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Partitioning of Olive Oil Antioxidants between Oil and Water Phases

PANAYOTIS S. RODIS,* VAIOS T. KARATHANOS,† AND ANTONIA MANTZAVINOU

Department of Food Science and Technology, Agricultural University of Athens, Iera Odos 75, Votanikos 11855, Athens, Greece

The partition coefficient (K_p) of the natural phenolic antioxidant compounds in the olive fruit between aqueous and olive oil phases was determined. The antioxidants of olive oil are either present in the olive fruit or formed during the olive oil extraction process. The antioxidants impart stability to and determine properties of the oil and are valuable from the nutritional point of view. The olive oil antioxidants are amphiphilic in nature and are more soluble in the water than in the oil phase. Consequently, a large amount of the antioxidants is lost with the wastewater during processing. The determination of antioxidants was performed using HPLC, and the K_p was estimated to be from as low as 0.0006 for oleuropein to a maximum of 1.5 for 3,4-DHPEA−EA (di-hydroxy-phenyl-ethanol−elenolic acid, oleuropein aglycon). Henry's law fitted very well to the experimental data. The partition coefficients were also estimated by applying the activity coefficients of the antioxidants in the two phases using a predictive group contribution method, the UNIFAC equation. The K_p values estimated with UNIFAC method were of the same order of magnitude but varied from the experimental values. Nevertheless, this method may be a rough predictive tool for process optimization or design. Because the K_p values were very low, some changes in the process are recommended in order to achieve a higher concentration of antioxidants in the oil. A temperature increase may lead to increasing the partition coefficient. Also, limiting the quantity of water during oil extraction could be a basis for designing alternative processes for increasing the antioxidant concentration in the olive oil.

KEYWORDS: Partition coefficient; phenolic compounds; UNIFAC; activity coefficient

INTRODUCTION

The phenolic compounds of virgin olive oil show several functional, nutritional, and sensory properties: inhibition of blood platelet aggregation (*1*) and phospholipid oxidation (*2*), protection of human erythrocytes against oxidative damage (*3*), correlation with the pungent and bitter taste of oil, reduction of the oxidative process of fruity flavored aromatic compounds, and improvement of the olive oil shelf life (*4*−*6*). These phenolic compounds are either originally present in the olive fruit (*7*) or formed during processing of olive oil extraction.

The phenolic compounds, once released or formed during processing of olives, are distributed between the water and oil phases. Another part of the phenolics is trapped in the solid phase: the "pomace". The distribution of the released amount of the phenolics between water and oil is dependent on their solubilities in these two phases. Consequently, only a fraction of the phenolics enters the oil phase. In this way, an upper limit on the kind and amount of the phenolic compounds entering the oil phase is set. In general, the concentration of the phenolics in the olive oil ranges from 50 to 1000 μg/g of oil depending on the olive variety (*8*). This amount of antioxidants in the olive oil is 1−2% of the available pool of antioxidants in the olive fruit. The rest is lost with the wastewater (approximately 53%) and the pomace (approximately 45%) depending on the extraction system (*9*).

As aforementioned, some of the phenolic compounds present in the olive oil possess antioxidant activity, thus imparting higher stability to the oil. The most important of the phenols of the olive oil (in terms of having the highest protection factor (*10*)) that possess antioxidant activity is the 3,4-dihydroxyphenylethanol (3,4-DHPEA) (*3, 10*−*12*). The 3,4-DHPEA is present in different oleosidic forms: the 3,4-DHPEA form, the form linked to the dialdehyde form of elenolic acid (3,4-DHPEA−EDA), and as an isomer of oleuropein aglycon (3,4-DHPEA-EA) (*12*). All of these three forms are degradation products of oleuropein. Oleuropein is a glucoside that contributes to the bitterness of olives. The antioxidant activity of all three forms is equivalent (*13*). Their antioxidant activity is also dependent on the concentration of these compounds, and more specifically, it is proportional to the antioxidant concentration in the oil phase (*14*).

During processing for the extraction of olive oil, almost 80% of all oleuropein is degraded upon crushing the olives (*4*). In the olive paste, which is a multiphasic system, the antioxidants

* To whom correspondence should be addressed. Tel. and Fax: ++-(30-1) 529-4702. E-mail: prodis@aua.gr.
† Department of Agricultural Machinery, Technological Educational Institute of Larissa, Larissa 41110, Greece; e-mail: vkarath@tee.gr.

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partition into the different phases thermodynamically according to their affinities toward these phases. The proportions of antioxidants residing in the three different phases (oil, water, and solids) depend on the relative polarities of the antioxidants, presence of surfactants, temperature, and the composition and relative amounts of the phases.

