

Differential Partitioning of Bioantioxidants in Edible Oil–Water and Octanol–Water Systems: Linear Free Energy Relationships

J. Freiría-Gándara,[†] S. Losada-Barreiro,^{*,†,‡} F. Paiva-Martins,[‡] and C. Bravo-Díaz[†]

[†]Fac. Química, Departamento Química Física, Universidad de Vigo, 36310 Vigo, Spain

[‡]REQUIMTE-LAQV, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, 4169-007 Porto, Portugal

ABSTRACT: Partition coefficients, P_W^{O} , of antioxidants (AOs) between edible oils and water are scarce in the literature, despite that AOs are widely used to control lipid oxidation and the oxidative stress in cells. P_W^{O} values have a great importance to predict the efficiency and distribution of bioactives at different levels of biological organization from binary oil–water systems to living cells. Here, we determined the partition coefficients, P_W^{O} , of four series of potent, natural AOs of increasing lipophilicity between vegetable oils (olive, soybean, and corn) and water and, for the sake of comparison, between octanol–water, P_W^{OCT} . Results indicate that the contribution of the –CH₂ groups to the overall lipophilicity of the AO is the same irrespective of the oil employed. The P_W^{O} values were compared with P_W^{OCT} values, and linear relationships were obtained for each series of AOs. Results indicate that, in general, P_W^{O} values cannot be predicted from the P_W^{OCT} values, making necessary to determine the P_W^{O} values for each oil and antioxidant.

4 Hydroxytyrosol 2 Caffeic 2 Ca

Gallic

 $(P_{W^{0}})$

) bo Protocatechuic

INTRODUCTION

Antioxidants (AOs) present in the human body can be either biosynthesized or obtained from the diet.^{1,2} They play an important role in controlling the oxidative stress of cells, helping to minimize the production of harmful radicals, and the intake of antioxidants is recommended because they also have well-recognized beneficial health effects, including anticancer, antitumor, and anti-inflammatory properties.^{3–3} AOs are also added to lipid-based foods to control the oxidative stability of the lipids.⁶ Most AOs are polyphenolic in nature and inhibit the oxidation of lipids because they are excellent hydrogen donors that are accepted by reactive radicals to yield less reactive radical and nonradical species.^{1,2}

Knowledge on how AOs partition in different systems and the effects of lipophilicity of AOs on their distribution in binary oil—water systems is important in relevant economic areas such as medicine, pharmacy, and food technology.^{7,8} For example, the preparation of olive oil requires the use of large amounts of water which are subsequently discarded, leading to a significant loss of important endogenous antioxidants present in the olive fruit.^{9,10} Olive oil formulations are prime examples of lipidbased delivery systems such as emulsions and nanoemulsions,^{11,12} and bio-oils are receiving attention as green solvents for liquid—liquid extractions.¹³

Partitioning of AOs in multiphasic systems such as living cells is important for understanding their efficiency in inhibiting lipid oxidation and their bioavailability in the human body.^{1,14} The partition coefficient values between octanol and water, $P_W^{\rm OCT}$, are widely use in predictive environmental and pharmaceutical studies⁸ and are important for a rational description of the partitioning behavior in multiphasic biological systems. However, the partition

coefficients between edible oils (e.g., olive) and water, P_w^{O} , correlate pretty well with the partitioning of drugs into rats and human tissues and, in some instances, they are even superior for the prediction of lipid tissue plasma partition coefficients over the widely used octanol—water partition coefficient, $P_W^{OCT, 15-17}$ Note that partition coefficient values are determined by all intermolecular interactions, including electrostatic, hydrogen bonding, and dispersion forces between the solute and the two phases in which it is dissolved. Its value thus depends on the balance of all intermolecular forces involving a solute and the two phases between which it partitions.^{7,18,19}

The differential partitioning of AOs makes them to be present at a wide range of concentrations in body fluids, tissues, and in the different regions of lipid-based emulsions, affecting largely their efficiency in the system.^{20,21} In general, water-soluble AOs react with oxidants in the cell cytoplasm, the blood plasma, or in the aqueous and interfacial regions of lipid-based emulsions; meanwhile, lipid-soluble antioxidants are oil-soluble and protect the lipids in the cell membranes or in the food emulsions because they are mostly located in the interfacial or oil regions of the system.^{20–22}

In this work, we determined the distribution in olive–, soybean–, and corn oil–water binary systems of series of homologous AOs of different hydrophobicity derived from potent, natural AOs. For the purpose, we grafted gallic (GA), caffeic (CA), and protochateuic (PT) acids and hydroxytyrosol (HT) with alkyl chains of increasing number of carbon atoms

Received: March 29, 2018 Accepted: July 3, 2018

Table 1. Composition	(Given As Percentage)	of the Main Fatt	y Acids Found in the	Oils Employed in This Work

	palmitic (C16:0)	palmitoleic (C16:1)	octadecanoic (C18:0)	oleic (C18:1)	linoleic (18:2)	linolenic (18:3)	arachidonic (C20:4)
corn	12.6	0.0	2.3	30.2	53.4	0.8	0.6
olive	13.4	0.9	2.4	72.1	2.9	3.7	0.5
soybean	9.0	0.0	3.7	27.6	53.7	5.1	0.0

Table 2	. Partition	Coefficients	Values fo	or the	Distributions	of AOs of	Different	Hydrophobicities	in the	Oil-V	Nater and
Octano	l–Water M	lixtures at T	=25°C								

