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1 Surface-Active Properties of Lipophilic Antioxidants

2 Tyrosol and Hydroxytyrosol Fatty Acid Esters: a

3 Potential Explanation for the Nonlinear Hypothesis of

4 the Antioxidant Activity in Oil-in-Water Emulsions

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11 Running Title. Surface-active properties of tyrosol and hydroxytyrosol esters

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2 **ABSTRACT**

3 Our group has recently observed a nonlinear tendency in antioxidant capacity of different
4 hydroxytyrosol fatty acid esters in fish oil-in-water emulsions, where a maximum of antioxidant efficiency
5 appeared for hydroxytyrosol octanoate. These results appear to disagree with the antioxidant polar
6 paradox. Since the physical location of the antioxidants in an oil-water interface has been postulated as an
7 important factor in explaining this behaviour, we have prepared a series of tyrosol and hydroxytyrosol
8 fatty acid esters with different chain length and studied their surface active properties in water, since
9 these physicochemical parameters could be directly related to the preferential placement at the interface.
10 We have found that tyrosol and hydroxytyrosol fatty acid esters are relevant surfactants when the right
11 hydrophilic-lipophilic balance (HLB) is attained, and in some cases, as efficient as emulsifiers commonly
12 used in industry, such as Brij 30® or Tween 20®. Moreover, a nonlinear dependency of surfactant
13 effectiveness is observed with the increase in chain length of the lipophilic antioxidants. This tendency
14 seems to fit quite well with the reported antioxidant activity in emulsions, and the best antioxidant of the
15 series (hydroxytyrosol octanoate) is also a very effective surfactant. This potential explanation of the
16 nonlinear hypothesis will help in the rational design of antioxidants used in oil-in-water emulsions.

17

18

19 **KEYWORDS** Tyrosol, hydroxytyrosol, antioxidants, lipophilic, emulsions, surface activity, surfactants.

20

1 INTRODUCTION

2
3 Lipid oxidation is still today an important problem for cosmetic and food industries. This is especially
4 relevant when the lipidic substrates are composed of unsaturated or poly-unsaturated fatty acids that are
5 very sensitive to oxidation processes (1, 2) such as in fish lipids. Lipid oxidation alters their organoleptic
6 properties (taste, odor, color and texture), depletes their nutritional properties and at the same time toxic
7 compounds are produced. This phenomenon is much more accentuated in oil-in-water emulsions since a
8 large interfacial area is produced during the emulsification process where the lipid oxidation has been
9 suggested to be initiated (3, 4).

10 Antioxidants have been used to control food oxidation for years. Their effectiveness depends on their
11 chemical reactivity (as radical scavengers or metal chelators), the interaction with other food
12 components, the environmental conditions (such as pH or concentration) and the physical location of the
13 antioxidant in different food systems (4-6). A general working hypothesis for antioxidant activity was
14 formulated two decades ago, the antioxidant polar paradox: hydrophilic antioxidants are more effective in
15 bulk oils whereas lipophilic antioxidants are more effective in systems of high surface-to-volume ratio,
16 such as emulsions, micelles or membranes (7, 8). This behaviour was explained by the concept of
17 interfacial oxidation (9). Accordingly, lipophilic antioxidants would have more affinity for the oil-water
18 interface in emulsions and therefore, would inhibit lipid oxidation more efficiently (10). In contrast, polar
19 antioxidants would concentrate into the air-oil interface in bulk oils and would be more efficient in this
20 type of matrices. An alternative explanation proposes that bulk lipids contain surface active minor
21 components (e.g., free fatty acids, monoacylglycerols) which form reverse micelles that stabilize water
22 droplets. In this scenario, the polar antioxidants are thought to aggregate at these microemulsion
23 droplets (11). Since many natural phenolic antioxidants are highly polar, their lipophilization could extend
24 their application in oil-based foods and cosmetics, and make them more efficient in emulsions.

1 Among the natural polyphenols, olive oil phenols, and particularly tyrosol and hydroxytyrosol, have
2 shown highly potent antioxidant activity in oils and oil-in-water emulsions, even higher than several
3 commonly used food antioxidants such as α -tocopherol, BHT or ascorbyl palmitate (12-14). Moreover,
4 hydroxytyrosol displays interesting biological properties such as inhibition of human low-density
5 lipoprotein (LDL) oxidation (a critical step in atherosclerosis) (15) and anticancer properties (16). Our
6 group and others have prepared lipophilic derivatives of hydroxytyrosol (17-20) and tyrosol (21). These
7 new compounds also display remarkable antioxidant capacity when tested in cell lines (17, 19) and in
8 food matrices such as oils and oil-in-water emulsions (18, 19, 22). The antioxidant polar paradox
9 explains the antioxidant capacity of these phenolic antioxidants from a general perspective but some
10 discrepancies were found especially in emulsions. An increase in the chain length of hydroxytyrosol fatty
11 acid esters correlated with an increase in antioxidant activity in a fish oil-in-water emulsion system but
12 only up to a certain length. In fact, hydroxytyrosol octanoate exhibited the highest antioxidant capacity,
13 higher than the butyl and the lauroyl hydroxytyrosol esters, indicating a nonlinear tendency (22). Indeed,
14 a “nonlinear trend hypothesis” has been recently proposed Laguerre et al. (23). They observed a
15 nonlinear dependency on antioxidant capacity in emulsion systems for chlorogenic acid alkyl esters where
16 maximum antioxidant efficiency was detected for the corresponding phenolic dodecyl ester (23, 24), and
17 also for rosmarinic acid alkyl esters where a maximum was detected for the corresponding phenolic octyl
18 ester (25). All these series of lipophilic phenolic antioxidants display a parabolic shape when antioxidant
19 capacity was plotted against alkyl chain length.

