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Surface-Active Properties of Lipophilic Antioxidants Tyrosol and Hydroxytyrosol Fatty Acid Esters: A Potential Explanation for the Nonlinear Hypothesis of the Antioxidant Activity in Oil-in-Water Emulsions

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

KEYWORDS: Tyrosol; hydroxytyrosol; antioxidants; lipophilic; emulsions; surface activity; surfactants

INTRODUCTION

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

Antioxidants have been used to control food oxidation for years. Their effectiveness depends upon their chemical reactivity (as radical scavengers or metal chelators), interaction with other food components, environmental conditions (such as pH or concentration), and physical location of the antioxidant in different food systems (*4*−*6*). A general working hypothesis for antioxidant activity was formulated 2 decades ago, the antioxidant polar paradox: hydrophilic antioxidants are more effective in bulk oils, whereas lipophilic antioxidants are more effective in systems of high surface/volume ratio, such as emulsions, micelles, or membranes (*7*, *8*). This behavior was explained by the concept of interfacial oxidation (*9*). Accordingly, lipophilic antioxidants would have more affinity for the oil−water interface in emulsions and, therefore, would inhibit lipid oxidation more efficiently (*10*). In contrast, polar antioxidants would concentrate into the air−oil interface in bulk oils and would be more efficient in this type of matrix. An alternative explanation proposes that bulk lipids contain surface-active minor components (e.g., free fatty acids and monoacylglycerols), which form reverse micelles that stabilize water droplets. In this scenario, the polar antioxidants are thought to aggregate at these microemulsion droplets (*11*). Because many natural phenolic antioxidants are highly polar, their lipophilization could extend their application in oil-based foods and cosmetics and make them more efficient in emulsions.

Among the natural polyphenols, olive oil phenols and, particularly, tyrosol and hydroxytyrosol have shown highly potent antioxidant activity in oils and oil-in-water emulsions, even

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

Our working hypothesis is based on the suggestion by Heins et al. (*26*) that an antioxidant with notable surface-active properties would possess better ability to inhibit lipid oxidation in emulsions because it would concentrate at the oil−water interface. Therefore, the antioxidant would act as a shield for the oil placed in the interior of the micelle. The amphiphilic character of these recently called "phenolipids" (*25*) could envisage certain surface-active properties that would lead to a non-ionic surfactant. In fact, it is important to mention that different polar head groups have been used in non-ionic surfactants, such as carbohydrates and amino acids, leading to *n*-alkyl polyglucosides (*27*), sorbitan esters (*28*), sugar fatty acid esters (*29*), and amino-acid-based surfactants (*30*). Nevertheless, phenols have barely been used as such polar heads of surfactants. To the best of our knowledge, the only examples where the polar head is a phenol group are the alkyl esters of *p*-hydroxyphenylacetic acid (*31*) and, more recently, the chlorogenic fatty acid esters (*24*). In both cases, a decrease of the interfacial tension in a water−hexadecane interface has been described.

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

MATERIALS AND METHODS

Materials. All chemicals were obtained from chemical suppliers and used without further purification, unless otherwise noted. Vinyl alkyl esters were provided by TCI Chemicals. Tyrosol was provided by Sigma-Aldrich, and hydroxytyrosol was prepared from their corresponding carboxylic acid by reduction with lithium aluminum hydride (*32*).

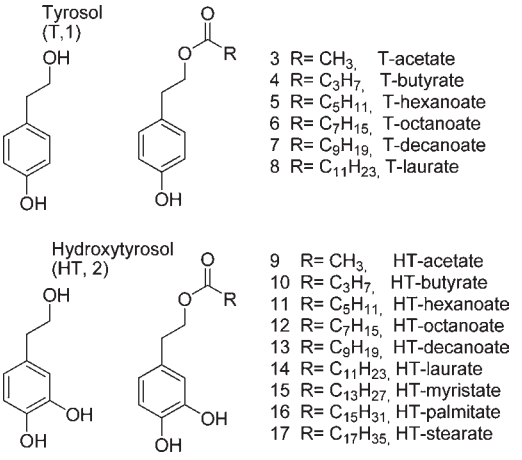

Figure 1. Chemical structures of tyrosol, hydroxytyrosol, and their corresponding fatty acid esters.