ANTIC	Log Pw ^o					
Structure	-R	n(CH ₂)	Olive	Soybean	Corn	Octanol
HOOR	-CH ₃ (C1)	0	0.40	0.45	0.52	1.56
HO	-CH ₂ CH ₃ (C2)	1	0.89	0.92	1.04	1.93
	-(CH ₂) ₂ CH ₃ (C3)	2	1.45	1.48	1.58	2.44
Caffeates	-(CH ₂) ₇ CH ₃ (C8)	7	2.23	2.25	2.81	5.02
0	-CH ₃ (C1)	0	-1.30	-1.40	-0.94	0.85
HO	-CH ₂ CH ₃ (C2)	1	-0.70		-0.60	1.23
HO	-(CH ₂) ₂ CH ₃ (C3)	2	-0.07	-0.07	0.10	1.73
ÓH	-(CH ₂) ₃ CH ₃ (C4)	3	0.48	0.51	0.60	2.29
Gallates	-(CH ₂) ₇ CH ₃ (C8)	7	1.88	2.29		4.31
	CH ₃ (C2)	0	-0.20			1.22
HOR	$CH_2 CH_3$ (C3)	1	0.34			1.58
о́н	(CH ₂) ₄ CH ₃ (C6)	4	1.50			3.15
Hydroxytyrosol Derivatives	(CH ₂) ₆ CH ₃ (C8)	6	1.61			4.16
0	-CH ₃ (C1)	0	-0.11	-0.11		1.14
OR	-CH ₂ CH ₃ (C2)	1	0.27	0.36		1.52
НО	-(CH ₂) ₂ CH ₃ (C3)	2	0.91	0.91		2.02
OH	-(CH ₂) ₃ CH ₃ (C4)	3		1.60		2.58
Protocatechuates	-(CH ₂) ₅ CH ₃ (C6)	5	1.72	1.71		3.59

(1-8) so that four series of homologous AOs bearing the same reactive group but of different hydrophobicities were prepared. Data on partitioning of bioantioxidants in vegetable oils, and particularly in olive oil, are scarce and much more sparse than partitioning data in octanol-water systems.²³ Determining the partition coefficients and the distribution of AOs in oil-water systems may be of general interest because analyses of the P_w^{O} values contribute to the general understanding of the interactions of the molecules in the different oil phases. All the oils employed here are premium functional foods of enormous economic and social importance. Particularly, olive oil is important in both the Mediterranean region and in the healthy Mediterranean "lifestyle" because of its well-balanced fatty acid composition/profile with oleic acid as the main component, Table 1, and because of the presence of minor compounds such as polar phenols, polyphenols, tocopherols, and sterols. The composition of the oils employed in this work is given in Table 1, and the chemical structures of the AOs are shown in Table 2.

It may be particularly important for predictive purposes to investigate if there is any relationship between P_w^{O} and P_W^{OCT} values. Thus, we also determined the P_W^{OCT} values for the same series of AOs and explored if any extrathermodynamic

relationship can be established between both parameters. P_W^{OCT} values can be determined by a variety of experimental^{24,25} and theoretical methods, including ACD/log *D* software, ADMET predictors etc., which provide P_W^{OCT} values very close to the experimental ones.^{7,26} We employed the free (online) computer software Molinspiration^{27,30} to determine the P_W^{OCT} values. The software estimates P_W^{OCT} values on the basis of the group contribution concept. This method assumes that each molecule is considered a mixture of simple groups (-CH₂, -COOH, -OH, etc.) whose thermodynamic property parameters are calculated by the summation of the contributions of the various groups.²⁴

Moreover, some of the parent AOs employed here are phenolic acids that may be partially ionized at the typical pH (4-6) range of foods,⁶ affecting their partition coefficients. Thus, we analyzed the effects of acidity on the P_w^{O} values of two representative phenolic acids, GA and CA, and of ascorbic acid, representative of nonphenolic antioxidants.

EXPERIMENTAL SECTION

Materials. All chemicals were of the highest purity available and used as received. Aqueous buffered (citric acid/citrate sodium; 0.04M; pH 2–6) solutions were prepared by using



Figure 1. (A) Spectra of aqueous solutions of GA in aqueous solutions at different pH values. (B) Calibration curves of CA in BuOH/EtOH mixtures obtained from independent experiments carried out on different days. The slopes are essentially the same (differences less than 7%). (C) Standard addition curves obtained from aliquots (40 μ L) of the oil phase of oil/acidic water mixtures obtained after phase separation by centrifugation.



Figure 2. Variations of the natural $\log(P_W^O)$ with the number of $-CH_2$ groups in the alkyl chain (\bullet) for AO ester derivatives of gallic (A), caffeic (B), and protocatechnic (C) acids and hydroxytyrosol (D). For the sake of comparisons, the partition coefficients P_W^{OCT} between octanol and water (\blacksquare) are also displayed.

Mili-Q grade (conductivity $<0.1 \text{ mS} \cdot \text{cm}^{-1}$). Commercial antioxidants were purchased from Sigma-Aldrich or Acros.

Gallic acid (GA), methyl gallate (C_1), ethyl gallate (C_2), propyl gallate (C_3), butyl gallate (C_4), octyl gallate (C_8), and lauryl gallate (C_{12}) were purchased from Sigma-Aldrich and

used as received. Caffeates were synthesized from manomalonates through Verley–Doebner modification of Knoevenagel condensation with the 3,4-dihydroxybenzaldehyde and 4dihydroxybenzaldehyde.²⁸ Protocatechuates (C_1 – C_{16}) were synthesized by chemical acylation of the carboxylic group

> DOI: 10.1021/acs.jced.8b00258 J. Chem. Eng. Data XXXX, XXX, XXX–XXX

Article

following literature procedures.¹⁴ The hydroxytyrosol esters were synthesized by direct esterification of HT with the corresponding fatty acid (C_1-C_6 derivatives) or by transesterification with the corresponding fatty ester (C_8-C_{16}) in the presence of Novozym 435 according to published procedures.²⁹ Final products were purified by chromatography with silica gel 60 (0.040–0.063 mm, Merck). Nuclear magnetic resonance (NMR) spectra were recorded on 100 or 400 MHz NMR spectrometers, and the ¹H and ¹³C NMR spectra of the synthesized AOs (in CDCl₃) were in accordance with the literature reports.

