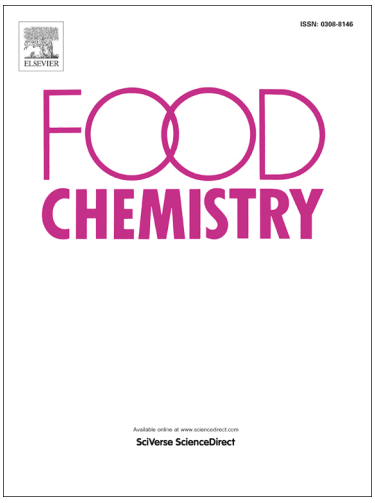

Antioxidant ability of Tyrosol and derivative-compounds in the presence of $O_2(^1\Delta_g)$-species. Studies of synergistic antioxidant effect with commercial antioxidants

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PII: S0308-8146(19)30236-5
DOI: https://doi.org/10.1016/j.foodchem.2019.01.161
Reference: FOCH 24256

To appear in: *Food Chemistry*

Received Date: 1 September 2018
Revised Date: 29 December 2018
Accepted Date: 29 January 2019

Please cite this article as: Casadey, R., Challier, C., Senz, A., Criado, S., Antioxidant ability of Tyrosol and derivative-compounds in the presence of $O_2(^1\Delta_g)$-species. Studies of synergistic antioxidant effect with commercial antioxidants, *Food Chemistry* (2019), doi: https://doi.org/10.1016/j.foodchem.2019.01.161

Antioxidant ability of Tyrosol and derivative-compounds in the presence of $O_2(^1\Delta_g)$-species. Studies of synergistic antioxidant effect with commercial antioxidants.

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ABSTRACT

The exposure of fatty products to environmental light can trigger lipid oxidation in food through a sensitized-photooxidation process, which involves the participation of the species singlet oxygen ($O_2(^1\Delta_g)$). Therefore, preservation of food quality represents a subject of great economic interest to the food industry. In this sense, the phenolic compounds are natural antioxidants widely used in food industry.

In this contribution we studied the interactions of phenolic derivatives (Phd), tyrosol and tyrosol derived isomers, with $O_2(^1\Delta_g)$ and their possible protective effect against the oxidative degradation of unsaturated fatty acids and amino acids. Besides, a potential synergistic interaction between Phd and antioxidants used in food industry were investigated.

Phd substrates showed properties as antioxidant additives due to their high ability deactivating $O_2(^1\Delta_g)$ through a physical process and synergistic effect in the presence of commercial antioxidants. Phd presented an antioxidant protective effect toward $O_2(^1\Delta_g)$-mediated degradation of methyl linoleate and tryptophan.

Keywords

singlet oxygen; phenolic antioxidants; photodegradation

Chemical Compounds

Rose Bengal (PubChem CID: 69438); (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) (PubChem CID: 40634); Methyl linoleate (PubChem CID: 5284421); 4-hydroxyphenethyl alcohol (PubChem CID: 10393); 2-hydroxyphenethyl alcohol (PubChem CID: 82200); 3-hydroxyphenethyl alcohol (PubChem CID: 83404); L-Triptophan (PubChem CID: 6305); Furfuryl alcohol (PubChem CID: 7361); L-Ascorbic acid (PubChem CID: 54670067).

1. Introduction

Lipids play an important role in food quality related to nutrition facts and health care. Unfortunately, they are considered the principal target of oxidative reactions in foodstuff, which produces serious injuries in both natural and processed food.

Oxidative reactions can occur in the presence of molecular oxygen. It is known that exposure of a fatty product to environmental light can begin or accelerate the lipid oxidation in food (Rosenthal, 1985). In these cases, a sensitized-photooxidation process can occur, with the participation of one of the reactive oxygen species, singlet oxygen ($O_2(^1\Delta_g)$). In this process, $O_2(^1\Delta_g)$ indirectly generated from a pigment present in the food product, can trigger the oxidation of fatty acids (Straight & Spikes, 1985). This process and the alterations produced by microorganisms are the main causes of food spoilage. This topic is a subject of great economic interest to the food industry as it gives rise to the appearance of unpleasant flavours and rancid odours, resulting in loss of food quality and nutritional value. Besides, products formed during lipid oxidation, in some cases, may be toxic and carcinogenic (Chong, Chang, Mei Sia, & Yim, 2015; Vhangani & Van Wyk, 2016). Therefore, the foodstuffs exposed to this type of alteration reduce their shelf life or become unacceptable to the consumer. As a consequence, the food industry is making great efforts in order to find ways to retard or inhibit these processes. In this sense, the use of different techniques such as microencapsulation of fatty acids, freezing, vacuum or active packagings represent some examples of such techniques (Teets, Sundararaman, & Were, 2008). In cases where these procedures are expensive or not practical, industries employ antioxidant compounds which can act through different mechanisms, including radical scavengers, singlet oxygen quenchers, photosensitizer inactivators, metal chelators (Kristinova, Mozuraityte, Storrø, & Rustad, 2009). A common technique used in the food industry is the combination of different types of

antioxidants since they can lead to synergistic effects. However, additive or antagonistic effects may occur within this antioxidant combination. Nevertheless, industry is trying to find an antioxidant mixture that produces synergistic effects in order to achieve greater effectiveness in the preservation of food, as well as a decrease in the number of synthetic antioxidants needed (Tsao, 2015).

