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Chemical Characterization and Kinetic parameter determination under Rancimat test conditions of four monovarietal virgin olive oils grown in Morocco

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Abstract – The aim of the present investigation is to compare the chemical characterization of four monovarietal virgin olive oils obtained from fruits of olive trees grown in Morocco (Picholine, Picual, Arebiquine, Koroneiki) with kinetic parameters of oxidation based on Rancimat measurements and finally to assess the oxidative stabilities. The examined oils from different varieties showed a chemical composition within the regulatory limits. Rancimat measurements of induction times were carried out under isothermal conditions in an air atmosphere at temperatures from 373 to 423 K with intervals of 10 K. Using the Arrhenius-type correlation between the inverse induction times and the absolute temperature of the measurements, E_a, Z, and k values for oil oxidation under Rancimat conditions were calculated. The primary kinetic parameters derived from this method were qualitatively consistent and help to evaluate the oxidative stabilities of oils at increased temperatures.

Keywords: Chemical composition / kinetics of oxidation / olive oils / oxidative stability / rancimat

Résumé – Caractérisation chimique et la détermination des paramètres cinétiques sous le test Rancimat de quatre huiles d'olive vierges monovariétales cultivées au Maroc. Le but de ce travail est d'abord de comparer les caractéristiques chimiques de quatre huiles d'olive vierges monovariétales obtenues à partir de fruits d'oliviers cultivés au Maroc (Picholine, Picual, Arbequina, Koroneiki) à l'aide des paramètres cinétiques d'oxydation basés sur des mesures Rancimat et ensuite évaluer leur stabilité à l'oxydation. Ainsi les huiles examinées de différentes variétés ont montré une composition chimique dans les limites réglementaires. Les mesures de Rancimat des temps d'induction ont été effectuées dans des conditions isothermiques, dans une atmosphère d'air à des températures de 373 à 423 K. En utilisant la corrélation de type Arrhenius entre les temps d'induction inverse et de la température absolue des mesures, E_a, Z et les valeurs de k pour l'oxydation de l'huile dans les conditions de Rancimat ont été calculées. Les paramètres cinétiques primaires dérivés de cette méthode étaient qualitativement cohérents et aident à évaluer la stabilité d'oxydation des huiles à des températures élevées.

Mots clés : Composition chimique / cinétique d'oxydation / huiles d'olive / stabilité à l'oxydation / rancimat

1 Introduction

Olives from the olive tree (*Olea europaea* L.) are one of the most important fruits throughout the Mediterranean Basin (Mataix *et al.,* 2008) and in Morocco, with agriculture as one important economic pillar, the cultivation of olive trees constitutes one of the principal economical and agricultural sectors. Olive groves in Morocco are characterized by the predominance of the Moroccan variety Picholine, which represents more than 96% of the national heritage (Gharby *et al.,* 2013). The rest consists of several varieties original from Spain, Greece and Italy (Picual, Arebiquine, Koroneiki).

Olive oil is extensively consumed due to its nutritional value and its organoleptic characteristics (Manai *et al.,* 2008). Besides, it is also used in medicine, recommended since ancient times, for the prevention of cardiovascular diseases and for its anti-oxidative capacity (Allalout *et al.,* 2009; Djebali *et al.,* 2012). Some of these effects are associated with the high

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content of phenolic compounds as well as the high amounts of oleic acid, tocopherols and phytosterols (Allalout *et al.,* 2009; Dabbou *et al.* 2010; Owen *et al.,* 2000). The amount of these compounds in olive oil is influenced to a large extent by the cultivar, soil, climate conditions, irrigation, degree of ripeness, processing methods and lipid oxidation (Allalout *et al.,* 2009; Djebali *et al.,* 2012; Gharby *et al.,* 2011; Morello *et al.,* 2004).