Methods. The partition coefficients P_W^O (at constant acidity) in binary olive oil-water systems were determined by employing the shake-flask method.³⁰ Hydrophilic AOs were dissolved in water, while the hydrophobic ones were dissolved directly in the oil. 1:9 or 4:6 (oil:water, v/v) mixtures ($V_T = 10$ mL) were prepared, stirred with a high-speed rotor for 1 min and allowed stand for at least 4 h to permit phase separation and to reach thermal equilibrium (T = 25 °C). The phases were separated by centrifugation, and the AO concentrations in the aqueous and oil phases were determined by UV spectrometry with the aid of a previously prepared calibration curves. The calibration curves were obtained by measuring the absorbance of increasing amounts of the antioxidants ($[AO] = 10^{-4} - 10^{-2}$ M) both in the water and in the oil phase of the binary mixtures.

Figure 1A is representative and show the spectrum of aqueous solutions of GA at different pH values . The observed spectral changes are due to the ionization of the carboxylic group because the first pK_a of the –COOH groups of phenolic acids range 4–5, meanwhile that of the aromatic –OH groups range 8.3–8.7.^{31,32} We note that the –OH groups of the alkyl ester derivatives do not significantly ionize at the typical physiological food acidities (2–7).

Calibration curves were obtained at selected pH values by measuring the absorbance of increasing amounts of the antioxidants. Vegetable oils absorb strongly in the same UV region as that of AOs, making the determination of the percentage of some AOs in this region difficult if not impossible. In some cases (for example, caffeic acid), the maximum in the absorbance is at higher wavelengths than that of the components of the oils, and in these cases, the fraction of CA in the oil, $%CA_{\Omega}$, could be determined directly in the oil phase after dilution in a BuOH/EtOH mixture. However, after dilution, the measured absorbance values are very low, and this creates some uncertainty in %CAo values. To minimize inasmuch as possible this problem, %AOo was determined by employing two complementary methods, (a) interpolation in a calibration curve and (b) a standard addition method. Both gave similar results, yielding values of the percentage of AO in the oil phase with differences less than 5%. Figures 1B and 1C are illustrative and show the calibration curves obtained at selected pH. Values of the intercepts and the slopes (molar extinction coefficients, ε), not shown here, were obtained by least-squares fitting of the (A, [AO]) pairs of data obtained under the different experimental conditions. In all cases, square correlation coefficients, r^2 , were higher than r^2 = 0.999.

A. Interpolation in a Calibration Curve. The absorbance of an aliquot $(40 \ \mu\text{L})$ of the oil fraction of the binary oil/acidic water mixtures prepared without antioxidant was measured after dilution in a 50:50 (v/v) EtOH/BuOH mixture. Calibration curves for the AOs were prepared in triplicate by adding increasing amounts of a AO stock solution ([AO] = 6.8 $\times 10^{-3}$ M) to EtOH/BuOH mixtures containing 40 μ L of oil (added to minimize a potential solvent effect). Figure 2B shows two of the typical calibration curves obtained. The average values of molar extinction coefficient for CA is $\varepsilon_{CA} = 13700 \pm 88 \text{ M}^{-1} \text{ cm}^{-1}$.

B. Standard Addition Method. After the centrifugation of the binary oil/water mixtures (different pH and prepared with AO), an aliquot (40 μ L) of the oil phase was removed and diluted in a 50:50 (v/v) EtOH/BuOH mixture. Addition standard curves were prepared by adding increasing volumes of a CA stock solution ([CA] = 6.8×10^{-3} M) as illustrated in Figure 2C, from where %CA_O was calculated.

For those AOs with low oil solubility, the fraction of AO in the oil region (%AO_o) could not be determined by interpolation in a calibration curve (as the percentage in the aqueous phase, %AO_W) because of the high absorption of the oils. In these cases, %AO_O values were estimated by difference between the total added amount of AO to the determined amount in water, i.e., $%AO_{O} = 100 - %AO_{W}$. On the contrary, for the most hydrophobic AOs (with alkyl chains of eight or more carbon atoms), the fraction of AO in the aqueous region could not be determined accurately because of their extremely low solubility in water. For example, the solubility of OG and LG have been reported to be less than 0.001 g/L.^{33,34} For these AOs, the reported partition coefficients need to be taken with extreme caution because values of (AO_W) in the oil/water mixture will also be very small, and small errors in the denominator of eq 1 may have a large effect on the value of $P_{\rm W}^{\rm O}$ of the large errors in their determinations.

The partition coefficients P_W^O (at constant acidity) in binary oil–water systems were determined by employing a shake-flask method³⁰ and by employing eq 1, where (hereafter), parentheses, (), indicate molar concentrations in moles per liter of a particular region's volume, and V_W and V_O are the volumes of the aqueous (W) and oil (O) regions, respectively.

$$P_{\rm W}^{\rm O} = \frac{(\rm AO_{\rm O})}{(\rm AO_{\rm W})} = \frac{\% \rm AO_{\rm o}}{\% \rm AO_{\rm W}} \times \frac{V_{\rm W}}{V_{\rm O}}$$
(1)

When investigating the effects of acidity, the apparent partition coefficients $P_W^{O}(app)$ for gallic, caffeic, and ascorbic acids, chosen as representatives of phenolic and nonphenolic acids, were determined at different acidities by employing the same shake-flask method. The percentages of GA in the aqueous phase, %AO_W were determined by interpolation of the absorbance of aliquots (30 μ L) of the aqueous phase of the binary olive oil–water mixtures by using previously prepared calibration curves at each pH as described elsewhere,^{35,36} and the $P_W^O(app)$ values were calculated by employing eq 2. Reported P_W^O and $P_W^O(app)$ values in Table 2 were obtained in duplicate or triplicate with deviations less than 5%.

$$P_{\rm W}^{\rm O}({\rm app}) = \frac{({\rm AO}_{\rm O})}{({\rm AO}_{\rm W}) + ({\rm AO}_{\rm W}^-)}$$
(2)

Statistical Analysis. Reported partition coefficients in binary oil-water systems were the average of 6–8 replicates. The Dixon's Q-test was employed in deciding whether to accept or reject the datum before calculating the average of the set of replicates. Data are presented as means \pm confidential interval. Square correlation coefficients, r^2 , of linear relationships were in all cases $r^2 > 0.999$.

