

The Role of Water in the Oxidation Process of Extra Virgin Olive Oils

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ABSTRACT: Different emulsifying techniques were used to study the influence of water on the oxidative degradation of extra virgin olive oil. The emulsions of water/extra virgin olive oil, oxidized with O₂, UV radiation, and Air, were prepared by dispersing water under different conditions. Oil oxidation, monitored by measuring the PV and polyphenolic content, was greater for emulsions obtained with low dispersing power. A linear model was used to correlate the PV and the polyphenolic content with dispersing energy. An important result was that the dispersed water exerted a positive antioxidant effect on the oil.

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Olive oil has received a great deal of attention lately since industrialized nations have realized that obesity, arteriosclerosis, high blood pressure, and diabetes are correlated to poor eating habits (1). Olive oil not only is highly digestible but also possesses excellent nutritional values (2).

Before becoming suitable for marketing, from a technological viewpoint, it undergoes a decanting or centrifugal step, during which water and other material in suspension are separated from the oil in order to make it transparent (3).

In recent years, some authors have shown that the water and the small particles dispersed in the oil act as antioxidants. In other words, the depletion of such substances, which occurs during the filtering process, may favor oil degradation (4).

It must be stressed that in addition to the water, other factors, e.g., phenolic compounds, prooxidants, and enzymes, contribute to the oxidative process (5). Further, olive oil is a natural product, and the chemical composition of its nonpolar part and of the liposoluble antioxidants may differ significantly from oil to oil. To emphasize the role of the dispersed water as a stabilizing agent against the oxidative deterioration of the olive oil, we decided to check this effect by looking at the conditions under which the water can be dispersed into the apolar matrix.

For this reason, we did not analyze the natural oil as a whole, but as samples treated by filtering the particulate solid and adding water under controlled conditions. An important result was that the antioxidantizing function took place anyway.

Such a system represents a multiphase (heterogeneous) system, in which one phase (water) exists in the form of small droplets (dispersed phase) and the rest constitutes the so-called continuous phase. In general, the area of the interface between the dispersed and continuous phases is rather large, and the more numerous the small-sized droplets, the larger the interfacial area. This is the reason the interfacial properties are of crucial importance for the stability of emulsions. Moreover, the large interface is the boundary where physicochemical processes of great interest occur (6,7).

The way in which water can be dispersed in the oil matrix affects the interfacial properties of the emulsion itself. Thus, a correlation between the oxidation rate and the structure of the water/olive oil emulsion might be expected.

In order to test this hypothesis, differently prepared emulsions of water/olive oil were oxidized in different ways. The final mixtures were analyzed using both chemical and physical methods. In the present paper we show the results of some chemical investigations that addressed the influence of dispersed water in the oxidative process.

EXPERIMENTAL PROCEDURE

Chemicals. All the chemicals used were reagents of analytical grade of 99.9% purity purchased from Carlo Erba Company (Milan, Italy) and used without any further purification.

Oil sampling. The oil was taken directly from the crusher and treated for 15 min with an N₂ current and stored at 4°C. Olives of mixed cultivars (Cima di Mola, Cima di Bitonto, and others) were picked during the first 10 d of the 1999–2000 November oil campaign and pressed a few days later. The oil, produced with a discontinuous process, was characterized using conventional analytical methods.

Analytical methods. PV, total acidity, spectrophotometric indexes, and refractive index were determined by the official method recommended by the European Community (8).

Analysis of polyphenols consisted of two steps: (i) The compounds were extracted by a methanol/water mixture, as described by Montedoro *et al.* (9), and then (ii) a spectrophotometric assay was used to estimate their relative amounts. The Folin–Ciocalteu reagent and the Arnou mixture were utilized to discriminate between the total phenols and orthodiphenols, respectively. The content of phenolics was expressed as ppm of caffeic acid equivalents.

A spectrophotometric assay, together with the Emmerie–Engel procedure, was used to determine the tocopherol con-

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tent in the oil sample, which was expressed as ppm of α -tocopherol equivalents (10).

A gas chromatograph with a Supelcowax 10 capillary column (Supelco, Bellefonte, PA) (length 30 m, i.d. = 0.32 mm, film 0.50 μ m) was used for the analysis of FA composition, and the experimental conditions were designed as described in Reference 11.

The same instrument equipped with an Rtx-65 TG Restek capillary column (Restek Corp., Bellefonte, PA) (length 15 m, i.d. = 0.32 mm, film 0.10 μ m) was used to determine the TG composition, and the experimental conditions were designed according AOAC official methods (12).