20 Our working hypothesis is based on the suggestion by Heins et al. (26) that an antioxidant with notable
21 surface-active properties would possess better ability to inhibit lipid oxidation in emulsions since it would
22 concentrate at the oil-in-water interface. Therefore, the antioxidant would act as a shield for the oil
23 placed in the interior of the micelle. The amphiphilic character of these recently called “phenolipids” (25)
24 could envisage certain surface-active properties that would lead to a non-ionic surfactant. In fact, it is
25 important to mention that different polar head groups have been used in non-ionic surfactants such as

1 carbohydrates and amino acids, leading to n-alkyl polyglucosides (27), sorbitan esters (28), sugar fatty
2 acid esters (29) and amino acid-based surfactants (30). Nevertheless, phenols have barely been used as
3 such polar heads of surfactants. To the best of our knowledge, the only examples where the polar head is
4 a phenol group are the alkyl esters of p-hydroxyphenylacetic acid (31) and, more recently, the
5 chlorogenic fatty acid esters (24). In both cases, a decrease of the interfacial tension in a water-
6 hexadecane interface has been described.

7 In this work we have prepared a series of tyrosol and hydroxytyrosol fatty acid esters and studied their
8 surfactive properties in water (**Figure 1**). Different chain lengths from C2 to C18 have been utilized in
9 the synthesis of the new derivatives in order to obtain a diversity of hydrophilic-lipophilic balance values
10 (HLB) and examine the tendency of surfactant efficiency with chain length. At the same time, this study
11 has allowed us to evaluate phenols as the polar head groups of non-ionic surfactants, and compare phenol
12 and di-orthophenol structures. Critical micelle concentration (CMC) and surface tension in water have
13 been analyzed and discussed.

14

15 **MATERIALS AND METHODS**

16

17 **Materials.** All chemicals were obtained from chemical suppliers and used without further purification,
18 unless otherwise noted. Vinyl alkyl esters were provided by TCI Chemicals. Tyrosol was provided by
19 Sigma-Aldrich and hydroxytyrosol was prepared from their corresponding carboxylic acid by reduction
20 with lithium aluminum hydride (32). Immobilized lipase Novozym435® was a gift from Novozymes. All
21 reactions were monitored by TLC on precoated Silica-Gel 60 plates F254, and detected by heating with
22 Mostain (500 ml of 10% H₂SO₄, 25g of (NH₄)₆Mo₇O₂₄• 4H₂O, 1g Ce(SO₄)₂•4H₂O). Products were
23 purified by flash chromatography with silica gel60 (200-400 mesh). NMR spectra were recorded on 300,
24 400 or 500 MHz NMR equipment, at room temperature for solutions in CDCl₃ or CD₃OD. Chemical
25 shifts are referred to the solvent signal and are expressed in ppm.

1 **Synthesis of tyrosol and hydroxytyrosol fatty acid esters.** Tyrosol fatty acid esters **3, 4** and **8** have
2 been previously described (21). Hydroxytyrosol fatty acid esters **9, 10, 12, 13, 14, 16** and **17** have been
3 previously described (17, 19, 20, 22). **General procedure for the synthesis of 3-17.** *Candida antarctica*
4 lipase (Novozym 435) (180 mg) was added to a mixture of tyrosol or hydroxytyrosol (1 eq) and the
5 acylating agent (20 eq.) in 45 mL of *t*-butyl methyl ether using a dry round bottom flask, and the mixture
6 was stirred for 60 min at 40 °C. The enzyme was decanted and separated. The solvent was evaporated
7 and the product was purified by flash column chromatography. Characterization data for the new
8 prepared compounds (**5, 6, 7, 11** and **15**) can be found in the supporting information.

9 **Surface tension and CMC determination.** Surface tension measurements were performed at 23 °C
10 by means of the Wilhelmy plate method in a Krüss K12 tensiometer. Samples were prepared by
11 successive dilutions of an initial concentrated solution. Prior to each surface tension measurement
12 samples were left 30 min in repose to attain the equilibrium. The possible aggregation properties of
13 tyrosol and hydroxytyrosol derivatives were evidenced from the adsorption isotherms obtained when
14 surface tension is plotted graphically against logarithm of concentration. The typical surfactant profile
15 consists in a linear decrease of the surface tension when the compound concentration increases, followed
16 by a surface tension stabilization when the concentration corresponding to the saturation of the interface
17 is attained. The intersection of the two linear portions in the graph determines the critical micelle
18 concentration (CMC).

19 **Determination of HLB values.** The hydrophilic-lipophilic balance (HLB) values were calculated
20 following the equation described by Griffin (33) for non-ionic surfactants. $HLB = 20 \times (\text{Hydrophilic}$
21 $\text{group molecular weight}) / (\text{surfactant molecular weight})$.

22 **Calculation of aggregation parameters.** The area occupied per molecule adsorbed at the water/air
23 interface (in Å²) can be obtained from the equation: $A = 10^{16} / N_A \cdot \Gamma$, where N_A is the Avogadro's
24 number and Γ is the adsorption at the saturated interface expressed in mol/cm², calculated according the
25 Gibbs equation: $\Gamma = -(dy/d\log C) / 2.303 n RT$, where n is the number of molecular species in solution ($n =$

- 1 1 for non-ionic compounds as in our case) and $(dy/d\log C)$ is the slope of the linear portion of the graph
- 2 before the CMC concentration.
- 3
- 4

1 **RESULTS**

2

3 **Preparation of tyrosol and hydroxytyrosol fatty acid esters.** Some of the compounds for both series
4 have been synthesized and characterized previously by our group and others (17, 19, 20), using an
5 organic acid catalyst, such as p-toluenesulfonic acid, or using an enzymatic catalyst such as a lipase. We
6 have prepared the full series by enzymatic acylation of tyrosol and hydroxytyrosol using immobilized
7 lipase B from *Candida antarctica* (Novozym435®). The reactions were carried out in tert-butylmethyl
8 ether and the acylating agents were the corresponding vinyl esters of the different alkyl chains. Yields
9 were very high in all cases after enzyme filtration and short column chromatography of the reaction
10 mixture. New compounds tyrosol hexanoate **5**, tyrosol octanoate **6**, tyrosol decanoate **7**, hydroxytyrosol
11 hexanoate **11** and hydroxytyrosol myristate **15** have been fully characterized by NMR spectroscopy and
12 mass spectrometry.

