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ScienceDirect
Talanta 74 (2008) 1287–1298
Talanta
www.elsevier.com/locate/talanta

Biosensor analysis for the kinetic study of polyphenols deterioration during the forced thermal oxidation of extra-virgin olive oil

Luigi Campanella [a], Adriano Nuccilli [a], Mauro Tomassetti [a,*], Stefano Vecchio [b]

[a] *Department of Chemistry, "La Sapienza" University of Rome, P.le A. Moro 5, I-00185 Rome, Italy*
[b] *Department of Chemical Engineering (I.C.M.A.), "La Sapienza" University of Rome, Via del Castro Laurenziano 7, I-00161 Rome, Italy*

Received 27 April 2007; received in revised form 28 August 2007; accepted 30 August 2007
Available online 5 September 2007

Abstract

The process of artificial rancidification of extra-virgin olive oil due to heating in an oxidizing atmosphere was studied by testing an actual kinetic model of the process and monitoring the thermal oxidative degradation of the polyphenols contained in it. To this end, a series of oxidative degradation experiments were carried out on extra-virgin olive oil samples under isothermal conditions at 98, 120, 140, 160, and 180 °C using a thermostatic silicon oil bath. The experimental procedure used in this study carefully followed the recommendations regarding the study of olive oil rancidification set out in the AOM procedure. The change in polyphenol concentration with time was monitored at selected temperatures using a tyrosinase biosensor operating in an organic phase (*n*-hexane). The activation energy for the polyphenol degradation process determined using the MacCallum method was found to be practically constant throughout most of the process.

Furthermore, the application of the so-called "model-fitting" method to this process enabled the specific constant rates to be determined at the above-mentioned selected temperatures. In addition, a confirmation of the activation energy value was obtained by the "model-fitting" method and the algorithm of the kinetic model equation best-fitting the experimental curve representing the whole process was checked.

Finally, further very interesting observations were made, for instance, the half-life concentration values of polyphenols at selected temperatures between 98 and 180 °C.

Keywords: Extra-virgin olive oil; Artificial rancidification; Tyrosinase biosensor; Kinetic analysis

1. Introduction

As part of a dietary tradition linked to the territory, olive oil represents one of the fundamental products of Mediterranean agriculture; it has undisputed food value due to its chemical composition, as well as its distinctive (organoleptic) features, enhanced by its everyday use as a condiment [1].

At the international level the importance of extra-virgin olive oil (EVOO) is universally recognized in the food, agricultural and industrial sectors. Italy plays a leading role as far as EVOO is concerned both as regards quantity (it is the second world producer after Spain) and quality, i.e. large number of DOP "denominazione di origine protetta" trade-marks (in English, PDO "protected designation of origin") trade-marks recognized within the European Union [2].

The consumption of olive oil is linked mainly to its alimentary use. Nevertheless, mention should also be made of its use in medicine, recommended already in ancient times and today for its anti-oxidative capacity and for the prevention of cardio-circulatory diseases [3].

One fundamental aspect of EVOO is the proper conservation of the product, as in the case of all fats [4].

The study of the phenomena and auto-oxidative kinetics underlying the rancidification of the product is therefore of great importance [5–7]. In a recent research [8] we studied the process of artificial rancidification of EVOO, at the same time determining both the variation in the more traditional index which is the result of the peroxide value (PV) and that of a more innovative index determined by the concentration of the "total polyphenols" (expressed in mol l^{-1} of phenol), however polyphenols

* Corresponding author. Tel.: +39 06 49913722; fax: +39 06 490631.
E-mail address: mauro.tomassetti@uniroma1.it (M. Tomassetti).

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doi:10.1016/j.talanta.2007.08.036

are known to be the main natural anti-oxidant contained in abundance in the EVOO [9,10]. In this research, EVOO rancidification was studied by subjecting the oil to an isothermal process of artificial oxidation. In order to artificially oxidize the EVOO test samples, the AOM (Active Oxygen Method) of the American Oil Chemist Society [11] was employed; the oxidative process was therefore conducted at 98 °C, under a constant air flow.

The EVOO rancidification process rate displayed good inverse correlation between the two indexes considered regarding the total polyphenols concentration, as well as the PV values. However, in this preliminary research [8] no considerations regarding kinetic degradation could be made. Furthermore, in literature, kinetic data on this process are both incomplete and scarce [12,13]. To fill this gap, in the present research the kinetic parameters of the process were determined through an isothermal study carried out at different temperatures (between 98 and 180 °C). This also allowed important considerations to be made concerning the stability of EVOO at the high temperatures (180 °C) to which EVOO is sometimes subjected, for example in the frying process.

2. Experimental

2.1. Materials

Silicon oil 350 Cps (Crual srl); *n*-hexane RPE (Carlo Erba Reagents); phenol RPE (Carlo Erba Reagents); Kappa-Carrageenan (Fluka); dialysis membrane (art. D-9777) (Sigma–Aldrich); gas permeable membrane in PTF (YSI model 5775, Yellow Springs); monobasic and dibasic potassium phosphate RPE (Carlo Erba Reagents); tyrosinase (EC 1.14.18.1) from mushroom 3216 U/mg (Fluka); potassium chloride supplied by Riedel-de Haen (Seelze, Germany); chloroform RPE (Carlo Erba Reagents); tyrosol (Sigma–Aldrich); vanillin (Sigma–Aldrich); caffeic acid (Extrasynthese); ferulic acid (Extrasynthese); oleuropein (Extrasynthese); glyceryl trioleate (solvent) about 65% (w/w) (Sigma–Aldrich); commercial extra-virgin olive oil, purchased from local shops and produced by the most important industrial Italian olive oil producer firms, was contained in sealed glass bottles.

2.2. Instruments

The degradation of the polyphenols contained in the sample examined in this study took place in a silicon oil-based thermostatic bath (Fig. 1) (with temperature ranging from 0 to 250 °C and an associated uncertainty of ±0.1 °C), in an air stream of 140 ml min^{-1} produced by a Unistar mod. AIR 1000–1 pump. The transducer employed for the biosensor determination of the polyphenols in EVOO samples was an amperometric electrode for oxygen supplied by Universal Sensor Inc., New Orleans (USA), Mod. 4000-1. Measurements were performed using a potentiostat supplied by Radelkis Mod. OP 960. The current signal was converted into potential and read off on a Mitek multimeter mod. MK 5001, connected to an Amel analog recorder, mod. 868.

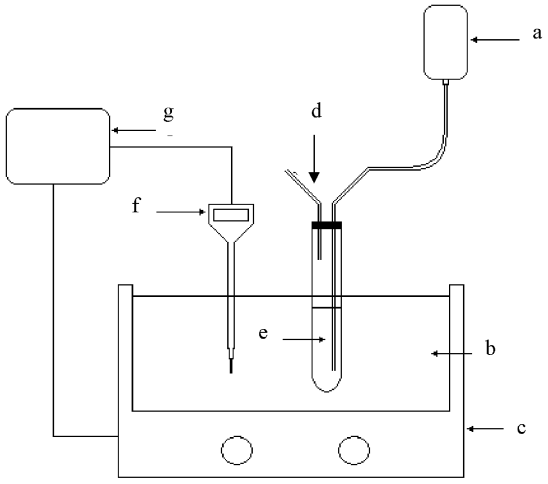

Fig. 1. Apparatus used to study the isothermal degradation of extra-virgin olive oil samples under in an oxidizing atmosphere at different temperatures between 98 and 180 °C. (a) Stream of air pump; (b) silicon oil; (c) adjustable heating device for the constant-temperature chamber; (d) overflow tube; (e) extra-virgin olive oil sample in a pyrex tube with a perforated Teflon plug; (f) vertex thermometer; and (g) electronic temperature controller.