Various natural and synthetic antioxidants have been successfully applied to different food formulations in order to prevent lipid oxidation (Shahidi, 2000). Within natural antioxidants, phenolic compounds are the most used in the food industry. Due to the fact that phenolic compounds have wide impacts on the quality of food products and furthermore, may have a substantial influence on human health, there are many researches on this topic (Boudet, 2007). Particularly interesting are the phenolic compounds present in olive oil which are related to oil stability and to its biological properties (Boskou, Blekas, & Tsimidou, 2005). Many reports have demonstrated that phenolic compounds present in olive oil (tyrosol, hydroxytyrosol and its derivatives) are powerful antioxidants and that they retain their biological activities after ingestion (Visioli, Bogani, Grande, & Galli, 2004). These substrates have been mainly reported as being beneficial to reduce several risk factors for coronary heart disease and certain cancers (Visioli, Bogani, Grande, & Galli, 2004). On the other hand, previous studies have demonstrated that the antioxidant capacity of phenolic compounds is pH-dependent and it increases with increasing pH of the medium. This is an interesting fact since the pH range of different fluids and tissues of the human body varies widely, from a pH of 1 in the stomach to a pH of 8.6 in the pancreas (Rubinstein, 2007). Furthermore, the pH of food products, in which the Phd substrates could act as antioxidants, also shows significant variations.

While polyphenols represent a class of compounds commonly associated with high antioxidant capacity, the pro-oxidant ability of some phenolic compounds has been widely documented. Zhou and Elias (2012) investigated the factors that promote the oxidation of a model polyphenol, (−)-epigallocatechin-3-gallate, which is commonly added to foods. They reported that this substrate generates hydrogen peroxide, which can potentially compromise the oxidative stability of foods and beverages. Flavonoids, important components of the human diet, are poly-phenolic compounds known for their antioxidant properties. However, under certain circumstances they act as pro-oxidants and, hence, promote the oxidation of other compounds (Procházková, Boušová, & Wilhelmová, 2011). Flavonoids pro-oxidant properties seem to be concentration-dependent and directly proportional to the total number of hydroxyl groups in the molecule (Yen, Duh, Tsai, & Huang, 2003). Hydroxycinnamic acids are phenolic compounds widely distributed in foods of plant origin, and they act as antioxidants scavenging free radicals (Manach, Scalbert, Morand, Rémésy, & Jiménez, 2004). However, depending on their structure, concentration and the medium-properties, they could also behave as pro-oxidants. Previous investigations have demonstrated the ability of phenolic compounds to generate reactive oxygen species upon direct irradiation. Considerable values of O_2 ($^1\Delta_g$)-production for phenolic substrates such as hydroxybenzenes and hydroxybiphenyls, under UV-irradiation, have been reported (Mártire, Braslavsky & Garcia, 1991; Mártire, Evans, Bertolotti, Braslavsky & Garcia, 1993).

In this context, the aim of this paper has been focused on the study of the potential interactions of tyrosol (4-hydroxyphenethyl alcohol, 4-OH) and two tyrosol derived isomers (2-hydroxyphenethyl alcohol, 2-OH and 3-hydroxyphenethyl alcohol, 3-OH) with $O_2(^1\Delta_g)$ and the possible protective effect of such substrates against the oxidative degradation of unsaturated fatty acids and amino acids. Moreover, synergistic interactions between the phenolic

compounds (Phd) and the antioxidants which have widespread use in the food industry, were investigated. Furthermore, pH-medium influence was studied upon $O_2(^1\Delta_g)$-scavenging capacity by Phd and commercial antioxidants. The pro-oxidant capacity of Phd was also evaluated under direct UV-irradiation.

The chemical structures of the substrates studied in the present contribution are shown in Tables 1 and 2.

2. Materials and methods

2.1. Materials

Rose Bengal (RB) and (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox, TX) were purchased from Aldrich Chemical Company. Methyl linoleate (ML), 4-hydroxyphenethyl alcohol (4-OH), 2-hydroxyphenethyl alcohol (2-OH), 3-hydroxyphenethyl alcohol (3-OH), deuterated water 99.9 % (D_2O), L-Triptofano (Trp), furfuryl alcohol (FFA), ascorbic acid (AAc), NaH_2PO_4 and NaOH were provided by Sigma-Aldrich. $K_2Cr_2O_7$ and methanol (HPLC quality) were obtained from Ciccarelli and Sintorgan, respectively.

2.2. Methods

O_2 ($^1\Delta_g$) species was photogenerated indirectly through the use of the xanthenic dye Rose Bengal (RB). The absorbance of RB was adjusted to 0.5-0.7 at the maximum absorption in the visible-band in water (549 nm). The stationary photolysis experiments of solutions containing the sensitizer and Phd were carried out in a home-made photolyser (Bertolotti, Arguello, & García, 1991). Briefly, this device consists of a 150 W quartz-halogen lamp, which is focused on an absorption cell or a reaction cell containing an oxygen-specific electrode. The solutions were continuously stirred. Light at λ<361 nm was filtered using a cut-off filter in order to ensure that the light was only absorbed by the sensitizer.

Experiments of oxygen uptake were performed with a specific oxygen electrode *Orion* 97-08, coupled to a pH meter *Orion* 720.

Changes in substrate concentration were followed by absorption spectroscopy with a double beam spectrophotometer *Shimadzu* 2401 or through stationary fluorescence measurements with *a Fluor Max*™ spectrofluorometer. Quartz cells of 1 cm optical path were used and the temperature was maintained at 25±1°C.

