Oxidation kinetics in olive oil triacylglycerols under accelerated shelf-life testing (25–75 °C)

A kinetic study of the autoxidation reaction in olive oil triacylglycerols stored in darkness at different temperatures (25, 40, 50, 60 and 75 °C), in absence of pro- and anti-oxidant compounds to avoid confounding effects, is described. After the induction period (IP) the decrease in the oxidizing substrate and the formation of primary oxidation products followed a pseudo-zero-order kinetic, and the calculated $E_a$ from the Arrhenius equation for the formation of hydroperoxides was 32.1 kJ·mol$^{-1}$. The formation of secondary oxidation products followed a pseudo-first-order kinetic whose rate reaction constant also increased exponentially with temperature. The first oxidation index to exceed the upper limit in the EU regulations was PV, followed by $K_{232}$ and $K_{270}$. The time required reaching these limits and the rancidity threshold showed a potential dependence on temperature, and therefore with accelerated storage at 75 °C, POO shelf-life in ambient conditions (25 °C) can be predicted. Finally, there was a good linear relationship between the time required to reach the rancidity threshold and the IP of the formation of the 2,4-decadienal, and hence this instrumental determination could be useful to measure sensory recognition of the rancid defect in POO.

Keywords: Oxidation kinetic, olive oil, triacylglycerols, sensory rancidity.

1 Introduction

One of the most fundamental reactions in lipid chemistry is oxidation. In the course of the autoxidation reaction a series of compounds are formed, causing off-flavors and rancidity, loss of nutritional value and finally consumer rejection of the food product. Autoxidation is therefore the main cause of olive oil quality deterioration and its reaction rate determines the shelf-life of this product [1].

Lipid oxidation is a process which occurs fairly slowly at room temperature, and hence accelerated methods should be employed to estimate the oxidative stability of the product or the induction period of the autoxidation reaction in a relatively short period of time [1]. Several physical or chemical parameters can be used to increase the rate of the reaction, and consequently the development of rancidity, such as the temperature, metal catalysis, rise in oxygen partial pressure, shaking to increase the reaction interface surface, etc. [2]. However, since the rate of the reaction increases exponentially with the absolute temperature [3] this parameter is usually chosen to accelerate the oxidation process.

To establish the conditions for an accelerated shelf-life test (ASLT) is not an easy or straightforward task, since it is necessary to choose what conditions are to be used to increase the oxidation rate and the analytical determinations to follow the progress of the reaction, preferably by measuring both primary and secondary oxidation products. In choosing the method used to determine the induction period of the reaction, which should correspond to a level of rancidity detectable by the consumer, a specific value of an oxidation index or a sudden change in the rate of oxidation must also be selected. In the case of virgin olive oil, the EU regulation (EC 796/2002) establishes upper limit values for different oxidation indexes (peroxide value, 20 meq/kg; $K_{232}$, 2.50 and $K_{270}$, 0.20) which could be employed as end points for an ASLT. Finally, the most difficult step is to correlate the value of the induction period obtained under accelerated conditions with the shelf-life of the food product in terms of maximum storage period [3]. To this end, it is necessary to carry out kinetic studies and apply the Arrhenius equation or calculate the increase in oxidation rate produced by a 10 °C increase in temperature ($Q_{10}$) [1]. Due to the difficulties and limitations involved in this methodology, accurate estimation of the shelf-life of edible fats and oils in normal storage conditions by ASLT is still nowadays a goal for the scientific community [4–9].

As a previous and necessary step it is essential to carry out a basic study on the oxidation process in purified olive oil, in absence of pro- and antioxidants compounds, to
fully understand the complex influence of accelerated test conditions, and therefore the effect of temperature, on the oxidation of olive oil triacylglycerol matrix before approaching the research in virgin olive oil. In fact, although in the last decade some studies concerning the effect of temperature and storage time on quality indexes of olive oil have been published, probably due to the complex interactions between pro- and antioxidants compounds, the results about virgin olive oil shelf-life estimated by ASLT are not yet conclusive. One of the few pieces of research reported was by Kaya et al. [4], who observed that the extrapolation of the induction periods, determined at temperatures over 100 °C, led to an over-prediction of those obtained from long term storage at 10 and 20 °C. More recently, virgin olive oil shelf-life was described by the relation between the increase in the K232 parameter and the tyrosol content [10], despite the known poor antioxidant activity of this compound [11], or by means of the time required to reach the upper legal limit of K270 from the initial Rancimat oxidative stability [12].