The partitioning coefficient, K_p, between oil and water phases can be determined experimentally by measuring the concentration of the phenolic compounds-antioxidants, in the two phases at equilibrium. Prediction of partitioning coefficients, K_p, between phases, though, may be feasible by using a general group contribution methods for prediction of activity coefficients in a liquid-phase, such as the UNIFAC method. This method has enabled the prediction of vapor/liquid or liquid/liquid equilibrium, or the solubility of several substances, in aqueous or nonaqueous phases (*15*−*17*). The group contribution method is based on the concept of the solution of groups instead of molecules. Each molecule is considered as a mixture of simple groups (−CH2−, −COOH, −OH, etc.) whose thermodynamic property parameters (described in the UNIFAC model below) are known in the literature (*15*, *17*), and the various properties are found by the summation of the contributions of the various groups. Thus, the group contribution method has the advantage of predicting various thermodynamic properties through estimation of the effects of the various groups. The UNIFAC method was based on the universal quasi chemical activity coefficient (UNIQUAC) method, which is another method derived from an extension of Guggenheim's quasi-chemical theory of liquid mixtures (*18*).

In food systems, the UNIFAC equation has been applied for the prediction of water activity in sugar solutions and for the prediction of volatility of aromatic compounds in sugar solutions (*19*−*23*). Torres and Meirelles (*24*) predicted the oil vapor pressures, as well as vapor−liquid equilibrium of oil, by using the UNIFAC set of equations.

In the present study, the partition coefficient, K_p, between olive oil and water phases of selected phenolic compounds related to olive oil, some of which are powerful antioxidants, was determined experimentally and predicted using the UNIFAC method.

MATERIALS AND METHODS

Commercial olive oil was purchased from the local supermarket. Its phenolic content was stripped off with a methanol/water (80:20) mixture and used as such for experimentation. Oleuropein was obtained from Extrasynthase (Genay, France); protocatechuic acid and caffeic acid were from Sigma Chemical Co. (St. Louis, MO); tyrosol was from Fluka (Bunchs, Switzerland); and gallic acid was from Aldrich (Steinheim, Germany). 3,4-DHPEA, 3,4-DHPEA-EDA, and 3,4-DHPEA-EA were gifts from Professor G. Montedoro of University of Perugia, Italy.

The partition coefficient, K_p, of antioxidants between the olive oil and water phases was determined according to Archer et al (*25*). The methodology involved the formation of 1:1 (w/w) oil/water mixtures containing the antioxidant or phenolic compound. The water or oil phase containing the antioxidants at various concentrations was mixed with either olive oil or water, respectively, and homogenized with an Ultra Turrax (IKA Werke, Germany) at 6,000 rpm for 1 min after purging with nitrogen and then centrifuged at 27000*g* to break down the emulsion. Specific information for each substance tested is as follows: concentrations of oleuropein in water before mixing [0.6, 1, 3, 5, 10, 15, and 20 mg/g], of protocatechuic acid [0.1, 0.2, 0.5, and 1 mg/g], and of caffeic acid [0.05, 0.1, 0.25, and 0.5 mg/g]. 3,4-DHPEA-EA and 3,4-DHPEA were first dissolved in water at concentrations of 1.94 μg eqGA/g (eqGA, equivalent of gallic acid) and 254 μg eqGA/g, respectively, and then mixed with olive oil at the following ratios: 10:2, 10:5, 10:10, and 10:20 water/oil (w/w). Tyrosol and 3,4-DHPEA-EDA, though, were first dissolved in olive oil at concentrations of 1.74 μg eqGA/g and 6.8 μg eqGA/g, respectively, and then mixed with distilled water at the following ratios: 10:1, 10:2, 10:3, 10:4, 10:5, 10:6, 10:10, 10:15, and 10:20 oil/water (w/w) for tyrosol, and 10:2, 10:5, 10:10, and 10:20 oil/water (w/w) for 3,4-DHPEA-EDA. The concentrations of the antioxidants (phenolic compounds) in the oil phase were determined quantitatively with high-performance liquid chromatography (HPLC). HPLC analyses were conducted according to the Montedoro procedure (*8, 13*) which has been modified as described in the following. The qualitative and quantitative analysis of the antioxidants was carried out by reversed-phase high-performance liquid chromatography (RPHPLC) on a Waters 600E pump controller (Waters, Milford, MA) equipped with a C18 NovaPak column (4.6 × 250 mm) (Waters) in combination with a guard column. The chromatograms were monitored using a Waters 486 tunable absorbance detector at 280 nm. The column was eluted at room temperature with a gradient mobile phase consisting of methanol and 1% acetic acid in water as follows: gradient time (min), 1% acetic acid (%), methanol (%): 2, 95, 5; 5, 75, 25; 15, 60, 40; 25, 50, 50; 40, 40, 60; respectively. The flow rate was set at 1 mL/min and the backpressure was set below 2000 psi. The system was controlled by Waters baseline 815 data acquisition and control software. The concentrations of all antioxidants were expressed as equivalents of gallic acid.