The sample under investigation was added to the initial solution (10 ml *n*-hexane) contained in a constant-temperature chamber at 23 ± 0.2 °C and kept under stirring using an Amel magnetic stirrer, mod. 291/lf.

3. Methods

3.1. Artificial rancidification process

The artificial oxidation of the EVOO was obtained by placing the sample in a pyrex glass tube (containing 25 ml of sample under a constant stream of air at 140 ml min^{-1}) immersed in a silicon oil thermostatic bath both at 98 °C, according to the method proposed by the AOM [11], and at the following set temperatures of 120, 140, 160, and 180 °C, using the apparatus illustrated in Fig. 1. During the artificial rancidification process under isothermal conditions at the above-mentioned selected temperatures, fixed amounts of the sample contained in the test glass tube were collected at regular time intervals and immediately analyzed. The determination of the pool of polyphenols was carried out using an enzymatic tyrosinase biosensor both on the pure EVOO sample and on the degraded samples [8,14].

3.2. Tyrosinase OPEE

The biosensor method adopted was based on the oxidation of phenol to quinone catalyzed by the tyrosinase enzyme. The recorded signal was proportional to the variation of the partial vapor pressure of oxygen dissolved in solution, which was consumed in the course of the enzymatic reaction (1):

$$\text{phenol} + O_2 \xrightarrow{\text{tyrosinase}} o\text{-quinone} + H_2O \quad (1)$$

For this purpose, a tyrosinase biosensor made of polytetrafluoroethylene (PTFE) capable of measuring the decrease in

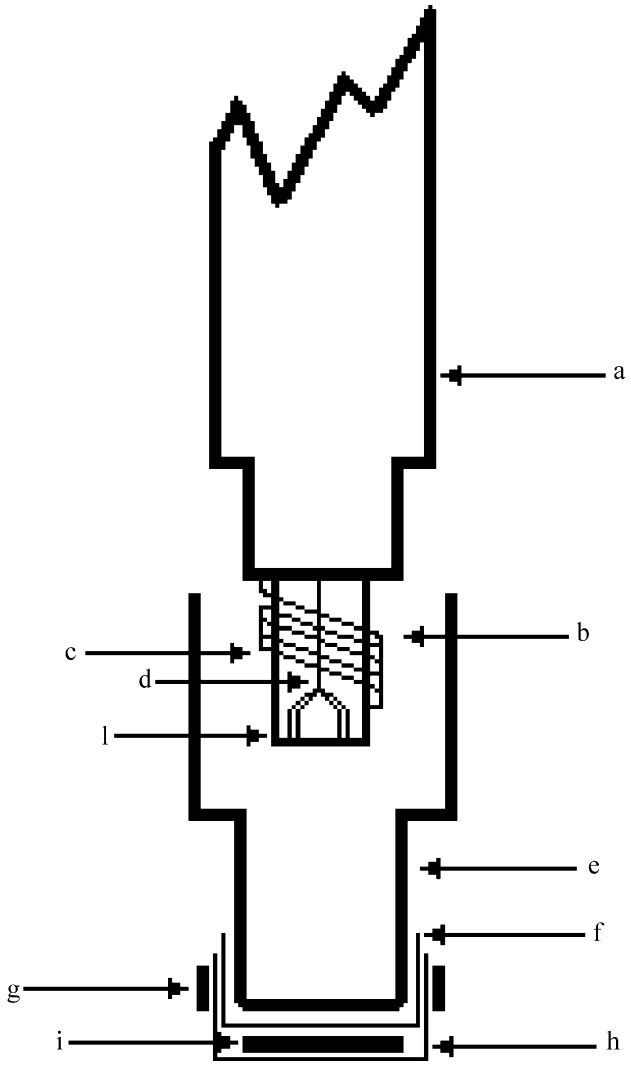

Fig. 2. Scheme of the tyrosinase biosensor used: (a) external structure of the electrode; (b) internal solution (phosphate buffer 0.067 mol l^{-1} at pH 6.6 and KCl 0.1 mol l^{-1}); (c) reference inner electrode: Ag/AgCl (anode); (d) Pt electrode (cathode); (e) Teflon cap; (f) gas-permeable membrane; (g) Teflon O-ring; (h) dialysis membrane; (i) tyrosinase immobilized in Kappa-Carrageenan gel; and (l) glass insulator.

dissolved oxygen concentration due to the oxidation of a phenolic compound to a *o*-quinone operating in *n*-hexane was used (this kind of biosensor is usually denoted as an organic phase enzyme electrode, OPEE) (Fig. 2). The variation of the signal recorded is proportional to the species reduced at the cathode (O_2), the amount of which depends on the concentration of the oxidized phenols in the sample. The decision to carry out the determination of the polyphenols in an organic solvent (*n*-hexane) was based on the very high solubility of the oil matrix in this organic solvent and to its very low solubility in aqueous solution [8,15].

The tyrosinase enzyme was immobilized in a Kappa-Carrageenan gel [16,17] prepared as follows: 3.75 mg of tyrosinase (3216 U mg^{-1}) was placed in an Eppendorf vial and dissolved in 100 μl of phosphate buffer 0.06 mol l^{-1} at pH 7. To obtain the enzyme membrane 50 μl of the enzyme solution was added to each disk of Kappa-Carrageenan, which was then stored for 48 h, at 4 °C.

The gel disk containing the enzyme was sandwiched between a PTFE membrane and a dialysis membrane; the whole assembly was fixed to the electrode head by a PTFE O-ring, as shown in Fig. 2.

3.3. Polyphenol measurement

At selected time intervals the oil samples to be analyzed were taken from the pyrex glass tube, where they had previously undergone thermal degradation under isothermal conditions at each of the above-mentioned five temperatures. These samples were placed in an Eppendorf vial which was cooled very rapidly by dipping it in cold water. Before performing the measures, both in order to reduce the viscosity of the sample in the successive preparations and to facilitate its immediate dissolution in the organic solvent (*n*-hexane) in which the measure was performed, the samples were diluted using the same solvent (*n*-hexane) in the ratio of (1:4 v/v).

The biosensor measurement was carried out under constant magnetic stirring in a glass cell thermostated at 23 °C and containing 10 ml of *n*-hexane, in which the bionsensor was immersed for the time necessary to achieve a stable signal [8]. When the signal of the biosensor immersed in *n*-hexane became stabilized, 25 μl of a standard solution of phenol 10^{-3} mol l^{-1} in *n*-hexane were added and the corresponding signal variation measured. When the new stationary state was reached, a second addition of 25 μl of the standard solution of phenol was made and after the new corresponding variation of the signal had been recorded, a further addition of 50 μl of the *n*-hexane solution of the oil sample, diluted (1:4 v/v), was made as described in a previous section and the corresponding variation of the signal was measured after the last addition. The polyphenol concentration in the sample examined was obtained using the following algorithm:

$$C_{\mathrm{EVOO}} = \left[\frac{\Delta S_{\mathrm{EVOO}}(\mu \mathrm{l}_{\mathrm{EVOO}})}{\Delta S_{\mathrm{standard}}(\mu \mathrm{l}_{\mathrm{standard}})}\right] C_{\mathrm{standard}} \times 4 \quad (2)$$

where 4 is the multiplier factor due to dilution and ΔS is the recorded signal variation, respectively, for EVOO and for the reference standard solution of phenol in *n*-hexane. The C_{EVOO} thus obtained, expressed in mol l^{-1} of phenol, clearly represents an index of the total polyphenols concentration in the sample.