This paper describes a kinetic study of the autoxidation reaction in olive oil triacylglycerols stored in darkness at temperatures ranging from 25–75 °C, as measured by the rate of formation of hydroperoxides and their decomposition products, in absence of pro- and anti-oxidant compounds to avoid confounding effects. Finally, the time required reaching the limits established by the EU regulations and the sensory rancidity threshold were also evaluated in order to address the effect of temperature on shelf-life and flavor stability.

2 Materials and methods

2.1 Purified olive oil (POO) preparation

Cornicabra virgin olive oil was stripped of pro- and anti-oxidants and trace metals by adsorption chromatography [13]. 100 g of virgin olive oil in 1000 mL distilled hexane were passed through a column (i.d. 2 cm) filled with 70 g alumina (type 507c, neutral, Fluka, Buchs, Switzerland) activated for 4 h at 180 °C, and collected in darkness. The absence of antioxidants in the POO was verified using the AOCS official method (Ce 8–89) for α-tocopherol and the method previously described by these authors for phenolic compounds [14].

2.2 Oxidation experiments

Twelve 36.6 g (40 mL) samples of POO were stored in darkness at different temperatures (25, 40, 50, 60 and 75 °C) in 125-mL open amber glass bottles (i.d. 4.2 cm; surface area exposed to the atmosphere: 13.85 cm²). One bottle was taken from the incubator for analysis at scheduled times.

2.3 Analytical determinations

All reagents used were of analytical, HPLC or spectroscopic grade, and were supplied by Merck (Darmstadt, Germany).

2.3.1 Peroxide value

Peroxide value (PV) and UV absorption characteristics (K232 and K270) were measured following the analytical methods described in European Regulation EEC 2568/91. Peroxide value is expressed as milliequivalents of active oxygen per kilogram of oil (meq O₂/kg), K232 and K270 extinction coefficients were calculated from absorption at 232 and 270 nm respectively, p-Anisidine value (AnV) was determined following the AOCS official method (Cd 18–90), using a UV-Visible spectrophotometer.

2.3.2 Polar compounds

The altered triacylglycerol (TG) compounds that constitute the polar fraction of the oxidized oil were separated into TG dimers and polymers, oxidized TG, diacylglycerols and free fatty acids by high performance size-exclusion chromatography (HPSEC) according to Márquez-Ruiz et al. [15]. HPLC was used, equipped with a refractive index detector working at 35 °C, and two serially-connected PLgel columns (300 × 7.5 mm, 5 µm particle and 100 Å and 500 Å pore size, respectively; Agilent, Winnersh, UK) at 25 °C. The mobile phase was tetrahydrofuran (THF) at 1 mL/min; the injection volume was 20 µl; and monoolein (Sigma Chemical Co., St. Louis, MO) was added as internal standard.

2.3.3 Fatty acid composition

Fatty acid composition was analyzed according to European Regulations EEC 2568/91 and following amendments, corresponding to the AOCS method Ch 2–91. To determine fatty acid composition, the methyl esters were prepared by vigorous shaking of a solution of oil in hexane (0.2 g in 3 mL) with 0.4 mL of 2 M methanolic potassium hydroxide and analyzed by GC with a FID detector. A fused silica column (50 m length × 0.25 mm i.d.) was used, coated with SGL-1000 phase (0.25 µm thickness; Sugerlabor, Madrid, Spain). The carrier gas was helium, at a flow through the column of 1 mL/min. The tempera-
ture of the injector and detector was set at 250 °C and the oven temperature at 210 °C. The injection volume was 1 µL.

