Natural Phenolic Acids as Effective Bulk Oil Antioxidants: Oxidative Stability Modeling Using Olive Kernel Oil as a Case Study

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Abstract: Natural polyphenols may be very effective lipid antioxidants; however, the studies pertaining to specific commodities, such as refined olive kernel oil (OKO), are extremely limited, and thus there is a lack of relevant information. To provide coverage for this gap in the literature, this study was performed with the view of testing structurally related natural hydroxycinnamates, namely caffeic acid (CA), ferulic acid (FA), and p-coumaric acid (CouA), as effective OKO antioxidants, and establishing concentration–activity relationships. First, a ranking concerning the antioxidant potency of the three polyphenols was attempted using the radical probe DPPH, and then the compounds were assayed using OKO as the lipid substrate. Employing a concentration–activity model, the potency of the three polyphenols to stabilize OKO, based on Rancimat measurements, was CA > CouA ≈ FA. A further simulated long-term trial revealed that all three polyphenols may be equally effective in inhibiting peroxide onset over a period of 40 days, but the determination of thiobarbituric acid reactive substances (TBARS) formation evidenced that FA may be a more efficient antioxidant. The outcome was that all three polyphenols tested may behave as very effective agents against oxidative OKO rancidity, and the prospect of producing such compounds from agri-food wastes may provide unprecedented opportunities for replacing the controversial synthetic antioxidants.

Keywords: hydroxycinnamates; lipid antioxidants; oil oxidation; polyphenols

1. Introduction

Nowadays, contemporary dietary trends dictate the consumption of foods manufactured for providing health benefits beyond nutritional adequacy. Such consumer demands have been the driving force for the food industry to develop commodities with increased functionality, yet also devoid of additives which could, in the long term, have a questionable impact on the health status [1,2]. Antioxidants are some of the commonest food additives, destined to protect mainly lipid-containing foods from oxidative deterioration. This problem is of particular concern to fats and oils, where rancidity is manifested by both organoleptic degradation and loss of nutritional value [3].

Lipid oxidation can be brought about through various pathways, and it is a rapid process involving the generation of lipid radicals, which, via chain reactions, may cause severe effects on fatty acid structure and composition with detrimental consequences to their biological properties, but also the generation of toxic compounds [4]. At the same time, the decomposition of certain lipid oxidation products results in the formation of an assortment of volatile substances, which lend lipid-containing foods highly undesirable
sensory attributes. Therefore, battling oxidation onset and progression is of paramount significance for producing palatable and safe foods [5].

Antioxidants are regularly added to lipid foods to provide an effective shield against oxidation and rancidity development. A common practice in the food industry is the use of the so-called synthetic antioxidants, which are mainly represented, but not limited to, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tert-butyl hydroquinone (TBHQ). These additives are permitted in a spectrum of foods, such as vegetable oils, animal fats, cured meats, etc., and their levels usually vary from 25 to 200 mg kg\(^{-1}\) [6]. By virtue of the low price, high carry-over effect, exceptional efficacy, and ease of procurement, this family of compounds has been the tool of preference in the food industry for many years. However, a significant number of recent studies have raised serious concerns on the safety of these additives, concerning their involvement in adverse effects on human health and the environment [6–8]. On this ground, consumer pressure has shifted food industries to adopt alternative policies for manufacturing lipid-containing foods, by lowering or even eliminating the use of synthetic antioxidants.

In this line, the necessity of natural antioxidants equally efficient or even superior to synthetic ones is pressing, and there has been a significant number of studies reporting on the use of natural products as food antioxidants [9–11]. The most prominent class of compounds that could effectively replace synthetic antioxidants is polyphenols, a category which embraces a bewildering diversity of chemical structures. Polyphenols are notorious for expressing antioxidant activity, and owing to this property, thorough examinations have been carried out on several food matrices [12–14]. Nevertheless, most of these studies have dealt with plant extracts rather than pure compounds, and this is an important shortcoming in the relevant field. This is because the species and the relevant proportions of various polyphenolic substances in a plant extract may vary largely, and therefore the control and appropriate regulation of the antioxidant protection required might be particularly problematic. On the other hand, the isolation of pure compounds may be laborious, time-consuming, and of a high cost, thus hindering their wide applicability [15].