Prediction of Partitioning in Oil/Water System. The partition coefficient was calculated by dividing the equilibrium concentration of antioxidant in the oil and water phases, respectively.

$$K_p = C_{oil}/C_{water} \quad (1)$$

At equilibrium, the activity of any component is the same in both phases by definition, therefore, for an antioxidant−oil/antioxidant−water system the following relationship may apply:

$$(\gamma_1^{\infty} y)_{oil} = (\gamma_2^{\infty} x)_{water} \quad (2)$$

where γ_1^{∞}, γ_2^{∞} are the activity coefficients of a substrate (i.e., an antioxidant) in the oil or in the water phase at infinite dilution, respectively; and x and y are the (molecular) concentrations of the substrate in the two phases.

The partition or distribution coefficient may be expressed as the ratio of molecular concentrations in the two liquid streams (oil and water) by the following equation:

$$K_p^{o} = y_{oil}/x_{water} \quad (3)$$

The eq 2 may result in the following:

$$K_p^{o} = y_{oil}/x_{water} = \gamma_2^{\infty}/\gamma_1^{\infty} \quad (4)$$

where y_{oil} is the molecular concentration of the substance (antioxidant) in the oil phase and x_{water} is the corresponding concentration in the aqueous phase.

The relationship between eqs 1 and 4 is the following (*26*):

$$K_p = K^{o}_p(MW_{water}/MW_{oil}) = (y_{oil}/x_{water})(MW_{water}/MW_{oil}) = (\gamma_2^{\infty}/\gamma_1^{\infty})(MW_{water}/MW_{oil}) \quad (5)$$

where MW_{oil} and MW_{water} are the molecular weights of oil and water, respectively.

Therefore, by calculating or predicting the activity coefficients one may be able to predict the partition coefficient of a substrate (i.e., antioxidant) between the two phases (oil and water) according to eq 5. The prediction of the activity coefficients γ_1^{∞}, γ_2^{∞} may be done using the UNIFAC method for small concentrations of antioxidants.

UNIFAC Model. According to this model the activity coefficient of the liquid phase may be found by adding two terms: a combinatorial term and a residual term.

$$\ln \gamma_i = \ln \gamma_i^C + \ln \gamma_i^R \quad (6)$$

where the combinatorial part of the activity coefficient is derived from pure component properties such as group volume and area constants, and it is given by the equation:

$$\ln(\gamma_i^C) = \ln(\Phi_i/x_i) + 5q_i \ln(\theta_i/\Phi_i) + I_i - (\Phi_i/x_i)\sum_j x_j I_j \quad (7)$$

$$\theta_i = q_i x_i / \sum_j q_j x_j y \quad (8)$$

$$\Phi_i = r_i x_i / \sum_j r_j x_j \quad (9)$$

$$r_i = \sum_k \nu_k^{(i)} R_k \quad (10)$$

$$q_i = \sum_k \nu_k^{(i)} Q_k \quad (11)$$

$$l_i = 5(r_i - q_i) - (r_i - 1) \quad (12)$$

The residual part of the activity coefficient is a function of group area fractions and their interactions in pure components and in mixtures, and it is given by the equation:

$$\ln(\gamma_i^R) = \sum_k \nu_k^{(i)} (\ln\Gamma_k - \ln\Gamma_k^{(i)}) \quad (13)$$

where Γ_k is the group residual activity coefficient and $\Gamma_k(i)$ is the residual activity coefficient of a group k in a reference solution containing only molecules of i (i.e., water or oil).

$$\ln\Gamma_i = Q_k[1 - \ln(\sum_m \theta_m \psi_{mk}) - \sum_m (\theta_m \psi_{km} / \sum_n \theta_n \psi_{nm})] \quad (14)$$

$$\theta_m = Q_m X_m / \sum_n Q_n X_n \quad (15)$$

$$X_m = \sum_i \nu_m^{(i)} x_i / \sum_j \sum_i \nu_j^{(i)} x_i \quad (16)$$

$$\theta_{mn} = \exp(-a_{mn}/T) \quad (17)$$

The parameter table data reported by Magnussen et al. (*16*) was used for the determination of the activity coefficients. These data are considered appropriate for use in liquid−liquid equilibrium calculations. The various substances which were considered in this work (water, oil, antioxidants) were analyzed in various groups whose thermodynamic constants are known. For simplicity reasons the olive oil was considered as being made exclusively of tri-ester of glycerol, with oleic acid ($CH_3(CH_2)_6CH{=}CH(CH_2)_6CH_2COOH$) being the only fatty acid of the ester. The oleic acid is usually approximately 85% of all fatty acids of the olive oil. The groups which were considered for each molecule are shown in Table 1. The thermodynamic parameters group volume (R_i) and area (Q_i) as well as the group interaction parameters (a_{mn}) were taken from Magnussen et al. (*16*) and Reid et al. (*17*).