Loss from oxidation in the unsaturated fatty acids was quantified on the basis of the ratio between each fatty acid and the palmitic acid peak areas, since saturated fatty acids are not altered by autoxidation [16].

2.3.4 Volatile compounds

Volatile compounds were determined by the following method, adapted from Jelen et al. [17]. Three grams of oil sample were placed in a 10-mL headspace vial and kept at 28 °C for 1 h. The Teflon-lined septum covering the vial was pierced with a SPME (Solid Phase Micro-Extraction) needle and a fiber (100 µm divinylbenzene/carboxene/poly(dimethylsiloxane) (DVB/CAR/PDMS) (Supelco Inc., Bellefonte, PA) was exposed to the oil headspace for 20 min. The fiber was then retracted into the needle and immediately transferred and desorbed for 5 min in the gas chromatograph injection port. A gas chromatograph equipped with a FID detector was used. Compounds were resolved on a HP-5 fused silica column (30 m × 0.32 mm × 0.25 µm, Agilent Technologies, Wilmington, USA) under the following conditions: injection port temperature 240 °C; helium flow 2 mL/min; oven temperature ramp 35 °C for 5 min, 4 °C/min up to 100 °C and then 15 °C/min up to 220 °C (maintained for 5 min). Volatile compounds were identified by comparison with standard substances. The following reference compounds were used: hexanal from Sigma, heptanal, octanal, nonanal, t-2-hexenal, t-2-heptenal, t-2-octenal and t,t-2,4-decalienal from Aldrich Chemie (Steinheim, Germany) and t-2-decanal from Fluka.

2.3.5 Sensory analysis

POO samples were assessed, for aroma changes only, at twelve different stages of the oxidation process by a Sensory Panel of eight assessors from the University of Castilla-La Mancha and the Protected Designation of Origin “Montes de Toledo” (Toledo, Spain).

The purpose of the sensory analysis was to determine the recognition threshold of rancid defect and to correlate it with the chemical composition of the oil at that stage of the oxidation process. The recognition threshold is defined as the level of a stimulus at which the specific stimulus can be recognized and identified. A rank probability plot is a useful tool for testing whether a set of individual thresholds are normally distributed; this graph also serves to locate the group threshold of the stimulus (75% of the correct answers by the panel). Assessors were therefore asked to evaluate differences between the aroma of the fresh POO (reference oil) and the twelve samples of POO removed from the incubator at different times. 15 mL of the oil were poured into a standard olive oil tasting glass (COI/T.20/Doc.no.5, 1987; corresponding to UNE 87021:1992), and the panelists marked the oil samples in which they could recognize the defect on an appropriate form (Method of Investigating Sensitivity of Taste; ISO 3972:1996, corresponding to UNE 87003–2000).

All experiments and analytical determinations were carried out at least in duplicate.

2.3.6 Statistical analysis

Statistical analyses were performed using SPSS 11 statistical software (SPSS Inc. Chicago, IL).

3 Results and discussion

The purified olive oil (POO) presented very low levels of both primary (fatty acid hydroperoxides, measured as peroxide value; K232, and oxidized triacylglycerols) and secondary (fatty acid hydroperoxides decomposition products, detected as K270; anisidine value; and dimers and polymers of triacylglycerols) oxidation products. In terms of fatty acids, the POO employed presented high oleic acid content (80.2%) and low polyunsaturated fatty acid content (5.3%), as previously reported in more details [18].

3.1 Primary oxidation products

The kinetic behavior of the oxidizing substrate, the unsaturated fatty acids (UFAs), and of the primary products of the autoxidation reaction, the FA hydroperoxides measured as peroxide value (PV) are depicted in Fig. 1. This figure plots the data obtained at 25 °C; however, the same behavior was observed at all of the experimental temperatures (25, 40, 50, 60 and 75 °C).