3.4. Preparation of synthetic mixture of polyphenols

The lack of quantitative kinetic data available in literature concerning the thermal oxidative degradation process of polyphenols contained in EVOO led us to investigate a comparison system consisting in a synthetic mixture (SM) of five of the more readily available polyphenols contained in the EVOO dissolved in glyceryl trioleate. The composition of this mixture is shown in Table 1. Also this mixture was subjected to an isothermal degradation process at the same temperatures and in the same experimental conditions as used for the EVOO and analyzed with the biosensor method described above in order to

Table 1
Composition of the synthetic mixture (SM) of polyphenols in glyceryl trioleate

Compounds	MW	C (mol l^{-1})
Tyrosol	138	1.1
Vanillin	152	3.1
Caffeic acid	180	4.4
Ferulic acid	194	2.5
Oleuropein	540	1.4

evaluate quantitatively the kinetics of the polyphenol degradation process and to compare it with the same process occurring in the EVOO sample.

3.5. MacCallum method

The MacCallum method [18] was selected for this study from among the several kinetic methods reported in literature. It enables the activation energy related to the process to be determined by carrying out isothermal experiments at different pre-fixed temperatures using the following equation:

$$\ln(t_{iso})_\alpha = a + \frac{b}{(T_{iso})_\alpha} \quad (3)$$

where t_{iso} is the time necessary to achieve a given degree of conversion α under isothermal conditions at a given temperature T_{iso}. The kinetic parameters (pre-exponential factor, A and activation energy, E_a) are calculated from the regression parameters of Eq. (3) via the following relation: $a = \ln[f(1-\alpha) - \ln A]$ and $b = E_a/R$, where $f(1-\alpha)$ is a function representing the fraction of the sample not converted during the isothermal degradation process and R is the gas constant. From the slope b of Eq. (3), calculated at each degree of conversion α, the corresponding activation energy value is derived for the process considered. The time dependence of the degree of conversion at each isothermal temperature enables the $\ln(t_{iso})_\alpha$ values corresponding to given $(T_{iso})_\alpha$ values to be derived at selected α values ranging from 0 to 0.20, in accordance with the recommended literature interval for the application of the method [18]. Moreover, this method requires the construction of interpolated α_i *versus* t_i curves based on the experimental data at each T_{iso} in order to determine the time values necessary to achieve pre-fixed α values.

3.6. "Model-fitting" method

In order to complete the kinetic data and to validate more satisfactorily the results obtained using the above-mentioned method, another method denoted as "model-fitting" [19] was also applied to the experimental data obtained using the tyrosinase biosensor. This method was used to seek the "model function" that represents the best equation for describing the process; it also enabled the most important kinetic parameters of the process considered to be determined. It involved selecting from among the most suitable kinetic models described by appropriate mathematical functions the one that shows the best (linear) fit with the experimental data. To this end, the mathematical expressions of the most common differential and integral model functions ($f(\alpha)$ and $g(\alpha)$, respectively) found in literature [19] are given in Table 2. Since only the actual integral function $g(\alpha)$, which represents the process examined, depends linearly on time, according to the following relationship [19]:

Table 2
Most commonly used differential $f(\alpha)$ and integral $g(\alpha)$ model functions of the degree of conversion $\alpha = ((C_i - C_t)/(C_i - C_f))$, which are used to describe the degradation process examined

Reaction model	$f(\alpha)$	$g(\alpha) = kt$
P1	$4\alpha^{3/4}$	$\alpha^{1/4}$
A1.5	$1.5(1-\alpha)(-\ln(1-\alpha))^{1/3}$	$(-\ln(1-\alpha))^{2/3}$
A2	$2(1-\alpha)(-\ln(1-\alpha))^{1/2}$	$(-\ln(1-\alpha))^{1/2}$
A3	$3(1-\alpha)(-\ln(1-\alpha))^{2/3}$	$(-\ln(1-\alpha))^{1/3}$
A4	$4(1-\alpha)(-\ln(1-\alpha))^{3/4}$	$(-\ln(1-\alpha))^{1/4}$
B1	$\alpha(1-\alpha)$	$\ln(\alpha/(1-\alpha))$
R2	$2(1-\alpha)^{1/2}$	$1-(1-\alpha)^{1/2}$
R3	$3(1-\alpha)^{1/3}$	$1-(1-\alpha)^{1/3}$
D1	$1/2\alpha$	α^2
D2	$(-\ln(1-\alpha))^{-1}$	$(1-\alpha)\ln(1-\alpha)+\alpha$
D3	$1.5(1-(1-\alpha)^{1/3})^{-1}(1-\alpha)^{1/3}$	$(1-(1-\alpha)^{1/3})^2$
D4	$1.5((1-\alpha)^{1/3}-1)^{-1}$	$1-(2\alpha/3)-(1-\alpha)^{2/3}$
F1	$(1-\alpha)$	$-\ln(1-\alpha)$
F2	$(1-\alpha)^2$	$1/(1-\alpha)-1$
F3	$0.5(1-\alpha)^3$	$1/(1-\alpha)^2-1$

$$g(\alpha) = \int_0^\alpha \frac{d\alpha}{f(\alpha)} \quad (4)$$

A plot of the $g(\alpha)$ values *versus* time values corresponding to the fixed α value was made at each isothermal temperature. At each isothermal temperature the $g(\alpha)$ values were obtained by inserting in the expressions reported in Table 2 the regression α values (obtained from non-linear regression of the $\alpha = f(t)$ experimental values) for the thermal degradation of both EVOO and SM. The selection of the best $g(\alpha)$ function was obtained from the best linear fit of the $g(\alpha)$ *versus* t curves. Once the best $g(\alpha)$ function was selected, the specific rate constant k_{iso} values at each isothermal temperature were obtained from the slopes of the corresponding "best-fitting" $g(\alpha)$ *versus* t regression line. The temperature dependence of the specific rate constants is usually represented by the Arrhenius equation [20]:

$$\ln k_{iso} = c - \frac{b}{T_{iso}} \quad (5)$$

where $c = \ln A$ and $b = E_a/R$. Thus, by applying the linear regression to the Arrhenius equation, the pre-exponential factor $\ln A$ and the activation energy E_a were determined, respectively, from the intercept and the slope of the corresponding regression line as far as the best linear-fitting model function was concerned.

4. Results and discussion

4.1. Kinetic analysis of polyphenol degradation using the MacCallum method

Once the samples of EVOO and of SM had been subjected to the isothermal oxidative degradation process, at the different

Table 3
Concentration values in the "total polyphenols" of the extra-virgin olive oil (EVOO) sample for selected time values during the degradation as obtained by isothermal measurements at different temperatures using the tyrosinase biosensor

T (°C)	t (min)	C ($\times 10^3$ mol l^{-1})	t (min)	C ($\times 10^3$ mol l^{-1})	t (min)	C ($\times 10^3$ mol l^{-1})
98	0	2.00	480	0.47	1980	0.26
	10	1.96	1080	0.45	2820	0.23
	20	1.94	1380	0.42	3120	0.18
	30	1.40	1620	0.38	3360	0.13
	60	0.81	1800	0.29	3600	0.05
	120	0.77				
120	0	2.00	30	1.07	450	0.25
	3	1.95	60	0.94	570	0.21
	6	1.82	90	0.91	720	0.18
	10	1.79	150	0.59	840	0.15
	15	1.46	210	0.52	960	0.08
	20	1.28	330	0.41		
140	0	2.00	30	0.95	450	0.21
	3	1.86	60	0.87	570	0.15
	6	1.60	90	0.86	720	0.13
	10	1.54	150	0.53	840	0.11
	15	1.31	210	0.48	960	0.04
	20	1.12	330	0.40		
160	0	2.00	15	1.17	90	0.51
	3	1.67	20	1.01	210	0.30
	6	1.36	30	0.90	330	0.18
	10	1.35	60	0.67	450	0.07
180	0	2.00	15	0.61	90	0.26
	3	1.56	20	0.56	120	0.18
	6	0.71	30	0.41	210	0.11
	10	0.67	60	0.27		