However, innovative technologies implemented on biomass valorization have paved the way to establishing sustainable and cost-effective routes for the recovery of antioxidant polyphenols from industrial side streams. This is the most promising strategy of producing high value-added food antioxidants, since their production would be based on abundant waste materials, and therefore the recovery and marketability would be economically viable [16,17]. Examples of materials that may be regarded in this context are wheat bran and corn stover, from which extracts with a well-defined composition and antioxidant activity could be produced. Recent works have illustrated that, upon implementation of appropriate treatments, the efficacious recovery of extracts enriched in ferulic acid and/or \(p\)-coumaric and ferulic acid may be feasible, making these materials an ideal candidate in this regard [18,19].

Based on this concept, this investigation had as its objective the study of natural, abundantly occurring hydroxycinnamates, including caffeic acid, ferulic acid, and \(p\)-coumaric acid, as antioxidants in edible oils (Figure 1). For this purpose, olive kernel oil, a refined oil free from synthetic antioxidants, was used as the lipid substrate, and a novel concentration-based model was used to assessing the efficiency of these compounds to act as stabilizers against oxidative rancidity. To the best of the authors’ information, this is the first study employing such an approach, and may be a valuable tool in appraising natural antioxidants as oil additives.
2. Materials and Methods

2.1. Chemicals

1,1,3,3-Tetraethoxypropane (TEP), triacetin, trans-caffeic acid (CA), trans-p-coumaric acid (CouA), trans-ferulic acid (FA), 2,2-diphenylpicrylhydrazyl (DPPH), thiobarbituric acid, ammonium thiocyanate, and iron chloride hexahydrate were obtained from Sigma-Aldrich (Steinheim, Germany). Butylated hydroxytoluene (BHT) and ammonium iron(II) sulfate hexahydrate were obtained from Thermo Scientific (Dreieich, Germany). Trichloroacetic acid (TCA) was obtained from Riedel-de Haen (Seelze, Germany).

2.2. Olive Kernel Oil (OKO)

The OKO (3 bottles of 1 L) was produced and packaged locally (Chania, Crete, Greece), and was purchased from a local grocery store. Upon receipt, it was stored at ambient temperature, in a dark and dry chamber, and it was used at the latest 3 days after opening. All experiments were conducted with OKO from the same batch, to avoid result variations attributed to variations in oil composition. The OKO examined in this study was also free from synthetic antioxidants.

2.3. OKO Enrichment with Antioxidants

The compounds examined were the natural hydroxycinnamates CA, CouA, and FA, and also the well-investigated BHT, which served as a positive control antioxidant. An exact amount of 200 mg of each compound to be tested was dissolved in 10 mL triacetin, to make up stock solutions. Each stock solution was diluted as appropriate with triacetin prior to enriching OKO. Then, 9.9 g of OKO was mixed with 0.1 g of triacetin solution to provide the desired antioxidant content (25, 50, 100, and 200 mg kg\(^{-1}\)). Thus, triacetin in the samples assayed never exceeded 1% (w/w). After adding the triacetin stock solution in OKO, the mixture was stirred for 48 h in the dark, to ascertain full homogenization. The enriched OKO samples were then tested for stability.
2.4. Rancimat Test

Thermal oil resilience against oxidative deterioration was assessed using a Rancimat 679 (Metrohm Ltd., Herisau, Switzerland) device. Duplicate samples were analyzed at a temperature of 110 °C and an air flow rate of 15 L h⁻¹. The induction period (IP, h) was determined from the inflection point of the conductivity curve.

2.5. Accelerated Oxidation Treatment

An amount of 20 g of oil enriched with each phenolic acid and BHT (200 mg kg⁻¹), and neat oil as a blank, were placed in 50 mL screwcap, glass vessels, and stored in a laboratory oven at 65 °C, in the dark, for 40 days. Sampling was accomplished every 3 days to monitor oxidation indices (peroxide value—PV and thiobarbituric acid reactive substances—TBARS).