Journal of Chemical & Engineering Data

Theory. On thermodynamic grounds, the transfer of a solute from an aqueous (W) to an organic (oil, O) phase (both in equilibrium) can be described by the change in the Gibbs free energy, $\Delta G^{W \rightarrow O}$, that accompanies the transfer, providing a quantitative measurement of the relative hydrophobicity of the phases.³⁷ At a constant temperature T and pressure P, $\Delta G^{0,W \to O}$ is given by the difference in the chemical potentials of the compound *i* being transferred, $\Delta G^{0,W \to O} = \mu_i^W - \mu_i^O$, where μ_i is the chemical potential of the component *i*. At equilibrium, $\Delta G^{0,W\to O} = 0$, and the chemical potentials equal each other. The chemical potentials depend on the activities of the solute, and at the low concentration limit, the activity coefficients can be assumed to be close to the unit. Therefore, it is safe to use concentrations instead of activities, and eq 3 can be derived. Eq 3 relates the Gibbs free energy of transfer of the component *i* from one phase to another with its partition coefficient between both phases. A deeper explanation on the assumptions and equations involved can be found elsewhere. 19,37,38

$$\Delta G^{0,W \to 0} = -RT \ln P_W^O \tag{3}$$

Contributions to partition coefficient values can be factorized into various parameters, including the molar volume, which comprises hydrophobic and dispersion forces, solvatocromic parameters, defined as the solute's H-bond donor acidity, H-bond acceptor basicity, and polarizability, which embraces the orientation and induction forces.^{7,24} Thus, studies of the partitioning of solutes between water and nonpolar solvents provide estimates for the energy cost of exposing nonpolar molecules to water creating hydrophobic water contacts.^{19,23}

When low solute concentrations are employed, the distribution of the AOs is described by the partition coefficient $P_{\rm W}^{\rm O}$ defined by eq 1. Hereafter, parentheses, (), indicate molar concentrations in moles per liter of a particular region's volume, and $V_{\rm W}$ and $V_{\rm O}$ are the volumes of the aqueous (W) and oil (O) regions, respectively, meanwhile stoichiometric concentrations in moles per liter of total volume of the oilwater mixture will be indicated by the commonly employed brackets []. If the AO is partially ionized under the experimental conditions (e.g., a phenolic acid), then an apparent partition coefficient, $P_W^O(app)$, eq 2, may be defined in terms of all ionized and neutral forms present at a particular pH.³⁹ The ionization of weak acids in the oil phase is negligible because literature reports indicate that the ionization constants of weak acids in oils are typically 5-6 orders of magnitude smaller than those in the aqueous phase,³² (i.e., $pK_a(O) \gg$ $pK_a(W)$). Thus, the distribution of the AO can be illustrated as in Scheme 1, and the apparent (measured) partition coefficient is defined by eq 2. Note that at the low pH limit, the

antioxidant is completely neutral, and $P_W^{O}(app) = P_W^{O}$. Importance of Determining P_W^{O} Values in Oil–Water Systems to Estimate the Distribution of AOs in Multiphasic Systems. Partitioning in multiphasic systems is more complex and requires definition of new distribution constants.^{40,41} For example, addition of surfactants to oil– water binary systems to create kinetically stable emulsions makes the antioxidant to partition between the oil, water, and interfacial regions, and their distribution is now described by two partition coefficients, Scheme 2, that between the oilinterfacial, P_O^{I} , and aqueous-interfacial, P_w^{I} , regions, eqs 4 and 5. Because of the physical impossibility of separating the interfacial region from the oil and aqueous ones, any attempt Scheme 1. Oil–Water Biphasic System (Employed in This Work) Showing the Equilibrium Contribution of an Ionizable Antioxidant AO, Its Conjugated Base, AO^{-a}



 ${}^{a}P_{w}{}^{O}$ is the partition coefficient, and K_{a}^{W} is the aqueous ionization constant of the AO. Ionization is assumed to take place exclusively in the aqueous region, and the formation of potential ion pairs is neglected.

Scheme 2. Distribution of an Antioxidant AO between the Oil, Interfacial, and Aqueous is Described by the Partition Coefficients between the Aqueous–Interfacial, P_W^{I} , and Oil–Interfacial, P_O^{I} , Regions of the Emulsion^{*a*}



"For the sake of clarity, the potential ionization of the AO in the aqueous and interfacial regions is not shown.

to determine the partition coefficients $P_{\rm O}^{\ I}$ and $P_{\rm w}^{\ I}$ needs to be done in the intact emulsions.^{40,41} We note that the ratio between $P_{\rm O}^{\ I}$ and $P_{\rm w}^{\ I}$ is equal to the partition coefficient in the absence of surfactant, $P_{\rm W}^{\ O}$, eq 6, highlighting the importance of determining partition coefficients in binary systems. Therefore, determining partition coefficients in binary systems provides useful information on the behavior of chemicals of interest in both binary oil–water systems and in food emulsions.

$$P_{\rm W}^{\rm I} = \frac{(\rm AO_{\rm I})}{(\rm AO_{\rm W})} \tag{4}$$

$$P_{\rm O}^{\rm I} = \frac{(\rm AO_{\rm I})}{(\rm AO_{\rm O})} \tag{5}$$

$$\frac{P_{\rm W}^{\rm I}}{P_{\rm O}^{\rm I}} = \frac{({\rm AO}_{\rm I})/({\rm AO}_{\rm W})}{({\rm AO}_{\rm I})/({\rm AO}_{\rm O})} = \frac{({\rm AO}_{\rm O})}{({\rm AO}_{\rm W})} = P_{\rm W}^{\rm O}$$
(6)