Lipid oxidation has a negative impact on the functionality of raw materials, sensory and nutritional quality of food, and causes economic losses (Matthäus *et al.,* 2010). The most noticeable result of lipid oxidation is the appearance of an unpleasant flavor often referred to rancid, which modifies the sensory characteristics of the food and thus the acceptance of food by the consumer (Matthäus *et al.,* 2010; Velasco and Dobarganes, 2002; Frankel, 2007; Gharby *et al.,* 2012). A number of accelerated methods have been developed to test the resistance of edible oils to oxidation. All these accelerated methods involve the use of elevated temperatures because it is known that the reaction rate is exponentially related to temperature (Reynhout, 1991). Among these methods, nowadays the Rancimat method is very popular and it is frequently used and reviewed due to its ease of use and reproducibility (Anwar *et al.,* 2003; Hasenhuettl and Wan, 1992; Matthäus, 1996; Mendez *et al.,* 1996). Based on induction times from Rancimat measurements it is very easy to rank the oxidative stability of oils, but any kinetic characterization of their oxidation needs at least additional determinations. Rancimat experiments performed at various temperatures for given oil can be used for the kinetic analysis of the oxidation (Farhoosh *et al.,* 2008; Kowalski, 1989). The efficiency of antioxidants can be compared by Rancimat measurements (Ratusz, 2002). There are also some trials to use data from Rancimat measurements to calculate shelf life predictions, but such trials have recently been questioned (Mancebo-Campos *et al.* 2007; Marques-Ruiz *et al.,* 2008). It was underlined that the mechanisms of lipid oxidation under Rancimat conditions and at ambient temperature are substantially different. Evaluation of oil stability based on induction periods derived from the Rancimat test and from the analysis of oxidation products from the oil phase can lead to inconsistent results.

The purpose of this paper is to compare the chemical characterization of four monovarietal virgin olive oils grown in Morocco, to calculate the kinetic parameters of oxidation based on Rancimat measurements and finally to assess the oxidative stabilities of these oils.

2 Materials and methods

2.1 Quality parameter

Acidity index, peroxide value (PV), and extinction coefficients (K_{232} and K_{270}) determination were carried out following the analytical methods described in the Regulations EEC/2568/91 of the European Union Commission (1991). Acidity was expressed as amount of oleic acid. PV was expressed as milliequivalents of active oxygen per kilogram of oil (meq O_2/kg oil), and extinction coefficient K_{232} and K_{270} were expressed as the specific extinctions of a 1% (w/v) solution of oil in 2,2,4-trimethylpentane measured in a 1 cm cuvette.

For the determination of the fatty acid composition (ISO 5508, 1990), the methyl esters were analyzed on a CP-Wax 52CB column (30 m × 0.25 mm i.d.) using helium (flow rate 1 ml/min) as carrier gas. Initial oven temperature was set at 170 °C; injector temperature 200 °C; detector temperature 230 °C. Injected volume was 1 µl for each analysis.

Sterol composition was determined using the International Standard Organisation method (ISO 6799, 1991). Sterol composition was determined after trimethylsilylation of the crude sterol fraction using a Varian 3800 instrument equipped with a VF-1 ms column (30 m and 0.25 mm i.d.) and using helium (flow rate 1.6 ml/mn) as carrier gas. Column temperature was isothermal at 270 °C, injector and detector temperature was 300 °C. Injected quantity was 1 µl for each analysis. Data were processed using Varian Star Workstation v 6.30 (Varian Inc., Walnut Creek, CA, USA).

Tocopherol composition was determined using the International Standard Organisation method (ISO 9936, 2006). High performance liquid chromatography (HPLC) was used for the determination of tocopherols, using a solution of 250 mg of oil in 25 ml of n-heptane and a Shimadzu CR8A HPLC instrument (Champ sur Marne, France) equipped with a C18-Varian column (25 cm × 4 mm; Varian Inc., Middelburg, The Netherlands). Detection was performed using a fluorescence detector (excitation wavelength 290 nm, detection wavelength 330 nm). Eluent used was a 99:1 isooctane/isopropanol (V/V) mixture, flow rate of 1.2 ml/min.

The polyphenol content was determined using the Folin-Ciocalteu spectrophotometrically according to the Singleton method (Singleton *et al.,* 1999) using caffeic acid as standard.

2.2 Rancimat test

Induction time was determined using the International Standard Organisation method (ISO 6886, 2006). The oxidative stability of each sample was determined as the induction period (IP, h) recorded by a 743 Rancimat (Metrohm, Herisau, Switzerland) apparatus using 3 g of oil sample. Samples placed into Rancimat standard tubes were subjected to the normal operation conditions of the test by heating at 373 K, 383 K, 393 K, 403 K, 413 K, 423 K with an air flow of 20 L/h.

2.3 Kinetic data analysis

Temperature coefficients (T_{Coeff}, K^{-1}) were determined from the slopes of the lines generated by plotting $\ln(k)$ *vs.* the absolute temperature (T, K):

$$\ln(k) = a(T) + b \tag{1}$$

where a and b are the equation parameters.