2.6. Peroxide Value (PV) Determination

Peroxide value (PV) was determined using the official ferric thiocyanate method of the International Dairy Federation (IDF), with modifications [20,21]. A volume of 0.1 mL oil sample (0.02 g oil in 1 mL hexane) or 0.1 mL control (hexane) was mixed with 5 mL ethanol, 0.1 mL of a 30% ammonium thiocyanate solution, and 0.1 mL ammonium iron(II) sulfate hexahydrate reagent (100 mg in 10 mL of 10 M HCl). The mixture was vortexed, and the absorbance at 500 nm was obtained exactly 3 min after adding the iron(II) reagent, using ethanol as blank. A calibration curve was constructed using iron(III) chloride hexahydrate (1–20 µg Fe³⁺). The PV was computed as follows:

\[
PV \left( \text{mEq O}_2 \text{ kg}^{-1} \text{ oil} \right) = \frac{(A_{500} - A_{500}) \times L \times V}{55.845 \times S \times 0.1} \times \frac{1}{2} \quad (1)
\]

where \( A_{500} \) and \( A_{500} \) are the absorbencies of the sample and the control, respectively; \( L \) represents the slope of the calibration curve; \( V \) represents the volume hexane used for dissolving the oil (in mL); and \( S \) is the amount of oil (in g). The number 55.845 is the molecular weight of iron; 0.1 is the volume of the oil sample (dissolved in hexane) used in the assay; and 1/2 is a correction factor.

2.7. Thiobarbituric Acid Reactive Substances (TBARS) Determination

A well-established methodology was employed to determine the TBARS [22]. First, the thiobarbituric acid (TBA) reagent was prepared, by mixing 15 g trichloroacetic acid, 0.375 g TBA, and 1.76 mL concentrated HCl into a volumetric flask of 100 mL, made up to the volume with deionized water. Then, freshly prepared TBA reagent was combined with accurately weighted 0.1 g of oil in a 15 mL falcon tube, and incubated in a water bath, at 95 °C, for 20 min. Following this, the mixture was cooled down to ambient temperature with tap water, 0.2 mL of chloroform was added, and the mixture was vortexed and centrifuged at 5000 × g, for 5 min. Finally, the absorbance of the supernatant was recorded at 532 nm, using suitable blank. The results were expressed as 1,1,3,3-tetraethoxypropane (TEP) equivalents (TEPEs), using a TEP calibration curve, constructed by using standard TEP solutions with concentration varying from 30 to 300 µM.

2.8. Determination of the Antiradical Activity

The protocol used was based on previously published ones [23,24]. A volume of 0.025 mL of methanol was combined with 0.975 mL of DPPH solution (100 µM in methanol) and the absorbance at 515 nm (\( A_{515} \)) was measured immediately after mixing. This was defined as initial absorbance (\( A_i \)). Likewise, 0.025 mL of antioxidant solution (final concentration in the reaction mixture 1–64 µM) was mixed with 0.975 mL of DPPH solution and
the $A_{515}$ was measured after exactly 30 min. This was defined as the final absorbance ($A_f$). Then, the % decrease in $A_{515}$ was determined as follows:

$$\% \Delta A = \left( \frac{A_i - A_f}{A_i} \right) \times 100$$

(2)

2.9. Data Processing and Statistics

All treatments with oil samples were carried out at least in duplicate. Non-linear regressions were established using SigmaPlot 12.5 (Systat Software Inc., San Jose, CA, USA). All spectrophotometric determinations were carried out in triplicate. A Shapiro–Wilk test was applied to check the normality of the experimental data, which were normally distributed. Therefore, IBM SPSS Statistics™ 29 (SPSS Inc., Chicago, IL, USA) was used to examine statistically significant differences, based on the Kruskal–Wallis test.