The photooxidation experiments were performed in a buffer solution of neutral and alkaline pH, in order to compare the kinetic results obtained under different ionization conditions of the phenolic substrates. The experiments with AAc were not performed at alkaline-pH due to low stability of the antioxidant under these conditions.

The determination of the values of the reactive deactivation rate constants of $O_2(^1\Delta_g)$ by Phd, k_r, was carried out through an actinometric method (Foote & Ching, 1975), which compares the slopes of the first order graphs for the oxygen consumption *vs*. irradiation time by the photooxidizable substrate and a reference compound, both determined at identical concentration. The ratio of these slopes is equal to the ratio k_r/k_{rRef}. FFA was employed as reference compound, which is a current reference for $O_2(^1\Delta_g)$ reactions, with a reported pH-independent rate constant k_{rRef} of 1.2 x 10^8 $M^{-1}s^{-1}$ in water (Wilkinson, Helman, & Ross, 1995). This method assumes that only the reaction of the photooxidizable substrate with $O_2(^1\Delta_g)$ leads to oxygen consumption. In order to avoid possible interference from photogenerated products, conversions lower than 10% were employed.

The overall rate constant of deactivation of O_2 $(^1\Delta_g)$ by Phd, k_t, which takes into account the physical and reactive contribution to the global process of $O_2(^1\Delta_g)$-deactivation by a photooxidizable substrate (processes (5) and (6), Figure S1), was determined by Time Resolved Phosphorescence Detection of $O_2(^1\Delta_g)$ at 1270 nm (TRPD). This technique has

been previously described (Criado, Bertolotti, & García, 1996a). Briefly, an Nd:YAG laser (*Spectron Laser System*, SL400) was used as excitation source. The output at 532 nm was employed to excite RB. The emitted radiation was detected at right angle using an amplified *Judson* J16/8Sp germanium detector, which is provided with an appropriate internal filter system. The absorbance of the sensitizer RB was adjusted to 0.2-0.3 at the excitation wavelength, to avoid saturation of the detector. The kinetic decays of the phosphorescent signals were first order in all cases. Experiments were made in D_2O as solvent instead of H_2O, in order to enhance the $O_2(^1\Delta_g)$-lifetime (Nonell et al., 1995). The k_t values were graphically obtained through Stern-Volmer treatment (Eq. 1)

$$1 / \tau = 1/\tau_0 + k_t\,[\text{Phd}] \qquad \text{Eq. 1}$$

where τ_o and τ represent the $O_2(^1\Delta_g)$ phosphorescence lifetime in the absence and in the presence of different Phd concentrations, respectively.

The quantum yields of $O_2(^1\Delta_g)$ production (Φ_Δ) by Phd were determined by Laser Flash Photolysis in D_2O solutions (pD 7). The 266-nm output of the already mentioned Nd:Yag laser (*Spectron Laser System*, SL400) was used as excitation source. Φ_Δ values were determined through an actinometric method, employing RB as a reference compound. The initial intensities of the emission of $O_2(^1\Delta_g)$ obtained at 1270 nm (I_0) were measured as a function of laser fluence, for optically matched solutions of Phd and RB. The laser fluence was varied employing neutral density filters. From I_0 *vs.* transmitance percentage plots for Phd and RB, the respective slopes were obtained (I_{0E} and $I_{0E\,Ref}$). The Φ_Δ values were determined through Eq. 2, employing a Φ_Δ value (Gandin, Lion, & Van de Vorst, 1983) for $O_2(^1\Delta_g)$-generation by RB of 0.75 in D_2O.

$$\Phi_\Delta / \Phi_{\Delta\,Ref} = I_{0E} / I_{0E\,Ref} \qquad \text{Eq. 2}$$

where Φ_{Δ} and $\Phi_{\Delta\ Ref}$ are the quantum yield of $O_2(^1\Delta_g)$ production by Phd, and RB, the reference compound, respectively.

The antioxidant capacity of 2-OH against O_2 ($^1\Delta_g$) was evaluated using methyl linoleate (ML) as a model compound of lipid oxidation. In the presence of O_2 ($^1\Delta_g$), ML gives rise to the formation of hydroperoxides, which can be detected from an increase in absorbance at 233 nm (Salehi, Pages, Furó, Henriksson, & Johansson, 2011; Xie, Ji, & Wang, 2007). Experimentally, the change in absorbance at 233 nm of ML, sensitized by RB, was followed as a function of the irradiation time, in the presence and in the absence of 2-OH. Due to the low solubility of ML in aqueous solution, these determinations were performed in methanol.

3. Results and discussion

3.1. General

Results in this contribution will be interpreted and discussed according to Figure S1.

This figure describes a generic photosensitized process, in which the absorption of incident light promotes the sensitizer (S, in ground state) to electronically excited singlet and triplet states (process (1) and (2), respectively). From the latter an energy transfer process to molecular oxygen ($O_2(^3\Sigma^-_g)$) dissolved in the medium can take place (process (3)), yielding the excited state oxygen species $O_2(^1\Delta_g)$. This can decay either by collision with surrounding solvent molecules (process (4)) or by interaction with Phd through a physical (step (5)) and/or reactive (step (6)) process.