13 **Surface tension measurements for tyrosol fatty acid esters.** The graphs of surface tension/log of
14 compound concentration for the tyrosol series are represented in **Figure 2**. It can be observed that for
15 tyrosol **1**, tyrosol acetate **3** and tyrosol butyrate **4**, surface tension decreases but does not show a plateau.
16 Higher concentrations could not be tested since these samples become insoluble. Whereas these
17 compounds show surface activity reducing the surface tension, they do not behave as surfactants since no
18 self-aggregation (micelle formation) occurs. In contrast, when the alkyl chain length of the acyl group
19 increases, an adequate hydrophilic/lipophilic balance is attained and consequently, a typical curve shape
20 of surfactant is observed for tyrosol hexanoate **5**, octanoate **6** and decanoate **7**. Tyrosol laurate **8** showed
21 very low solubility in water, not allowing the corresponding surface tension measurements.

22 **Surface tension measurements for hydroxytyrosol fatty acid esters.** The representation of surface
23 tension/log of product concentration for the series of hydroxytyrosol fatty acid esters can be observed in
24 **Figure 3**. For these series even hydroxytyrosol esters with short alkyl chains (hydroxytyrosol acetate **9**
25 and hydroxytyrosol butyrate **10**) show a certain surface-active behaviour although the decrease in the

1 surface tension is just moderate. The most outstanding surfactant properties are shown by hydroxytyrosol
2 derivatives with acyl chain length between C6 and C12 (hydroxytyrosol hexanoate **11**, octanoate **12**,
3 decanoate **13** and laurate **14**).

4 **Physicochemical parameters for tyrosol and hydroxytyrosol fatty acid esters.** The
5 physicochemical parameters obtained for all tyrosol and hydroxytyrosol compounds are summarized in
6 **Table 1**. Besides the CMC values, the following data were also calculated: (1) the surface tension at the
7 CMC (γ_{cmc}) related to the surfactant effectiveness, (2) pC_{20} (corresponding to $-\log C_{20}$, being C_{20} the
8 necessary concentration to decrease in 20 unities the surface tension of the pure water, i.e. 52 mN/m)
9 related to the surfactant efficiency, (3) the maximum surfactant adsorption (Γ_{max}), and (4) the area
10 occupied per molecule in the saturated interface (A). Other data such as molecular weight and HLB
11 values are also included. Considering the tyrosol series, it can be observed that the effectiveness of
12 compounds **5** to **8** (in terms of the minimum surface tension available for the surfactant) are very similar,
13 being the best for tyrosol octanoate **6** and tyrosol decanoate **7** (41.1 and 41.5 mN/m, respectively). With
14 regard to the efficiency (in terms of the pC_{20} parameter), the larger the alkyl chain the higher are the pC_{20}
15 values, indicating that a lower concentration for tyrosol decanoate is required to decrease the surface
16 tension of the aqueous phase in 20 unities (52 mN/m).

17 Concerning the hydroxytyrosol series, it is remarkable that the best effectiveness values (γ_{cmc}),
18 observed for hydroxytyrosol octanoate **12** and hydroxytyrosol decanoate **13** (30.5 and 28.0 mN/m,
19 respectively, **Table 1**), are in the same range of those observed for commonly used non-ionic surfactants,
20 such as Brij 30®, Tween 20® or n-octyl glucoside (34-36). When longer fatty acids such as myristic acid
21 and palmitic acid are attached to hydroxytyrosol (compounds **15** and **16**, respectively), a surfactant
22 behaviour is observed but with a dramatic decrease in surfactant effectiveness (59.5 and 62.0 mN/m,
23 respectively, Table 1). Finally, hydroxytyrosol stearate **17** exhibited very low water-solubility and did not
24 decrease surface tension at any concentration.

25

26

1 DISCUSSION

2
3 Compounds of both series, tyrosol and hydroxytyrosol fatty acid esters, have been synthesized in high
4 yield using an enzymatic approach and the corresponding vinyl esters as acylating agents. All the new
5 compounds have been fully characterized (see supporting information).

6 The surface tension measurements carried out for the tyrosol and hydroxytyrosol fatty acid esters have
7 revealed that only at certain HLB values (between 8 and 11), these lipophilic phenolic antioxidants show
8 adequate surfactant properties. Therefore, these compounds could be considered as antioxidant
9 surfactants. Actually, scarce examples of antioxidants with surface active character have been described
10 so far, such as alkanoyl-6-O-ascorbic acid esters (37), alkyl ammonium ascorbate salts (38), tocopheryl
11 polyethylene glycol succinate (39) and BHT alkyl ammonium salts (40).

12 It can also be observed for both tyrosol and hydroxytyrosol series (**Table 1**) that an increase in the
13 length of the acyl alkyl chain leads to a decrease of CMC, similarly to other surfactants. When log CMC
14 is plotted against the number of carbons of the acyl alkyl chain (**Figure 4**) for compounds displaying the
15 optimal surfactant properties (appropriate HLB range), a continuous decrease close to linearity can be
16 observed as occurs for conventional surfactants. It can also be noticed that tyrosol derivatives show
17 lower values than the equivalent hydroxytyrosol ones. The reason must be the lower hydrophilic
18 character of tyrosol compounds, given that they have only one hydroxyl group in the aromatic ring
19 against the two of the hydroxytyrosol derivatives.