3. Results and Discussion

3.1. Investigation Based on the Model DPPH• Radical

The reaction of antioxidants with the stable radical DPPH• is a non-competitive scheme to draw information regarding their radical scavenging potency [25]:

$$AH + n \text{ DPPH•} \rightarrow \text{products}$$

(3)

Such a scheme has been widely used to build up several stoichiometric and kinetic hypotheses and evaluate a high number of naturally occurring polyphenols as antioxidants [25]. The phenolic acids investigated herein were assayed not only for stoichiometry, but also efficiency, by determining the efficient concentration (IC$_{50}$), that is, the molar concentration of an antioxidant to provoke a 50% reduction in the initial molar DPPH• concentration.

To compute the total stoichiometry, the following equation was used [25–27]:

$$n_t = \frac{A_i - A_f}{\varepsilon C}$$

(4)

where $A_i$ and $A_f$ are the initial and final absorbance of the reaction mixture (DPPH• + antioxidant), at 517 nm, and $\varepsilon$ the molar DPPH• absorptivity in methanol (11,240 M$^{-1}$ cm$^{-1}$). C is the antioxidant molar concentration, which in this case was set at 16 µM, to give an approximate DPPH•/antioxidant molar ratio of 6 [25,28]. This was deemed necessary in order to eliminate variations arising from different concentrations, since $n_t$ was shown to be concentration dependent [29,30]. The $n_t$ is a measure of how many radicals can be trapped by one antioxidant molecule and may provide valuable information regarding the effectiveness of an antioxidant.

After $n_t$ determination, and assuming Equation (3) represents a complete reaction, the IC$_{50}$ was estimated using the following function [31]:

$$\text{IC}_{50} \text{ (µM)} = \frac{[\text{DPPH•}]}{2n_t}$$

(5)

where [DPPH•]$_0$ is the initial molar DPPH• concentration (µM). The $n_t$ and IC$_{50}$ values determined experimentally are analytically provided in Table 1. The IC$_{50}$ found for CA was in absolute accordance with 19.8 µM previously reported [32], although other authors gave a value of 12.2 µM [27]. Likewise, the IC$_{50}$ of 45.3 µM [33], 41.8 µM [34], and 44.6 µM [35] determined for FA was in close agreement with the one determined in this examination, although values as high as 61.9 µM [32] and as low as 22 µM [36] and 24.7 µM [27] have also been reported. For CouA, all available studies reported IC$_{50} > 100$ µM [32,33,35].
Table 1. Total stoichiometry (nt) and IC_{50} values determined for the hydroxycinnamates considered. Each compound was tested at C = 16 µM, with [DPPH•]₀ = 102.3 µM.

<table>
<thead>
<tr>
<th>Antioxidant</th>
<th>nt</th>
<th>IC_{50} (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHT</td>
<td>0.31</td>
<td>160.6</td>
</tr>
<tr>
<td>CA</td>
<td>2.63</td>
<td>20.0</td>
</tr>
<tr>
<td>FA</td>
<td>1.10</td>
<td>47.8</td>
</tr>
<tr>
<td>CouA</td>
<td>0.28</td>
<td>187.7</td>
</tr>
</tbody>
</table>

Regarding nt, the value determined in this study was 2.63, in near-perfect agreement with 2.65 found in an earlier investigation [25] and 2.6 determined in a recent study [30], but also in close match with the values of 2.3 [27] and 2.2 [29]. Similarly, the nt of 1.10 for FA was in agreement with the values of 1.16 [23], 1.2 [29], and 1.3 [27,30] found in previous studies. For CouA, discrepancies have been observed, with nt values estimated to be either 1 [29] or >1 [23].

Taking into account both nt and IC_{50}, the ranking of the hydroxycinnamates tested was CA > FA > CouA. This outcome was in absolute agreement with previous findings, which demonstrated the same order of efficiency [35,37–40]. It should be noted that the ranking based on any testing with DPPH• may be considered as merely indicative, since the quenching mechanisms of DPPH• and ROO• by a given antioxidant may differ significantly. In alcoholic solutions, such as methanol, where the DPPH• test is usually carried out, phenols ionize and thus react with DPPH• via electron transfer (ET) from their anions [41], whereas in lipids, antioxidants act as H-donating molecules [31]. Therefore, no direct extrapolation of the effectiveness of an antioxidant could be made for its lipid-stabilizing effect against oxidation.