Immobilized lipase Novozym435 was a gift from Novozymes. All reactions were monitored by thin-layer chromatography (TLC) on precoated silica-gel 60 plates F254 and detected by heating with Mostain (500 mL of 10% H_2SO_4, 25 g of $(NH_4)_6Mo_7O_{24}\bullet 4H_2O$, and 1 g of $Ce(SO_4)_2\bullet 4H_2O$). Products were purified by flash chromatography with silica gel 60 (200−400 mesh). Nuclear magnetic resonance (NMR) spectra were recorded on 300, 400, or 500 MHz NMR equipment, at room temperature for solutions in $CDCl_3$ or CD_3OD. Chemical shifts are referred to the solvent signal and are expressed in parts per million (ppm).

Synthesis of Tyrosol and Hydroxytyrosol Fatty Acid Esters. Tyrosol fatty acid esters **3**, **4**, and **8** have been previously described (*21*). Hydroxytyrosol fatty acid esters **9**, **10**, **12**, **13**, **14**, **16**, and **17** have been previously described (*17*, *19*, *20*, *22*).

General Procedure for the Synthesis of 3−17. *Candida antarctica* lipase (Novozym435) (180 mg) was added to a mixture of tyrosol or hydroxytyrosol (1 equiv) and the acylating agent (20 equiv) in 45 mL of *t*-butyl methyl ether using a dry round-bottom flask, and the mixture was stirred for 60 min at 40 °C. The enzyme was decanted and separated. The solvent was evaporated, and the product was purified by flash column chromatography. Characterization data for the new prepared compounds (**5**, **6**, **7**, **11**, and **15**) can be found in the Supporting Information.

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

Determination of HLB Values. The HLB values were calculated following the equation described by Griffin (*33*) for non-ionic surfactants. HLB = 20(hydrophilic group molecular weight)/(surfactant molecular weight).

Calculation of Aggregation Parameters. The area occupied per molecule adsorbed at the water−air interface (in $Å^2$) can be obtained from the equation: $A = 10^{16}/N_A\Gamma$, where N_A is Avogadro's number and Γ is the adsorption at the saturated interface expressed in mol/cm^2, calculated according the Gibbs equation: $\Gamma = -(d\gamma/d \log C)/2.303nRT$, where n is the number of molecular species in solution ($n = 1$ for non-ionic compounds, as in our case) and $(d\gamma/d \log C)$ is the slope of the linear portion of the graph before the cmc.

RESULTS

Preparation of Tyrosol and Hydroxytyrosol Fatty Acid Esters. Some of the compounds for both series have been synthesized and

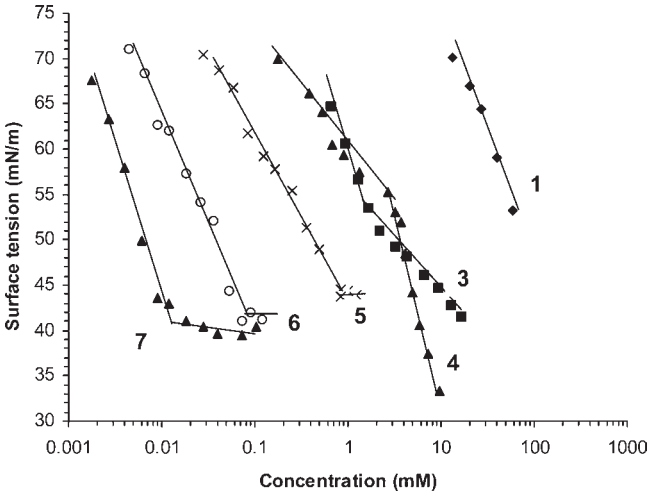

Figure 2. Surface tension versus log of the concentration plots for the series of tyrosol fatty acid esters.