S.D. $\leq 0.04 \times 10^{-3}$ mol l^{-1}.

pre-set temperatures, that is, at 98, 120, 140, 160, and 180 °C, the "total polyphenols" concentrations in the EVOO and in the SM were determined as a function of the heating time. The relevant experimental values found have been summarized in Tables 3 and 4. The comparison of degradation trends at the same temperatures is shown graphically in Fig. 3; the concentration of the "total polyphenols" as a function of heating time does not appear significantly different for the EVOO and the SM samples. It may thus be stated that the SM, although made up of a polyphenol mixture that is simpler than that contained in the EVOO, proves to be sufficiently representative of the EVOO samples. On the other hand, further confirmation was obtained by observing the time trends of total polyphenols disappearance in the EVOO and in the SM at the five different temperatures examined, shown in Fig. 4, which were very similar. This also confirms that it is correct to affirm that the trend observed for the degradation process of EVOO may essentially be ascribed to the degradation of the total polyphenols contained therein.

Moreover, comparison of the histograms illustrated in Fig. 4 allows some more interesting results to be harvested, as may be inferred from the trends shown in Fig. 3, such as the time required to achieve complete disappearance of the polyphenols, (A) in the EVOO and (B) in the SM, at the different temperatures considered. For example, at 100 °C, about 60–65 h are necessary, while at 180 °C only about 6–7 h are required. This

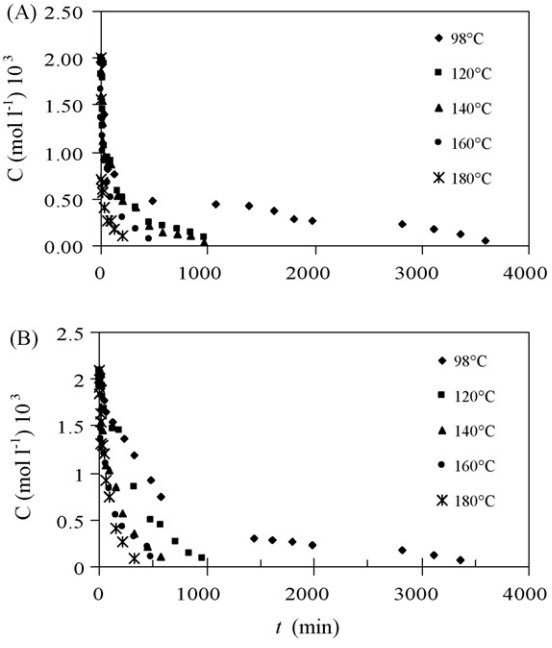

Fig. 3. Total polyphenol concentration trend in (A) extra-virgin olive oil and (B) synthetic polyphenols mixture concentration trend in glyceryl trioleate as a function of time for isothermal measurements at different temperatures.

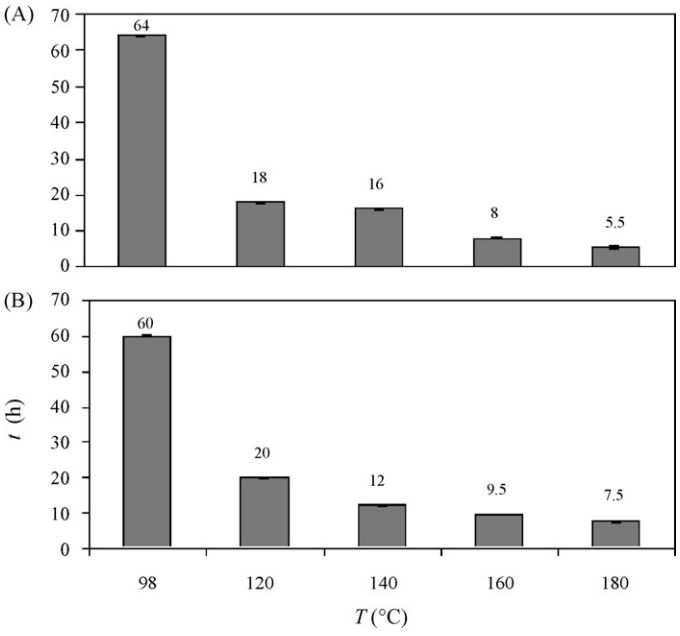

Fig. 4. Time taken to achieve complete disappearance of total polyphenols in extra-virgin olive oil and in the synthetic mixture in glyceryl trioleate due to the isothermal heating at different temperatures. (A) Extra-virgin olive oil and (B) synthetic mixture in glyceryl trioleate.

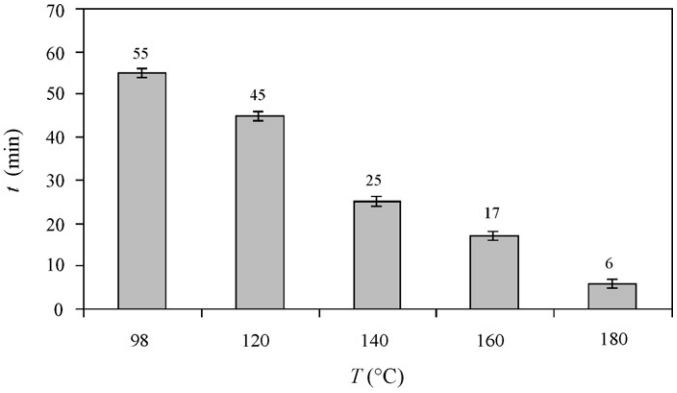

Fig. 5. Half-life values for total polyphenols concentration in extra-virgin olive oil samples at the different isothermal temperatures considered.

is because the polyphenols disappear completely both from the EVOO and from the SM subjected to the artificial rancidification process under the same conditions as prescribed by the AOM method [11], although of course individually at each of the five different temperatures considered. Also of great interest are the values of the polyphenol concentration half-life of the EVOO at the different isothermal heating temperatures considered, shown as a histogram in Fig. 5. The half-life is seen to decrease almost constantly between 100 and 180 °C, plunging from about 1 h to about 5 min on going from the

Table 4
Concentration values in the "total polyphenols" of the synthetic mixture (SM) sample for selected time values during the degradation as obtained by isothermal measurements at different temperatures using the tyrosinase biosensor

T (°C)	t (min)	C ($\times 10^3$ mol l^{-1})	t (min)	C ($\times 10^3$ mol l^{-1})	t (min)	C ($\times 10^3$ mol l^{-1})
98	0	2.09	120	1.55	1620	0.29
	10	2.05	240	1.36	1800	0.26
	20	2.02	330	1.18	1980	0.23
	30	1.94	480	0.92	2820	0.18
	45	1.78	570	0.74	3120	0.13
	60	1.65	1440	0.31	3360	0.07
120	0	2.09	45	1.69	570	0.45
	3	2.03	120	1.48	720	0.26
	6	1.98	180	1.45	840	0.15
	10	1.95	330	0.85	960	0.08
	30	1.80	480	0.50		
140	0	2.09	20	1.54	210	0.56
	3	2.02	30	1.46	330	0.36
	6	1.96	60	1.09	450	0.22
	10	1.94	90	1.02	570	0.10
	15	1.71	150	0.85		
160	0	2.09	20	1.37	150	0.55
	3	1.97	30	1.31	210	0.43
	6	1.92	45	1.20	330	0.32
	10	1.83	60	1.10	450	0.22
	15	1.48	90	0.83	480	0.10
180	0	2.09	20	1.32	90	0.75
	3	1.92	30	1.30	150	0.40
	6	1.84	45	1.20	210	0.26
	10	1.63	60	0.92	330	0.09
	15	1.55				

S.D. $\leq 0.04 \times 10^{-3}$ mol l^{-1}.

first to the second temperature. Finally, it should be noted how the temperature to which the oil is heated is the decisive factor, as it determines polyphenol persistence time and therefore the time of rancidification of the extra-virgin olive oil.