RESULTS

Experimental Results on Partitioning. During oil extraction from the olives the phenolic compounds are distributed between the oil and aqueous phases. It is important to determine the partition coefficients of these compounds in oil/water mixtures because their concentration has beneficial effects on the stability, aroma, and nutritional properties of the oil.

The ratio of concentration of antioxidants in the oil phase against the concentration in the aqueous phase attained a maximum value after some time of mixing. This time was approximately 17 min for most antioxidants, and it was considered as the time to attain equilibrium. Figure 1 shows the ratio of concentrations of tyrosol in the oil and water phases against time. It may be seen that the ratio $[tyrosol]_{oil}/[tyrosol]_{water}$ attained a maximum value of 0.09 after 17 min of mixing of the two phases (oil/water). Similar curves were obtained for the other antioxidants. Thus, all measurements of antioxidants took place after equilibrium was reached (>17 min).

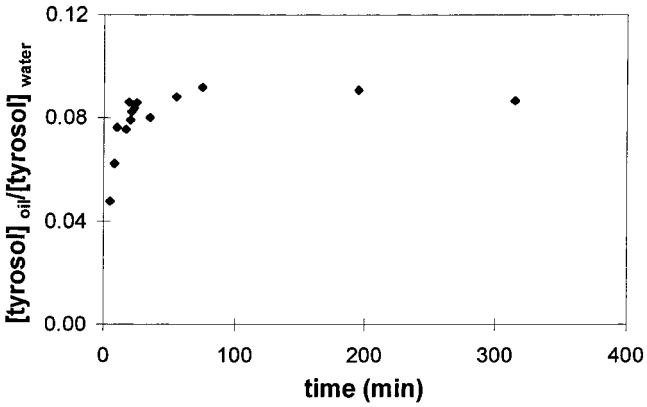

Figure 1. Distribution of tyrosol in the oil and aqueous phases against time of mixing. Equilibrium was attained after 17 min.

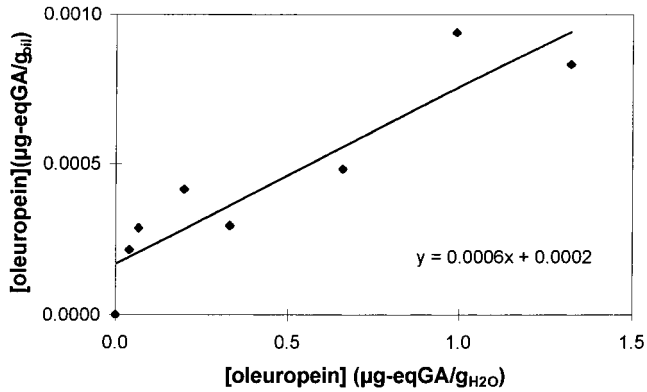

Figure 2. Partitioning of oleuropein between oil and water phase at equilibrium at 20 °C (eqGA, equivalent of gallic acid). Number of repetitions, 5.

Table 1. Type and Number of Groups of Various Molecules Involved in Liquid–Liquid Equilibria (oil, water, antioxidants)

molecule	UNIFAC groups
olive oil	3 groups CH_3, 41 CH_2, 1 CH, 3 CH═CH, 3 CH_2COO
water	1 group H_2O
oleuropein	1 group CH_3, 2 CH_2, 6 ACH, 1 $ACCH_2$, 2 ACOH, 2 CHO, 1 CH_2COO, 1 CH_3COO, 1 –O-, 1 AC, 1 C═CH
tyrosol	4 groups ACH, 1 $ACCH_2$, 1 CH_2, 1 OH, 1 ACOH
protocatechuic acid	3 groups ACH, 1 AC, 2 ACOH, 1 COOH
3,4-DHPEA	3 groups ACH, 1 $ACCH_2$, 1 CH_2, 1 OH, 2 ACOH
3,4-DHPEA-EDA	1 group CH_3, 2 CH_2, 1 CH, 1 C═CH, 3 ACH, 1 $ACCH_2$, 2 ACOH, 2 CHO, 1 CH_2COO, 1 CHO
3,4-DHPEA-EA	1 group CH_3, 2 CH_2, 6 ACH, 1 $ACCH_2$, 2 ACOH, 2 CHO, 1 CH_2COO, 1 CH_3COO, 1 –O-, 1 AC
caffeic acid	1 group CH═CH, 3 ACH, 1 AC, 2 ACOH, 1 COOH