Experimental methods. The role of water was studied by performing all the analyses "simultaneously" on three different oil samples and comparing the results. The first sample constituted oil without further treatments (natural oil), the second was composed of completely filtered oil (filtered oil), and the third was a sample with 3% (w/w) of dispersed water (emulsified oil).

Preparation of emulsions. Olive oil (45 g) was first filtered in the presence of anhydrous Na_2SO_4 and then 3% (w/w) of water was added. To obtain emulsions under controlled conditions, the mixtures were shaken for 30 min using different methods. We used an Ultraturrax T8 IKA Labortechnik (Janke & Kunkel GmbH, Staufen, Germany), a Vortex ZX3 VELP Scientifica (Milan, Italy), and a Carlo Erba ASAL (Milan, Italy) Model 708 rotatory mixer, which supplied 100, 45, and 40 W of power, respectively. These procedures will be referred to as (U), (V), and (R), respectively, and defined as emulsifying techniques.

Oxidizing conditions. The oil samples were oxidized under controlled conditions with three different modalities. A first aliquot of the sample, placed in a polyvinyl chloride vessel, was exposed to UV light (wavelength 254 nm) for 24 h. A second one was kept in an atmosphere saturated with pure oxygen and exposed to white light under magnetic stirring for 24 h. The third one, also magnetically stirred, was oxidized with air and in darkness for 24 h. All these oxidizing conditions were carried out at room temperature. The terms UV, O_2 , and air will be used to designate these three conditions.

To realize controlled conditions for all samples (natural, filtered, and emulsified) before oxidizing them, all samples underwent the same emulsifying conditions. The precision of the results was estimated from several runs (not less than three), and the SD were evaluated.

RESULTS AND DISCUSSION

Before investigating the effect of the added water, the olive oil used was characterized analytically. Results of these analyses were as follows: PV (meq O_2/kg) 5.90 ± 0.05 (eight repeated measurements); acidity (%) 0.262 ± 0.009 (six measurements); total tocopherols (mg/kg) 335 ± 50 (three measurements); refractive index (n_D) 1.4677 ± 0.0005 (four measurements); phenols (mg/kg) 484 ± 40 (three measurements); ortho-diphenols (mg/kg) 416 ± 40 (three measurements); K_{232} 1.62 ± 0.01 (three measurements); K_{270} 0.10 ± 0.03 (three measurements); $\Delta K = K_{270} - 0.5 (K_{266} - K_{274})$ 0.006 . (The means and SD, σ , refer to

repeated measurements. K_λ is the specific extinction coefficient at wavelength λ ; for the details of symbolism see Ref. 8.) The oil composition data regarding the TG and FA distributions are displayed in Table 1. These results indicated that the oil sample used was a typical Italian extra virgin olive oil. Furthermore, the data shown above were used to evaluate the precision and sensitivity of each experimental determination. The experimental uncertainty was estimated by calculating the SD, σ , of repeated measurements. We also investigated whether the experimental differences between different samples were significant. This was carried out by calculating the mean value and the SD of each set of measurements and then comparing the single deviations with respect to σ . The measurements were repeated three times, and an ANOVA was used to test the differences in treatment means for each oxidizing condition. Mathematical computations were performed on Matlab version 5.3 for PC (The MathWorks, Natick, MA), using the subroutine Anova1, whose mathematical details are reported by Hogg and Ledolter (13). The probability of the hypothesis that the population means were the same was calculated for all sets of data. For PV results, this probability was always extremely small (less than 0.01%), in contrast with that of total phenols and ortho-diphenols in the presence of air, where it was higher (about 7%). In any case, the analyses of phenols were used only as a control, and this greater uncertainty in the experimental method did not affect the conclusions of the work.

The oxidation phenomenon is very complex and involves various factors (14). This reaction leads to the formation of volatile products that change the original flavor. Therefore, understanding the factors that influence such a process is extremely important. In Figure 1 the experimental results for PV are shown in the form of histograms, with their relative errors, for natural, emulsified, and filtered oil samples. One can see that the oil oxidized by UV light presented a constantly higher average PV independently of the emulsifying techniques used. That is, the exposure to UV radiation was the most efficient way to oxidize the oil. These results indicate that the UV radiation acted as "a sort of catalyst." This experimental evidence is in agreement with the mechanism of photooxidation observed in lipidic matrices (15–17).