In order to determine the energy of activation of the EVOO and SM degradation processes, first of all the MacCallum method [18] was applied. To this end, a set of values of the natural logarithm of t (i.e. $\ln t$) were determined that corresponded to pre-established values of the conversion degree α, over the interval $\alpha \leq 0.20$, for each of the temperatures considered (see Fig. 6 and Table 5). After inserting these values in Eq. (3), and performing the linear regressions of each ($\ln t_{iso}$ *vs.* $1/T_{iso}$) set of data using the least squares method at the five different isothermal temperatures considered, the values of the activation energy (E_a) corresponding to the different α values selected were drawn from the value of the slope of the straight line obtained (Fig. 7) and have been set out in Table 6. The associated errors were obtained starting with the error on the slope (b) of the regression reported in Table 6. Observing the behaviour of the values of the activation energy of the EVOO and of the SM at the different a values considered, which are shown graphically in Fig. 8, it can be concluded that the E_a values, at least over the range of α values examined, can be considered constant within the limits of experimental error. For this reason the mean activation energy values (38.0 ± 0.8 and 33.8 ± 0.1 kJ mol^{-1}, respectively, for the thermal degradation of the EVOO and of the SM), which are also reported, together with their standard deviations, in Table 6, can be considered constant for all the first part (i.e. about a quarter of the entire process) and as likely to be representative of the entire degradation process.

4.2. Kinetic analysis of polyphenol degradation using the model-fitting method

As neither the values of the kinetic constants nor the mathematical model representing the mechanism of the degradation process were obtained via the previous method, and in order to stress the value of the results of the activation energy achieved, it was decided to apply another kinetic method, namely the "model-fitting" method [19], by processing the data reported in Tables 3 and 4. First of all, to this end, the values of the degree of conversion for the entire isotherm degradation process of the EVOO and of the SM at the five temperatures selected (see Tables 7 and 8) were computed; these values have

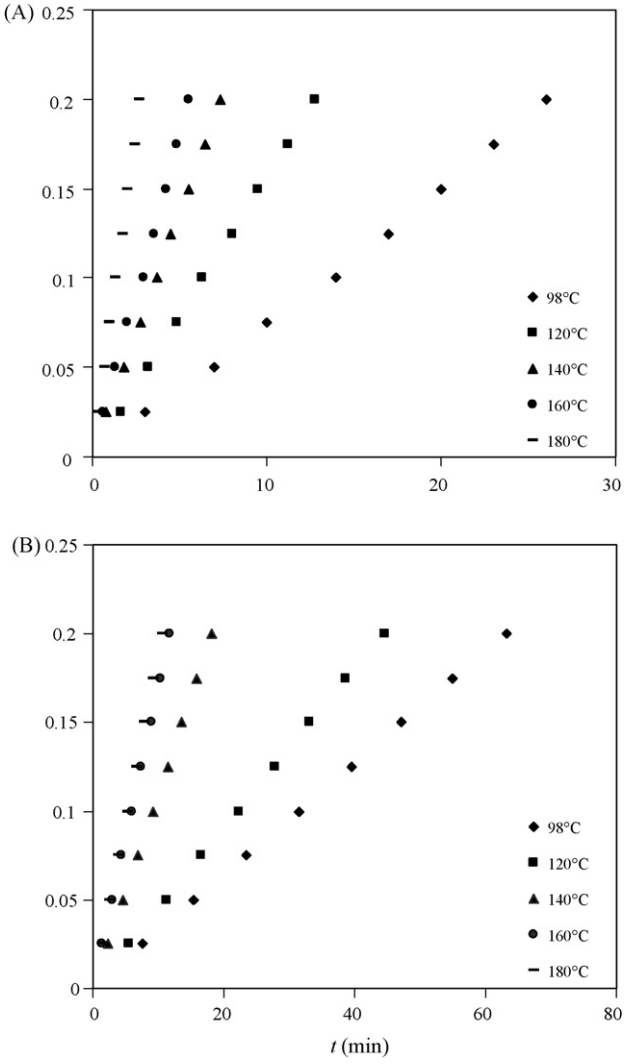

Fig. 6. Regression degree of conversion (α) values obtained from the linear fit of experimental data regarding total polyphenols degradation in (A) extra-virgin olive oil and (B) the synthetic mixture at different isothermal temperatures during heating over the range $0.025 \leq \alpha \leq 0.200$.

Table 5
$\ln t$ (min) values for selected values of the degree of conversion $\alpha = ((C_i - C_t)/(C_i - C_f))$, obtained by isothermal measurements corresponding to defined temperatures

α	EVOO					SM				
	98 °C	120 °C	140 °C	160 °C	180 °C	98 °C	120 °C	140 °C	160 °C	180 °C
0.025	1.099	0.470	−0.223	−0.511	−1.204	2.015	1.705	0.789	0.337	0.262
0.050	1.946	1.163	0.588	0.262	−0.431	2.741	2.425	1.504	1.065	0.956
0.075	2.303	1.569	1.030	0.693	−0.051	3.157	2.803	1.932	1.482	1.361
0.100	2.639	1.841	1.308	1.065	0.262	3.447	3.100	2.230	1.775	1.668
0.125	2.833	2.079	1.504	1.253	0.531	3.676	3.325	2.434	2.002	1.887
0.150	2.996	2.251	1.705	1.447	0.693	3.852	3.503	2.610	2.186	2.067
0.175	3.136	2.416	1.872	1.569	0.876	4.007	3.656	2.766	2.332	2.219
0.200	3.258	2.549	1.988	1.705	0.993	4.146	3.798	2.901	2.468	2.361

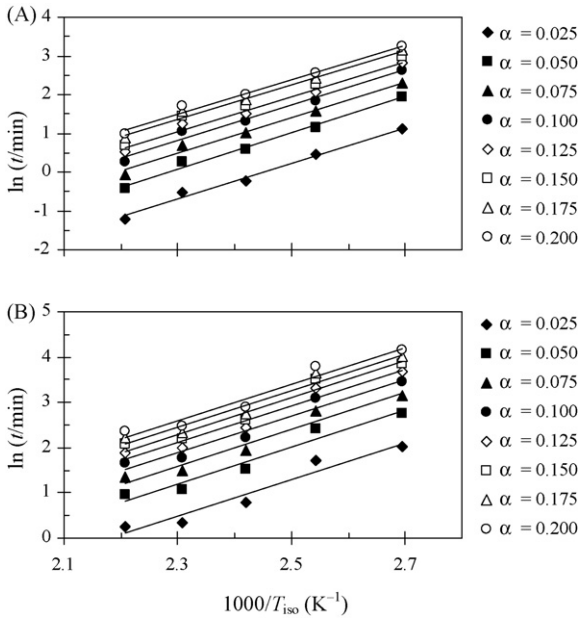

Fig. 7. Linear trend of the $\ln(t_{iso})_\alpha$ values as a function of T_{iso}^{-1} at the given values of the degree of conversion (α) for the thermal degradation of (A) extra-virgin olive oil and (B) synthetic mixture of polyphenols in glyceryl trioleate.

