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# Influence of Crystallization on the Oxidative Stability of Extra Virgin Olive Oil

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The aim of this study was to evaluate the influence of the extra virgin olive oil (EVOO) physical state on the kinetics of oxidative reactions. To this purpose, EVOO was stored at increasing temperatures from 3 to 60 °C and the oxidation was followed by measuring both primary and secondary oxidation products. Results highlighted that crystallization plays an important role in determining EVOO stability. Below the melting point, the oxidation rate was found to be higher than that expected on the basis of the Arrhenius equation. The observed deviation from the Arrhenius equation was attributed to the physicochemical changes occurring as a consequence of phase transitions. In particular, the increase in unsaturated triacylglycerol concentration and the decrease of polyphenol content in the liquid phase surrounding fat crystals were indicated as the main factors causing the deviation. By taking into account these changes it was possible to describe the temperature dependence of the oxidation rate in the entire range of temperatures considered. This model appears to be promising in the challenge to find mathematical models able to predict the stability and, hence, the shelf life of lipid-containing foods.

**KEYWORDS: Extra virgin olive oil; lipid oxidation; phase transition; shelf life; ASLT**

## INTRODUCTION

In Mediterranean countries extra virgin olive oil (EVOO) represents the major edible vegetable oil. Recently, due to the evidence of its beneficial health effect, olive oil is becoming a widespread food product. Nowadays, EVOO is used not only by the end consumer as a flavoring and cooking fat but also by the food industry as an ingredient in a wide range of products. For instance, besides the use as filling medium of canned products, it represents the lipid phase of a number of food formulates, such as salad dressings, sauces, and chilled and frozen ready-to-eat products.

The shelf life of EVOO-containing food greatly depends on the oil's stability. As is well-known, among vegetable oils, EVOO is one of the more resistant to oxidation because of its low unsaturation level and its high natural antioxidant content (i.e., phenolic compounds) (*1*−*3*). Because EVOO oxidation proceeds fairy slowly at room temperature, accelerated shelf-life tests (ASLT) could be employed to predict the oil's oxidative stability as well as that of the EVOO-containing products (*4*−*6*). Generally, temperature being the most critical environmental factor affecting the reaction rate, this parameter is usually chosen to accelerate the oxidation process (*7*, *8*). Hence, by measuring the rate of the quality index changes at three different temperatures, the reaction rate at a desired temperature can be calculated by the application of the well-known Arrhenius equation.

The successful application of an ASLT to oxidative reactions is strictly dependent on the statement of the proper working conditions for shelf-life prediction. For instance, in choosing the temperature range to be used, some temperature upper limitations have been reported. In particular, Kaya et al. (*9*) reported that the calculation of the induction period determined at temperatures >100 °C led to an overprediction of those obtained for long-term storage at room temperature. In addition, a kinetic study of olive oil triacylglycerol autoxidation indicates that the Arrhenius equation could be employed to describe the temperature dependence of primary and secondary oxidation product formation between 25 and 75 °C (*6*). Besides these data, no temperature lower limitations seem to be present in the literature. However, if phase changes occur in the temperature range studied, a nonlinearity in the Arrhenius plot can be expected (*8*).

Recently, the phase transition of lipids was indicated as being able to cause an abrupt change in the Arrhenius behavior of oxidative reactions (*10*, *11*). In particular, Calligaris et al. (*11*) reported that the occurrence of sunflower oil crystallization led to a curvature in the Arrhenius plot that gives higher reaction rates than those expected on the basis of the Arrhenius equation. The changes in viscosity and unsaturated fatty acid concentration in the liquid phase surrounding fat crystals were indicated as the most critical parameters able to affect the kinetics of lipid oxidation. This information suggests that the application of accelerated tests to predict the stability of lipid-containing foods should be carefully used because it may cause an overestimation

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of the product shelf life. This can be the case of refrigerated and frozen EVOO-containing products. In fact, even if EVOO is liquid in ambient stable foods, its storage at low temperatures can cause its crystallization, leading to the presence of different solid/liquid ratios.

On the basis of these observations, the aim of this study was to evaluate the influence of EVOO physical state on the kinetics of oxidative oxidations. To this purpose, EVOO was stored at increasing temperatures from 3 to 60 °C and the oxidation was followed by measuring both primary and secondary oxidation products.