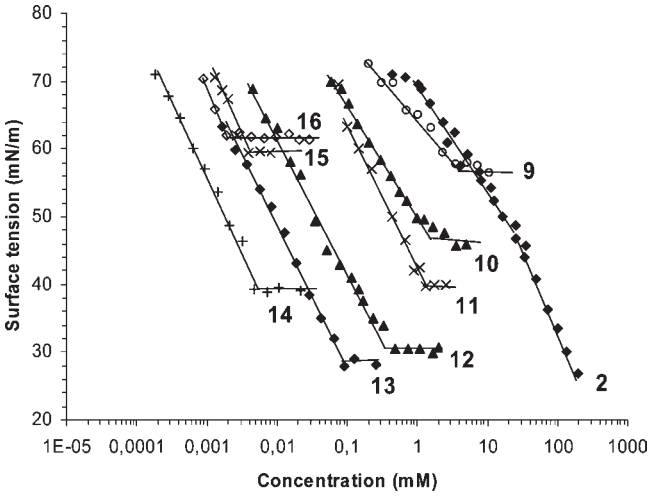

Figure 3. Surface tension versus log of the concentration plots for the series of hydroxytyrosol fatty acid esters.

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

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

Surface Tension Measurements for Hydroxytyrosol Fatty Acid Esters. The representation of surface tension/log of the product concentration for the series of hydroxytyrosol fatty acid esters can be observed in **Figure 3**. For these series, even hydroxytyrosol esters with short alkyl chains (hydroxytyrosol acetate **9** and hydroxytyrosol butyrate **10**) show a certain surface-active behavior, although the decrease in the surface tension is just moderate. The most outstanding surfactant properties are shown by hydroxytyrosol derivatives with acyl chain length between C6 and C12 (hydroxytyrosol hexanoate **11**, octanoate **12**, decanoate **13**, and laurate **14**).

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

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

DISCUSSION

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

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

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	3.240×10^{-10}	51.3
T octanoate (**6**)	264.4	9.2	0.073	41.1	0.029	4.54	4.712×10^{-10}	35.3
T decanoate (**7**)	292.4	8.3	0.013	41.5	0.0055	5.26	5.175×10^{-10}	32.0
HT acetate (**9**)	196.2	14.0	3.0	57.7			2.187×10^{-10}	76.0
HT butyrate (**10**)	224.3	12.2	1.5	45.8	0.75	3.12	3.50×10^{-10}	47.5
HT hexanoate (**11**)	252.3	10.9	1.2	39.6	0.34	3.47	3.91×10^{-10}	42.5
HT octanoate (**12**)	280.4	9.8	0.38	30.5	0.03	4.52	3.43×10^{-10}	48.4
HT decanoate (**13**)	308.4	8.9	0.09	28.0	0.007	5.15	4.21×10^{-10}	39.4
HT laurate (**14**)	336.5	8.1	0.0055	39.0	0.0015	5.82	3.97×10^{-10}	41.9
HT myristate (**15**)	364.5	7.5	0.0035	59.5			4.034×10^{-10}	41.2
HT palmitate (**16**)	392.6	7.0	0.002	62.0			4.286×10^{-10}	38.8
Brij 30 (polyoxyethylene (**4**) lauryl ether)[a]	362.5	9.4	0.0035	30.0	0.0024	5.62	3.80×10^{-10}	44.0
Tween 20 (polyoxyethylene sorbitan monolaurate)[b,c]	1227.5	16.6	0.0169	35.0	0.0025	5.61	3.560×10^{-10}	46.6
n-octyl glucoside[c,d]	292.4	11.1	25	~30.0	2.792	2.55	4.0×10^{-10}	41.0

[a] From ref *34*. [b] From ref *35*. [c] C_{20} and pC_{20} were calculated as noted in the Materials and Methods. [d] From ref *36*.

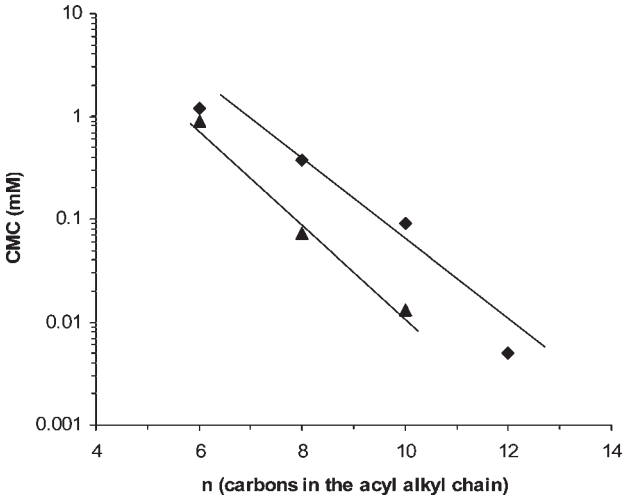

Figure 4. Relationship between log of cmc and the length of the acyl alkyl chain in the tyrosol (▲) and hydroxytyrosol (◆) ester series for compounds displaying the optimal surfactant properties.