Figure 2 shows the distribution of oleuropein between the oil and the water phase after the equilibrium was reached. Oleuropein is the parent molecule of powerful antioxidants in the oil such as 3,4-DHPEA. The solubility of oleuropein in the aqueous phase is much higher than in the oil phase resulting in a K_p=0.0006 (mg of oleuropein in the oil phase per g of oil/mg of oleuropein in the water phase per g of water). This low K_p

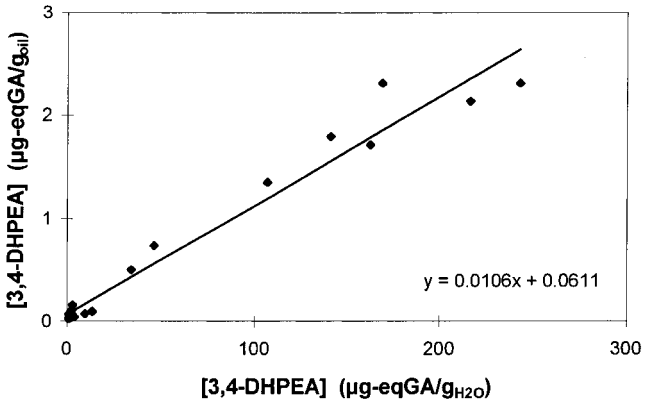

Figure 3. Partitioning of 3,4-DHPEA between oil and water phase at equilibrium at 20 °C (eqGA, equivalent of gallic acid). Number of repetitions, 5.

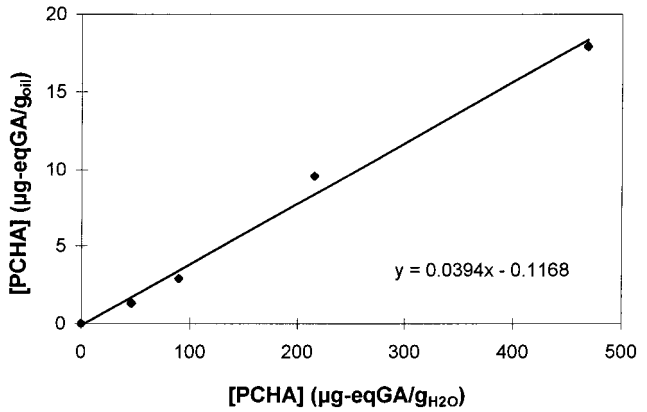

Figure 4. Partitioning of protocatechuic acid between oil and water phase at equilibrium at 20 °C (eqGA, equivalent of gallic acid). Number of repetitions, 5.

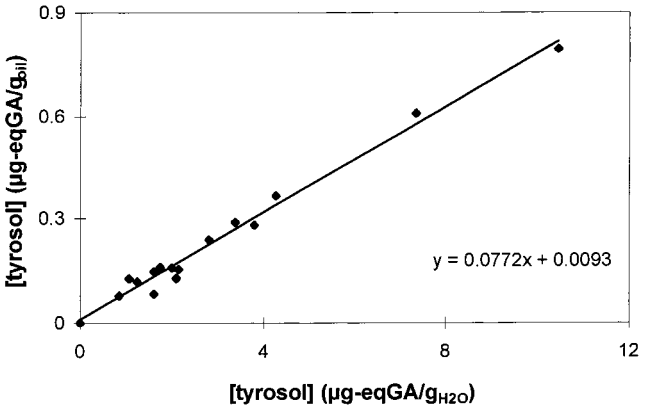

Figure 5. Partitioning of tyrosol between oil and water phase at equilibrium at 20 °C (eqGA, equivalent of gallic acid). Number of repetitions, 5.

value of oleuropein, obviously due to its structure, explains the observation that oleuropein is not practically found in the oil phase.

Similar curves showing the partitioning of other antioxidants between the aqueous and the oil phases at 20 °C is given in Figures 3, 4, 5, 6, 7, and 8 for 3,4-DHPEA, protocatechuic acid, tyrosol, caffeic acid, 3,4-DHPEA-EDA, and 3,4-DHPEA-EA, respectively. The partition coefficients of these antioxidants are significantly higher than the partition coefficient of oleuropein, ranging from 0.01 to 1.49, but still the K_p values for tyrosol, protocatechuic acid, caffeic acid, and 3,4-DHPEA were very low, pointing to their hydrophilic nature.