## MATERIALS AND METHODS

**Materials.** Aliquots of 3 g of EVOO, purchased at a local market, were inserted in 10 mL capacity vials, sealed with butyl septa and metallic caps. Samples were then stored in darkness in a refrigerator (MF1500/2EF, Perani, Milan, Italy) at 3 ± 1 °C and in an oven (Salvis Thermocenter, Oakton, Vernon Hills, IL) at 25, 40, and 60 °C (±1 °C) for up to 15 months.

**Oil Fractionation.** Aliquots of 50 g of oil were bottled in 100 mL screw-capped flasks, melted in a water bath at 40 °C for 15 min, and then stored at 3 °C in a refrigerator (MF1500/2EF, Perani). After 2 days, the liquid phase was separated at crystallization temperature by percolation. The liquid phase was analyzed for total polyphenol content, antioxidant activity, and fatty acid composition. It should be noted that the separation process of the oil fractions cannot be considered to be complete because part of the uncrystallized oil was physically trapped in the fat crystals.

**Analytical Determinations.** *Quality Characteristics of Olive Oil Samples.* The determinations of the peroxide values (PV) and the fatty acid composition (FA) of the oil samples were carried out according to the European Official Methods of Analysis (*12*).

Oxidative stability was determined by using the Rancimat test at 120 °C with an air flow of 20 L/h using a model 679 Metrohm Rancimat (Metrohm, Herisau, Switzerland).

*Total Phenol Content.* Phenol content was determined following the methodology proposed by Capannesi et al. (*13*). Aliquots of 2.5 g of EVOO were diluted with 2.5 mL of *n*-hexane (Carlo Erba, Milano, Italy) and extracted three times with 2.5 mL of a $CH_3OH/H_2O$ mixture (80:20 w/w) by 5 min of centrifugation (Heraeus Sepatech Megafuge 1.0, Hanau, Germany) at 5000 rpm. The extract was added to 2.5 mL of Folin−Ciocalteu reagent and 5 mL of $Na_2CO_3$ (Carlo Erba) (7.5% w/w) in a 50 mL volumetric flask, reaching the final volume with deionized water. Samples were stored overnight at room temperature, and spectrophotometric analysis was performed at 765 nm. Results were expressed as gallic acid equivalents (GAE).

*Measurement of Hexanal by Static Headspace GC.* Hexanal concentration was measured by static headspace GC following the methodology described by Elizalde et al. (*14*). An HGRC Mega 2 series gas chromatograph (Fisons Instruments, Milan, Italy) equipped with a headspace sampler (Carlo Erba HS 250, Carlo Erba Strumentazioni, Milan, Italy) and a thermal conductivity detector (Fisons HWD Control, Fison Instruments, Milan, Italy) was used. Hexanal was separated isothermally at 80 °C on a glass column (2 m × 2 mm) packed with 6.6% Carbowax 20 M on Carbopack B 60−80 mesh. The GC conditions were as follows: sample temperature, 35 °C; injector and detector temperatures, 180 °C; nitrogen flow rate, 35 L/min. Before analysis, samples were stored at 35 °C for 40 min in a temperature-controlled bath to reach equilibrium conditions. The chromatograms were integrated using Chromcard (ver. 1.18, 1996, CE Instrument, Milan, Italy) chromatography data system software.

*Chain-Breaking Activity.* The chain-breaking activity was measured following the methodology described by Brand-Williams et al. (*15*) whereby the bleaching rate of a stable free radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH•), is monitored at a characteristic wavelength in the presence of the sample. A volume of 2 mL of $6 \times 10^{-5}$ M DPPH• acetone solution was used. The reaction was started by the addition of 50 μL of samples. The bleaching of DPPH• was followed at 515 nm by a Uvikon 860 spectrophotometer (Kontron Instruments, Milano, Italy) at 25 °C for at least 10 min. In all cases the bleaching rate was proportional to the sample concentration added to the medium. The following equation was chosen to obtain the reaction rate of DPPH• bleaching, $k$ (*16*):

$$\frac{1}{A^3} - \frac{1}{A_0^3} = 3kt$$

$A_0$ is the initial optical density, and $A$ is the optical density at increasing time, $t$. The chain-breaking activity was expressed as $k$ ($OD^{-3}$ $min^{-1}$ $mg^{-1}$).