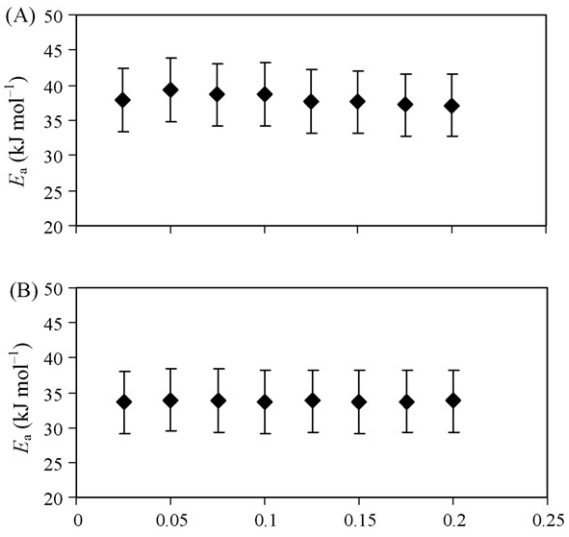

Fig. 8. Activation energy trends found for the thermal degradation of (A) the extra-virgin olive oil and (B) the synthetic mixture of polyphenols in glyceryl trioleate as a function of the degree of conversion α.

been shown graphically in Fig. 9. Lastly, the trends obtained for the corresponding regression values of conversion degree α are shown in Fig. 10. Hence, by reporting the values of $g(\alpha)$, calculated starting from the regression values of α represented in Fig. 10 as a function of the time values t_α. For each of the equations corresponding to the various kinetic models considered (reported in Table 2) and for each of the five different temperatures of degradation examined, trends were obtained (see, for example, those reported in Fig. 11) that were found to have different degree of linearity, depending on the greater or lesser correspondence between the trends of the experimental data and the kinetic model used to represent them. The slopes of the values for the straight line representing the best fit of these behaviours provide the values of the kinetic constants (k) at the five different temperatures at which the isotherms of Fig. 10 were obtained. The selection of the model that best represents the kinetic behaviour of the thermal degradation of the polyphenols among all the kinetic models considered, both for the EVOO and for the SM in glyceryl trioleate, was carried out by determining the values of the correlation coefficients r^2 calculated for all the (more or less straight-line) curves obtained by inserting the expressions given in Table 2 in Eq. (4). In Table 9 the values of r^2 for all the kinetic models and for all the temperatures considered have been reported and compared. This table indicates that for the degradation of polyphenols in extra-virgin olive oil the most representative kinetic model was the one marked in Table 9 with the abbreviation F2, while, for the degradation of the "total polyphenols" in glyceryl trioleate, two models proved to be

Table 6
Slope and intercept values of the linear regressions applied to the MacCallum equation for the isothermal degradation of the "total polyphenols" in both the extra-virgin olive oil (EVOO) and synthetic mixture (SM) samples

α	EVOO				SM			
	a[a]	b[b] ($\times 10^3$ K)	r^2	E_a (kJ mol^{-1})	a[a]	b[b] ($\times 10^3$ K)	r^2	E_a (kJ mol^{-1})
0.025	−11.2 ± 0.9	4.6 ± 0.4	0.9886	37.9 ± 3.0	−9.5 ± 1.4	4.0 ± 0.6	0.9499	33.6 ± 4.8
0.050	−10.8 ± 0.8	4.7 ± 0.3	0.9899	39.3 ± 2.7	−8.1 ± 1.4	4.1 ± 0.6	0.9515	33.9 ± 4.7
0.075	−10.2 ± 0.9	4.6 ± 0.4	0.9868	38.6 ± 3.2	−7.6 ± 1.2	4.1 ± 0.5	0.9515	33.8 ± 4.3
0.100	−9.9 ± 1.2	4.6 ± 0.5	0.9771	38.6 ± 4.0	−7.2 ± 1.3	4.0 ± 0.5	0.9514	33.6 ± 4.3
0.125	−9.4 ± 1.0	4.5 ± 0.4	0.9841	37.7 ± 3.4	−7.7 ± 1.3	4.1 ± 0.5	0.9527	33.8 ± 4.4
0.150	−9.2 ± 1.0	4.5 ± 0.4	0.9819	37.6 ± 3.6	−7.5 ± 1.3	4.0 ± 0.5	0.9547	33.7 ± 4.4
0.175	−8.9 ± 0.8	4.5 ± 0.3	0.9881	37.2 ± 2.9	−7.4 ± 1.3	4.1 ± 0.5	0.9461	33.8 ± 4.4
0.200	−8.8 ± 0.9	4.5 ± 0.4	0.9864	37.1 ± 3.2	−7.3 ± 1.3	4.1 ± 0.5	0.9412	33.8 ± 4.5
				38.8 ± 0.8[c]				33.8 ± 0.1[c]

E_a = activation energy.
[a] $a = \ln[f(1-\alpha)] - \ln A$, where $f(1-\alpha)$ is a function representing the degree of degradation trend.
[b] $b = E_a/R$.
[c] Average.

Table 7
Degree of conversion (α) values for the thermal degradation of the "total polyphenols" of the extra-virgin olive oil (EVOO) sample obtained by isothermal measurements at different temperatures

T (°C)	t (min)	α	t (min)	α	t (min)	α
98	0	0.00	480	0.76	2820	0.88
	10	0.02	1380	0.77	3120	0.91
	20	0.03	1620	0.81	3360	0.94
	30	0.30	1800	0.86	3600	0.97
	60	0.60	1980	0.87	3840	1.00
	120	0.62				
120	0	0.00	30	0.55	450	0.88
	3	0.07	60	0.56	570	0.90
	6	0.09	90	0.86	720	0.91
	10	0.11	150	0.74	840	0.93
	15	0.27	210	0.76	960	0.96
	20	0.49	330	0.80		
140	0	0.00	30	0.53	330	0.90
	3	0.17	60	0.55	450	0.93
	6	0.20	90	0.71	570	0.94
	10	0.23	150	0.74	720	0.98
	15	0.41	210	0.79	840	1.00
	20	0.47				
160	0	0.00	15	0.34	90	0.74
	3	0.02	20	0.36	210	0.85
	6	0.32	30	0.53	330	0.91
	10	0.32	60	0.67	450	0.97
180	0	0.00	15	0.70	90	0.89
	3	0.22	20	0.72	120	0.91
	6	0.65	30	0.80	210	0.95
	10	0.67	60	0.87		