TABLE 1
Extra Virgin Olive Oil Sample: FA and TG Distributions

FA	%	TG ^a	%
16:0	10.8	P-P-P	0.168
16:1	0.138	P-Po-P	0.243
17:0	0.594	P-O-P	4.38
18:0	1.74	P-L-P	1.25
18:1	74.5	P-O-S	0.558
18:2	8.27	P-O-O	26.4
18:3	0.693	P-L-O	5.21
20:0	0.316	P-L-L	0.995
20:1	0.325	S-O-O	1.79
Others	2.62	O-L-L	1.88
		O-L-Ln	0.948
		O-L-O	6.51

^aO, oleic acid; P, palmitic acid; Po, palmitoleic acid; L, linoleic acid; Ln, linolenic acid; S, stearic acid.

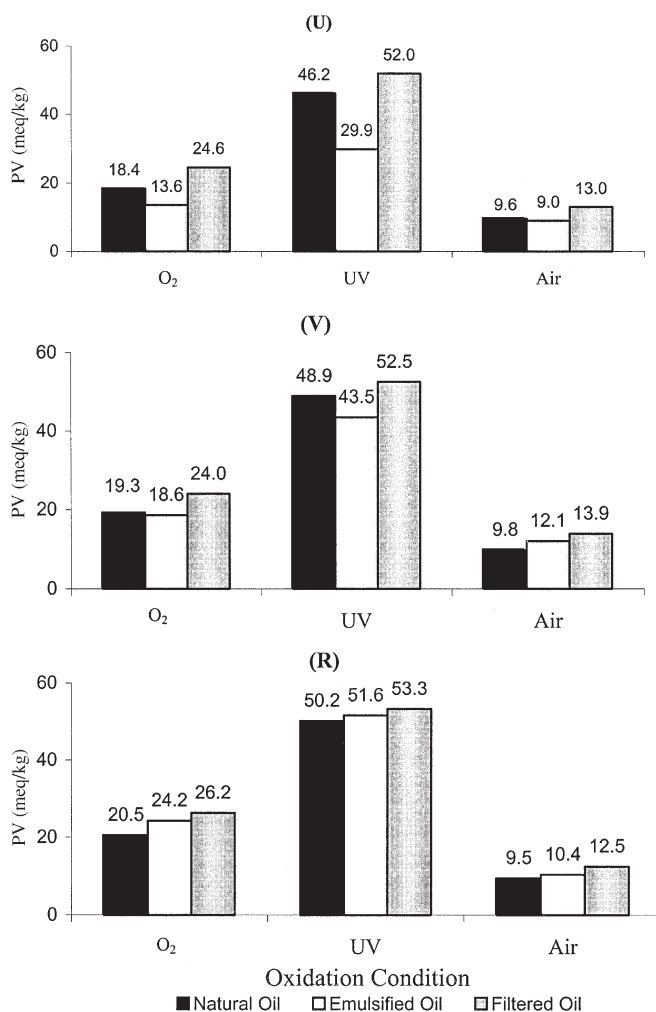


FIG. 1. PV of natural, filtered, and emulsified extra virgin olive oil as a function of oxidizing conditions and emulsifying techniques [(U), Ultraturax T8 IKA Labortechnik, Janke & Kunkel GmbH, Staufen, Germany, supplying 100 W of power; (V), Vortex ZX3, VELP Scientifica, Milan, Italy, supplying 45 W of power; and (R), Carlo Erba ASAL Model 708 rotary mixer, Milan, Italy, supplying 40 W of power.]: (O₂) is the sample exposed to white light under continuous stirring for 24 h in atmosphere saturated with oxygen; (UV) is the sample exposed to UV light ($\lambda = 254$ nm) for 24 h; and (Air) is the sample kept in the dark and exposed to the air. All the oxidations were carried out at room temperature.

To gain information about the extent to which the water/oil interface affected oil oxidation, we analyzed the PV values for fixed oxidizing conditions vs. the three emulsifying techniques. In the case of UV oxidation and emulsified oil, we found that PV increased according to the order (R) > (V) > (U) (Fig. 1). In contrast, for the filtered samples the differences were not significant; PV remained practically constant. The trend was the same for all three oxidizing conditions. It was evident that the dispersed water in emulsified oil had the capability to reduce the oxidation rate.