Activation energies (E_a, kJ/mol) and pre-exponential or frequency factors (A, h^{-1}) were determined from the slopes and intercepts, respectively, of the lines generated by plotting $\ln(k)$ *vs.* $1/T$ using the Arrhenius equation:

$$\ln(k) = \ln(A) - (E_a/RT) \tag{2}$$

where k is the reaction rate constant or reciprocal OSI (h^{-1}), and R is the molar gas constant (8.3143 J/mol K). Also, a temperature acceleration factor, based on the increase in oxidation rate per 10 °C increase in temperature, known as Q_{10} number, was calculated from the slopes of the lines.

Enthalpies (ΔH) and entropies (ΔS) of activation were determined by plotting $\ln(k/T)$ *vs.* $1/T$ *via* the equation derived from the activated complex theory:

$$\ln(k/T) = \ln(k_{rmB}/h) + (\Delta S/R) - (\Delta H/RT) \quad (3)$$

Where k_B is the Boltzmann constant ($1.3806586 \times 10^{-23}$ J/K) and h is the Planck's constant ($6.62607556 \times 10^{-34}$ J s). From the slopes and intercepts of the lines, ΔH and ΔS were calculated.

2.4 Statistical analysis

Values reported in tables and figures are the means ± SE of two to three replications. The significance level was set at $P = 0.05$. Separation of means was performed by Tukey's test at the 0.05 significance level.

3 Results and discussion

3.1 Initial quality of olive oils

3.1.1 Quality indices

Olive oil quality can be classified into different categories by use of chemical, physical and sensory parameters according to the definitions and standards defined by the Commission Regulation (EEC) No. 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis (Commission Regulation, 1990). The European Commission has defined the quality of olive oil based on certain parameters and indicators, mainly the degree of acidity, peroxide value, values of specific extinction in the UV absorbance at 232 nm and 270 nm (E_{232} and E_{270}). The acidity of oil is evaluated from the amount of free fatty acids, expressed as grams of oleic acid per 100 g of oil. It is a simple and effective method to assess and classify the grade of olive oil (Commission Regulation, 1990). Generally, if the oil is extracted from fresh and sound fruits by best practices crushing, oil has a very low acidity (Gharby *et al.,* 2013; Gutierrez *et al.,* 2000). However, during storage, the oil may deteriorate and its acidity increases due to the release of fatty acids by hydrolysis of triacylglycerols.

The highest initial acidity was for Arbequina sample, it reached 0.8/100 g. Moroccan picholine olive oil presented the second highest initial acidity (0.6/100 g). Koroneiki and picual olive oils presented a low initial acidity (0.4/100 and 0.2/100 g, respectively). However, the high level of free fatty acids in Arbequina olive oil (0.8/100 g,) could be the result of hydrolysis of triacylglycerols during oil extraction (Manai *et al.,* 2008; Rigane *et al.,* 2013).

The second criterion for the quality of olive oil is the peroxide value (PV). This index is used to evaluate the oxidation state of oil during storage and must not exceed 20 meq O_2/kg for all categories of olive oil (Commission Regulation. 1991). The PV of the analysed olive oils (Tab. 1) is between 1.06 and 3.2 meq O_2/kg being lower than the maximum values indicated by the regulations (Commission Regulation, 1991).

Measurements of absorbance at specific wavelengths (K_{232} and K_{270}) in the UV region are used to provide information on the oxidative state (K_{232}) and a forbidden bleaching (K_{270}) of olive oil (Hadorn and Zurcher, 1966). The absorbance E_{232} showed low values for all oils ranging from 1.4 to 2.1 without exceeding the limit (2.5) defined by the European regulations (Commission Regulation, 1991). The absorption at 270 nm which provides information on the performance of a bleaching step showed for all virgin olive oil samples values below the limit of 0.22 given by the European regulations (Commission Regulation, 1991).

These results show that the type cultivar had no significant influence on these analytical quality parameters. These results are in agreement with data reported in the literature (Ben Temime *et al.,* 2006).

3.1.2 Fatty acid composition

Table 1 shows the results of the main fatty acids of the four olive oil cultivars. Major fatty acid components present in all virgin olive oil samples were oleic acid (C18:1), linoleic acid (C18:2) and palmitic acid (C16:0). A low amount was found for palmitoleic acid (C16:1), stearic acid (C18:0), and linolenic acid (18:3). The fatty acid composition of the four oils was found to be in agreement with the European Regulations. On the other side significant differences were observed between the different cultivars.