The kinetic of the oxidation process corresponded to an autocatalytic reaction [1], in which the degradation rate of the UFAs and the formation rate of the FA hydroperoxides, very low at the beginning of the storage, increased very rapidly in the induction period (IP). After the IP, the behavior of the autoxidation reaction was apparently pseudo-zero-order, where the rate of the reaction does not depend on the concentration of the oxidizing substrate:

\[ C = C_0 \times e^{-kt} \]
where \( C_0 \) and \( C \) are respectively the concentration of hydroperoxides in the fresh POO and after time \( t [\text{h}] \) of storage and \( k [\text{mol l}^{-1} \text{s}^{-1}] \) is the rate constant of the reaction. Under the experimental conditions, the factor limiting the oxidation reaction is the diffusion of atmospheric oxygen into the oily phase [19].

A previous paper by this research group showed that the behavior during the autoxidation reaction of conjugated dienes (K232) and of oxidized triacylglycerols (oxTGs) was very similar to that observed for PV [18]. The kinetic behavior of these indexes of the primary oxidation products is therefore presumably also of pseudo zero-order.

Values of the oxidation rate constants \( k \) and of the regression coefficients for PV and K270 at the experimental temperatures studied are reported in Tab. 1. The value of \( k \) for PV increased with the temperature from \( 1.10 \times 10^{-8} \text{ mol l}^{-1} \text{s}^{-1} \) at 25°C up to \( 7.52 \times 10^{-8} \text{ mol l}^{-1} \text{s}^{-1} \) at 75°C, which confirmed that this factor considerably influences the rate of the autoxidation reaction. The regression coefficient \( (r^2) \) for PV was always higher than 0.985 for all the temperatures studied and showed a good linear relationship with storage time, as observed in Fig. 1 at 25°C.

The plot of the natural logarithm (ln) of the PV rate constant versus the inverse of the temperature (1/T) showed a good linear relationship \((r^2 = 0.990)\), as depicted in Fig. 2. This kinetic behavior thus followed the Arrhenius equation:

\[
\ln k = \ln A + \frac{E_a}{RT}
\]

where \( A \) is a constant known as the pre-exponential factor, \( E_a [\text{kJ mol}^{-1}] \) is the activation energy, \( R [\text{kJ K}^{-1} \text{mol}^{-1}] \) is the gas constant and \( T [\text{K}] \) is the absolute temperature. The values of \( E_a \) and \( A \) for the formation reaction of the primary oxidation products as calculated from the experimental data were 32.1 kJ mol\(^{-1}\) and 3.22 \( \times 10^{-8} \) mol l\(^{-1}\) s\(^{-1}\) respectively.

**Tab. 1.** Values of the oxidation rate constants \( k \) and of the regression coefficient \( (r^2) \) for PV and K270 at the experimental temperatures.

<table>
<thead>
<tr>
<th>( T [\text{C}] )</th>
<th>Peroxide value</th>
<th>( K_{270} )</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>( k [10^{-8} \text{ mol l}^{-1} \text{s}^{-1}] )(^2)</td>
<td>( k [10^{-7} \text{ s}^{-1}] )</td>
</tr>
<tr>
<td>25</td>
<td>1.10±0.06</td>
<td>0.988</td>
</tr>
<tr>
<td>40</td>
<td>2.47±0.07</td>
<td>0.992</td>
</tr>
<tr>
<td>50</td>
<td>3.22±0.13</td>
<td>0.985</td>
</tr>
<tr>
<td>60</td>
<td>4.38±0.09</td>
<td>0.995</td>
</tr>
<tr>
<td>75</td>
<td>7.52±0.15</td>
<td>0.996</td>
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</table>

### 3.2 Secondary oxidation products

The K270 index was chosen to study the kinetic behavior of the secondary oxidation products. In fact, as shown in a previous paper, the behavior of this oxidation parameter is very similar to that of the formation of dimers and polymers of triacylglycerides (DTGs+PTGs), and therefore...
the conclusions of the analysis of K270 kinetic behavior (i.e. the order of the reaction and the compliance of the Arrhenius equation) can also be applied to the other oxidation index [18].