3.2. Rancimat Trials—Concentration-Dependent Effects in a Lipid Matrix (Olive Kernel Oil)

The first approach to evaluate the effect of the addition of increasing amounts of antioxidants in OKO was to examine the correlation between the induction period (IP) and antioxidant concentration (C). To this end, IP was determined using increasing concentrations of antioxidants, and plotted as a function of C (Figure 2).

![Figure 2](image-url) Changes in induction period (IP) as a response to increasing antioxidant concentration (C). Assignments: BHT, butylated hydroxytoluene; CA, caffeic acid; CouA, p-coumaric acid; FA, ferulic acid.
Model fitting was performed with SigmaPlot™ 12.5 software, and revealed that IP may be correlated with C following a three parameter power function:

$$IP(C) = IP(0) + kC^a$$  \hspace{1cm} (6)

where \( IP(C) \) represents the IP recorded with any C, and \( IP(0) \) represents the one determined without antioxidants added. The terms “\( k \)” and “\( a \)” are fitting parameters. For all antioxidants tested, both \( k \) and \( a \) were determined from the non-linear regression and are provided in Table 2.

**Table 2.** The \( k \) and \( a \) value determined for each hydroxycinnamate considered, using the model described by Equation (5).

<table>
<thead>
<tr>
<th>Antioxidant</th>
<th>( R^2 )</th>
<th>( k )</th>
<th>( a )</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHT</td>
<td>0.99</td>
<td>0.155</td>
<td>0.469</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>0.98</td>
<td>0.056</td>
<td>0.774</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>0.91</td>
<td>0.164</td>
<td>0.349</td>
</tr>
<tr>
<td>( p )-Coumaric acid</td>
<td>0.96</td>
<td>0.132</td>
<td>0.378</td>
</tr>
</tbody>
</table>

The rearrangement of Equation (6) would give the following:

$$IP(C) - IP(0) = kC^a$$  \hspace{1cm} (7)

The term \( IP(C) - IP(0) \) represents the extension in IP provoked by a given concentration of antioxidant under the Rancimat conditions employed, and it is assigned as “induction period extension” (IPE) from now on. Thus, Equation (7) may be written as the following:

$$IPE = kC^a$$  \hspace{1cm} (8)

The parameter \( k \) is a constant that might be associated with the nature of the antioxidant. The parameter \( a \) shows the sensitivity of IPE as a response to modifying C, that is, the higher the \( a \) for a given antioxidant, the lower the concentration required to achieve a certain IPE for a given oil. Thus, \( a \) may be regarded as a measure of antioxidant effectiveness.

Furthermore, to shed more light regarding the stabilizing potency of the antioxidants tested, the mean rate \( (R_m) \) of inhibitor (antioxidant) consumption was also determined \([42]\) as follows:

$$R_m \ (mg \ kg^{-1} \ h^{-1}) = \frac{C}{IP}$$  \hspace{1cm} (9)

If \( R_m \) is plotted as a function of C, a linear correction may be obtained, as illustrated in Figure 3.

The analytical data derived from the linear regressions are given in Table 3. According to these correlations, \( R_m \) is directly proportional to C. Therefore, increasing C would provoke a monotonous effect on \( R_m \). The slope of the linear function depicted in Figure 2 has units of h\(^{-1}\) and represents a first-order reaction constant, termed as \( k_s \) (instability constant) from now on. The higher the \( k_s \), the higher the rate of antioxidant depletion. Therefore, an antioxidant that exhibits low \( k_s \) might be more stable under the conditions employed, provoking higher IPE. In this regard, the ranking of the compounds tested would be CA > CouA ≈ FA. Additionally, based on the \( a \) values presented in Table 2, the ranking would be CA > CouA > FA.