According to their action mode, phenolic antioxidants are included in the category of free radical terminators (17). It is well known that the antioxidant action of the phenolic compounds depends on the type and the position of their sub-

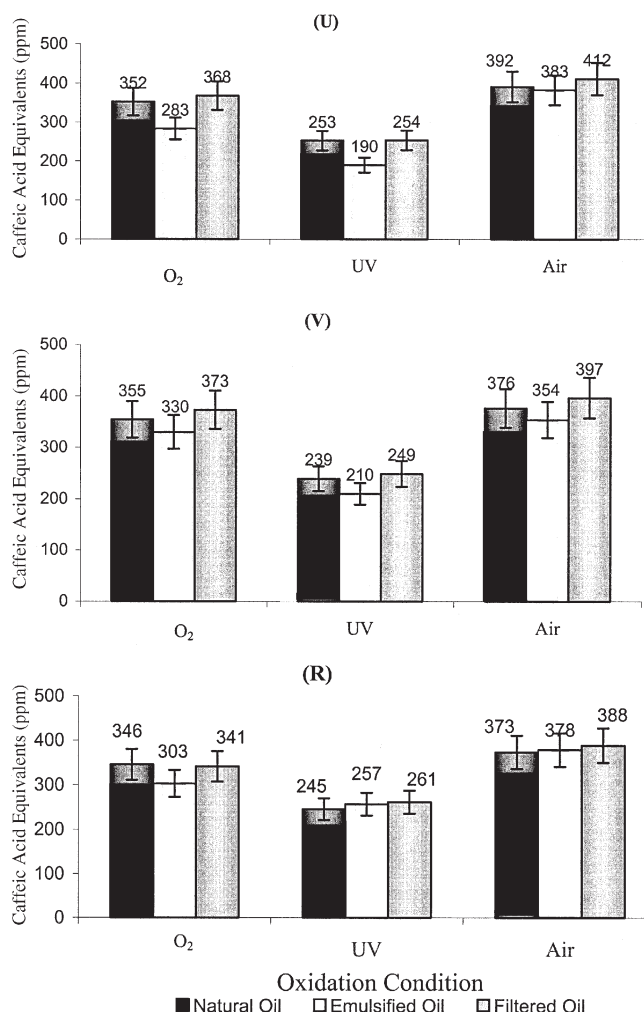


FIG. 2. Total polyphenols content, expressed as ppm of caffeic acid equivalents, of extra virgin olive oil as function of oxidizing conditions and emulsifying techniques (U, V, R). See Figure 1 for experimental details. Error bars represent SD ($n \geq 3$).

stituents (18). In the samples oxidized with O₂, UV, and air and emulsified with (U), (V) and (R) techniques, we also monitored the total phenols and ortho-diphenols contents. These results are illustrated in Figures 2 and 3. It is evident that for natural, filtered, and emulsified samples, both total phenols and ortho-diphenols were lower than the values reported in Table 1. The phenolic content in the samples oxidized with oxygen was significantly greater than in those oxidized with UV; this seems in agreement with the idea that the phenolic antioxidants do not act as singlet oxygen scavengers (19).

In any case, the largest variation was verified in the UV oxidation and (U) emulsification. Once more, it was evident that the water/oil interface was involved in the rate of the oxidation processes.

In order to understand the extent to which the emulsified water influenced oil acidity, it is sufficient to analyze Figure 4. Here we observe that the differences in total acidity were not significant; thus, the acidity was essentially independent of the oxidizing conditions and unperturbed by the emulsifying techniques. This evidence fits the observation that during the in-