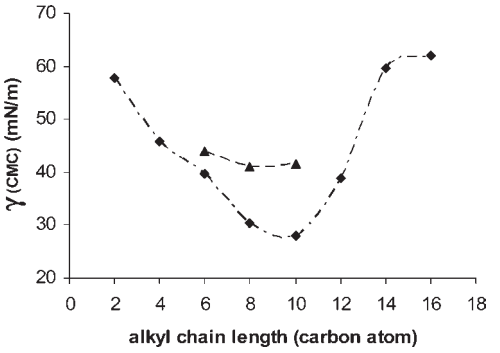

Figure 5. Surfactant effectiveness versus the length of the acyl alkyl chain in the tyrosol (▲) and hydroxytyrosol (◆) ester series for compounds displaying surfactant properties.

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

Concerning the surfactant effectiveness (γ_{cmc}), hydroxytyrosol fatty acid esters show better values than their tyrosol ester homologues (**Table 1**). When the surfactant effectiveness (γ_{cmc}) is plotted against the alkyl chain length for the tyrosol and hydroxytyrosol ester series (**Figure 5**), a parabolic shape with a maximum around 8−10 carbon atoms can be observed, especially clear for the hydroxytyrosol series. This nonlinear dependency seems to fit quite well with the nonlinear behavior our group has recently found for the antioxidant activity of the hydroxytyrosol ester series in a fish oil-in-water emulsion system (*22*). The rate of oxidation was monitored by the formation of lipid oxidation products (conjugated diene and triene hydroperoxides) during controlled sample storage. The antioxidant capacity (measured as the percentage of inhibition in the formation of conjugated diene hydroperoxides with respect to the control on day 7) increased with the alkyl chain length in the series hydroxytyrosol **2** (65%), hydroxytyrosol acetate **9** (73%), and hydroxytyrosol butyrate **10** (74%) to reach a maximum for hydroxytyrosol octanoate **12** (92%) and then decreased for hydroxytyrosol laurate **14** (85%). Because the phenolic antioxidant moiety is responsible for most of the antioxidant activity of these lipophilic compounds and this unit is the same for all of the compounds of the series, it seems that the better location on the oil−water interface in the emulsions would inhibit lipid oxidation more efficiently, as suggested previously (*9*, *23*, *26*). An effective surfactant would prefer to be placed right at the oil−water interface (**Figure 6b**), the best place and with the best orientation of the phenolic moiety to counteract the effect of the radicals that initiate the oxidation process. Most likely, the antioxidant surfactant would be sharing this specific location with the emulsifier used to prepare the emulsion, producing some sort of mixed micelles, as already proposed by Laguerre et al. (*23*) with a homologous series of chlorogenate esters. If the phenolipid is not such a good surfactant and is more polar because of its short alkyl chain, it would preferentially be placed in the aqueous solution (**Figure 6a**). This would probably mean that its antioxidant activity would be less efficient because it is not close to the optimum location for the shielding of the oil droplets from oxidation, the oil−water interface. Finally, if the phenolipid is not such a good surfactant and is quite apolar because of its long alkyl chain, it would most likely be placed at the interior of the emulsion dissolved in the oil droplet (**Figure 6c**)

