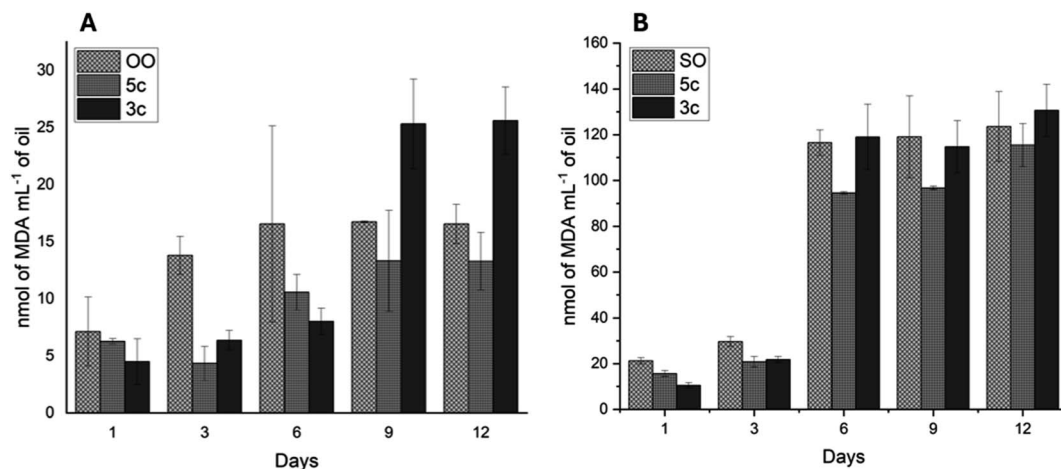


Fig. 10 Lipid peroxidation process was measured using the thiobarbituric acid reactive substance (TBARS) assay on olive oil (A) and sunflower oil (B) supplemented with additives 5c and 3c at 60 °C in the dark. Data points represent mean ( $n = 3$ )  $\pm$  standard error of the mean.

Table 3 *In vitro* cytotoxicity of compounds on Caco-2, HUVEC, and Vero cell lines

| Compound | $IC_{50} \pm SD$ ( $\mu M$ ) |                  |      |                  |                  |      |                  |                  |      |
|----------|------------------------------|------------------|------|------------------|------------------|------|------------------|------------------|------|
|          | 24 h                         |                  |      | 48 h             |                  |      | 72 h             |                  |      |
|          | Caco-2                       | HUVEC            | Vero | Caco-2           | HUVEC            | Vero | Caco-2           | HUVEC            | Vero |
| 3a       | >100                         | >100             | >100 | >100             | >100             | >100 | >100             | >100             | >100 |
| 3b       | >100                         | >100             | >100 | >100             | >100             | >100 | >100             | >100             | >100 |
| 3c       | >100                         | >100             | >100 | >100             | >100             | >100 | >100             | >100             | >100 |
| 3d       | >100                         | >100             | >100 | >100             | >100             | >100 | >100             | >100             | >100 |
| 3e       | >100                         | 90.02 $\pm$ 1.03 | >100 | >100             | 53.08 $\pm$ 1.02 | >100 | 96.19 $\pm$ 1.10 | 54.39 $\pm$ 1.05 | >100 |
| 3f       | >100                         | 55.61 $\pm$ 1.05 | >100 | 88.72 $\pm$ 1.08 | 26.44 $\pm$ 1.06 | >100 | 76.77 $\pm$ 1.08 | 25.37 $\pm$ 1.08 | >100 |
| 5a       | >100                         | >100             | >100 | >100             | >100             | >100 | >100             | >100             | >100 |
| 5b       | >100                         | >100             | >100 | >100             | >100             | >100 | >100             | >100             | >100 |
| 5c       | >100                         | >100             | >100 | >100             | >100             | >100 | >100             | >100             | >100 |
| 5d       | >100                         | >100             | >100 | >100             | >100             | >100 | >100             | >100             | >100 |
| 5e       | >100                         | >100             | >100 | >100             | 55.94 $\pm$ 1.06 | >100 | >100             | 53.34 $\pm$ 1.05 | >100 |
| 5f       | >100                         | 47.25 $\pm$ 1.04 | >100 | 69.96 $\pm$ 1.06 | 35.44 $\pm$ 1.04 | >100 | 62.79 $\pm$ 1.07 | 29.31 $\pm$ 1.09 | >100 |