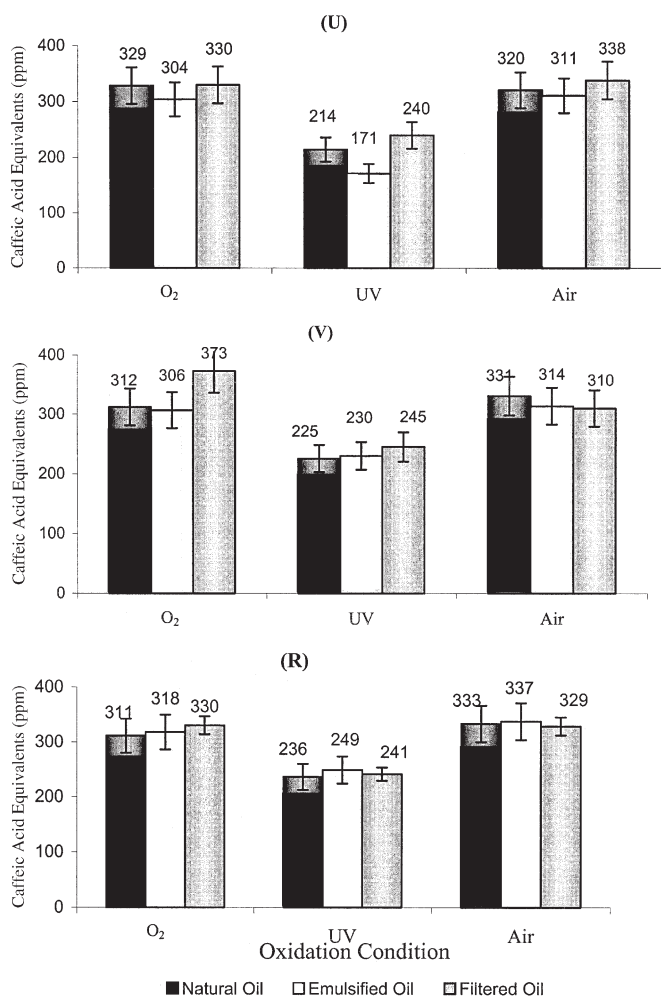


FIG. 3. Ortho-diphenols content, expressed as ppm of caffeic acid equivalents, of extra virgin olive oil as function of oxidizing conditions and emulsifying techniques (U, V, R). See Figure 1 for experimental details. Error bars represent SD ($n \geq 3$).

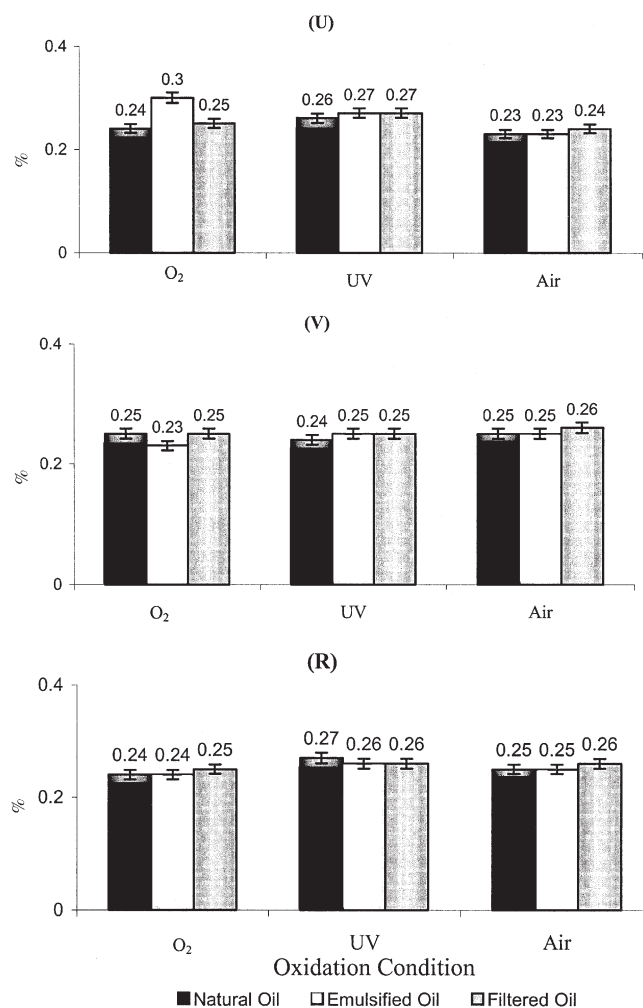


FIG. 4. Total acidity of extra virgin olive oil as function of oxidizing conditions and emulsifying techniques (U, V, R). See Figure 1 for experimental details. Error bars represent SD ($n \geq 3$).

duction period compounds that do not affect the total acidity (e.g., aldehydes, ketones, and alcohols) increase (20).

Further relevant information about the role of water in the oxidative process can be obtained by comparing the results of different emulsifying procedures for the same oxidizing conditions. To do this, a method of quantifying the (U), (V), and (R) techniques is needed.

To disperse the volume, V , of a liquid in the form of droplets of radius R in another liquid of surface tension γ , it needs energy, E , as given by

$$E = 3\gamma V/R \quad [1]$$

Assuming, as a first approximation, that the energy supplied to the system by shaking is completely utilized to disperse the water droplets, we may characterize each emulsifying technique by this energy value.