*Calorimetric Analysis.* Calorimetric analyses were made using a TA4000 differential scanning calorimeter (Mettler-Toledo, Greifensee, Switzerland) connected to GraphWare software TAT72.2/5 (Mettler-Toledo). Heat flow calibration was achieved using indium (heat of fusion = 28.45 J/g). Temperature calibration was carried out using hexane (mp = −93.5 °C), water (mp = 0.0 °C), and indium (mp = 156.6 °C). Samples were prepared by carefully weighing 10−15 μg of the material in 40 μL aluminum DSC pans, closed without hermetic sealing. An empty pan was used as a reference. Samples were heated under nitrogen flow (10 mL/min) at 40 °C for 15 min to destroy crystallization memory, cooled to −80 °C, and then heated from −80 to 20 °C. The scanning rate was 2 °C/min. Additional scanning was performed at 2 °C/min from −80 to 20 °C after annealing at −18 °C for 30 min. The liquid fraction (LF), defined as the percentage of the liquid mass of oil at selected temperatures, was calculated from melting curves. LF calculation is based on the assumption that at −80 °C the lipid mass, after annealing, is totally crystallized (LF = 0). The start and end of melting transition were taken as on-set ($T_{on}$) and off-set ($T_{off}$) points of transition, which are the points at which the extrapolated baseline intersects the extrapolated tangent of the calorimetric peak in the transition state. Total peak enthalpy and LF were obtained by integration. DSC thermograms were converted into ASCII format for trace deconvolution according to the methodology described by Riva and Schiraldi (*17*).

At each temperature the concentration factor of the liquid phase ($C_{oil}$) was defined as the ratio between the liquid fraction originally present in the sample ($C_0$) and the liquid fraction at selected temperature ($C_T$):

$$C = \frac{C_0}{C_T} \quad (1)$$

*Viscosity.* The viscosity ($\eta$) of EVOO as a function of temperature was determined by a Stresstech Rheometer (Reologica Instruments AB, Milnrow-Rochdale, U.K.) using 4 cm plate geometry. In all cases the volume of the samples was 1 mL. The temperature control was maintained by a circulation ethanol bath (Julabo 70, Julabo, Labortechnik, Seelbach, Germany) with an accuracy of ±0.1 °C.

Before the analysis, oil samples were conditioned at 40 °C for 15 min to destroy crystallization "memory". Viscosity was measured at selected temperatures during cooling at 1 °C/min from 20 to 0 °C. Samples showed a Newtonian behavior in the shear-rate range from 5.96 to 119 $s^{-1}$. During $\eta$ measurement, the oil was stirred and data were recorded after 25 s by the Stresstech software (v. 2.90, 1993−1996, Reologica Instruments).

**Kinetic Data Analysis.** Apparent zero-order rate constants of peroxide ($k_{PV}$) and hexanal ($k_{hexanal}$) formation were calculated, excluding the lag phase if present, by linear regression of at least six points from the initial part of the curves, excluding the lag phase when present. The effect of temperature on the rate of lipid oxidation was evaluated by means of the Arrhenius equation (eq 2)

$$k = k_0\, e^{(-E_a/RT)} \quad (2)$$

where $k$ is the reaction rate constant, $R$ is the molar gas constant (8.31 J $K^{-1}$ $mol^{-1}$), $T$ is the absolute temperature (K), $E_a$ is the activation energy (J $mol^{-1}$), and $k_0$ is the pre-exponential factor of frequency factor. To make a better estimate of the apparent activation energy a one-step nonlinear regression was applied to all data by using the reparametrized Arrhenius equation (*18*)

$$k = k_{ref}\, e^{-E_a/R(1/T-1/T_{ref})} \quad (3)$$

where $k$ is the reaction rate constant, $k_{ref}$ is the rate constant at reference temperature, $R$ is the molar gas constant (8.31 J $K^{-1}$ $mol^{-1}$), $T$ is the absolute temperature (K), $T_{ref}$ is the reference temperature, and $E_a$ is the apparent activation energy (J $mol^{-1}$). As reference temperature was used 301 K, the middle point between 276 and 333 K.

**Data Analysis.** The results reported here are the average of at least three measurements, and the relative standard deviations (RDS%), expressed as the percentage ratio between the standard deviations (SD) and the mean values, were lower than 6 for enthalpy data, 7 for peroxide value, 8 for hexanal formation, and 5 for total polyhenol content. Oxidation kinetics were performed in duplicate, and the coefficients of variation were <5%.

Least squares and nonlinear linear regression analyses were performed by using Statistica for Windows (ver. 4.5, 1993, Stat Soft Inc., Tulsa, OK).