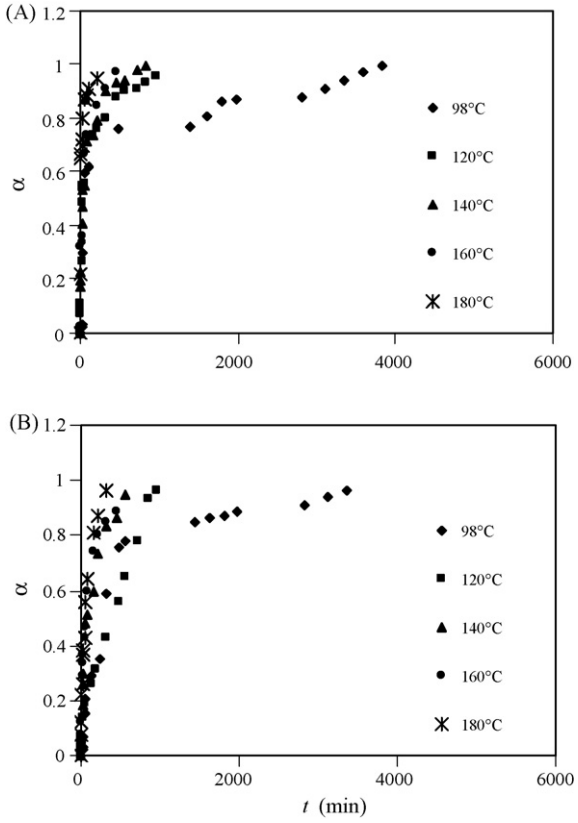

Fig. 9. Trend of the degree of conversion (α) values for the thermal degradation of total polyphenols pool under isothermal conditions at different temperatures in (A) extra-virgin olive oil and (B) synthetic mixture of polyphenols in glyceryl trioleate. R.S.D. $\leq 2\%$.

equally significant, that is, the ones indicated with the abbreviations D2 and F1 (see the corresponding equations reported in Fig. 11).

As stated previously, the slopes of the $g(\alpha)$ *versus* t lines represent the specific constant rates k_{iso} related to the five given T_{iso} values. The resulting k_{iso} values for the thermal degradation of polyphenols contained in the EVOO at 98, 120, 140, 160, and 180 °C related to the "model function" F2, which shows the best linear fit (Table 9), are displayed as a histogram in Fig. 12. It should be noted that at 180 °C k_{iso} is about 20 times greater than at 98 °C. However, once the specific constant rates have been determined for the most suitable model functions (F2 for thermal degradation of EVOO and D2 and F1 for that of SM in glyceryl trioleate, as emerged from the comparison of values in Table 9), single activation energy (E_a) values for the polyphenol degradation process over the temperature range of 98–180 °C were easily obtained from the slope of the logarithmic form of the Arrhenius equation (Eq. (5)).

The $\ln k_{iso}$ values used in Eq. (5) for the thermal degradation of both EVOO and SM in glyceryl trioleate at the five T_{iso} temperatures considered are summarized in Table 10 for the most probable "model functions". The E values thus obtained referred to the thermal degradation of polyphenols in EVOO, as described by the F2 model function, and SM to polyphenol degradation in glyceryl trioleate, as described by the D2 and F1 model functions presented, are 36 ± 4 and

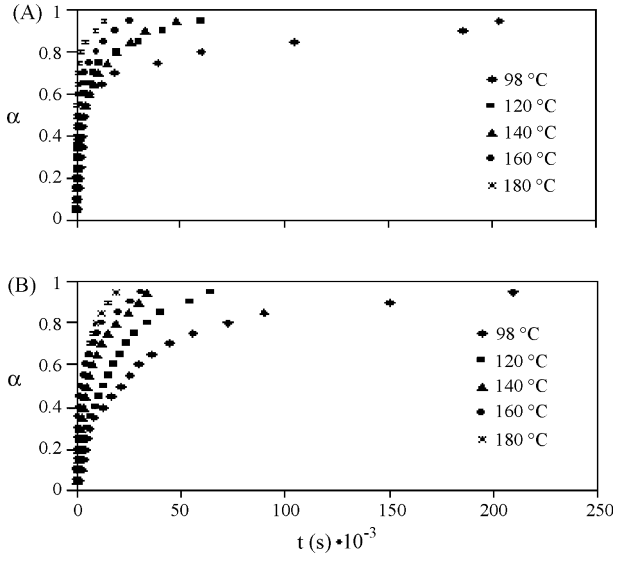

Fig. 10. Trend of the regression α values for the thermal degradation of total polyphenols under isothermal conditions at different temperatures in (A) extra-virgin olive oil and (B) synthetic mixture of polyphenols in glyceryl trioleate. The isothermal experiments were carried out at selected temperatures.

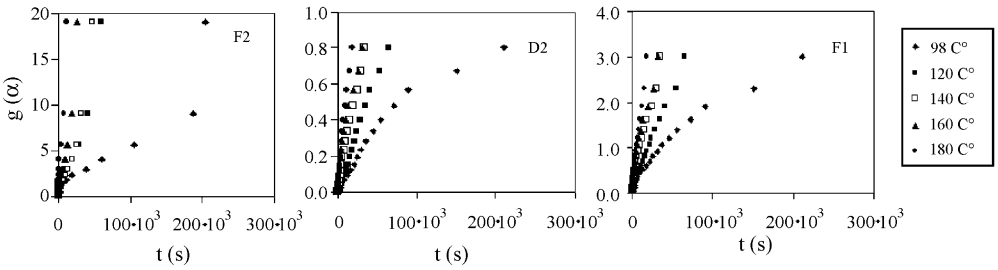

Fig. 11. Trend of the values of the integral function $g(\alpha)$ as a function of time t for the most suitable selected model functions (F2, D2 and F1) at the different selected temperatures for the thermal degradation of total polyphenols contained in extra-virgin olive oil and in the synthetic mixture in glyceryl trioleate.

Table 8
Degree of conversion (α) values for the thermal degradation of the "total polyphenols" of the synthetic mixture (SM) sample obtained by isothermal measurements at different temperatures

T (°C)	t (min)	α	t (min)	α	t (min)	α
98	0	0.00	120	0.29	1620	0.86
	10	0.02	240	0.35	1800	0.87
	20	0.03	330	0.59	1980	0.89
	30	0.07	480	0.76	2820	0.91
	45	0.15	570	0.78	3120	0.94
	60	0.21	1440	0.85	3360	0.96
120	0	0.00	45	0.19	570	0.65
	3	0.03	120	0.26	720	0.78
	6	0.06	180	0.31	840	0.93
	10	0.07	330	0.43	960	0.96
	30	0.14	480	0.56		
140	0	0.00	20	0.26	210	0.73
	3	0.03	30	0.30	330	0.83
	6	0.06	60	0.48	450	0.86
	10	0.07	90	0.51	570	0.95
	15	0.18	150	0.60		
160	0	0.00	20	0.34	150	0.74
	3	0.06	30	0.37	210	0.80
	6	0.08	45	0.43	330	0.85
	10	0.12	60	0.47	450	0.89
	15	0.29	90	0.60		
180	0	0.00	20	0.37	90	0.64
	3	0.08	30	0.38	150	0.81
	6	0.12	45	0.43	210	0.87
	10	0.22	60	0.56	330	0.96
	15	0.26				

38 ± 6 or $38 \pm 5\,\mathrm{kJ\,mol^{-1}}$, respectively. A comparison of the activation energy values found for the thermal degradation processes in both EVOO and SM, obtained using the MacCallum and model-fitting kinetic methods, is presented in Table 11. It may be observed that the agreement among the E_a values found for EVOO ($36 \pm 4\,\mathrm{kJ\,mol^{-1}}$) is reasonably good if compared with that obtained for the synthetic mixture of polyphenols (SM) ($38.8 \pm 0.8\,\mathrm{kJ\,mol^{-1}}$). This result confirms that the MacCallum method applied in this study is able to provide realistic kinetic data not only for relatively simple synthetic mixtures but also for real complex samples like extra-virgin olive oil. On the other hand, in the case of the SM the most realistic E_a value (closer to that of EVOO) seems to be that obtained with the model-fitting method, even if the precision is higher for the values calculated by the MacCallum method.