The addition of the respective rate constants for physical (k_q) and reactive (k_r) deactivation of O_2 ($^1\Delta_g$) is equal to the overall rate constant of deactivation of O_2 ($^1\Delta_g$) by a photooxidizable substrate ($k_t = k_q + k_r$).

3.2. O_2 ($^1\Delta_g$)-scavenging capacity of Phd

The scavenging capacity of Phd towards O_2 ($^1\Delta_g$) was investigated. In this sense, the dye RB was used in order to generate the species O_2 ($^1\Delta_g$) under irradiation.

Irradiation with light (λ > 361nm) of the system Phd/sensitizer (RB)/oxygen/buffer solutions produced modifications in the UV-absorption spectra of the mixture, due to the formation of products on RB-sensitized photooxidation of Phd (Figure 1). Furthermore, as can be seen in Figure 1, the perturbations in the spectra are more meaningful at alkaline pH than at neutral pH. Similar results were observed for 2-OH and 3-OH. Simultaneously with Phd consumption, oxygen uptake was observed at both pHs assayed. These experiments were also dependent on the pH of the medium. It is known that $O_2(^1\Delta_g)$-mediated degradation of phenolic compounds is more effective when the -OH group is found as phenolate (García, 1994). The reactivity of these substrates is related to the acidity dissociation constants (pKa) for Phd, which are in the range of 11-12. Therefore, the higher reactivity of Phd observed in the alkaline medium is due to the elevated percentage of Phd molecules with their -OH ionized. All these results suggest chemical interaction between Phd and $O_2(^1\Delta_g)$-species.

On the other hand, the RB-sensitized photodegradation of Phd does not produce modifications in the visible-absorption zone of the sensitizer (500-600 nm). These results allow us to discard specific interactions between Phd and electronically excited states (singlet and/or triplet) of RB.

3.3. Quantification of the interaction Phd-O_2 ($^1\Delta_g$)

The reactive contribution of O_2 ($^1\Delta_g$) (process (6), Figure S1) to the global degradation process of Phd was quantified through the k_r values, in buffered solutions of pH 7 and 11, for

the substrates 3-OH and 2-OH. In case of 4-OH, k_r values were evaluated in buffered solutions of pH 7 and 12. Different alkaline pHs were selected in accordance with the acidity dissociation constants values of –OH group (pKa).

The k_r values were obtained applying Foote and Ching method (Foote & Ching, 1975), as described in section 2.2 (Figure S2). k_r values are shown in Tables 1 and 2.

As can be seen from both Tables, the ionization of the phenolic group produces a significant increase of k_r values. This dependence of k_r values on the degree of ionization of –OH group has been reported (Barua, Escalada, Bregliani, Pajares, & Criado, 2016; Bertolotti, Arguello, & García, 1991; García, 1994;). As mentioned above, taking into account the pKa values for Phd, at pH 11-12 a significant percentage of molecules would have their phenolic group ionized. For some substrates (phenols, amines, indoles and furans) a substrate-$O_2(^1\Delta_g)$ encounter complex, with partial charge-transfer character, has been postulated (Palumbo, Garcia & Argüello, 1990; Gorman, Lovering & Rodgers, 1979; Young, Martin, Feriozzi, Brewer & Kayser, 1973). The formation of this complex is favoured by the higher electron-donor ability of the ionized species and its deactivation could produce the oxidation of the substrate or lead to physical deactivation (Gorman, Gould, Hamblett, & Standen, 1984), as can be seen in Figure S3.

The overall rate constants of deactivation of O_2 ($^1\Delta_g$) by Phd, k_t, were determined through the technique (TRPD) as described in section 2.2. The k_t values were obtained through a graphical Stern-Volmer treatment of data. Experiments were made in buffered solutions at pD 7 and 11 for the substrates 3-OH and 2-OH, while the substrate 4-OH was measured at pD 7 and 12 (Figure S4, Tables 1 and 2).

As shown in Tables 1 and 2, the k_t values were also strongly influenced by the pH-environment. In alkaline conditions, these constants are approximately two orders of

magnitude higher than at neutral pH, due to the presence of the phenolate group, as already discussed. This result is consistent with previous published data (García, 1994). Mártire, Braslavsky and Garcia (1991) reported that for hydroxyaromatic derivatives, the k_t and k_r values of the ionized species (ionization of the -OH groups) are higher than those of the non-ionized forms by at least one order of magnitude. Criado, Gutierrez, Avila, Bertolotti, and Garcia (1996b) demonstrated that the ionization of the -OH groups in hydroxyflavones has a great influence on their reaction kinetics. Similar results have been reported for (+)-catechin and derivatives (Barua, Escalada, Bregliani, Pajares, & Criado, 2016).

According to the deactivation mechanism of O_2 ($^1\Delta_g$) proposed in Figure S3, the stabilization of the encounter complex will depend on the charge delocalization within the complex. At alkaline pH, the charge will be more localized due to the presence of the phenolate group; therefore, the encounter complex will stabilize. Under these conditions, it will be more reactive towards the electrophilic species O_2 ($^1\Delta_g$) than at neutral pH.

In Tables 1 and 2, the k_r/k_t ratio for the substrates Phd was included. This ratio can be interpreted as a relative measure of the overall fraction of substrate-O_2 ($^1\Delta_g$) collisions that effectively lead to a chemical reaction. From the values of k_r/k_t it is observed that the substrate 3-OH presents a bigger physical contribution to the overall O_2($^1\Delta_g$)-deactivation process than 4-OH and 2-OH substrates, at both pH assayed.