## RESULTS AND DISCUSSION

**Chemical and Physical Properties of EVOO. Table 1** reports the FA composition and other qualitative indices of the EVOO used in the present research. Besides the chemical characteristics, the oil physical properties were studied by using DSC analysis and viscosity measurements. **Figure 1** shows the DSC heating curves from −60 to 20 °C of the sample. Two endothermic peaks (1, 2) were detected. According to Tan and Che Man (*19*), the presence of multiple peaks in DSC thermograms of vegetable oils can be attributed to the complex feature of triacylglycerol (TAG) distribution in lipids. In fact, saturated TAGs melt at higher temperatures than the highly unsaturated ones, whereas the partially saturated TAG fraction shows an intermediate melting temperature. Thus, peak 1 (from −18 to 0 °C) can be reasonably attributed to the melting of highly unsaturated TAG crystals, whereas peak 2 (from 0 to 10 °C) is attributed to the phase transition of the oil fraction rich in saturated TAGs. By integration of DSC data, EVOO LF as a function of temperature was calculated (**Figure 2**). As expected, the LF as a function of temperature presented a sigmoid shape, which can be well described ($R^2 = 0.99$, $P < 0.01$) by the following sigmoidal model, as proposed by Calligaris et al. (*11*):

$$LF = \frac{a}{1 + e^{-(T-b)/c}} \quad (4)$$

In eq 4, $a$, $b$, and $c$ are experimental parameters of regression, which were found to be 0.94, −5.63, and 2.95, respectively. This equation was used to evaluate the liquid fraction of EVOO at any temperature of interest and to calculate the concentration factor of the liquid phase ($C_{oil}$), defined according to eq 1. This factor indicated how many times the concentration of compound involved in oxidative process (i.e., unsaturated TAG) in the liquid phase was increased as a consequence of crystallization. The concentration factors at interesting temperatures are reported in **Table 2**. A $C_{oil}$ value equal to 1 indicates that, at the given temperature, crystallization did not occur and sample concentration remained unchanged. By contrast, $C_{oil}$ values higher than 1 indicate that oil partially crystallized at the given temperature, leading to a $C$-times increase in the concentration of the liquid phase.

Besides oil thermal properties, the changes of EVOO viscosity as a function of temperature were evaluated (**Figure 3**). The temperature dependence of oil viscosity is generally described by the Andrade equation (*20*)

$$\ln \eta = q + \frac{m}{T} \quad (5)$$

**Table 1.** Qualitative Characteristics and Fatty Acid Composition of Extra Virgin Olive Oil Considered in This Research

| | |
|---|---|
| qualitative characteristics | |
| PV (mequiv of $O_2$ $kg_{oil}^{-1}$) | 9.38 ± 0.60 |
| total phenols (mg $kg^{-1}$ as gallic acid) | 772.1 ± 35.8 |
| induction time[a] (h) | 16.4 ± 0.8 |
| FA composition | |
| $C_{16:0}$ | 13.38 ± 0.20 |
| $C_{16:1}$ | 1.35 ± 0.08 |
| $C_{18:0}$ | 2.61 ± 0.02 |
| $C_{18:1}$ | 71.17 ± 0.40 |
| $C_{18:2}$ | 10.12 ± 0.05 |
| $C_{18:3}$ | 0.60 ± 0.04 |
| $C_{20:0}$ | 0.39 ± 0.01 |
| $C_{20:1}$ | 0.32 ± 0.01 |
| $C_{22:0}$ | 0.06 ± 0.10 |
| $C_{18:1}/C_{18:2}$ | 7.03 |

[a] Induction time to oxidation measured by the Rancimat test apparatus.