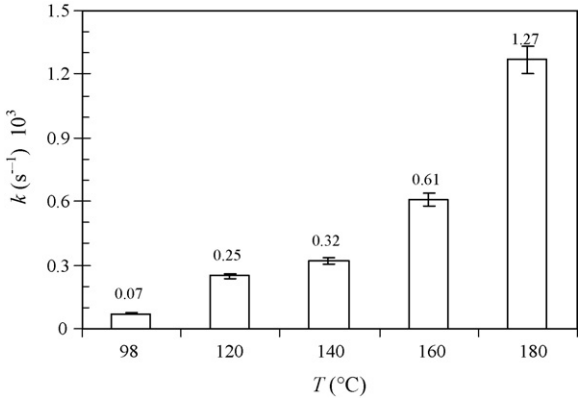

Fig. 12. Trend of the kinetic constant k_{iso} values as a function of temperature for the thermal degradation of total polyphenols in extra-virgin olive oil according to the F2 model function.

Table 9
r^2 values for the $g(\alpha)$ *vs.* t linear equations at the different temperatures considered for both the extra-virgin olive oil (EVOO) and the synthetic mixture (SM) samples

$g(\alpha)$	r^2 values for EVOO					r^2 values for SM				
	98 °C	120 °C	140 °C	160 °C	180 °C	98 °C	120 °C	140 °C	160 °C	180 °C
P1	0.3589	0.3991	0.4939	0.4476	0.2969	0.4826	0.6615	0.6106	0.5077	0.6080
A1.5	0.7445	0.795	0.8763	0.8342	0.6755	0.8573	0.9656	0.9373	0.8793	0.9428
A2	0.6788	0.7289	0.8182	0.7726	0.6067	0.7996	0.9305	0.8965	0.8244	0.8996
A3	0.6045	0.6534	0.7476	0.7003	0.5324	0.7306	0.8799	0.8399	0.7564	0.8415
A4	0.5649	0.6128	0.7081	0.6607	0.4941	0.6925	0.8487	0.8060	0.7180	0.8070
B1	0.5988	0.6476	0.7361	0.6933	0.5349	0.7249	0.8660	0.8220	0.7440	0.8268
R2	0.6970	0.7455	0.8388	0.7890	0.6133	0.8113	0.9453	0.9208	0.8464	0.9190
R3	0.7501	0.7988	0.883	0.8382	0.6725	0.8579	0.9701	0.9481	0.8876	0.9498
D1	0.7288	0.7746	0.8688	0.8152	0.6315	0.8289	0.9581	0.9491	0.8772	0.9411
D2	0.8280	0.8711	0.9421	0.9022	0.7441	**0.9091**	**0.9906**	**0.9871**	**0.9459**	**0.9858**
D3	0.5855	0.6333	0.7398	0.6825	0.4964	0.7093	0.8780	0.8481	0.7514	0.8412
D4	0.7288	0.7746	0.8688	0.8152	0.6315	0.8289	0.9582	0.9491	0.8772	0.9411
F1	0.8454	0.8948	0.9541	0.9229	0.7912	**0.9388**	**0.9956**	**0.9762**	**0.9501**	**0.9880**
F2	**0.8797**	**0.9400**	**0.9142**	**0.9272**	**0.9587**	0.9477	0.8276	0.7953	0.8699	0.8467
F3	0.6833	0.7604	0.7073	0.7300	0.8478	0.7637	0.5844	0.5379	0.6370	0.6057

The most significant models and the related r^2 values are given in bold.

Table 10
$\ln k$ values for the thermal degradation of both extra-virgin olive oil (EVOO) and synthetic mixture (SM) in glyceryl trioleate obtained using the most probable model functions selected from the best linear fits of $g(\alpha)$ *vs.* t in Table 9

T_{iso} (°C)	$\ln k$ (min^{-1})		
	EVOO	SM	
	F2	D2	F1
98	−8.93	−12.39	−11.17
120	−8.23	−11.24	−10.04
140	−8.03	−10.67	−9.48
160	−7.40	−10.58	−9.37
180	−6.67	−10.01	−8.81

Table 11
Comparison of the activation energy (E_a) values for the thermal degradation of the total polyphenols contained in both the extra-virgin olive oil (EVOO) and the synthetic mixture (SM) obtained using the two kinetic methods proposed

	E_a (kJ mol^{-1})	
	MacCallum method	Model-fitting method
EVOO	38.8 ± 0.8	36 ± 4[a]
SM	33.8 ± 0.1	38 ± 6[b]
		38 ± 5[c]

The associated uncertainties are standard deviations.
[a] Values obtained taking into account the reaction model F2.
[b] Values obtained taking into account the reaction model D2.
[c] Values obtained taking into account the reaction model F1.

5. Conclusions

As described in the previous section, for the purpose of monitoring the rancidification process of the extra-virgin olive oil carried out isothermally at different temperatures (98, 120, 140, 160, and 180 °C), the "total polyphenols" concentration was determined at regular time intervals. Polyphenol determination using the tyrosinase OPEE proved to be particularly suitable for the purpose of the present research as it allowed the analysis to be carried out more rapidly (only a few minutes) than when using other chemical methods, such as Folin Ciocalteu's [21].

The good solubility of the oily sample in *n*-hexane, i.e. the solvent used to carry out the measurement, allowed the problems of inaccuracy often encountered when using other methods [21] to analyse oily matrixes due to the low solubility of the oil in aqueous medium to be overcome successfully. Moreover, the solvent selected for carrying out the biosensor measurement, i.e. *n*-hexane, was found to be particularly suitable, also from a theoretical point of view, for carrying out measurements using an enzymatic biosensor; indeed, if the value of an index for hydrophobicity measurement, i.e. $\log p = 3.5$ (p is the distribution coefficient of the solvent used in a standard two-phase octanol/water system) and the value of the dielectric constant of this solvent (1.89) are considered, it is immediately evident that the first value is sufficiently high and the second sufficiently low to ensure a high enzymatic activity and therefore a good response by the (tyrosinase) biosensor [22,23].

Finally, the study of the thermal degradation in an oxidising atmosphere of the polyphenols contained in the extra-virgin olive oil was carried out isothermally at five different temperatures. This allowed both the kinetic model to be identified and the activation energy (E_a) to be calculated, which was found to be in the order of 38 kJ mol^{-1}. Furthermore, this value remains constant (and so also the degradation mechanism does not change) at least throughout whole of the first part, i.e. for practically a quarter, of the entire process. The value of E_a found was obtained for the thermal degradation of a typical commercial extra-virgin olive oil of good quality. Therefore, it can be considered valid in all likelihood way for the most commonly used commercial extra-virgin olive oils, most of which have common standard characteristics and are consequently very similar to each other. Naturally, in the case of extra-virgin olive oils obtained from very particular cultivars it cannot be stated that the value of E_a thus derived can be considered strictly representative also of the thermal degradation of this particular oil sample. From an applicative point of view, some significant aspects of the rancid-

ification process have been displayed: for instance it was found that, at 98 °C, the polyphenols disappear completely in about 60 h, while, at 180 °C, they disappear in about 6 h only; moreover, at 98 °C their concentration is halved after about 50 min, while at 180 °C it is halved in only 6 min. Finally, the kinetic study showed that the value of the kinetic constant of the process at 180 °C is about 20 times higher than at 98 °C.

It may thus be concluded that the temperature at which this important foodstuff is used is a crucial factor in establishing the maximum time for which it can be used safely for the cooking of food.

Acknowledgement

This work was financially supported by MIUR, Co-funded project Prin 2005.

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