RESULTS AND DISCUSSION

Table 2 shows the values of the partition coefficients for the distributions of AOs in the oil-water binary mixtures and, for the sake of comparisons, in octanol-water. To a first approximation, each of the homologous series of AOs employed have two distinct regions within their molecules that influence their partitioning: an aromatic residue, which is common to all individuals of each series, and an alkyl ester tail containing a variable number of methylene groups and the

Table 3. Slopes and	Intercepts for the	Variations of log(P _W ^O)	with the N	Number of Methylene	Groups According	g to E	lq 7
---------------------	--------------------	---	------------	---------------------	------------------	--------	------

oil		gallic	caffeic	protocatechuic	hydroxytyrosol
octanol (OCT)	<i>a</i> _{OCT}	0.77 ± 0.03	1.53 ± 0.05	1.06 ± 0.03	1.15 ± 0.04
	$b_{\rm OCT}$	0.51 ± 0.01	0.49 ± 0.01	0.50 ± 0.01	0.50 ± 0.01
olive (OL)	a _{OL}	-1.15 ± 0.04	0.40 ± 0.03	-0.18 ± 0.07	-0.16 ± 0.02
	$b_{\rm OL}$	0.50 ± 0.01	0.51 ± 0.01	0.55 ± 0.04	0.44 ± 0.02
soybean (SO)	a _{so}	-1.18 ± 0.06	0.44 ± 0.03		
	b_{SO}	0.58 ± 0.03	0.52 ± 0.03		
corn (CO)	a _{CO}	-1.03 ± 0.10	0.52 ± 0.01		
	b_{CO}	0.54 ± 0.06	0.53 ± 0.01		



Figure 3. Variations of $\log(P_W^{O})$ with the number of $-CH_2$ groups in the alkyl chain derivatives of caffeic (A) and gallic (B) acids in olive (\blacksquare), soybean (\bullet), and corn (\blacktriangle) oils. Data for the partition coefficients of gallic derivatives in corn oil were obtained from Stöckman et al.⁴³

terminal methyl group. The aromatic moiety and the methyl group make a constant contribution.

Using the basic group-contribution concept, the logarithm of the partition coefficient log P_W^O can be computed as the summation of two contributions, eq 7, that of the alkyl part of the molecule and that of the aromatic and methyl residues. In eq 7, usually called the Colander equation,⁴² n_{CH_2} is the number of methylene groups in the AO, and the parameter *a* stands for the contribution of the nonalkyl part of the molecule to P_W^O . Eq 7 predicts that an increase in the number of C atoms in the alkyl chain increases the hydrophobicity of the AOs and its solubility in oil.

$$\log(P_{\rm W}^{\rm O}) = a_{\rm O} + b_{\rm O} n_{\rm CH_2} \tag{7}$$

Figure 2 shows that plots of $\log(P_W^{O})$ (olive and octanol) vs the number of $-CH_2$ groups for the esters derived of gallic (2A), caffeic (2B), and protochateuic (2C) acids and of hydroxytyrosol (2D) are linear except for the most hydrophobic AOs (n > 7), where deviations from the linearity are evident. Deviations from linearity are probably caused by the uncertainty in their values, affected by experimental errors in the determination of their concentration or percentages in the water phase. These values were not considered in determining the intercepts and slopes collected in Table 3. For the sake of clarity, the variations of $\log(P_W^{O})$ with the number of $-CH_2$ groups for the caffeic and gallic esters in olive, corn, and soybean oils are shown in Figure 3.

Table 3 shows that the slopes *b* obtained are very similar to each other (with differences less than 10%, except for HT in olive, which is 20%) regardless of the oil or the AO series, with an average value of $b_0 = 0.53 \pm 0.02$. Remarkably, this average

value for the slope is quite similar to the average value obtained in octanol, $b_{oct} = 0.50 \pm 0.01$, suggesting that the contribution of the methylene group to the hydrophobicity of the AOs is very similar in both solvents.

However, the intercept values are different for each series as a consequence of the different structure of the aromatic moieties of the AOs employed, following the order $a_{GA} < a_{PT} \approx$ $a_{HT} < a_{CA}$. These a_O values cannot be compared directly to each other because the nonalkyl moieties contain different groups and refer to different oils with different compositions. However, for the same oil (olive), their relative values reflect the big effect of the addition of an –OH group to their chemical structure on the overall hydrophobicity of the nonalkyl portion of the AOs (compare, for instance, GA and PT) and also the big effect of the –OH group on the hydrophobicity of the molecule compared to the addition of a single (HT) or double bond (CA) to the alkyl chain.

Because $P_W^{\rm OCT}$ is a key physicochemical parameter for drug discovery, design, and development, databases with $P_W^{\rm OCT}$ values are available for several thousands of compounds in the literature (for example, logKoW, PHYSPROP, etc.). If not available for a given species, the values can be easily calculated with the aid of various computer programs that employ different descriptors. Thus, for predictive purposes, it may be useful to convert the partition coefficients from the octanol– water system to the olive oil-water system. This can be easily done bearing in mind that partition coefficients are equilibrium constants, and therefore, extrathermodynamic relationships between values in different solvents can be established.^{44,45}

The linearity in the variations of both P_W^O and P_W^{OCT} with the number of CH₂ atoms in the alkyl chain of each series of

AOs, Figures 2A–D, with very similar slopes suggests that there must be a linear relationship such as that given by eq 8 between P_W^{O} and P_W^{OCT} . Figure 4 shows such linear



Figure 4. Extrathermodynamic linear relationships between $log(P_W^O)$ and $log(P_W^{OCT})$ for homologous series of AOs.

relationship. The values for the intercept and slope were obtained by fitting the corresponding (P_W^{O}, P_W^{OCT}) pairs of data to eq 8 and are given in Table 4.