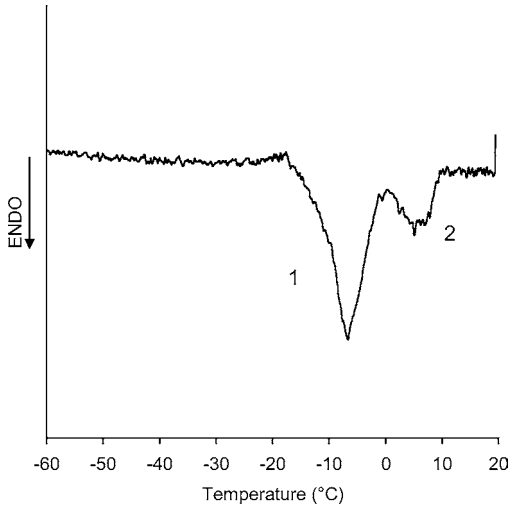


**Figure 1.** Melting thermogram of extra virgin olive oil.

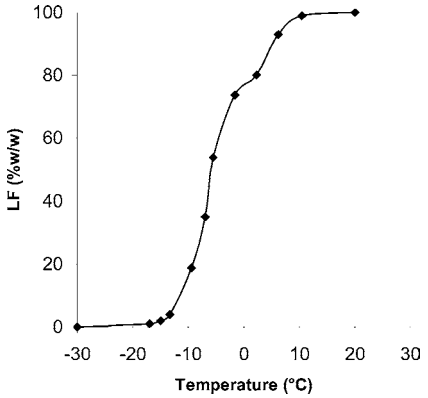


**Figure 2.** Liquid fraction (LF) of extra virgin olive oil as a function of temperature.

where $\eta$ is the viscosity, $T$ is the absolute temperature (K), and $m$ and $q$ are experimental parameters of regression. The linear regression results obtained by fitting the viscosity data indicate a good correlation ($R^2 = 0.99$, $P < 0.001$). The experimental parameters $m$ and $q$ were found to be 4203.8 and 15.1, respectively.

**Oxidation Kinetics.** EVOO oxidation kinetics were followed at 3, 25, 40, and 60 °C. As previously pointed out, the oil is totally liquid above 10 °C and at 3 °C the solid phase corresponds to ∼18% (w/w) (**Figure 2**). **Figure 4** shows the changes in peroxide value of the samples stored at increasing

**Table 2.** Concentration Factor ($C_{oil}$) of Oil Samples as a Function of Temperature

| temp (°C) | LF (% w/w) | concn factor ($C_{oil}$) |
|---|---|---|
| 3 | 82 | 1.22 |
| 25 | 1 | 1 |
| 40 | 1 | 1 |
| 60 | 1 | 1 |

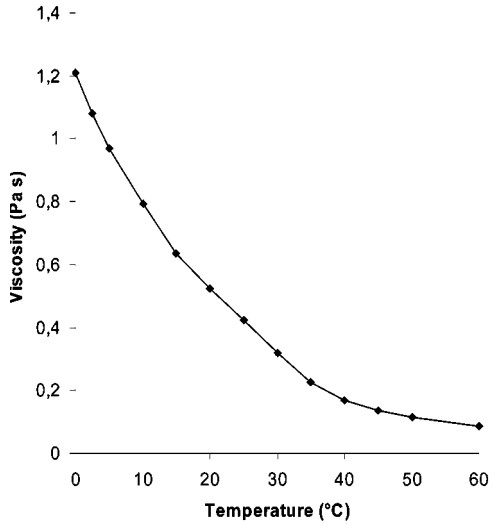


**Figure 3.** Viscosity of extra virgin olive oil as a function of temperature.

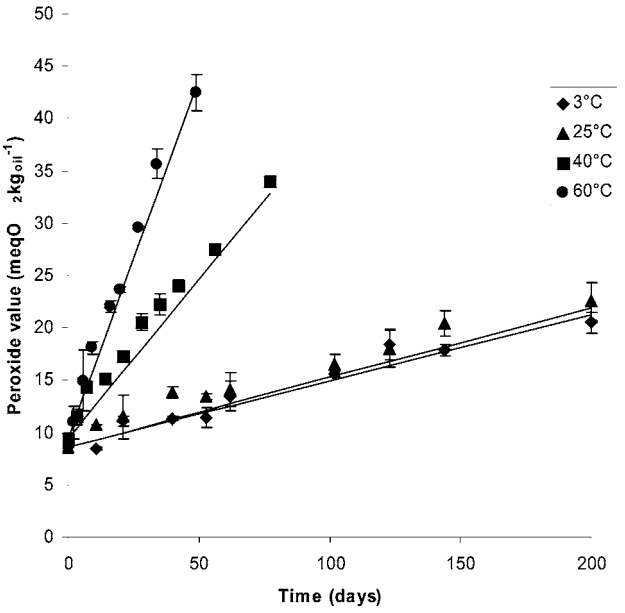


**Figure 4.** Peroxide value of extra virgin olive oil as a function of storage time at different temperatures (symbols, experimental data; solid line, regression results).

temperatures. As expected, a faster increase in PV was observed as temperature increases. However, surprisingly, data referring to samples stored at 3 and 25 °C appear to be not significantly different. Because the PV changes result from a balance between the hydroperoxides produced and those decomposed during propagation and termination steps of oxidation reactions (*21*−*23*), the evaluation of formation of secondary oxidation products could allow these data to be confirmed. Results obtained by analyzing the headspace hexanal concentration are reported in **Figure 5**. In this case, it is interesting to note that after prolonged storage at 25 and 3 °C, a decrease in hexanal