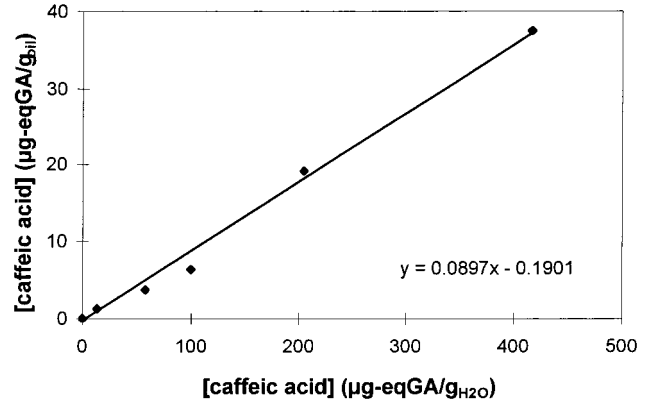

Figure 6. Partitioning of caffeic acid between oil and water phase at equilibrium at 20 °C (eqGA, equivalent of gallic acid). Number of repetitions, 5.

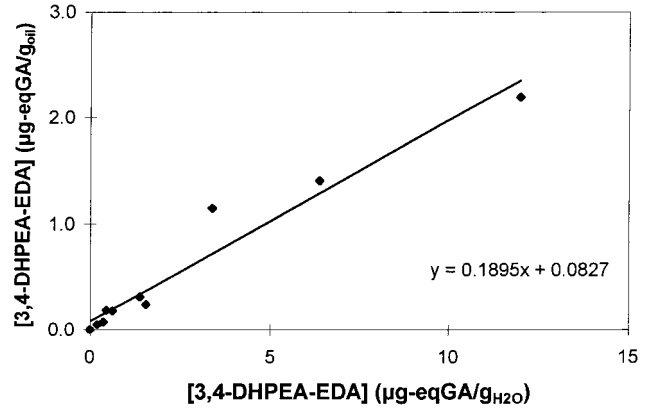

Figure 7. Partitioning of 3,4-DHPEA-EDA between oil and water phase at equilibrium at 20 °C (eqGA, equivalent of gallic acid). Number of repetitions, 5.

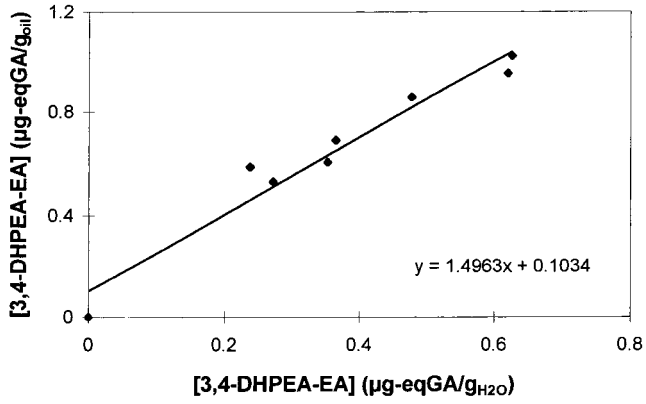

Figure 8. Partitioning of 3,4-DHPEA-EA between oil and water phase at equilibrium at 20 °C (eqGA, equivalent of gallic acid). Number of repetitions, 5.

The highest value of partition coefficient was found for 3,4-DHPEA-EA ($K_p = 1.49$). The partition coefficients for various antioxidants appear in Table 2 in the order of increasing K_p. As mentioned earlier, the most potent antioxidant is the three forms 3,4-DHPEA, 3,4-DHPEA-EDA, and 3,4-DHPEA-EA. The form 3,4-DHPEA has a $K_p = 0.01$ (approximately), thus, it is much less soluble in oil than in water. Thus, most of this form is lost during water treatment of the olive pulp (extraction). However, the two other forms, the 3,4-DHPEA-EDA and 3,4-DHPEA-EA, are 0.189 and 1.5 times, respectively, more soluble in oil than in water, thus having a considerable solubility in the oil phase.

Table 2. Experimentally Estimated Partition Coefficients of Olive Oil Antioxidants between Oil and Water Phases

antioxidant	partition coefficient (*K*p)
oleuropein	0.0006
3,4-DHPEA	0.0100
protocatechuic acid	0.0390
tyrosol	0.0770
caffeic acid	0.0890
3,4-DHPEA-EDA	0.1890
3,4-DHPEA-EA	1.4900

Table 3. Partition Coefficients of Olive Oil Antioxidants among Oil and Water Phases as Predicted by UNIFAC

antioxidant	*K*p (25 °C)	*K*p (45 °C)	*K*p (65 °C)
oleuropein	0.0012	0.0034	0.0087
3,4-DHPEA	0.0004	0.0012	0.0026
protocatechuic acid	0.0250	0.0430	0.0580
tyrosol	0.0970	0.1510	0.2220
caffeic acid	0.3600	0.5920	0.7970
3,4-DHPEA-EDA	0.1870	0.2990	0.4130
3,4-DHPEA-EA	11.8000	16.5000	20.0400

Figures 2−8 also show that Henry's law applied quite well to the experimental data in that the relationship of the solubility of antioxidants at various concentrations in the two phases obey a linear relationship equation.