It is known that in the substrate-O_2($^1\Delta_g$) encounter complex, the physical quenching pathway results from spin-orbit coupling- induced intersystem crossing within the complex, and therefore, is controlled by spin-orbit coupling factors. On the other hand, it has been proposed that the chemical deactivation is regulated by entropy factors. Hence, the balance between physical quenching (k_{ISC}) and chemical reaction (k_r) depends on spin-orbit coupling

and entropy factors (Gorman, Lovering & Rodgers, 1979; Gorman, Gould & Hamblett, 1982; Gorman, Gould, Hamblett & Standen, 1984).

As shown in Tables 1 and 2, Phd present different behaviour with respect to the physical deactivation of $O_2(^1\Delta_g)$. It is important to notice that this substrates are structural isomers, and this fact makes it very difficult to estimate or even calculate how the substituent position (-CH_2CH_2OH) on the phenolic ring may affect the spin-orbit coupling factors. According to our results, the spin-orbit coupling factors may be favoured with the substituent -CH_2CH_2OH in the *meta*-position (substrate 3-OH). Therefore the physical deactivation pathway prevails for the substrate with the substituent -CH_2CH_2OH in *meta*-position (substrate 3-OH) compared with the substituent in *ortho*-(substrate 2-OH) and *para*- (substrate 4-OH) positions. These results are in agreement with previous reports, where the $O_2(^1\Delta_g)$-deactivation by a series of salicylic acid derivatives (with the -NH_2 substituent in different positions respect to the phenolic group) has been studied. The authors also postulated that the deactivation of $O_2(^1\Delta_g)$ by such substrates occurs through an encounter complex formation and that the physical quenching is favoured for the substrate with the -NH_2 substituent in *meta*-position respect to the phenolic group.

Hence, this fact makes 3-OH substrate a better antioxidant than 4-OH and 2-OH. The poor chemical reactivity of 3-OH towards $O_2(^1\Delta_g)$ constitutes an important property for a potential antioxidant agent because it physically deactivates the species $O_2(^1\Delta_g)$ and remains intact (without chemical deterioration) to continue acting in the preservation of the food quality and nutritional value.

3.4. Antioxidant capacity of Phd on the $O_2(^1\Delta_g)$-mediated tryptophan oxidation

The amino acid tryptophan (Trp) is considered one of the target molecules against the attack of oxidizing agents such as $O_2(^1\Delta_g)$. Modifications in the structure of this amino acid can cause significant alterations in the physicochemical properties of proteins, which are one of the main nutritional components in foods. For this reason, Trp was chosen in order to study the protective effect of Phd and commercial antioxidants trolox (TX) and ascorbic acid (AAc), on their $O_2(^1\Delta_g)$-mediated oxidation.

The oxidation of Trp by $O_2(^1\Delta_g)$ was investigated in the absence and in the presence of Phd, TX and AAc through stationary fluorescence measurements. According to Trp spectroscopic properties, 300 nm and 355 nm were selected as excitation and emission wavelength, respectively.

Figure 2 shows Trp relative emission of fluorescence *vs.* irradiation time in the absence and in the presence of 3-OH, TX and AAc.

As can be seen in Figure 2, 3-OH showed a slight protective effect on $O_2(^1\Delta_g)$-mediated tryptophan oxidation. Similar results were found for the substrate 2-OH. Nevertheless, in the case of 4-OH an antioxidant protective effect on Trp degradation was not observed under the experimental conditions assayed ([Trp] = 5 $x10^{-4}$ M; [Phd] = 2.5 $x10^{-4}$ M). Besides, the commercial antioxidant AAc presented an excellent protective effect on the amino acid oxidation, efficiently deactivating O_2 $(^1\Delta_g)$ species. On the other hand, TX showed a lesser antioxidant ability than AAc on the Trp degradation, under working conditions ([Trp] = 5 $x10^{-4}$ M; [TX] = [AAc] = 2.5 $x10^{-4}$ M).

3.5. Antioxidant ability of Phd on methyl linoleate, a model compound of lipid oxidation

Lipid oxidation process is triggered in the presence of light, O_2 (Σ_g^-) and a molecule capable of absorbing this radiation (sensitizer). Under this scenario, the O_2 ($^1\Delta_g$) species is generated and reacts with the double bonds of unsaturated fatty acids.

In this assay methyl linoleate (ML) was used as a model compound of lipid oxidation process. As described in Section 2.2, the antioxidant capacity of 2-OH against O_2 ($^1\Delta_g$) was evaluated in the presence of ML, monitoring the increase in absorbance at 233 nm due to the generation of hydroperoxides.

Figure 3 shows the O_2 ($^1\Delta_g$)-mediated degradation of ML, monitoring the absorbance changes as a function of the irradiation time, in the absence and in the presence of 2-OH.

In this Figure 3 an increase in the absorbance of ML at 233 nm was observed. Likewise, in this figure it can be seen that in the presence of 2-OH there is a decrease in the absorbance change of ML regarding the measurements in the absence of the substrate. These results indicate that the phenolic derivative effectively acts as a protector of ML against O_2 ($^1\Delta_g$)-photoinduced degradation.

3.6. Synergistic antioxidant effect of Phd and commercial antioxidants

With the aim of investigating the potential synergistic antioxidant effect of Phd and commercial antioxidants against O_2 ($^1\Delta_g$), oxygen uptake experiments were performed.