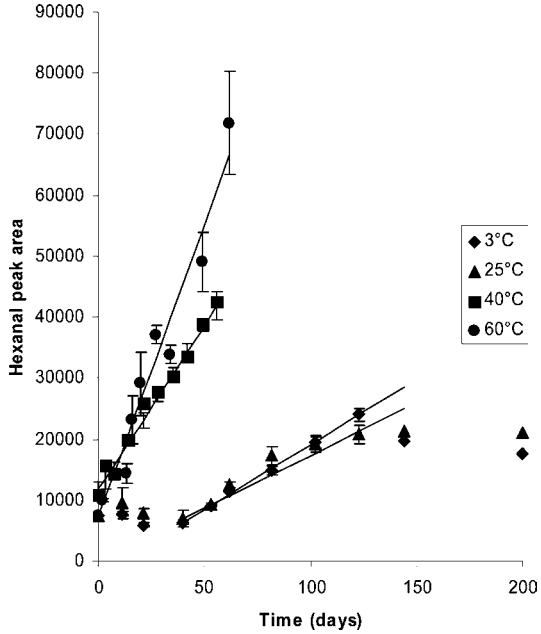


**Figure 5.** Changes in hexanal peak area of extra virgin olive oil as a function of storage time at different temperature (symbols, experimental data; solid line, regression results).

**Table 3.** Zero-Order Rate Constants Obtained by Linear Regression Analysis of Peroxide ($k_{PV}$) and Hexanal ($k_{hexanal}$) Formation in Extra Virgin Olive Oil Stored at Increasing Temperature[a]

| | peroxide value | | | hexanal | | |
|---|---|---|---|---|---|---|
| temp (°C) | $k_{PV}$ (mequiv of $O_2$ $kg^{-1}$ $day^{-1}$) | SE (Standard Error) | $R^2$ | $k_{hexanal}$ (peak area $day^{-1}$) | SE (Standard Error) | $R^2$ |
| 3 | 0.063 | 0.005 | 0.95 | 212.1 | 4.6 | 0.99 |
| 25 | 0.067 | 0.004 | 0.97 | 142.3 | 21.4 | 0.90 |
| 40 | 0.301 | 0.012 | 0.99 | 537.5 | 23.5 | 0.98 |
| 60 | 0.685 | 0.027 | 0.99 | 957.6 | 51.5 | 0.97 |

[a] $P < 0.05$.

peak area was detected. This behavior can be attributed to the development of oxidative reactions involving hexanal itself (*24*). Similar results were obtained by Di Giovacchino et al. (*25*) for long-term storage of EVOO.

**Figures 4** and **5** show also the regression results obtained according to the pseudo-zero-order reaction model. Apparent zero-order rate constants of peroxide and hexanal formation ($k_{PV}$ and $k_{hexanal}$) are reported in **Table 3**.

It is evident that the kinetics of both indices at 3 and 25 °C appear not so different as expected and that even at 3 °C the $k_{hexanal}$ value was even higher than that at 25 °C. The Arrhenius plot of $k_{PV}$ and $k_{hexanal}$ as a function of the reciprocal of temperature highlights this observation (**Figure 6**). The Arrhenius behavior seems to be fulfilled only between 25 and 60 °C. Below this temperature range a positive deviation from the linearity was found. The determination coefficient of the linear regression analysis of the data in the entire temperature range is lower than 0.84 for $k_{PV}$ and 0.66 for $k_{hexanal}$.

To calculate the activation energy of peroxide and hexanal formation from 25 to 60 °C, a one-step nonlinear regression analysis was applied to all data of peroxide and hexanal changes (*18*).

The apparent activation energies for peroxides and hexanal formation are reported in **Table 4**. These results are in agreement with previous literature data, reporting typical $E_a$ for lipid

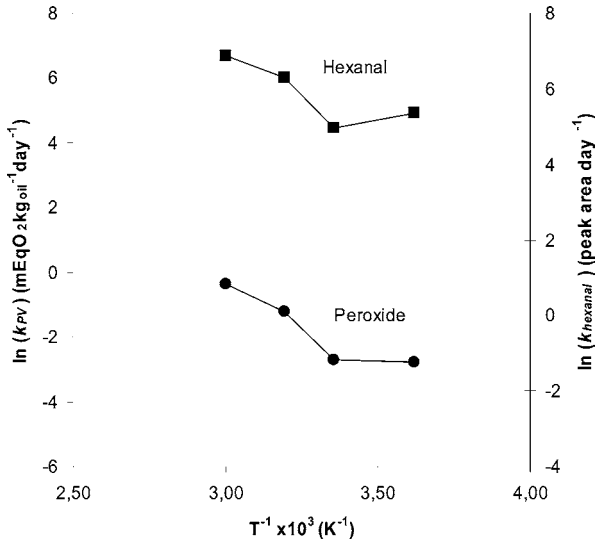


**Figure 6.** Apparent zero-order rate constants of peroxide and hexanal formation as a function of temperature$^{-1}$.

**Table 4.** Activation Energies Estimated Using One-Step Nonlinear Regression Analysis of Peroxides and Hexanal Formation in Extra Virgin Olive Oil between 25 and 60 °C