Palmitic acid is the major saturated fatty acid in olive oil (Djebali *et al.,* 2012) and its content was between 9.2% (Moroccan Picholine) and 14.3% (Arebiquina) according to cultivars with an mean value of 12.6%. For oleic acid, the main mono-unsaturated fatty acid of olive oil (Djebali *et al.,* 2012), the highest values (76.3% and 76.5%) were found in varieties Picual and Koroneiki, respectively while varieties Arebiquina (60.43%) and Moroccan Picholine (64.33%) showed significant lower amounts. Concerning linoleic acid, which is much more susceptible to oxidation than monounsaturated fatty acids (Manai *et al.,* 2008), the highest percentage was observed in variety Arebiquina (13.2%), whereas the lowest amount was found in variety Picual (5.4%). The other samples showed percentages at 6.4% and 10.7% in varieties Koroneiki and Moroccan Picholine, respectively (Tab. 1). Linolenic acid belongs to the minor fatty acids of olive oil and according to the European Regulations (Commission Regulation, 1991) the concentration must be less than 1%. The investigated oils were in agreement with European Regulations with values of linolenic acid between 0.7% and 0.9%. Also the amount of the other minor fatty acids, palmitoleic acid and stearic acid varied in the different oils. For almost all oils the oleic acid to linoleic acid ratio was superior to the minimum value of 7 (Kiritsakis and Markakis, 1987), only variety Arebiquina should a ratio of 5.08 (Tab. 1). This ratio can be useful for the characterization of olive cultivars and for the interpretation of stability effects (Aparicio *et al.,* 1999). Additionally the ratio between unsaturated and saturated fatty acids was found between 4.90 for olive oil from variety Arebequina oil and 7.00 for olive oil from Picholine.

Table 1. Physicochemical parameters of extra virgin olive obtained from four varieties: Moroccan Picholine, Picual, Koroneiki and Arebiquina oils.

	European Regulations (1991) for olive oil extra virgin	Moroccan Picholine	Picual	Koroneiki	Arebiquina
Acidity (%)	<0.8	0.6 ± 0.02^{c}	0.2 ± 0.05^{a}	0.4 ± 0.1^{b}	0.8 ± 0.1^{d}
PV (Meq O2/kg)	<20	3.2 ± 0.5^{c}	1.4 ± 0.5^{ab}	1.06 ± 0.50^{a}	2.1 ± 0.5^{bc}
E_{232}	<2.5	2.1 ± 0.01^{c}	1.70 ± 0.01^{b}	1.4 ± 0.01^{a}	1.7 ± 0.01^{b}
E_{270}	<0.22	0.1 ± 0.01^{a}	0.1 ± 0.01^{a}	0.1 ± 0.01^{a}	0.1 ± 0.01^{a}
Palmitic acid C16:0	7.5–20	9.2 ± 0.1^{a}	12.7 ± 1.5^{b}	12.5 ± 1.5^{b}	14.3 ± 0.1^{b}
Stearic acid C18:0	0.5–5	2.9 ± 0.1^{b}	2.9 ± 0.5^{b}	2.5 ± 0.1^{a}	2 ± 0.1^{a}
Oleic acid C18:1	55–83	74.6 ± 0.1^{b}	76.3 ± 2.5^{b}	76.5 ± 1.5^{b}	67.1 ± 0.1^{a}
Linoleic acid C18:2	3.5–21	10.7 ± 0.1^{b}	5.4 ± 1.5^{a}	6.4 ± 0.1^{a}	13.2 ± 0.1^{c}
Linolenic acid C18:3	<1	0.9 ± 0.1^{a}	0.7 ± 0.1^{a}	0.7 ± 0.1^{a}	0.8 ± 0.1^{a}
SFA[a] (mg/100 mg)		12.4 ± 0.1^{a}	15.6 ± 0.1^{c}	15 ± 0.1^{b}	16.6 ± 0.1^{d}
MUFA[a] (mg/100 mg)		75.0 ± 0.1^{b}	76.3 ± 2.5^{b}	76.5 ± 1.5^{b}	67.1 ± 0.1^{a}
PUFA[a] (mg/100 mg)		11.8 ± 0.1^{b}	6.1 ± 0.1^{a}	7.1 ± 0.1^{a}	14.3 ± 0.1^{c}
UFA[a]/ SFA[a]		7.0^{a}	5.28^{a}	5.57^{a}	4.9^{a}
oleic acid/linoleic acid		6.97	14.13	11.95	5.08
Campesterol	<4	2.7 ± 0.2^{a}	3.1 ± 0.5^{ab}	3.2 ± 0.2^{b}	3.1 ± 0.3^{ab}
Stigmasterol	<Campesterol	1.7 ± 0.1^{a}	2.1 ± 0.1^{a}	1.8 ± 0.2^{a}	1.9 ± 0.2^{a}
Beta-sterol (other sterols)	>93	93.8 ± 0.5^{a}	94.2 ± 7^{ab}	94.7 ± 1.1^{ab}	94.8 ± 0.5^{b}
7 Stigmastenol	<0.5	0.2 ± 0.1^{a}	0.4 ± 0.1^{c}	0.3 ± 0.1^{b}	0.3 ± 0.1^{b}
7 Avenasterol	–	0.1 ± 0.1^{a}	0.3 ± 0.1^{a}	0.5 ± 0.1^{a}	
Tocopherol (mg/kg)	–	202 ± 21^{a}	205 ± 33^{a}	360.5 ± 25^{b}	182 ± 30^{a}
α-Tocopherol (mg/kg)	–	166.3 ± 5^{a}	164 ± 15^{a}	324 ± 25^{b}	167 ± 5^{a}
Polyphenol (mg/kg)	–	275 ± 20^{b}	295 ± 25^{b}	320 ± 30^{c}	136 ± 25^{a}