The kinetic of the formation of secondary oxidation products followed a pseudo-first-order reaction ($r^2 = 0.957$):

$$\ln(C/C_0) = kt$$

Values of $k$ [s$^{-1}$] and $r^2$ for the increase of absorbance at 270 nm during storage at the different temperatures studied are reported in Tab. 1. The rate constant for K270 increased rapidly with temperature, ranging from $2.93 \times 10^{-7}$ s$^{-1}$ at 25 °C up to $34.74 \times 10^{-7}$ s$^{-1}$ at 75 °C. The behavior of the rate constant of the secondary oxidation products with temperature also followed an Arrhenius equation ($r^2 = 0.991$), as observed in Fig. 2.

3.3 Shelf-life prediction

From a practical point of view, it is far more useful to be able to predict shelf-life in normal storage conditions, which in virgin olive oil is determined by the time it takes to reach the upper limits of the EU quality indexes [20] using an accelerated shelf-life testing (ASLT) method [4, 8], than to determine the effect of temperature on kinetic behavior.

Tab. 2 shows the storage time required by the POO warmed at different temperatures to exceed the upper limits established by EU Regulation (EC 796/2002) in the case of extra virgin olive oil: for PV (20 meq/kg), K232 (2.50) and K270 (0.200). The Tab. also shows the time taken to reach the recognition threshold of the rancid defect in POO, as measured by a trained virgin olive oil sensory panel. The first oxidation index to exceed the legal upper limit was PV, followed by K232 and K270. The behavior of the rancidity threshold was closer to that of K232, particularly at lower temperatures.

![Fig. 3. Effect of temperature on the time taken to reach the limit values for PV, K232 and K270, and the rancid threshold.](image)

The observed relationship between the temperature and the time required to reach the upper legal limits followed a potential equation, and not an exponential one as suggested by other authors for olive oil [4]:

$$t = aT^b$$

where $t$ is the time [h] taken to reach the upper limit at temperature $T$ [°C], and $a$ and $b$ are respectively the regression constant and the coefficient.

Consequently, the plot of the natural logarithm of the time required ($t$) versus the logarithm of the temperature ($T$) gave a linear relationship, as depicted in Fig. 3:

$$\ln t = \ln a + b \ln T$$

The data plotted in Fig. 3 suggest that accelerated storage at 75 °C can predict POO shelf-life at room temperature (25 °C) in darkness. Moreover, while PV, K232 and K270 behaviors are almost parallel, the time required to reach the rancidity threshold was closer to that for PV and K232 at lower temperature. This means that the time required reaching the upper limits for PV and K232 is more affected by the temperature than the rancidity threshold. Moreover, at RT the times taken to reach the upper values for PV, K232 and the rancidity threshold are similar, and therefore the limits established by the EU for PV or K232 may be a useful index of the sensory stability of POO. Of course this must be evaluated in virgin olive oil, a subject currently under investigation.
It is important to note that equations (1) to (4) are not intended to define the chemical mechanisms of the reactions that actually take place during autoxidation as proposed by other authors [21–23]. They are simple empirical equations that could be used to predict with reasonable accuracy the concentration of relevant oxidized components, and hence the time required to reach a given value in an oxidation or quality index under normal storage conditions (shelf-life), from the results of an ASLT.

A previous paper by this research group suggested that the time required to reach the rancidity threshold was apparently related with the IP of the formation reaction of \( t,t \)-2,4-decadienal [18]. On the basis of the results for the range of temperatures studied (25–75 °C), it can now be shown that there was in fact a high linear correlation \( (r^2 = 0.999) \) between these two parameters, as reported in Fig. 4. This instrumental determination may therefore be useful for measuring sensory recognition of the rancid defect, at least in POO. The value of the slope of the linear regression (0.97) was close to 1, and therefore the time required to reach the rancidity threshold is approximately equal to the IP for formation of \( t,t \)-2,4-decadienal.

\[ r^2 = 0.999 \]

Fig. 4. Correlation between the time taken to reach the IP for the formation of \( t,t \)-2,4-decadienal and the rancid threshold at different temperatures (25–75 °C).

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References


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