| | $E_a$ (kJ/mol) | $R^2$ | $P$ |
|---|---|---|---|
| peroxide | 42.22 ± 8.84 | 0.97 | 0.01 |
| hexanal | 33.61 ± 6.00 | 0.95 | 0.03 |

oxidation ranging from 24 to 240 kJ mol$^{-1}$ (*26*−*28*). By using this equation to predict $k_{PV}$ and $k_{hexanal}$ at 3 °C, the values obtained were 2.1 and 2.7 times higher, respectively, than those predicted by the Arrhenius equation. This means that, considering 20 mequiv of $O_{2att}$ $kg_{oil}^{-1}$ as the upper limit established by the EU regulation for EVOO commercialization, the application of the ASLT to predict the achievement of this limit during storage at 3 °C indicates that 367 days was necessary. Actually, this limit was reached after just 190 days of storage. Such an indication appears to be of considerable interest not only for the prediction of bulk oil stability but especially for that of oil-containing products.

Deviation from the Arrhenius equation in the temperature range where oil crystallization takes place is in agreement with data reported by Calligaris et al. (*11*) on sunflower oil. Also in that case, a deviation from the Arrhenius equation was observed in the temperature range where sunflower oil presents different solid/liquid ratios. The authors attributed the existence of a critical temperature below which the Arrhenius equation is not fulfilled to the occurrence of temperature-dependent compositional changes upon crystallization phenomena. In particular, the changes in unsaturated TAG concentration in the liquid phase surrounding fat crystals and in viscosity were indicated as the most critical parameters able to affect the kinetics of lipid oxidation in the partially crystallized lipid matrix. These modifications could be able to counterbalance and even oppose the direct effect of temperature on oxidation rate. This hypothesis was supported by the development of a modified Arrhenius equation, which was able to accurately describe the temperature dependence of the oxidation rate by taking into account the changes in the physical state of oil

$$(k_{ox}) = (k_0 \Delta k)\, e^{-E_a/RT} \quad (6)$$

where $\Delta k$ is a corrective factor included in the Arrhenius equation (eq 2), which takes into account the influence of variables, other than temperature, that significantly affect the reaction rate in the partially crystallized matrix. In particular, in the case of sunflower oil, $\Delta k$ was defined as the ratio between the concentration factor of the liquid phase ($C_{oil}$) and the viscosity ($\eta$).

By applying reparametrized eq 6 ($T_{ref}$ = 301 K) on EVOO $k_{PV}$ and $k_{hexanal}$, the regression generated unsatisfactory results ($R^2$ < 0.13). Thus, the mechanisms involved in EVOO Arrhenius deviation seem to be different form those in sunflower oil.

Because the stability of EVOOs is strongly related to the antioxidant activity of some unsaponificable components, the influence of phase transition on the natural antioxidants distribution in olive oil cannot be underestimated. As is well-known, the main antioxidant activity is exerted by polyphenolic compounds (*2*, *3*, *29*, *30*). To study the possible factors causing Arrhenius deviations, EVOO was isothermally crystallized at 3 °C. The fractionation process allowed a liquid to be obtained, which was analyzed for its chemical composition, total phenol content, and antioxidant activity.

**Table 5** reports the composition in fatty acids, the polyphenol content, and the antioxidant activity relevant to the control oil and the liquid fraction separated at 3 °C. It can be noted that the crystallization of increasing percentages of oil caused the progressive concentration of unsaturated fatty acids in the liquid phase surrounding fat crystals. Much more surprising results were those of chain-breaking activity and total phenol content. It is evident that the antioxidant activity of the liquid fraction at 3 °C was lower than that of the oil. This result can be easily attributed to the decrease of protective antioxidant phenols in the liquid fraction at 3 °C. The latter could be physically entrapped in the solid phase during its crystallization. As a consequence, the highly unsaturated residual liquid fraction should be less protected against oxidative reactions than the corresponding liquid matrix.