the 12 days, demonstrating the great stability presented by OO without the presence of additives. For its part, additives **5c** and **3c** decrease TBARS values. The OO supplemented with **5c** and **3c** shows TBARS values below that of OO<sub>control</sub> from days 1 to 6. For 9 to 12 days, only **5c** presents values lower than those of the OO<sub>control</sub>. Regarding SO, additive **5c** decreased the TBARS values over 12 days although these values are far above those presented by OO (100 units above from days 6 to 12).

To assess the effect and potential health risk of the use of these lipophilic antioxidants as additives in edible oils, the cytotoxicity of these compounds was evaluated in three cell lines. The results of the cytotoxic effect on Caco-2, HUVEC, and Vero cell lines indicated that only compounds **3e**, **3f**, **5e** and **5f** showed an IC<sub>50</sub> value below 100 µM (Table 3). It is important to specify that the compounds were examined at quantities significantly higher than those used for additives, indicating their safety for use in edible oils.

In addition, the physicochemical and pharmacokinetic properties of the compounds were predicted. The results are displayed in the ESI (Tables S1 and S2†). The most active *O*-alkyl phenolic acid derivatives show very favorable physicochemical properties and no violations of Lipinski's rule. Additionally, lipophilicity (log *P*), topological polar surface area (TPSA), and water solubility (log *S*) parameters showed favorable values for adequate hydrophobic interaction between *O*-alkyl phenolic acid derivatives and triglycerides. Regarding the ADMET properties, the results show that these compounds have acceptable values of physicochemical properties, pharmacokinetic parameters, and low toxicity for use without risk in humans and animals.<sup>60</sup>

## 5. Conclusions

Despite the difficulty in stabilizing SO due to its high content of linoleic acid, the results of the present study indicated that the lipophilic antioxidants **3b–c** and **5b–c** exhibited satisfactory antioxidant capacities during the thermoxidation process of SO; this effect was greater when the physicochemical parameters, such as lipophilicity, melting, and boiling point, increased with *O*-alkyl chain-length. Thus, these parameters are 3.387, 155–157 °C, 358 ± 37 °C < 3.776, 128–129 °C, 385.8 ± 37 °C and 2.945, 123–125 °C, 390.1 ± 27.0 °C < 3.331, 105–106 °C, 414.2 ± 30 °C for **3b–c** and **5b–c**, respectively. Furthermore, additive **3c** presents an important antioxidant capacity compared to **3b** (*p* < 0.05) and **1**, and efficiently reduces SO lipoxidation compared to SO without additive, with the best TBARS values at 220 °C. The additive **5b–c** also exerted a protective effect on the thermoxidation of SO with a significant difference of *p* < 0.05, but unlike **3b–c**, there were no significant differences between **5b** and **5c**. The test for accelerated aging of oils of 12 days shows that additives **5c** and **3c** reduce TBARS values in olive oil (OO) and sunflower oil (SO). Additive **5c** is especially effective in OO<sub>control</sub> between days 9 and 12, keeping the TBARS value lower than that of OO<sub>control</sub>. This reduction tendency is also observed in the TBARS values in SO supplemented although it presents higher values than in OO supplemented.

This result demonstrated that increasing the lipophilic and thermal properties of natural antioxidants through *O*-alkyl phenolic acid derivatization consists of a promising and safe alternative approach in the search for lipophilic antioxidants with potential use as oil additives in food matrices.

## Data availability

The data supporting this article have been included as part of the ESI.†

## Conflicts of interest

There are no conflicts to declare.

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