20 Concerning the surfactant effectiveness (γ_{cmc}), hydroxytyrosol fatty acid esters show better values than
21 their tyrosol ester homologues (**Table 1**). When the surfactant effectiveness (γ_{cmc}) is plotted against the
22 alkyl chain length for the tyrosol and hydroxytyrosol ester series (**Figure 5**), a parabolic shape with a
23 maximum around eight to ten carbon atoms can be observed, especially clear for the hydroxytyrosol
24 series. This nonlinear dependency seems to fit quite well with the nonlinear behaviour our group has
25 recently found for the antioxidant activity of the hydroxytyrosol ester series in a fish oil-in-water

1 emulsion system (22). The rate of oxidation was monitored by the formation of lipid oxidation products
2 (conjugated diene and triene hydroperoxides) during controlled sample storage. The antioxidant capacity
3 (measured as the percentage of inhibition in the formation of conjugated diene hydroperoxides respect to
4 the control on day 7) increased with the alkyl chain length in the series hydroxytyrosol **2** (65%),
5 hydroxytyrosol acetate **9** (73%), and hydroxytyrosol butyrate **10** (74%) to reach a maximum for
6 hydroxytyrosol octanoate **12** (92%) and then decreased for hydroxytyrosol laurate **14** (85%). Since the
7 phenolic antioxidant moiety is responsible for most of the antioxidant activity of these lipophilic
8 compounds, and this unit is the same for all the compounds of the series, it seems that the better location
9 on the oil-water interface in the emulsions would inhibit lipid oxidation more efficiently as suggested
10 previously (9, 23, 26). An effective surfactant would prefer to be placed right at the oil-water interface
11 (**Figure 6b**), the best place and with the best orientation of the phenolic moiety to counteract the effect
12 of the radicals that initiate the oxidation process. Most probably, the antioxidant surfactant would be
13 sharing this specific location with the emulsifier used to prepare the emulsion, producing some sort of
14 mixed micelles, as already proposed by Laguerre et al. (23) with a homologous series of chlorogenate
15 esters. If the phenolipid is not such a good surfactant and is more polar due to its short alkyl chain, it
16 would preferentially be placed in the aqueous solution (**Figure 6a**). This would probably mean that its
17 antioxidant activity would be less efficient since it is not close to the optimum location for the shielding
18 of the oil droplets from oxidation, the oil-water interface. Finally, if the phenolipid is not such a good
19 surfactant and is quite apolar due to its long alkyl chain, it would most probably be placed at the interior
20 of the emulsion dissolved in the oil droplet (**Figure 6c**) and therefore far from the shielding layer. This
21 reasoning will take us to hypothesize that a good antioxidant for oil-in-water emulsions should be, at the
22 same time, a good antioxidant and an effective surfactant.

23 The nonlinear dependency observed for the antioxidant activity of chlorogenic acid alkyl esters in
24 emulsions, showing a maximum for the dodecyl ester derivative is a similar case (23). Here, dodecyl
25 chlorogenate is the best antioxidant most probably because is the best surfactant of the series and

1 therefore, is mainly placed at the oil-water interface. A similar reasoning would apply for the rosmarinic
2 acid alkyl esters where the maximum was observed for the phenolic octyl ester derivative (25). The
3 reason why there are different cutoff chain lengths could be due to the fact that to be an effective
4 surfactant depends on different parameters that may vary for each specific series of phenolipids, such as
5 the optimum HLB value or the specific polarity and geometry of the polar head. Other aspects may also
6 be important since the maximum observed for the chlorogenic acid alkyl esters in emulsions (dodecyl
7 chlorogenate) changes to the octyl chlorogenate when no methanol is added to the emulsion system and
8 different emulsifier concentration is employed (24).

9 The same authors have tried to correlate the nonlinear trend with the surfactant properties of these
10 lipophilic phenolic antioxidants measuring the decrease in the interfacial tension in a water-hexadecane
11 interface (24, 31). The results of these experiments give information on the actual capacity to produce an
12 emulsion, but they may not be a good indicator of the statistically more probable location of the
13 phenolipid in an emulsion that has already been formed with a specific emulsifier where the antioxidant is
14 added in a final step.

15 Partition coefficient measurements have been previously carried out for hydroxytyrosol fatty acid esters
16 (22), chlorogenic acid alkyl esters (23) and rosmarinic acid alkyl esters (25) trying to show a relation
17 with their antioxidant activity. In all the cases, a linear correlation between alkyl chain length and
18 phenolic concentration in the aqueous phase or with polarity was observed.

19 In conclusion, these data reveal that potent antioxidants such as tyrosol and hydroxytyrosol fatty acid
20 esters are relevant surfactants when the right hydrophilic-lipophilic balance (HLB) is attained, around 8-
21 11 in this specific case. Hydroxytyrosol fatty acid esters, which are better antioxidants than their tyrosol
22 homologues, have also shown better surfactant properties. Discussion of the results on surfactant
23 effectiveness of hydroxytyrosol fatty acid esters to the light of our previous results on their antioxidant
24 activity in fish oil-in-water emulsions, invite us to propose intrinsic surface-active properties of these
25 compounds, represented by this physicochemical parameter, to explain the nonlinear dependency found

1 when relating phenolipids hydrophobicity with their antioxidant activity in emulsions. This new
2 hypothesis will help in the rational design of antioxidants used in oil-in-water emulsions, where a good
3 antioxidant should also be an effective surfactant. At the same time, it may help us to better understand
4 basic aspects of the behaviour of lipophilic phenolic antioxidants in emulsions. These results open up
5 potential new applications for these surfactant antioxidants in the food, pharmaceutical or personal care
6 industries.

7

8 **ACKNOWLEDGMENT**

9 This work was supported by the Agencia Estatal Consejo Superior de Investigaciones Científicas
10 (CSIC) with an Intramural Frontier Project (200680F0132) and a JAE-Doc contract (RL).

11

1 **FIGURE LEGENDS**

2

3 Figure 1. Chemical structures of tyrosol, hydroxytyrosol and their corresponding fatty acid esters.

4 Figure 2. Surface tension vs log of concentration plots for the series of tyrosol fatty acid esters.

5 Figure 3. Surface tension vs log of concentration plots for the series of hydroxytyrosol fatty acid esters.

6 Figure 4. Relationship between log of CMC and the length of the acyl alkyl chain in the tyrosol (▲) and
7 hydroxytyrosol (◆) ester series for compounds displaying the optimal surfactant properties.