The measurements were carried out under different concentrations and pH, in order to evaluate the influence of these factors on the synergistic effect. The assays were performed at a fixed concentration of Phd and at two different concentrations of TX and AAc.

In Figure 4 oxygen consumptions plots of 3-OH in the absence and in the presence of TX and AAc, at pH 7, are shown.

From Figure 4A and 4B a synergistic antioxidant effect can be noticed, since the system 3-OH/TX and 3-OH/AAc present a higher oxygen consumption, relative to 3-OH. Furthermore, by comparing Figure 4A and 4B it can be seen that the higher the concentration, the greater synergistic effect. In general, similar results were obtained for 2-OH and 4-OH (data not shown).

Regarding pH-influence, a common pattern of Phd-behaviour on synergistic antioxidant effect was not observed. For example, results for 4-OH exhibit a particular behaviour as displayed in Figure S5. In this figure, oxygen uptake plots of the systems 4-OH and 4-OH/TX at pH 7 and 12 are shown.

As can be seen in Figure S5, under pH 12 the system 4-OH/TX shows a decrease in oxygen uptake compared with system 4-OH. This result suggests an antagonistic antioxidant effect. Therefore, depending on the experimental conditions (medium-pH, relative antioxidant-concentrations) some specific interaction between the antioxidants could be favoured, compromising the availability of Phd to act as scavengers of the $O_2(^1\Delta_g)$ species.

As previously described by other authors, the presence of a possible synergistic or antagonistic effect in a mixture of antioxidants depends on the properties of reaction-medium, the polarity of the compounds, the specific interaction that may involve the antioxidants and their relative concentrations (Lee, Li, Kim, Chung, Lee, & Oh, 2011; Marteau, Favier, Nardello-Rataj, & Aubry, 2014; Prieto, Murado, & Vázquez, 2014; Zanfini, Corbini, La Rosa, & Dreassi, 2010). In this sense, several researches have reported interactions between tocopherols and carotenes which produced a synergistic effect on lipid peroxidation-inhibition (Li, Wu, Ma, Liu, & Liu, 1995; Prieto, Murado, & Vázquez, 2014). Bohm, Edge, McGarvey, and Truscott (1998) demonstrated that the synergistic effect of these antioxidants can be observed only in the presence of vitamin C (α-tocopherol/vitamin C; β-carotene/vitamin C).

However, in a mixture of both antioxidants and vitamin C this synergistic effect was not observed. On the other hand, Handelman, van Kuik, Chaterjee, and Krinsky (1991) informed a lack of synergistic effect under α-tocopherol-concentration much lesser than β-carotene. Besides, an antagonistic effect was observed in mixtures of phenolic compounds with butylated hydroxytoluene, α-tocopherol, catechin, resveratrol or quercetin (Pinelo, Manzocco, Nuñez, & Nicoli, 2004). The experimental evidence derived from previous research clearly demonstrate that it is not possible to predict a common synergistic or antagonistic behaviour for the antioxidants. In fact, the interactions between antioxidants are strongly influenced by the properties of the food matrix.

3.7. Pro-oxidant capacity of Phd

In order to investigate the pro-oxidant ability of Phd, the O_2 ($^1\Delta_g$)-generation was evaluated through the determination of Φ_Δ values.

The O_2 ($^1\Delta_g$)-production by a substrate upon direct irradiation requests the previous generation of an electronically excited triplet state of this substrate. An energy transfer process from this state to O_2 (Σ_g^-) can yield O_2 ($^1\Delta_g$), as shown in Figure S1. The Phd-electronically excited triplet state was characterized by Laser Flash Photolysis (λ_{exc} = 266 nm), as mentioned in Section 2.2. Results confirmed the formation of Phd-electronically excited triplet state, in agreement with previous reports (Lind, Shen, Eriksen, & Merény, 1990). As a consequence, the O_2 ($^1\Delta_g$)-generation by Phd was evaluated through an actinometric method, and Φ_Δ values were determined (Tables 1 and 2).

According to these results, it can be noted that the generation of O_2 ($^1\Delta_g$) by Phd is much larger with respect to common sensitizers (Redmond & Gamlin, 1999), such as Riboflavin (Φ_Δ= 0.49 in buffer pH 7.4), Methylene Blue (Φ_Δ= 0.5 in methanol), Eosin B (Φ_Δ= 0.42 in methanol).

Therefore, Phd may act as pro-oxidant substrates through O_2 ($^1\Delta_g$)-production under UV-irradiation. This characteristic of Phd could be undesirable whilst considering these compounds as potential antioxidants additives. However, it is important to note that these substrates do not absorb in the visible region. As a consequence, O_2 ($^1\Delta_g$)-generation by Phd can be discarded if these substrates are used as food additive.

4. Conclusions

Phd substrates show encouraged properties as antioxidant additives due to their high ability to deactivate O_2 ($^1\Delta_g$) through a physical process. This fact keeps the compound intact and available to act in a new protection cycle. The substrate 3-OH can be considered the best antioxidant among the Phd studied. Furthermore, the O_2 ($^1\Delta_g$) deactivation by Phd is strongly influenced by pH-medium. This result is interesting since the pH range of different fluids and tissues of the human body varies widely.