Figure 5 shows the plot of PV vs. E . A linear correlation coefficient of 0.90 indicates a good correlation between the oxidation rate and the emulsifying techniques. In this correlation plot each straight line represents an oxidizing condition,

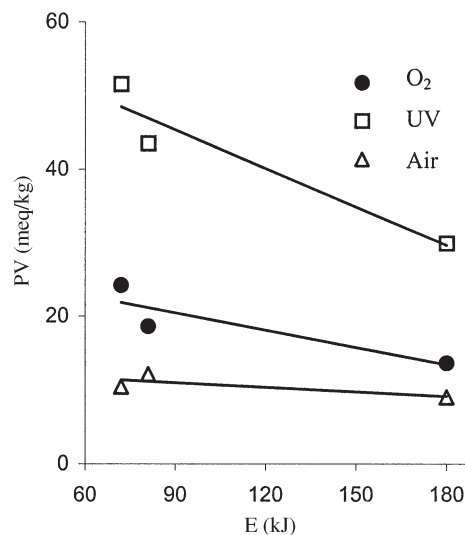


FIG. 5. PV of emulsified extra virgin olive oil as function of mechanical energy supplied to make the emulsions (see Fig. 1 for details). The symbols in the plot represent the oxidizing conditions.

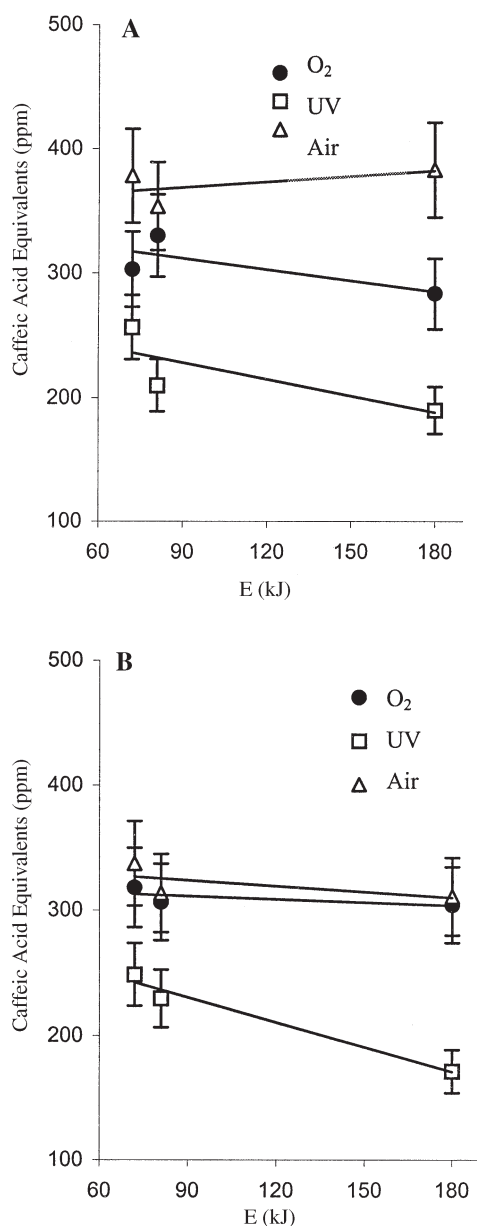


FIG. 6. Phenol content of emulsified extra virgin olive oil as a function of mechanical energy supplied to make the emulsions (see Fig. 1 for details): (A) total phenols; (B) ortho-diphenols. The symbols in the plots represent the oxidizing conditions. Error bars represent SD ($n \geq 3$).

and their relative positions are in the same order as discussed for Figure 1.

Equation 1 states that at higher energy, E , a smaller mean radius results in a larger surface/volume ratio. However, if the water/oil surface does alter the oxidation rate as the surface/volume ratio is varied, one might expect some influence of energy on the oxidation rate. Therefore, PV is expected to diminish at higher energy values, owing to the decrease in the droplet radius, as shown in Figure 5.

Strictly connected to the formation of hydroperoxides is the activity of the phenolic antioxidants. Figures 6A and 6B indicate that phenols also exhibit a strong correlation with the

energy, E . However, in this case, the relative position of the straight line is inverted in comparison with the ones of Figure 5, emphasizing the antioxidant character of these substances. A direct comparison between Figures 6A and 6B shows that the oil samples oxidized *via* air and O₂ do not show any significant differences in their ortho-diphenol content (Fig. 6B). In contrast with the total phenols, a net difference is found (Fig. 6A). The molecular structure of the ortho-diphenols is optimal for adsorbing onto the water/oil interface so that the activation energy is increased. Furthermore, for the oxidation *via* UV, one observes a slightly higher slope for the ortho-diphenols, indicating a larger antioxidant effect (Fig. 6B).

More information about the role of the dispersed water may be gathered by studying the droplet size time evolution in the emulsified oil.

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