8 Figure 5. Surfactant effectiveness vs the length of the acyl alkyl chain in the tyrosol (▲) and
9 hydroxytyrosol (◆) ester series for compounds displaying surfactant properties.

10 Figure 6. Putative scheme of the preferential location of the antioxidants in an emulsified system (inspired
11 by the previous proposal by Laguerre et al.(23)).

Figure 1.

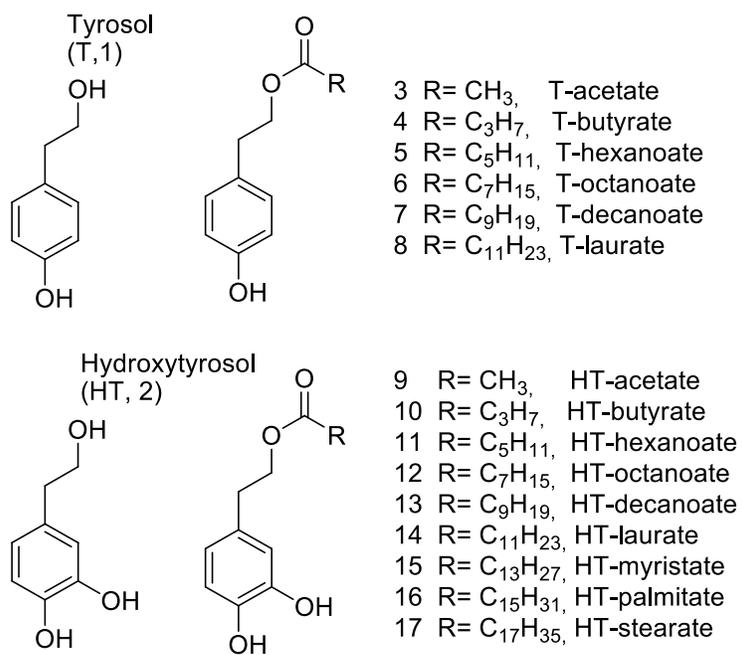


Figure 2.

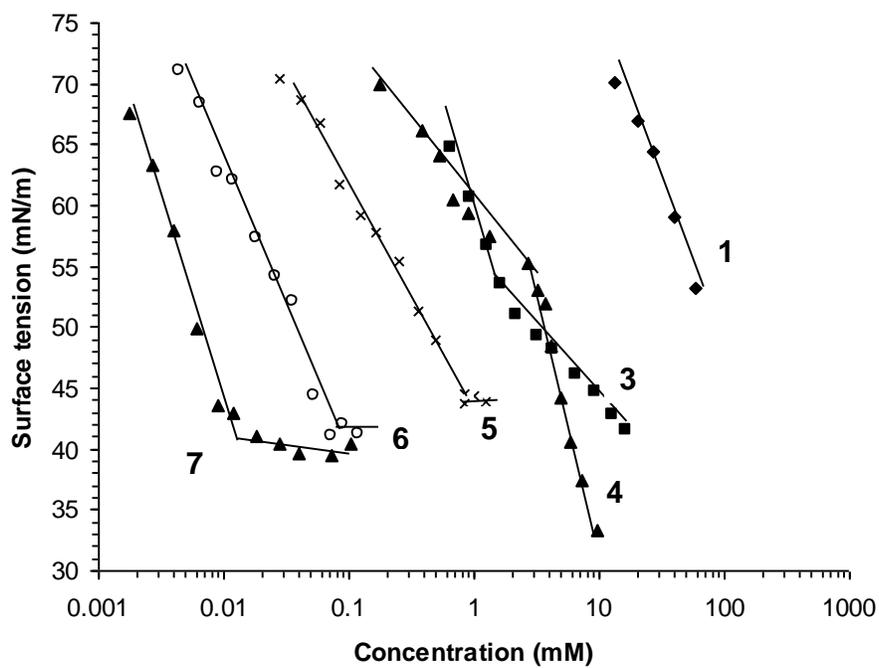


Figure 3.

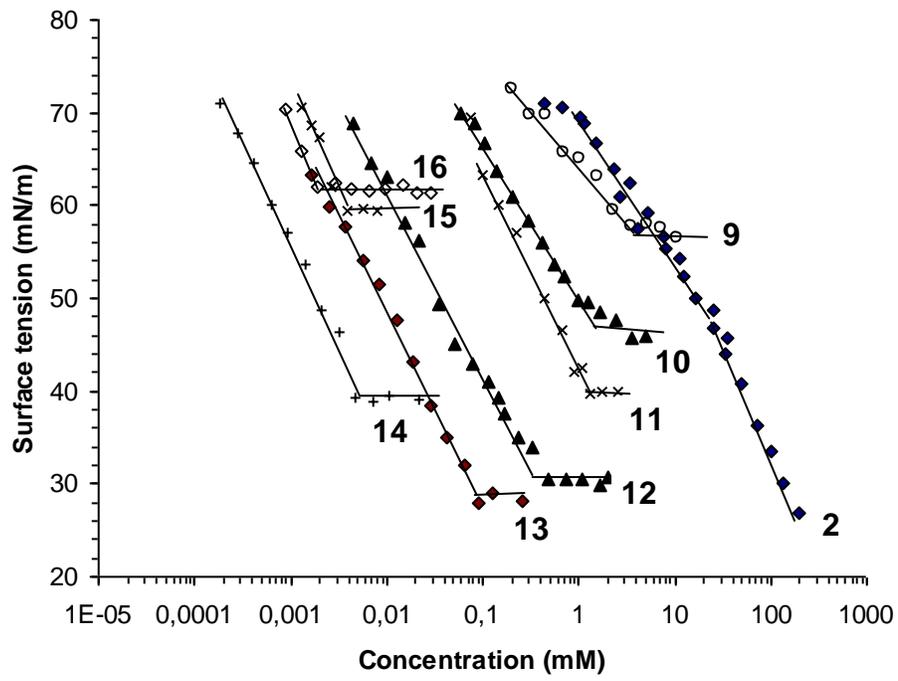


Figure 4.