Considering these findings, it was evident that CA was a superior antioxidant compared to the other two hydroxycinnamates tested, FA and CouA. This ranking was also roughly consistent with an early study, which employed only IP as a means of comparing the protective effectiveness of antioxidants against lard oxidation, giving a ranking of CA > FA > CouA \([43]\). A latter evaluation based on more complicated kinetic methodology demonstrated that the efficiency of these antioxidants to react with peroxy radicals during methyl linoleate oxidation followed the order CA > FA > CouA \([44]\). Likewise, the kinetic
investigation of lard and sunflower oil methyl ester oxidation showed that the order of efficiency was CA > FA > CouA [42].

![Graph showing correlation between mean rate (Rm) of inhibitor (antioxidant) consumption with inhibitor concentration. Assignments: BHT, butylated hydroxytoluene; CA, caffeic acid; CouA, p-coumaric acid; FA, ferulic acid.]

**Table 3.** Linear functions established after plotting the mean rate (Rm) of inhibitor (antioxidant) consumption with inhibitor concentration.

<table>
<thead>
<tr>
<th>Antioxidant</th>
<th>Equation</th>
<th>R²</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHT</td>
<td>$R_m = 0.128C + 1.089$</td>
<td>0.9981</td>
<td>0.128</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>$R_m = 0.104C + 2.026$</td>
<td>0.9999</td>
<td>0.104</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>$R_m = 0.150C + 0.373$</td>
<td>0.9999</td>
<td>0.150</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>$R_m = 0.148C + 0.656$</td>
<td>0.9990</td>
<td>0.148</td>
</tr>
</tbody>
</table>

Based on the above data, it could be supported that the mathematical approaches employed may be a simple and valuable tool in appraising the efficiency of natural antioxidants to protect oils from oxidative deterioration, as demonstrated by previous investigations. The similarity of the ranking of antioxidants with those from other studies that utilized very different methodologies might bear witness to the validity of this approach, and also provide further insights into the protective effect of polyphenolic antioxidants against oil rancidity. However, the results concerning FA and CouA were contradictory, a fact that merits further and more extensive examination.

### 3.3. Accelerated Storage Trials

To gain a full understanding of the efficiency of the antioxidants tested, oil samples enriched with 200 mg kg⁻¹ of each antioxidant were maintained at 65 °C, for a period of 40 days. Samples were periodically checked for rancidity development, by measuring the onset of peroxide value (PV), which represents the formation of primary lipid oxidation products, and the thiobarbituric acid reactive substances (TBARS), which gives an account of secondary oxidation product formation.

For the non-enriched sample (control), PV increased almost 2-fold at 20 days, and 3-fold up to the end of the examination period (40 days), pointing clearly to a gradual peroxide accumulation (Figure 4). On the contrary, for the oil samples enriched with antioxidants, the increases in PV at day 40 were 1.3 to 1.6 times higher than the initial value. Furthermore, it was observed that, after 20 days, the most effective antioxidants were CA and BHT, while at the end of the treatment (day 40), only BHT contributed to
a significantly lower PV. On the other hand, the differences in PV found for oil samples enriched with either CA, FA, and CouA were non-significant. Similarly, the oil sample with no antioxidants added showed a gradual TBARS accumulation over the examination period and, at day 40, the TBARS were increased by approximately 4.5-fold. On the contrary, all antioxidants tested were proven effective in delaying TBARS accumulation. Moreover, measurements at both day 20 and 40 indicated that FA exhibited significantly higher efficacy ($p < 0.05$) compared to all other compounds (Figure 5).

![Figure 4.](image1.png) **Figure 4.** Evolution of peroxide formation upon incubation of OKO at 65 °C. Assignments: BHT, butylated hydroxytoluene; CA, caffeic acid; CouA, $p$-coumaric acid; FA, ferulic acid. Each antioxidant was tested at 200 mg kg$^{-1}$ of oil. Bars indicate standard deviation. Columns denoted with different letters (a, b, c, d and e) represent significantly different values ($p < 0.05$).