$$\log(P_{\rm W}^{\rm O}) = A + B\log(P_{\rm W}^{\rm OCT}) \tag{8}$$

Table 4. Values of the Slopes and Intercepts of the LinearPlots in Figure 4

antioxidant series	Α	В
GA	-2.56 ± 0.16	1.22 ± 0.10
PT	-1.45 ± 0.04	1.18 ± 0.02
CA	-1.16 ± 0.18	1.04 ± 0.08
HT	-2.60 ± 0.09	0.84 ± 0.03

Both *A* and *B* values are different for each series of AOs but are in line with those reported by Leo et al.⁴⁵ when analyzing the distribution of a number of compounds between vegetable oils and octanol. Because the *A* and *B* values are different, the use of linear relationships such as that indicated by eq 8 seems to be limited to series of the same AOs, suggesting that, in

general, the values of the partition coefficients of a given antioxidant in vegetable oil—water systems cannot be predicted from the values of the partition coefficient in octanol—water.

pH Effects on the Partition Coefficients between Olive Oil and Water. Phenolic and nonphenolic AOs may be partially ionized under the typical pH of foods, and their degree of ionization depends, therefore, on the particular experimental conditions (acidity) employed. Because the neutral and ionic species exhibit different polarities, their partition coefficients are pH dependent, and an apparent partition coefficient, $P_W^{O}(app)$, may be defined in terms of all ionized and neutral forms present at a particular pH. Ionization constants of phenolic and nonphenolic AOs range 4-9, but the typical pH of foods is 4-7. The pK_a of aromatic -OH groups is around 8-9, and that of phenolic and nonphenolic acids is around 4-5. Thus, as a first approach and for the sake of simplicity, we consider only those AOs that can be ionized in the pH 4-6 range, e.g., phenolic and nonphenolic acids. The mathematical treatment can be easily extended to other situations, for example, if the aqueous pH is higher than 7, then the second ionization equilibria should be eventually considered.

Literature reports indicate that the ionization constants of weak acids in oils are typically 5–6 orders of magnitude smaller than those in the aqueous phase,³² (i.e., $pK_a(O) \gg pK_a(W)$). Thus, one can safely assume that the ionization of weak acids in the oil phase is negligible, and the distribution of the AO can be illustrated as in Scheme 2. The apparent (measured) partition coefficient $P_W^O(app)$ is defined by eq 1, which assumes that activity effects are negligible, i.e., the activity coefficients are very close to the unit. If the ionic species may be formed in the oil phase, a more complex approach is required.³⁹ Note that eq 9 predicts that at the low pH limit, the antioxidant is completely neutral, and $P_W^O(app) = P_W^O$, eq 1.

Bearing in mind the ionization of the AO in the aqueous phase, eq 9, the relationship between $P_w^{O}(app)$ and P_w^{O} is given by eq 10:

$$K_{a} = \frac{(AO_{W}^{-})(H^{+})}{(AO_{W})}$$
(9)



Figure 5. (A) Variation of $P_W^{O}(app)$ with pH for GA(\bullet), AA (\blacksquare), and CA (\blacktriangle) for olive oil in water mixtures. The solid line is the theoretical curve obtained by fitting the experimental data to a sigmoidal Henderson–Hasselbach type equation. (B) Linear plots of $1/P_W^{O}(app)$ with pH according to eq 11. (C) Theoretical variations of $P_W^{O}(app)$ in octanol–water mixtures with pH for GA(\bullet), AA (\blacksquare), and CA (\blacktriangle).

$$P_{\rm W}^{\rm O}(\rm{app}) = \frac{P_{\rm W}^{\rm O}}{1 + \frac{K_{\rm a}}{[\rm H^+]}}$$
(10)

$$\frac{1}{P_{\rm W}^{\rm O}(\rm app)} = \frac{1}{P_{\rm W}^{\rm O}} + \frac{K_{\rm a}}{P_{\rm W}^{\rm O}} \frac{1}{[H^+]}$$
(11)

Eq 10 predicts a sigmoidal variation of the apparent (measured) partition coefficient value with acidity, Figure 5A. When pH is, at least, two pH units below the pK_a of the ionizable AO (i.e., $[H^+] \gg K_{a'}$) $P_W^O(app) = P_W^O$, and therefore, there is an upper limit in the experimentally measured partition coefficients which corresponds to that of the neutral molecule. The reverse of eq 10 is eq 11, which predicts a linear variation of $1/P_W^O(app)$ with $1/[H^+]$, Figure 5B, from where value of $P_W^O = 0.096 \pm 0.009$ (GA), $P_W^O = 0.090 \pm 0.004$ (CA), and $P_W^O = 0.110 \pm 0.002$ (AA) can be determined for the partition coefficient P_W^O of the totally neutral AOs. The value for CA is very similar to a literature value reported by Rodis et al., $P_W^O = 0.089$. For the sake of comparisons, sigmoidal variation of the P_W^{OCT} values with acidity is illustrated in Figure 5C.

The variation of $P_W^{O}(app)$ with pH reflects the difference in lipophilicity between the neutral and ionic species. We note that the actual values of both P_W^{O} and $P_W^{O}(app)$ are determined by all intermolecular interactions which may include electrostatic, hydrogen bonding, and dispersion forces between the solute and the two phases in which it is dissolved. Thus, the difference between $P_W^{O}(app)$ (partially or totally ionized substrate) and P_W^{O} (totally neutral substrate) is a parameter containing important information on the intermolecular forces and intramolecular interactions of a given solute.

CONCLUSIONS

We determined the partition coefficients for series of potent, natural AOs of different hydrophobicity in oil-water mixtures and, for the sake of comparison, in octanol-water systems. The variations of the partition coefficients with the number of C atoms in the alkyl chain were linear and parallel to those obtained in octanol-water, indicating a similar contribution of the $-CH_2$ groups to the total hydrophobicity of the molecule in both solvents. As expected, the intercept values, which reflect the contribution of the nonalkyl portion of the molecule, are different as a consequence of the different groups in the chemical structures of the AOs. Extrathermodynamic linear relationships between the partition coefficients in olive oil-water and octanol-water systems could be established. They are only valid for a given series of compounds, suggesting therefore that, in general, P_W^O values cannot be predicted from P_W^{OCT} values and should be determined for each series of AOs employed.