* Values are means of three replicates ± standard deviation. Values in the same row with different superscripts are significantly different ($p \leqslant 0.05$). UFA: Unsaturated fatty acid; SFA: Saturated fatty acid, MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid.

3.1.3 Sterol composition

Sterols are important minor constituents in vegetable oils and they are widely used to verify the authenticity (Al-Ismail *et al.,* 2010; Rigane *et al.,* 2013). Besides, their determination is of major interest due to their health benefits, as discussed before (Koutsaftakis *et al.,* 1999). Table 1 shows the sterol composition obtained for the different olive oils. The sterol composition shown in this study is in contrast to the literature (Aparicio *et al.,* 2002), which stated that the cultivar of the olive tree influences the proportion of sterols. In the present work no significant differences in the composition of sterols between the different cultivars was found. According to our results, the olive oils studied are characterized by a high content of β-sitosterol, comprising more than 93% of the total sterols in the four varieties (Tab. 1). This is in agreement with other results already reported in the literature (Al-Ismail *et al.,* 2010; Allalout *et al.,* 2009; Aparicio *et al.,* 2002; Djebali *et al.,* 2012; Gharby *et al.,* 2012; Rigane *et al.,* 2013).

3.1.4 Tocopherols composition

Tocopherols are important molecules due to their role as vitamins in nutrition or their ability to intercept free radicals (Aparicio *et al.,* 2002, Canâbate-Dıaz *et al.,* 2007). α-tocopherol is the major vitamin-E-active compound in the olive oil (Gharby *et al.,* 2012). As shown in Table 1, significant differences between the cultivars were found for the total tocopherols content and the content of α-tocopherol. The highest amount of total tocopherols was observed in the variety Koroneiki (360 mg/kg); whereas, the lowest amount was recorded in variety Arebiquina cultivar (182 mg/kg), with amounts for varieties Moroccan Picholine and Picual of 166.3 mg/kg and 164 mg/kg respectively The amount α-tocopherol in virgin olive oil depends on several factors such as variety, fruit ripeness, and agro-climatic conditions. Among these factors, variety is the most important reason for variation (Krichene *et al.,* 2007).

3.1.5 Total phenol content

Phenolic compounds contribute to the nutritional importance and benefit to human health of virgin olive oil and they are responsible for bitter taste and the antioxidant activity of the oil (Garc *et al.,* 2003; Kharazi *et al.,* 2012). Therefore the content of phenolic compounds is an important parameter which determines the characteristics and quality of olive oil (Garcia *et al.,* 2002; Kowalski *et al.,* 2004). The total amounts of phenolic compounds in olive oil depend on various factors such as cultivar, climate and irrigation, altitude and technological conditions during extraction (Garc *et al.* 2003; Garcia *et al.,* 2002). The amounts of total phenols in the analyzed oils show significant differences between different varieties (Tab. 1). The highest content of these components was detected in oil from variety Koroneiki (320.5 mg/kg), whereas the lowest amount was recorded for oil from variety Arbequina (136 mg/kg).