Phd substrates display a synergistic effect in the presence of antioxidant commercial additives which allows improving their antioxidant ability when present in the same food matrix. Experimental evidence showed that it is not possible to establish a common pattern in synergistic or antagonistic behaviour for a combination of different antioxidants. In fact, the interactions between antioxidants are strongly influenced by the properties of the food matrix. Therefore, food industries invest a lot of effort investigating the interactions between antioxidant additives under different experimental conditions in order to select the best antioxidant combination for a specific food matrix.

The substrates 3-OH and 2-OH presented an antioxidant protective effect toward O_2($^1\Delta_g$)-mediated degradation of the oxidizable amino acid Trp. Modifications in the structure of Trp can cause significant alterations in the functional properties of proteins. Therefore, in

the presence of 3-OH or 2-OH, the proteins containing Trp-residues in food matrices may be protected against O_2 ($^1\Delta_g$)-attack. Besides, 2-OH showed a protective effect toward ML degradation in the presence of O_2 ($^1\Delta_g$). This result suggests that 2-OH could be an efficient antioxidant additive against lipid peroxidation, an undesirable degradation process in high fat foods.

Phd presented good properties as pro-oxidants substrates through an efficient O_2 ($^1\Delta_g$)-generation, under UV irradiation. However, this radiation is not involved in common food storage and usage conditions. Consequently, if Phd are used as food additive their pro-oxidant abilities can be dismissed.

From every result obtained for tyrosol and the two tyrosol derived isomers, we can conclude that it is not possible to establish a pattern of behaviour regarding the different -CH_2-CH_2OH position on the phenolic ring of Phd.

Finally, the results provided in the present contribution suggest that it is essential to investigate the particular behaviour of each substrate, under different experimental conditions, in order to yield knowledge in designing new molecules with improved antioxidant properties.

Acknowledgments

This work was supported by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) and Secretaría de Ciencia y Técnica de la Universidad Nacional de Río Cuarto (SECyT-UNRC) Argentina. R. Casadey thanks to CONICET for a PhD fellowship.

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FIGURES CAPTIONS

Figure 1. O_2 ($^1\Delta_g$)-mediated degradation of 4-OH, in aqueous solutions of pH 12 **(main)** and pH 7 **(inset)**. Numbers on the spectra represent irradiation time, in seconds.

Figure 2. $O_2(^1\Delta_g)$-mediated Trp oxidation in the absence **(a)** and in the presence of 3-OH **(b)**; TX **(c)** and AAc **(d)**. Solvent: aqueous solution of pH 7. [Trp] = $5x10^{-4}$ M; [3-OH] = [TX] = [AAc] = $2.5x10^{-4}$ M

Figure 3. $O_2(^1\Delta_g)$-mediated ML oxidation in the absence **(a)** and in the presence of 2-OH **(b)**. Solvent: aqueous solution of pH 7. [ML] = $3x10^{-3}$ M; [2-OH] = $5x10^{-4}$ M.

Figure 4. (A) Oxygen consumptions during O_2 ($^1\Delta_g$)-mediated degradation of 3-OH ($5x10^{-4}$ M) in the absence **(a)** and in the presence of [TX] = $1x10^{-4}$ M **(b)** and [AAc] = $1x10^{-4}$ M **(c)**, in aqueous solution of pH 7. **(B)** Oxygen consumptions during O_2 ($^1\Delta_g$)-mediated degradation of 3-OH ($5x10^{-4}$ M) in the absence **(a)** and in the presence of [TX] = $1x10^{-3}$ M **(b)** and [AAc] = $1x10^{-3}$ M **(c)**, in aqueous solution of pH 7.