When AOs contain ionizable groups (phenolic and ascorbic acids), the values of the partition coefficients depend on the actual value of the acidity, decreasing upon increasing pH following a sigmoidal-like curve. Literature reports indicate that $P_W^O(app)$ values can differ by more than one order of magnitude, reflecting the changes in the hydrophobicity of the AO because of its ionization. Thus, determination of their variation is highly recommended because once the P_W^O values for the neutral and fully ionized species are known, theoretical equations can be used to calculate the change in hydrophobicity with pH.

AUTHOR INFORMATION

Corresponding Author

*E-mail: sonia@uvigo.es.

ORCID 💿

C. Bravo-Díaz: 0000-0002-9468-0881

Funding

Financial support of the following institutions is also acknowledged: FEDER (COMPETE) and FCT—Fundação para a Ciência e a Tecnologia (UID/QUI/50006/2013 POCI-01-0145-FEDER-007265), Red de Uso Sostenible de los Recursos Naturales y Agroalimentarios (REDUSO, Xunta de Galicia, Grant number ED431D 2017/18), and University of Vigo and Porto. S.L.-B. thanks Xunta de Galicia for a postdoctoral grant (POS-B/2016/012).

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Losada-Barreiro, S.; Bravo-Díaz, C. Free radicals and polyphenols: the redox chemistry of neurodegenerative diseases. *Eur. J. Med. Chem.* **2017**, *133*, 379–402.

(2) Shahidi, F. Handbook of Antioxidants for Food Preservation, 1st ed.; Woodhead Pub.: 2015.

(3) Rajendran, P.; Nandakumar, N.; Rengarajan, T.; Palaniswami, R.; Gnanadhas, E. N.; Lakshminarasaiah, U.; Gopas, J.; Nishigaki, I. Antioxidants and human diseases. *Clin. Chim. Acta* **2014**, *436*, 332–347.

(4) Menaa, F.; Menaa, A.; Tréton, J., Chapter 63: Polyphenols against Skin Aging. In *Polyphenols in Human Health and Disease*; Academic Press: San Diego, 2014; pp 819–830.

(5) Morales-González, J. A. Oxidative Stress and Chronic Degenerative Diseases: A Role for Antioxidants; InTech: 2013.

(6) Frankel, E. N. *Lipid Oxidation*; The Oily Press, PJ Barnes & Associates: Bridgwater, England, 2005.

(7) Leo, A. J., Octanol/Water Partition Coefficients. In *Encyclopedia* of *Computational Chemistry*; John Wiley & Sons, Ltd: 2002.

(8) Korinth, G.; Wellner, T.; Schaller, K. H.; Drexler, H. Potential of the octanol-water partition coefficient (logP) to predict the dermal penetration behaviour of amphiphilic compounds in aqueous solutions. *Toxicol. Lett.* **2012**, *215* (1), 49–53.

(9) Rodis, P. S.; Karathanos, V. T.; Mantzavinou, A. Partitioning of olive oil antioxidants between oil and water phases. J. Agric. Food Chem. 2002, 50, 596-601.

(10) De Leonardis, A. Virgin Olive Oil: Production, Composition, Uses and Benefits for Man; Nova Science Publishers, Incorporated: 2014.

(11) McClements, D. J. Enhanced delivery of lipophilic bioactives using emulsions: a review of major factors affecting vitamin, nutraceutical, and lipid bioaccessibility. *Food Funct.* **2018**, *9* (1), 22–41.

(12) Kumar, K. S.; Dhachinamoorthi, D.; Saravanan, R.; Gopal, U.; Shanmugam, V. Microemulsions as carriers for novel drug delivery: A review. *Int. J. Pharm. Sci. Rev. Res.* **2011**, *10*, 37–45.

(13) Spear, S. K.; Griffin, S. T.; Granger, K. S.; Huddleston, J. G.; Rogers, R. D. Renewable plant-based soybean oil methyl esters as alternatives to organic solvents. *Green Chem.* **2007**, *9* (9), 1008–1015.

(14) Silva, R.; Losada-Barreiro, S.; Paiva-Martins, F.; Bravo-Díaz, C. Partitioning and antioxidative effect of protocatechuates in soybean oil emulsions: Relevance of emulsifier concentration. *Eur. J. Lipid Sci. Technol.* **2017**, *119* (6), 1600274.

(15) Chamberlin, A. C.; Levitt, D. G.; Cramer, C. J.; Truhlar, D. G. Modeling Free Energies of Solvation in Olive Oil. *Mol. Pharmaceutics* **2008**, *5* (6), 1064–1079.

(16) Coe, E. L.; Coe, M. H. A hypothesis relating oil: Water partition coefficients and vapor pressures of nonelectrolytes to their penetration rates through biological membranes. *J. Theor. Biol.* **1965**, 8 (2), 327–343.

Journal of Chemical & Engineering Data

(18) Liu, X.; Testa, B.; Fahr, A. Lipophilicity and Its Relationship with Passive Drug Permeation. *Pharm. Res.* **2011**, *28* (5), 962–977.

(19) Sangster, J. Octanol-Water Partition Coefficients, Fundamentals and Physcial Chemistry; J. Wiley & Sons: Chichester, 1997.

(20) Losada-Barreiro, S.; Sánchez-Paz, V.; Bravo-Díaz, C. Transfer of antioxidants at the interfaces of model food emulsions: distributions and thermodynamic parameters. *Org. Biomol. Chem.* **2015**, *13*, 876–885.

(21) Costa, M.; Losada-Barreiro, S.; Paiva-Martins, F.; Bravo-Diaz, C. Optimizing the efficiency of antioxidants in emulsions by lipophilization: tuning interfacial concentrations. *RSC Adv.* **2016**, *6* (94), 91483–91493.