Table 2. Rancimat measurements of extra virgin olive oils obtained from four varieties.

	373 K	383 K	393 K	403 K	413 K	423 K
Arebiquina	25.1 ± 1	11.9 ± 1.5	5.9 ± 1	3.1 ± 1	1.4 ± 0.5	0.6 ± 0.5
Moroccan Picholine	44.1 ± 2	18.4 ± 1.5	9.2 ± 1.5	4.4 ± 1	1.8 ± 0.5	0.9 ± 0.5
Koroneiki	59 ± 2.5	31.5 ± 2.5	16.6 ± 1.5	9.1 ± 1.5	4.4 ± 0.5	1.8 ± 1.5
Picual	49.6 ± 1.5	26.1 ± 1	12 ± 1.5	5.7 ± 1	2.3 ± 0.5	1.2 ± 0.5

Table 3. The reaction rate constants (k) of the olive oils at different temperatures.

	$k \pm SD\ (6 \times 10^3)\ (h^{-1})$					
	373 K	383 K	393 K	403 K	413 K	423 K
Arebiquina	39.8 ± 0.3	84.03 ± 0.6	169.5 ± 1.4	322.5 ± 3.2	714.3 ± 3.8	1666.6 ± 5.1
Moroccan Picholine	22.6 ± 0.2	54.3 ± 0.5	108.7 ± 1.5	227.3 ± 3.1	555.5 ± 4.5	1111.1 ± 5.6
Koroneiki	16.94 ± 0.2	31.7 ± 0.5	60.2 ± 0.8	109.9 ± 2.5	227.3 ± 3.5	555.5 ± 4.5
Picual	20.2 ± 0.2	38.3 ± 0.6	83.3 ± 1.2	175.4 ± 2.5	434.8 ± 3.6	833.3 ± 5

3.2 Kinetic analysis of the Rancimat data

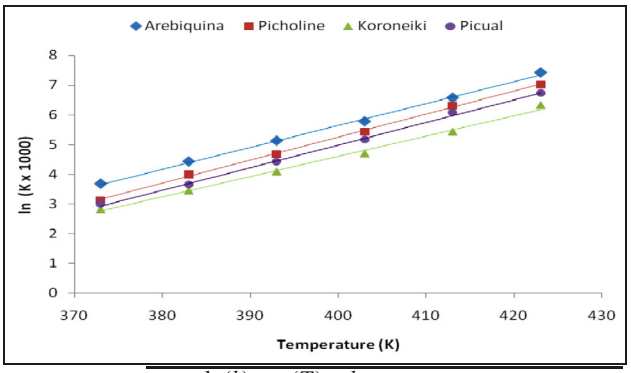
The oils studied displayed relatively high stabilities at 373 K (Tab. 2) in the range between 25.1 h (Arebiquina) and 59.0 h (Koroneiki) with longer induction times for the more stable oils. Heating of the oils under Rancimat conditions shows that the induction times as measures for the thermal-oxidative decomposition of the oils strongly depend on the temperature. The induction times for the oils from the four varieties can be ranked as Koroneiki > Picual > Moroccan Picholine > Arebquina in the temperature range from 373 K to 413 K while at 423 K no significant difference between the different oils was found. There are several reports that the ranking of oil and fat resistance to thermal-oxidative decomposition strongly depends on temperature (Kowalski *et al.,* 2004; Litwinienko, 2005).

The k values for lipid oxidation of each olive oil at each temperature are presented in Table 3. By studying the rates of lipid oxidation as a function of temperature, an increasing rate of oxidation can be observed as temperature increases. As revealed in Figure 1, the semi-logarithmic relationship between k and T values in all virgin olive oils showed a linear dependency with good correlation of determination ($R^2 > 0.99$) and they can be described by the following equation:

$$\ln(k) = a(T) + b$$

where a and b are adjustable coefficients and T is the temperature in K.