Consequently, besides the concentration in unsaturated TAGs, the physical separation of phenols could be considered as an additional driving force in the development of oxidation. Thus, the phenol concentration factor was defined as the ratio between the concentration of phenols in oil and in the liquid fraction ($C_{phenol}$) at the selected temperature.

On the basis of these observations, $\Delta k$ was defined as the product of $C_{oil}$ and $C_{phenol}$:

$$\Delta k = C_{oil} C_{phenol} \quad (7)$$

**Table 5.** Saturated and Unsaturated Fatty Acid Contents, Chain-Breaking Activity, and Polyphenol Content of the Liquid Fraction of Extra Virgin Olive Oil Obtained after Storage at 3 °C[a]

| sample | saturated fatty acid content (%) | unsaturated fatty acid content (%) | chain-breaking activity (OD$^{-3}$ min$^{-1}$ mg$^{-1}$) | polyphenol content (mg kg$^{-1}$) |
|---|---|---|---|---|
| oil | 16.68 ± 0.33a | 83.55 ± 0.32a | 0.68 ± 0.18a | 772.1 ± 35.8a |
| liquid fraction | 14.78 ± 0.11b | 85.43 ± 0.08b | 0.22 ± 0.05b | 156.4 ± 28.5b |

[a] Means in the same column with a common letter are significantly different ($P$ < 0.05).

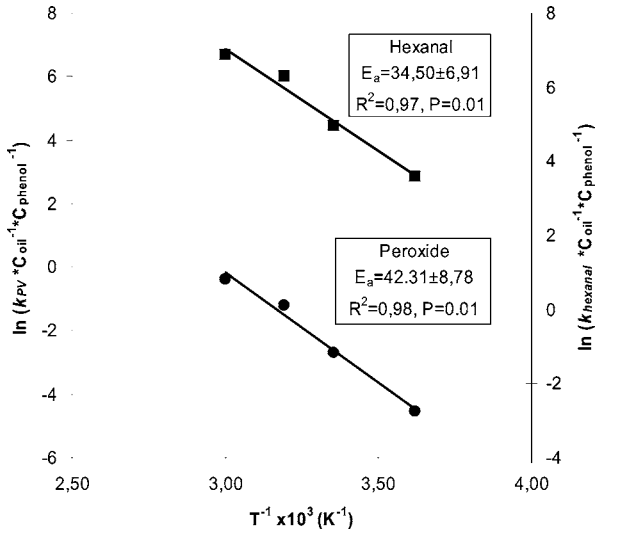


**Figure 7.** Apparent zero-order rate constants of peroxide and hexanal formation, expressed as (ln $k\ C_{oil}^{-1}\ C_{phenol}^{-1}$) as a function of temperature$^{-1}$.

Thus, eq 6 was modified as follows:

$$k_{ox} = (k_0 C_{oil} C_{phenol})\ e^{(-E_a/T)} \quad (8)$$

In eq 8 $k_{ox}$ represents $k_{PV}$ or $k_{hexanal}$. This equation allows one to obtain a new independent variable:

$$(k_{ox} C_{oil}^{-1} C_{phenol}^{-1}) = k_0\ e^{(-E_a/T)} \quad (9)$$

**Figure 7** shows Arrhenius plots of the new independent variables as a function of the reciprocal of temperature. Regression results of the reparametrized eq 9 are also reported. The goodness of fit is evident. It can be noted that $\Delta k$ is equal to 1 at temperatures >3 °C and becomes equal to 5.93 at 3 °C. It is interesting to observe that, in contrast to the sunflower oil case, the viscosity was not a limiting factor in the development of the reaction.

These results clearly indicate that relative concentration of reactants (UFA and polyphenols) in the liquid phase surrounding fat crystals is, besides temperature, the main variable affecting the oxidation rate in partially crystallized EVOO oil.

Results obtained in this study highlight that the oxidation rate below the EVOO melting point was higher than that predicted on the basis of the well-known Arrhenius equation. This positive deviation was related to some physicochemical changes occurring in the lipid matrix as a consequence of phase transition. In particular, the increase in UFA and the decrease of polyphenol content in the liquid-phase surrounding fat crystals were found to be the main changes that contribute, besides temperature, to the determinatin of the oxidation rate. By taking into account these changes, a feasible predictive model for shelf-life estimation of EVOO-containing product was set up.

The modified Arrhenius equation proposed appears to be promising in the challenge to find mathematical models able to predict the stability and, hence, the shelf life of lipid-containing foods, which nowadays is still a time- and cost-consuming process.

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