Results from the Application of the UNIFAC Model. The UNIFAC model was applied for the determination of the activity coefficients of the antioxidants at infinite dilution, γ_i^∞, in the two phases (oil and water). The estimated activity and partition coefficients (eq 5) are given in Table 3.

The reported values are for infinite dilution of antioxidants (practically $<10^{-4}$) in both phases at 25 °C, 45 °C, and 65 °C. The UNIFAC method resulted in higher γ_i^∞ of antioxidants for both phases (oil and water) as the temperature was raised from 25 to 65 °C with a much higher effect on the activity coefficient of the aqueous phase (a sharp increase of γ_i^∞). As a result, the partition coefficient overall increased at temperatures higher than 25 °C. This may be of interest when designing a process such as liquid extraction of oil, with consideration on product quality (antioxidant retention). The values of K_p, as predicted by the UNIFAC, are not very close to the experimental values of partition coefficient (Table 3), although there is some relevance. This is due to the model of UNIFAC which was applied and to the very complicated nature of antioxidants. This problem may be solved by introducing new interaction parameters between groups instead of the parameters given by Magnussen et al. (*16*) and Reid et al. (*17*). The new parameters may be obtained from an optimization technique applying the experimental partition coefficient or activity coefficient data to the model (*20*, *27*).

DISCUSSION

The difference in solubility explains why the 3,4-DHPEA, tyrosol, caffeic, and protocatechuic acid are found in small quantities in the oil phase.

The low partition coefficients of most olive oil antioxidants result in their sizable loss with the wastewater during processing. On the basis of the tool of the UNIFAC prediction model it is possible to predict the concentrations of the various substrates between the two phases at various process temperatures and pressures (*28*). As explained above, the model showed a relative increase (improvement) of the partitioning of antioxidants at higher process temperatures.

Limiting the quantity of water used during the oil extraction processes could be a basis for designing alternative processes for enriching olive oil with antioxidants. Indeed, use of the two-phase centrifugal decanter, where the volume of water added to dilute the paste is much less than that in the conventional three-phase unit, has resulted in increased concentration of *o*-diphenols in the oil (*5*).

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LITERATURE CITED

(1) Petroni, A.; Blasevich, M.; Salami, M.; Papini, N.; Montedoro, G. F.; Galli, C. Inhibition of platelet aggregation and eicosanoid production by phenolic components of olive oil. *Thromb. Res.* **1995**, *78* (2), 151−160.

(2) Visioli, F.; Bellomo, G.; Montedoro, G. F.; Galli, C. Low-density lipoprotein oxidation is inhibited *in vitro* by olive oil constituents. *Atherosclerosis* **1995**, *117*, 25−32.

(3) Manna, C. Olive oil hydroxytyrosol protects human erythrocytes against oxidative damages. *J. Nutr. Biochem*. **1999**, *10*, 159−165.

(4) Vazquez-Roncero, A.; Janer Del Valle, L.; Janer Del Valle, C. Componentes fenolicos de la acetuna. III. Polyfenoles del aceite. *Grasas Aceites* **1978,** *27*, 185−191.

(5) Vazquez-Roncero, A. Les polyphenols de l' huile d' olive et leur influence sur les characteristiques de l' huile. *Rev. Fr. Corps Gras* **1978**, *25*, 21.

(6) Montedoro, G.; Bertuccioli, M.; Anichini, F. Aroma analysis of virgin oil by headspace (volatiles) and extraction (polyphenols) techniques. In *Flavor of Foods and Beverages*; Charalambous, G., Ed.; Academic Press: New York, 1978; pp. 247−281.

(7) Pannelli, G.; Servili, M.; Baldioli, M.; Montedoro, G. F. Changes in the phenolic and pectic substances in olive fruit and oil as a function of ripening, cultivar and extraction technology. *OLEA* **1991**, *21*, 64.

(8) Montedoro, G. F.; Servili, M.; Baldioli, M.; Miniati, E. Simple and hydrolyzable phenolic compounds in virgin olive oil. 1. Their extraction, separation and quantitative and semiquantitative evaluation by HPLC. *J. Agric. Food Chem.* **1992**, *40*, 1571−1576.