The lipid oxidation at low and high temperatures may go through different steps or reaction pathways, depending on the reactivity of prooxidants such as metal ions and antioxidants at different temperatures (Tan *et al.,* 2001). Furthermore, the oil temperature affects the degree of oxygen solubility in vegetable oils which decreases by almost 25% for each 10 K rise in temperature (Robertson, 2000). The values for the temperature coefficient calculated from the linear functions in Figure 1 for the olive oils ranged from 6.8×10^{-2} to 7.7×10^{-2} K^{-1}. The lowest value was calculated for variety Koroneiki, while the highest value of T_{Coeff} (7.3×10^{-2} K^{-1}) was found for variety Arbequina. For varieties Picholine and Picual Figure 1 shows the same values (7.7×10^{-2} K^{-1}). Similar results were described by Farhoosh *et al.* (2008) for deodorized olive oil (6.65×10^{-2} K^{-1}) and for other oils (canola oil, soybean oil, sunflower oil and corn oil) they found values between 6.5×10^{-2} and 7.4×10^{-2} K^{-1}. In a recent study on argan oil, Zaanoun *et al.* (2014) found comparable values for roasted (7.2×10^{-2} K^{-1}) and for unroasted argan oil (7.5×10^{-2} K^{-1}).



	$\ln(k) = a(T) + b$			$T_{coef} \times 10^{-2}$ (K^{-1})
	a	b	R^2	
Arebiquina	0.073	−23.75	0.998	7.3
Picholine	0.077	−25.8	0.998	7.7
Picual	0.076	−25.45	0.998	7.7
Koroneiki	0.068	−22.76	0.994	6.8

Fig. 1. Semi-logarithmic relationship between k and T values for lipid oxidation of the four olive oils.

Table 3 provides the regression parameters for the Arrhenius relationships between the reaction rate constant and the temperature for the four virgin olive oils studied ($\ln(k) = \ln(A) - (E_a/R) \times (1/T)$). Using these regression parameters, frequency factors (A, h^{-1}), activation energies (E_a, kJ/mol), and Q_{10} numbers for the formation reaction of the secondary oxidation products under the Rancimat test conditions (volatile acids, mostly formic acids, with lower amounts of acetic acid, propionic acid, and other acids) were calculated (DeMan *et al.,* 1987). These values for the lipid oxidation of the olive oils under the Rancimat test conditions differed significantly. This implies that the formation of volatile acids under these conditions was dependent on the oil source which affects assessment of the relative stability of the olive oils (Mendez *et al.,* 1996).

The results obtained showed that a lower degree of polyunsaturation and the high content of polyphenols and tocopherols would improve the resistance to the lipid oxidation

Table 4. Regression parameters for Arrhenius relationships between the reaction rate constant and the temperature for the four olive oils.

	$\ln(k) = \ln(A) - E_a/R) \times (1/T)$					
	a	b	R^2	E_a	A	Q_{10}
Arebiquina	−11.58	34.65	0.994	−96.279594	1.11764E+15	2.1
Picholine	−12.24	35.91	0.997	−101.767032	3.94017E+15	2.2
Koroneiki	−10.76	31.57	0.988	−89.461868	5.13661E+13	2.0
Picual	−11.99	35.02	0.995	−99.688457	1.61805E+15	2.1

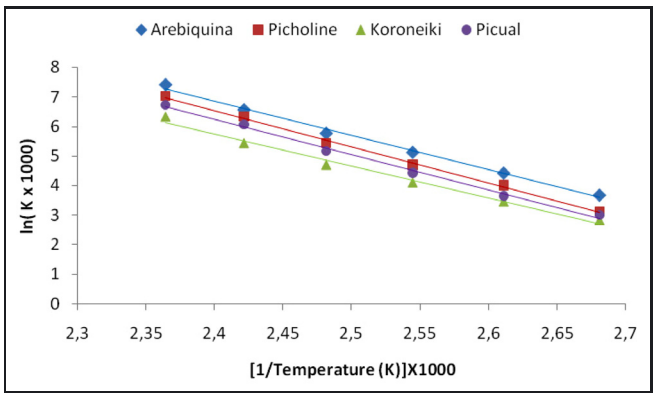


Fig. 2. Semi-logarithmic relationship between k and $1/T$ values for lipid oxidation of the four olive oils.