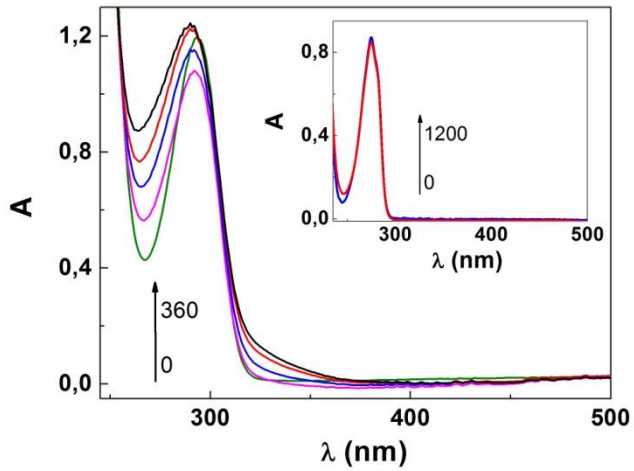


Figure 1 Casadey *et. al.*

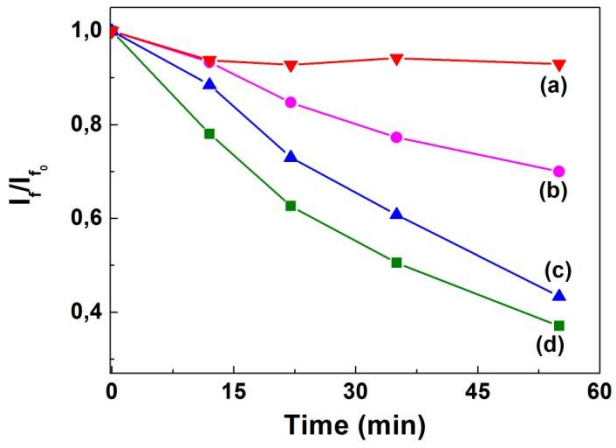


Figure 2 Casadey *et. al.*

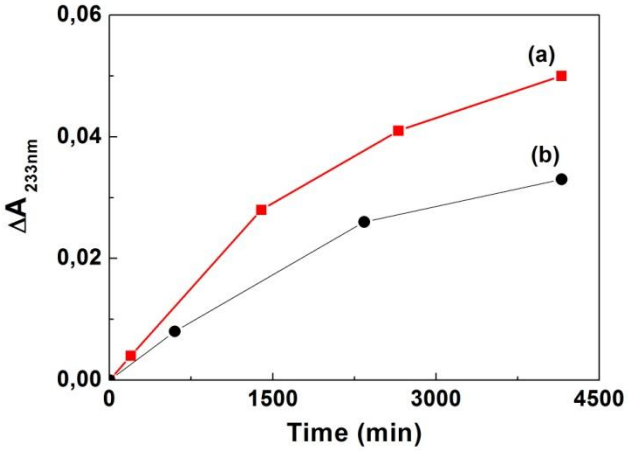


Figure 3 Casadey *et. al.*

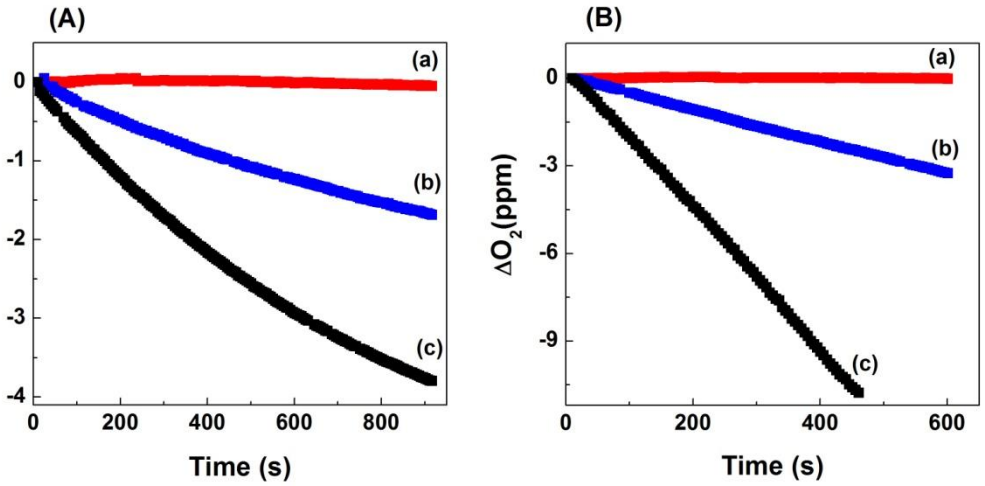


Figure 4 Casadey *et. al.*

Table 1: Rate constants for reactive (k_r) and overall (k_t) quenching of O_2 ($^1\Delta g$) by 4-hydroxyphenethyl alcohol (4-OH), ratio (k_r / k_t) and quantum yields of $O_2(^1\Delta_g)$ production (Φ_Δ).

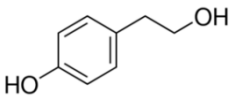
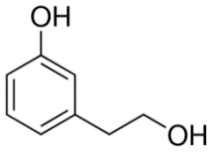
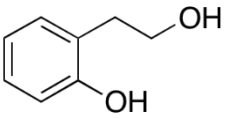
Compound	k_r x 10^{-7} (M^{-1} s^{-1}) **pH 7**	k_r x 10^{-7} (M^{-1} s^{-1}) **pH 12**	k_t x 10^{-7} (M^{-1} s^{-1}) **pD 7**	k_t x 10^{-7} (M^{-1} s^{-1}) **pD 12**	k_r/k_t **pH 7**	k_r/k_t **pH 12**	Φ_Δ
 **4-OH**	0.11±0.01	11±1	0.63±0.06	25±2	0.17	0.44	0.20±0.02

Table 2: Rate constants for reactive (k_r) and overall (k_t) quenching of O_2 ($^1\Delta g$) by 3-hydroxyphenethyl alcohol (3-OH) and 2-hydroxyphenethyl alcohol (2-OH), ratio (k_r / k_t) and quantum yields of $O_2(^1\Delta_g)$-production (Φ_Δ).

Compound	k_r x 10^{-7} (M^{-1} s^{-1}) **pH 7**	k_r x 10^{-7} (M^{-1} s^{-1}) **pH 11**	k_t x 10^{-7} (M^{-1} s^{-1}) **pD 7**	k_t x 10^{-7} (M^{-1} s^{-1}) **pD 11**	k_r/k_t **pH 7**	k_r/k_t **pH 11**	Φ_Δ
 **3-OH**	0.36±0.04	9.8±1.0	4.0±0.4	326±33	0.09	0.03	0.39±0.04
 **2-OH**	0.14±0.01	6.7±0.7	< 0.80	22±2	0.17	0.30	0.49±0.05

Highlights

Tyrosol and two isomers derived deactivate O_2 ($^1\Delta_g$) mainly through a physical process

The O_2 ($^1\Delta_g$) deactivation by the phenolic compounds is influenced by pH-medium

The phenolic compounds show a synergistic effect in presence of common antioxidants

The phenolic compounds could be efficient antioxidants against lipid peroxidation

Declarations of interest

Declarations of interest: none.