(22) Costa, M.; Losada-Barreiro, S.; Paiva-Martins, F.; Bravo-Díaz, C. Physical evidence that the variations in the efficiency of homologous series of antioxidants in emulsions are a result of differences in their distribution. *J. Sci. Food Agric.* **2017**, *97* (2), 564–571.

(23) Schwarzenbach, R. P.; Gschwend, P. M.; Imboden, D. M. Organic Liquid-Water Partitioning. In *Environmental Organic Chemistry*, 2nd Ed.; J. Wiley & Sons, Inc.: 2003.

(24) Molyneux, P. Octanol/water partition coefficients Kow: A critical examination of the value of the methylene group contribution to logKow for homologous series of organic compounds. *Fluid Phase Equilib.* **2014**, 368, 120–141.

(25) Cumming, H.; Rücker, C. Octanol–Water Partition Coefficient Measurement by a Simple 1H NMR Method. *ACS Omega* **2017**, *2* (9), 6244–6249.

(26) Klopman, G.; Zhu, H. Recent Methodologies for the Estimation of N-Octanol/Water Partition Coefficients and their Use in the Prediction of Membrane Transport Properties of Drugs. *Mini-Rev. Med. Chem.* **2005**, *5*, 127–133.

(27) Molinspiration. http://www.molinspiration.com/.

(28) Costa, M.; Losada-Barreiro, S.; Paiva-Martins, F.; Bravo-Díaz, C.; Romsted, L. S. A direct correlation between the antioxidant efficiencies of caffeic acid and its alkyl esters and their concentrations in the interfacial region of olive oil emulsions. The pseudophase model interpretation of the "cut-off" effect. *Food Chem.* **2015**, *175*, 233–242.

(29) Almeida, J.; Losada-Barreiro, S.; Costa, M.; Paiva-Martins, F.; Bravo-Díaz, C.; Romsted, L. S. Interfacial Concentrations of Hydroxytyrosol and Its Lipophilic Esters in Intact Olive Oil-in-Water Emulsions: Effects of Antioxidant Hydrophobicity, Surfactant Concentration, and the Oil-to-Water Ratio on the Oxidative Stability of the Emulsions. J. Agric. Food Chem. 2016, 64, 5274–5283.

(30) Berthod, A.; Carda-Broch, S. Determination of liquid-liquid partition coefficients by separation methods. *J. Chromatog. A* 2004, 1037, 3–14.

(31) CRC. Handbook of Chemistry and Physics, 78th ed.; CRC Press: Boca Raton, 1997.

(32) Dean, J. A. Langés Handbook of Chemistry; McGraw-Hill, Inc.: New York, 1992.

(33) Lu, L.-L.; Lu, X.-Y. Solubilities of gallic acid and its esters in water. J. Chem. Eng. Data 2007, 52, 37–39.

(34) Takai, E.; Hirano, A.; Shiraki, K. Effects of alkyl chain length of gallate on self-associationand membrane binding. *J. Biochem.* **2011**, *150* (2), 165–171.

(35) Galan, A.; Losada-Barreiro, S.; Bravo-Díaz, C. A Physicochemical Study of the Effects of Acidity on the Distribution and Antioxidant Efficiency of Trolox in Olive Oil-in-Water Emulsions. *ChemPhysChem* **2016**, *17*, 296–304.

(36) Losada-Barreiro, S.; Bravo-Díaz, C.; Romsted, L. S. Distributions of phenolic acid antioxidants between the interfacial and aqueous regions of corn oil emulsions: effects of pH and emulsifier concentration. *Eur. J. Lipid Sci. Technol.* **2015**, *117*, 1801–1813.

(37) Leo, A.; Hansch, C.; Elkins, D. Partition Coefficients And Their Uses. *Chem. Rev.* **1971**, *71* (6), 525–616.

(38) Silvério, S. C.; Rodríguez, O.; Teixeira, J. A.; Macedo, E. A. Gibbs free energy of transfer of a methylene group on {UCON +(sodium or potassium) phosphate salts} aqueous two-phase systems: Hydrophobicity effects. *J. Chem. Thermodyn.* **2010**, *42* (8), 1063–1069.

(39) Kah, M.; Brown, C. D. LogD: Lipophilicity for ionisable compounds. *Chemosphere* **2008**, 72 (10), 1401–1408.

(40) Bravo-Díaz, C.; Romsted, L. S.; Liu, C.; Losada-Barreiro, S.; Pastoriza-Gallego, M. J.; Gao, X.; Gu, Q.; Krishnan, G.; Sánchez-Paz, V.; Zhang, Y.; Ahmad-Dar, A. To Model Chemical Reactivity in Heterogeneous Emulsions, Think Homogeneous Microemulsions. *Langmuir* **2015**, *31*, 8961–8979.

(41) Romsted, L. S.; Bravo-Díaz, C. Modelling chemical reactivity in emulsions. *Curr. Opin. Colloid Interface Sci.* **2013**, *18*, 3–14.

(42) Beezer, A. E.; Gooch, C. A.; Hunter, W. H.; Volpe, P. L. O. A thermodynamic analysis of the Collander equation and establishment of a reference solvent for use in drug partitioning studies. *J. Pharm. Pharmacol.* **1987**, 39 (10), 774–779.

(43) Stöckmann, H.; Schwarz, K.; Huynh-Ba, T. The Influence of Various Emulsifiers on the Partitioning and Antioxidant Activity of Hydrobenzoic acids and Their Derivatives in Oil-in-Water Emulsions. *J. Am. Oil Chem. Soc.* **2000**, *77*, 535–542.

(44) Goss, K.-U.; Schwarzenbach, R. P. Linear Free Energy Relationships Used To Evaluate Equilibrium Partitioning of Organic Compounds. *Environ. Sci. Technol.* **2001**, *35* (1), 1–9.

(45) Leo, A. J.; Hansch, C. Linear free energy relations between partitioning solvent systems. J. Org. Chem. 1971, 36 (11), 1539–1544.