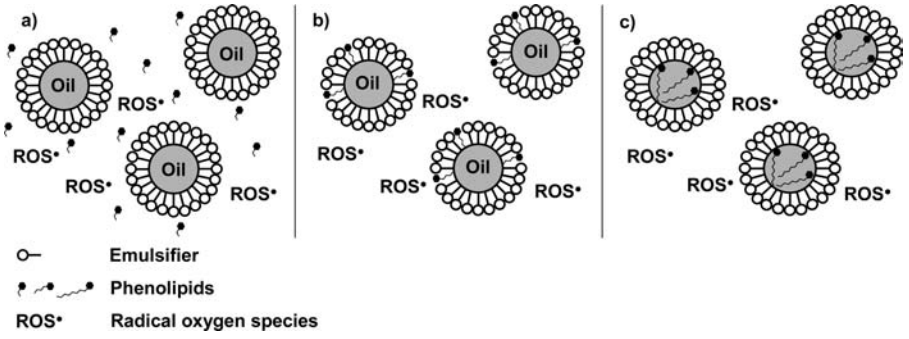

Figure 6. Putative scheme of the preferential location of the antioxidants in an emulsified system (inspired by the previous proposal by Laguerre et al.(*23*)).

and, therefore, far from the shielding layer. This reasoning will take us to hypothesize that a good antioxidant for oil-in-water emulsions should be, at the same time, a good antioxidant and an effective surfactant.

The nonlinear dependency observed for the antioxidant activity of chlorogenic acid alkyl esters in emulsions, showing a maximum for the dodecyl ester derivative, is a similar case (*23*). Here, dodecyl chlorogenate is the best antioxidant most likely because it is the best surfactant of the series and, therefore, is mainly placed at the oil−water interface. A similar reasoning would apply for the rosmarinic acid alkyl esters, where the maximum was observed for the phenolic octyl ester derivative (*25*). The reason why there are different cutoff chain lengths could be due to the fact that to be an effective surfactant depends upon different parameters that may vary for each specific series of phenolipids, such as the optimum HLB value or the specific polarity and geometry of the polar head. Other aspects may also be important because the maximum observed for the chlorogenic acid alkyl esters in emulsions (dodecyl chlorogenate) changes to octyl chlorogenate when no methanol is added to the emulsion system and different emulsifier concentrations are employed (*24*).

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

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

In conclusion, these data reveal that potent antioxidants, such as tyrosol and hydroxytyrosol fatty acid esters, are relevant surfactants when the right HLB is attained, around 8−11 in this specific case. Hydroxytyrosol fatty acid esters, which are better antioxidants than their tyrosol homologues, have also shown better surfactant properties. A discussion of the results on surfactant effectiveness of hydroxytyrosol fatty acid esters to the light of our previous results on their antioxidant activity in fish oil-in-water emulsions invite us to propose intrinsic surface-active properties of these compounds, represented by this physicochemical parameter, to explain the nonlinear dependency found when relating phenolipid hydrophobicity to their antioxidant activity in emulsions. This new hypothesis will help in the rational design of antioxidants used in oil-in-water emulsions, where a good antioxidant should also be an effective surfactant. At the same time, it may help us to better understand basic aspects of the behavior of lipophilic phenolic antioxidants in emulsions. These results open up potential new applications for these surfactant antioxidants in the food, pharmaceutical, or personal-care industries.

Supporting Information Available: Characterization of new compounds **5**, **6**, **7**, **11**, and **15**. This material is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED

(1) Lipid oxidation in food. In *Advances in Seafood Biochemistry: Composition and Quality*; Flick, G. J., Jr., Martin, R. E., Eds.; Technomic Publishing Co.: Lancaster, PA, 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*; Simic, M. G., Karel, M., Eds.; Plenum Press: 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), 2779–2787.

(11) Chaiyasit, 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, α-tocopherol, and other minor components in virgin olive oil. *J. Agric. Food Chem.* **2003**, *51* (24), 7170–7175.

(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*, 107–112.
(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), 137–152.
(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), 3779–3785.
(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), 10960–10966.
(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**, *57*, 9773−9779.
(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: Hoboken, NJ, 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), 11052–11056.
(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), 1745–1748.
(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: New York, 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* **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* **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) Przestalski, S.; Hladyszowski, J.; Kuczera, J.; Rózycka-Roszak, B.; Trela, Z.; Chojnacki, H.; Witek, S.; Fisicaro, E. Interaction between model membranes and a new class of surfactants with antioxidant function. *Biophys. J*. **1996**, *70* (5), 2203–2211.

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