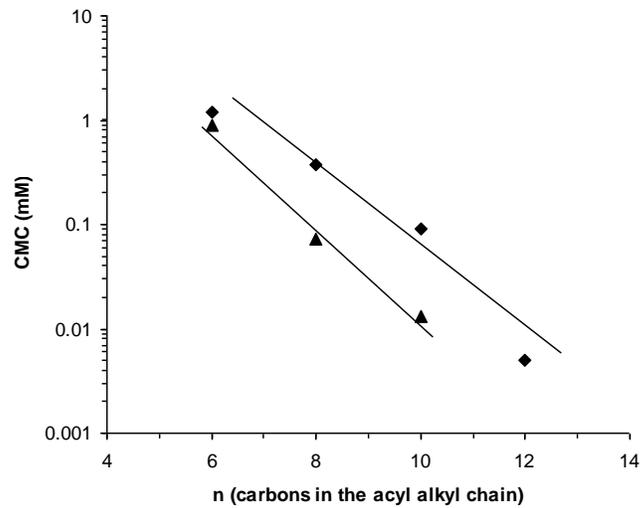


Figure 5.

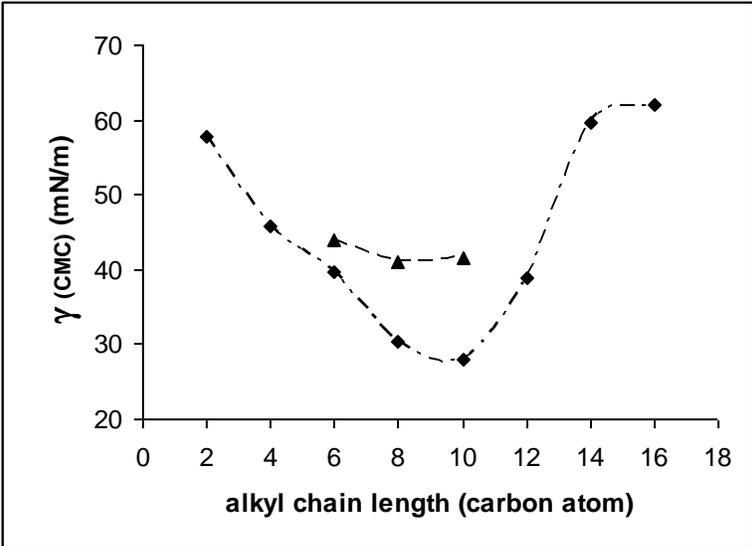


Figure 6.

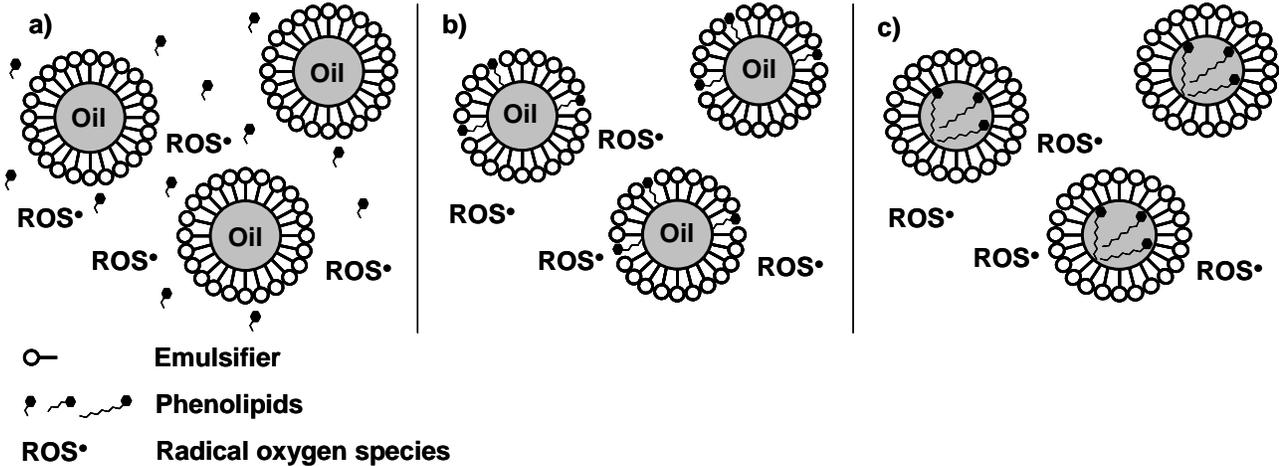


Table 1. MW, HLB, CMC, surface tension at the CMC, C_{20} , pC_{20} , Γ and area per molecule of prepared tyrosol and hydroxytyrosol fatty acid esters and several common non-ionic surfactants.