![Figure 5.](image2.png) **Figure 5.** Evolution of TBARS formation upon incubation of OKO at 65 °C. Assignments: BHT, butylated hydroxytoluene; CA, caffeic acid; CouA, $p$-coumaric acid; FA, ferulic acid. Each antioxidant was tested at 200 mg kg$^{-1}$ of oil. Bars indicate standard deviation. Columns denoted with different letters (a, b, c, d, e, f, g and h) represent significantly different values ($p < 0.05$).
Considering both assays, it was evident that all phenolic acids tested were demonstrated to provide significantly enhanced resilience against oxidative rancidity, as this can be manifested by both PV and TBARS. In the case of peroxide accumulation, CA appeared to provide higher stability after 20 days, but not after 40 days of treatment. This finding suggested that, in the long term, the structural features of the phenolic acids tested might not play a pivotal role. Such a phenomenon might be ascribed to the stability of the compounds tested, but also to the fact that peroxides are relatively unstable molecules, and gradually decompose, giving rise to secondary oxidation products [45].

Likewise, since the onset of TBARS is associated with the formation of carbonyl compounds as a result of peroxide degradation [5], the inhibition of the increase in TBARS could be attributed to limited peroxide formation. However, the supremacy of FA over the other phenolic acids tested might suggest that its involvement in TBARS formation could embrace complicated chemical pathways. In any case, the behavior of an antioxidant with regard to preventing lipid oxidation might be affected by several factors, such as its hydrogen-donating ability, the stability of the resonance form of the phenoxy radicals, the presence of other minor oil constituents, temperature, substrate structure (fatty acid unsaturation), etc. [4].

The activities of CA and FA could be attributed to their hydrogen-donating ability from the phenolic hydroxyls and stabilization of the radicals formed through resonance in a conjugated \(\pi\)-system. The methoxy group placed in the meta position in FA, or the lack of \(o\)-hydroxyl group in CouA, reduces the phenolic hydrogen availability and oxidized FA and CouA form fewer stable radicals compared to CA [46]. In this regard, it could be argued that the effectiveness of CA in increasing the IP is associated to the formation of \(o\)-quinone and CA regeneration by disproportionation of the semi-quinone radical, which is initially formed by the reaction between CA and the free radicals present in OKO.

Taking into account the diverse results obtained by testing natural antioxidants on a high number of lipid foods [10], it would then be anticipated that polyphenols may not afford the same protection for oils with significant differences in their composition. Although studies using simplified assays with model radicals (DPPH•) have long before attempted to correlated structural features (number of phenolic hydroxyl groups, \(o\)-dihydroxy structure, methylation) with the antioxidant activity of hydroxycinnamates [47,48], later findings have suggested that there may be major discrepancies in reliably ranking CA, FA, and CouA [40,49]. Thus, the estimation of the stability that an antioxidant may offer to a specific substrate is a subject of case experimentation.

4. Conclusions

The preliminary investigation of the hydroxycinnamates considered using the DPPH• radical probe evidenced a superiority of caffeic acid, which bears an ortho-hydroxy feature, over ferulic and \(p\)-coumaric acids, although discrepancies were observed in the efficacy ranking, compared to previously published data. A further insight into the potency of these antioxidants was achieved by using refined olive kernel oil as the substrate, where a novel mathematical approach revealed a relationship between antioxidant concentration and protection efficiency. Finally, the simulated long-term stability trial suggested that all three hydroxycinnamates tested may be equally effective in preventing peroxide formation, but with regard to secondary oxidation products accumulation, ferulic acid might display stronger effects. However, this behavior merits further clarification. The outcome of this study highlighted the prospect of using natural polyphenols as lipid stabilizers against oxidative rancidity. Considering that compounds such as ferulic and \(p\)-coumaric acid may be recovered in high amounts from agri-food waste biomass, then their low-cost production could enable their regular use as both effective antioxidants and functional additives in an array of lipid-containing foods. This perspective is of particular importance, given the urgent need for replacing the widely used synthetic antioxidants, in the light of findings which spotlight their adverse biological effects and possible harmful consequences to human health.
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