(9) EU. Improvement of olive oil quality by biotechnological means; Final Technical Report, Project AIR3-CT93-1355; European Union: Brussels, Belgium, 2000.

(10) Papadopoulos, G.; Boskou, D. Antioxidant effect of natural phenols on olive oil. *J. Am. Chem. Soc*. **1991**, *68*, 669−671.

(11) Kochhar, S. P.; Rossel, J. B. In *Food Antioxidants;* Hudson, B. J. F., Ed.; Elsevier Applied Science: London, 1990; pp 19−53.

(12) Montedoro, G. F.; Servili, M.; Baldioli, M.; Miniati, E.; Macchioni, A. Simple and hydrolyzable compounds in virgin olive oil. 3. Spectroscopic characterizations of the secoiridoid derivatives. *J. Agric. Food Chem*. **1993**, *41*, 2228−2234.

(13) Baldioli, M.; Servili, M.; Perretti, G.; Montedoro, G. F. Antioxidant activity of tocopherols and phenolic compounds of virgin olive oil. *J. Am. Oil Chem. Soc.* **1996**, *73* (3), 1589−1593.

(14) Ranalli, A.; Angerosa, F. Integral centrifuges for olive oil extraction. The quality characteristics of products. *J. Am. Oil Chem. Soc.* **1996**, *73* (4), 417−422.

(15) Fredenslund, A.; Gmehling, J.; Rasmussen, P. *Vapor-Liquid Equilibria Using UNIFAC*. Elsevier: Amsterdam, The Netherlands, 1977.

(16) Magnussen, T.; Rasmussen, P.; Fredenslund, A. UNIFAC parameter table for prediction of liquid−liquid equilibria. *Ind. Eng. Chem. Process Des. Dev*. **1981**, *20*, 331−339.

(17) Reid, R. C., Prausnitz, J. M., Poling, B. E. *The Properties of Gases and Liquids*, 4th edition; McGraw-Hill: New York, 1987.

(18) Abrams, D. S.; Prausnitz, J. M. Statistical thermodynamics of liquid mixtures. A new expression for the excess Gibbs energy of partly and completely miscible systems. *AIChE J.* **1975**, *21*, 116.
(19) Haralanpu, S. G. Using UNIFAC to predict aroma losses in juice concentrations. In *Drying '86*, vol. 2; Mujumdar, A., Ed.; Hemisphere Publishing Co.: Washington, DC, 1986; pp. 742−746.
(20) Saravacos, G. D.; Karathanos, V. T.; Marinos-Kouris, D. Volatility of fruit aroma compounds in sugar solutions. In *Flavors and Off-Flavors*; Charalambous, G., Ed.; Elsevier Science Publishers: B. V., Amsterdam, 1990; pp. 729−738.
(21) LeMaguer, M. Thermodynamics of vapor-liquid equilibria. In *Physical Chemistry of Foods*; Schwartzberg, H. G., Hartel, R. N., Eds.; Marcel Dekker: New York, 1992; pp. 1−42.
(22) LeThanh, M.; Lamer, T.; Voilley, A.; Jose, J. Determination des coefficients de partage vapeur-liquide et d' activite de composes d' arome a partir de leurs characteristiques physico-chimiques. *J. Chim. Phys.* **1993**, *90* (3), 545−560.
(23) Sorrentino, F.; Voilley, A.; Richon, D. Activity coefficients of aroma compounds in model food systems. *AIChE J.* **1986**, *32* (12), 1988−1993.
(24) Torres, M. B.; Meirelles, A. J. Prediction of fatty acids and fatty esters vapor pressure and of vapor-liquid equilibrium using UNIFAC. In *Proceedings of the 7th International Congress on Engineering and Food*; Jowitt, R., Ed.; Sheffield Academic Press: Sheffield, UK, 1997; pp. SA 35−38.
(25) Archer, M. H.; Dillon, V. M.; Campbell-Platt, G.; Owens, J. D. The partitioning of diacetyl between food oils and water. *Food Chem.* **1994**, *50*, 407−409.
(26) Perry, R. H.; Green, D. W.; Maloney, J. O. *Perry's Chemical Engineers' Handbook*, 6th edition; McGraw-Hill: New York, 1984; Ch. 15, pp 15/3−7.
(27) Skjold-Jorgensen, S.; Kolbe, B.; Gmehling, I.; Rasmussen, P. Vapor-liquid equilibria by UNIFAC group contribution. Revision and extension. *Ind. Eng. Chem. Process Des. Dev.* **1979**, *18* (4), 714−722.
(28) Bruin, S. Phase equilibria for food product and process design. *Fluid Phase Equil.* **1999**, *160*, 657−671.

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