Compound	MW	HLB	CMC(mM)	γ_{cmc} (mN/m)	C_{20} (mM)	pC_{20}	Γ (mol/cm ²)	A (Å ²)
T hexanoate (5)	236.3	10.2	0.9	44.0	0.32	3.49	3.240 x 10 ⁻¹⁰	51.3
T octanoate (6)	264.4	9.2	0.073	41.1	0.029	4.54	4.712 x 10 ⁻¹⁰	35.3
T decanoate (7)	292.4	8.3	0.013	41.5	0.0055	5.26	5.175 x 10 ⁻¹⁰	32.0
HT acetate (9)	196.2	14.0	3.0	57.7	-	-	2.187 x 10 ⁻¹⁰	76.0
HT butyrate (10)	224.3	12.2	1.5	45.8	0.75	3.12	3.50 x 10 ⁻¹⁰	47.5
HT hexanoate (11)	252.3	10.9	1.2	39.6	0.34	3.47	3.91 x 10 ⁻¹⁰	42.5
HT octanoate (12)	280.4	9.8	0.38	30.5	0.03	4.52	3.43 x 10 ⁻¹⁰	48.4
HT decanoate (13)	308.4	8.9	0.09	28.0	0.007	5.15	4.21 x 10 ⁻¹⁰	39.4
HT laurate (14)	336.5	8.1	0.0055	39.0	0.0015	5.82	3.97 x 10 ⁻¹⁰	41.9
HT myristate (15)	364.5	7.5	0.0035	59.5	-	-	4.034 x 10 ⁻¹⁰	41.2
HT palmitate (16)	392.6	7.0	0.002	62.0	-	-	4.286 x 10 ⁻¹⁰	38.8
Brij 30 ® (Polyoxyethylene lauryl ether) ^a	362.5	9.4	0.0035	30.0	0.0024	5.62	3.80 x 10 ⁻¹⁰	44.0
Tween 20 ® (polyoxyethylene sorbitan monolaurate) ^{b,d}	1227.5	16.6	0.0169	35.0	0.0025	5.61	3.560 x 10 ⁻¹⁰	46.6
n-octyl glucoside ^{c,d}	292.4	11.1	25	~30.0	2.792	2.55	4.0 x 10 ⁻¹⁰	41.0

^a From ref. 33. ^b From ref. 34. ^c From ref. 35. ^d C_{20} and pC_{20} were calculated as noted in the experimental section.

LITERATURE CITED

1. Flick, G. F.; Martin, J., Lipid oxidation in food. In *Advances in seafood biochemistry*, Company, T. P., Ed. 1992.
2. Hsieh, R. J.; Kinsella, J. E., Oxidation of polyunsaturated fatty acids: mechanisms, products, and inhibition with emphasis on fish. *Adv Food Nutr Res* **1989**, 33, 233-341.
3. Frankel, E. N., *Lipid oxidation*. Oily Press: Dundee, Scotland, 1998.
4. McClements, D. J.; Decker, E. A., Lipid Oxidation in Oil-in-Water Emulsions: Impact of Molecular Environment on Chemical Reactions in Heterogeneous Food Systems. *J Food Sci* **2000**, 65, (8), 1270-1282.
5. Decker, E. A.; Warner, K.; Richards, M. P.; Shahidi, F., Measuring Antioxidant Effectiveness in Food. *J Agric Food Chem* **2005**, 53, (10), 4303-4310.
6. Rice-Evans, C.; Miller, N.; Paganga, G., Antioxidant properties of phenolic compounds. *Trends Plant Sci* **1997**, 2, (4), 152-159.
7. Porter, W. L., Recent trends in food applications of antioxidants. In *Autoxidation in food and biological systems*, Press, P., Ed. New York, 1980; pp 295-365.
8. Porter, W. L.; Black, E. D.; Drolet, A. M., Use of polyamide oxidative fluorescence test on lipid emulsions: contrast in relative effectiveness of antioxidants in bulk versus dispersed systems. *J Agric Food Chem* **1989**, 37, (3), 615-624.
9. Frankel, E. N.; Huang, S.-W.; Kanner, J.; German, J. B., Interfacial Phenomena in the Evaluation of Antioxidants: Bulk Oils vs Emulsions. *J. Agric. Food Chem.* **1994**, 42, (5), 1054-1059.

10. Figueroa-Espinoza, M. C.; Villeneuve, P., Phenolic acids enzymatic lipophilization. *J Agric Food Chem* **2005**, *53*, (8), 2779-87.
11. Chaayasit, W.; Elias, R. J.; McClements, D. J.; Decker, E. A., Role of physical structures in bulk oils on lipid oxidation. *Crit. Rev. Food Sci. Nutr.* **2007**, *47*, (3), 299-317.
12. Mateos, R.; Dominguez, M. M.; Espartero, J. L.; Cert, A., Antioxidant effect of phenolic compounds, alpha-tocopherol, and other minor components in virgin olive oil. *J Agric Food Chem* **2003**, *51*, (24), 7170-5.
13. Pazos, M.; Alonso, A.; Sánchez, I.; Medina, I., Hydroxytyrosol Prevents Oxidative Deterioration in Foodstuffs Rich in Fish Lipids. *J Agric Food Chem* **2008**, *56*, (9), 3334-3340.
14. Torres de Pinedo, A.; Peñalver, P.; Morales, J. C., Synthesis and evaluation of new phenolic-based antioxidants: structure-activity relationship. *Food Chem* **2007**, *103*, 55-61.
15. Visioli, F.; Bellomo, G.; Montedoro, G.; Galli, C., Low density lipoprotein oxidation is inhibited in vitro by olive oil constituents. *Atherosclerosis* **1995**, *117*, (1), 25-32.
16. Owen, R. W.; Giacosa, A.; Hull, W. E.; Haubner, R.; Wurtele, G.; Spiegelhalder, B.; Bartsch, H., Olive-oil consumption and health: the possible role of antioxidants. *Lancet Oncol* **2000**, *1*, 107-12.
17. Grasso, S.; Siracusa, L.; Spatafora, C.; Renis, M.; Tringali, C., Hydroxytyrosol lipophilic analogues: enzymatic synthesis, radical scavenging activity and DNA oxidative damage protection. *Bioorg Chem* **2007**, *35*, (2), 137-52.
18. Torres de Pinedo, A.; Peñalver, P.; Pérez-Victoria, I.; Rondón, D.; Morales, J. C., Synthesis of new phenolic fatty acid esters and their evaluation as lipophilic antioxidants in an oil matrix. *Food Chem* **2007**, *105*, 657-665.
19. Trujillo, M.; Mateos, R.; Collantes de Teran, L.; Espartero, J. L.; Cert, R.; Jover, M.; Alcudia, F.; Bautista, J.; Cert, A.; Parrado, J., Lipophilic hydroxytyrosyl esters. Antioxidant activity in lipid matrices and biological systems. *J Agric Food Chem* **2006**, *54*, (11), 3779-85.
20. Torres de Pinedo, A.; Peñalver, P.; Rondón, D.; Morales, J. C., Efficient lipase-catalyzed synthesis of new antioxidants based on a catechol structure. *Tetrahedron* **2005**, *61*, 7654-7660.
21. Mateos, R.; Trujillo, M.; Pereira-Caro, G.; Madrona, A.; Cert, A.; Espartero, J. L., New lipophilic tyrosyl esters. Comparative antioxidant evaluation with hydroxytyrosyl esters. *J Agric Food Chem* **2008**, *56*, (22), 10960-6.
22. Medina, I.; Lois, S.; Alcantara, D.; Lucas, R.; Morales, J. C., Effect of Lipophilization of Hydroxytyrosol on Its Antioxidant Activity in Fish Oils and Fish Oil-in-Water Emulsions. *J Agric Food Chem* **2009**.
23. Laguerre, M.; López Giraldo, L. J.; Lecomte, J.; Figueroa-Espinoza, M.-C.; Baréa, B.; Weiss, J.; Decker, E. A.; Villeneuve, P., Chain Length Affects Antioxidant Properties of Chlorogenate Esters in Emulsion: The Cutoff Theory Behind the Polar Paradox. *J Agric Food Chem* **2009**, *57*, (23), 11335-11342.
24. Sasaki, K.; Alamed, J.; Weiss, J.; Villeneuve, P.; López Giraldo, L. J.; Lecomte, J.; Figueroa-Espinoza, M.-C.; Decker, E. A., Relationship between the physical properties of chlorogenic acid esters and their ability to inhibit lipid oxidation in oil-in-water emulsions. *Food Chem* **2010**, *118*, (3), 830-835.
25. Laguerre, M.; López Giraldo, L. J.; Lecomte, J.; Figueroa-Espinoza, M.-C.; Baréa, B.; Weiss, J.; Decker, E. A.; Villeneuve, P., Relationship between Hydrophobicity and Antioxidant Ability of "Phenolipids" in Emulsion: A Parabolic Effect of the Chain Length of Rosmarinate Esters. *J Agric Food Chem* **2010**, *58*, (5), 2869-2876.
26. Heins, A.; McPhail, D.; Sokolowski, T.; Stöckmann, H.; Schwarz, K., The Location of Phenolic Antioxidants and Radicals at Interfaces Determines Their Activity. *Lipids* **2007**, *42*, (6), 573-582.
27. von Rybinski, W.; Hill, K., Alkyl Polyglycosides - Properties and Applications of a new Class of Surfactants. *Angew. Chem. Int. Ed.* **1998**, *37*, (10), 1328-1345.
28. Cottrell, T.; van Peij, J., Sorbitan esters and polysorbates. In *Emulsifiers in Food Technology*, Whitehurst, R. J., Ed. Wiley-Blackwell: 2004; pp 162-185.

29. Plat, T.; Linhardt, R., Syntheses and applications of sucrose-based esters. *J. Surfactants Deterg.* **2001**, 4, (4), 415-421.
30. Moran, M. C.; Pinazo, A.; Perez, L.; Clapes, P.; Angelet, M.; Garcia, M. T.; Vinardell, M. P.; Infante, M. R., "Green" amino acid-based surfactants. *Green Chem.* **2004**, 6, (5), 233-240.
31. Yuji, H.; Weiss, J.; Villeneuve, P.; Lopez Giraldo, L. J.; Figueroa-Espinoza, M. C.; Decker, E. A., Ability of surface-active antioxidants to inhibit lipid oxidation in oil-in-water emulsion. *J Agric Food Chem* **2007**, 55, (26), 11052-6.
32. Capasso, R.; Evidente, A.; Avolio, S.; Solla, F., A highly convenient synthesis of hydroxytyrosol and its recovery from agricultural waste waters. *J Agric Food Chem* **1999**, 47, (4), 1745-8.
33. Griffin, W. C., Calculation of HLB values for non-ionic surfactants. *J. Soc. Cosmet. Chem.* **1954**, 5, 259-267.
34. Rosen, M. J., *Surfactant and Interfacial Phenomena*. Wiley and Sons: 1978.
35. Niño, M.; Patino, J., Surface tension of bovine serum albumin and tween 20 at the air-aqueous interface. *J. Am. Oil Chem. Soc.* **1998**, 75, (10), 1241-1248.
36. Shinoda, K.; Yamanaka, T.; Kinoshita, K., Surface chemical properties in aqueous solutions of nonionic surfactants: octyl glycol ether, α -octyl glyceryl ether and octyl glucoside. *J. Phys. Chem.* **1959**, 63, 648-650.
37. Palma, S.; Manzo, R. H.; Allemandi, D.; Fratoni, L.; Lo Nostro, P., Drugs solubilization in ascorbyl-decanoate micellar solutions. *Colloid Surf. A: Physicochem. Eng. Aspects* **2003**, 212, (2-3), 163-173.
38. Mounanga, T. K.; Gérardin, P.; Poaty, B.; Perrin, D.; Gérardin, C., Synthesis and properties of antioxidant amphiphilic ascorbate salts. *Colloid Surf. A: Physicochem. Eng. Aspects* **2008**, 318, (1-3), 134-140.
39. Yan, A.; Von Dem Bussche, A.; Kane, A. B.; Hurt, R. H., Tocopheryl polyethylene glycol succinate as a safe, antioxidant surfactant for processing carbon nanotubes and fullerenes. *Carbon* **2007**, 45, (13), 2463-2470.
40. Przystalski, S.; Hladyszowski, J.; Kuczera, J.; Rózycka-Roszak, B.; Z. Trela, H. C.; Witek, S.; Fiscaro, E., Interaction between model membranes and a new class of surfactants with antioxidant function *Biophys. J.* **1996**, Vol. 70, (5), 2203-2211.