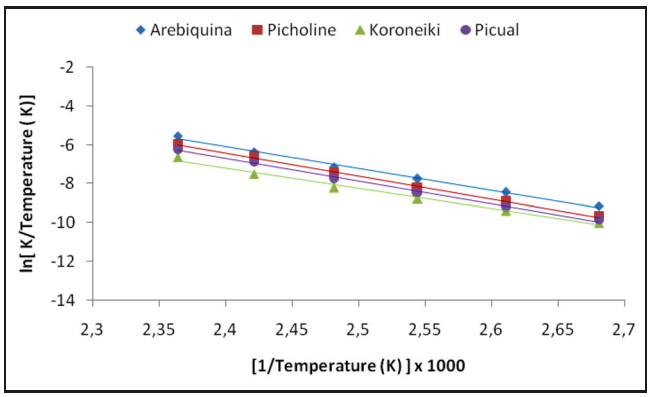


Fig. 3. Semi-logarithmic relationship between (k/T) and $(1/T)$ values for lipid oxidation of the four olive oils.

(raise the E_a value). Adhvaryu *et al.* (2000) showed that a high PUFA (linoleic acid and linolenic acid content) would lower the activation energies (E_a) value for lipid oxidation but a high oleic acid content would increase it. These would result in delaying the beginning of the initial oxidation process where bond scission takes place to form primary oxidation products. The polyphenol and tocopherols contents of the olive oils (Tab. 1) explain the observed trends in various activation energies (Tab. 4) to a certain extent. The olive oil from variety Koroneiki with the lowest content of polyunsaturated fatty acids, the highest ratio of oleic acid to linoleic acid and the highest content of antioxidants (tocopherols and polyphenols) showed the highest activation energy. However, it was observed that several other factors affecting the oxidative stability. Olive oil from variety Arebiquina had the lowest content of tocopherols and polyphenols and also the highest value of linoleic aicd, but the activation energy was the second-highest value. One reason could be the higher content of palmitic acid in comparison to the other oils. The frequency factors with a trend comparable to that of the activation energy values for the olive oils studied increased from 1.11×10^{15} for Arebiquina to 5.13×10^{13} for Koroneiki.

The magnitude of the temperature effect on the oxidation rate of the olive oils is evidenced by the Q_{10} numbers. In general, a higher Q_{10} number implies that a smaller temperature change is needed to induce a certain change in the rate of lipid oxidation. As can be seen in Table 4, the Q_{10} number increased from 2.0 for Koroneiki to 2.2 for Picholine, thus for olive oil from variety Koroneiki a higher temperature than for the other varieties is necessary to result in changes in the rate of lipid oxidation.

The ΔH and ΔS values estimated based on the activated complex theory (Labuza, 1980) and the corresponding regression parameters are summarized in Table 5. The high correlation of determination ($R^2 > 0.98$) indicated adequate fit and characterization of the temperature dependence of lipid oxidation when using the activated complex theory.

The ΔH values for the olive oils studied ranged from 86.21 kJ/mol for oil from variety Koroneiki to 98.44 kJ/mol for oil from variety Picholine. The ΔS values ranged from −50.55 J/mol K for oil from variety Koroneiki to −14.54 J/mol K for oil from variety Picholine. In their study on the determination of the oxidative stability of rapeseed, sunflower and soybean oils by the Rancimat test, Kowalski *et al.* (2004) calculated the ΔH- and ΔS-values as 82 kJ/mol and −52.7 J/mol K, 84 kJ/mol and −42.8 J/mol K, and 74.9 kJ/mol and −70.2 J/mol K, respectively. The negative values for ΔS indicate that the activated complexes are more ordered than the molecules of the reactants, as can be seen in Table 5. The greater negative ΔS value for oil from variety Koroneiki indicates fewer numbers of species in the activated complex state. Hence, the activated complex for lipid oxidation in oil from variety Koroneiki is less probable and therefore the rate is slower. So it can be stated that the oil from variety Koroneiki is the more stable oil.

Tan *et al.* (2001) evaluated the oxidative stability of vegetable oils by Differential Scanning Calorimetry and showed that the ΔH and ΔS values were greater for highly unsaturated oils than for oils with lower amounts of unsaturated fatty acids, which corroborate with our study. Olive oil from variety Koroneiki with the lowest ratio of unsaturated to saturated fatty acids showed the lowest values for ΔH and ΔS. Also for olive oil from varieties Arebiquina and Picual ΔH and ΔS values showed a good agreement with the ratio saturated to unsaturated fatty acids while for oil from variety Picholine the values were low although the ratio was high (Tab. 1).

Table 5. Activation enthalpies ΔH and entropies ΔS for lipid oxidation of the four olive oils.

	$\ln(k/T) = \ln(kB/h) + (\Delta S/R) - \Delta H/R) \times (1/T)$				
	a	b	R^2	ΔH (kJ/mol)	ΔS (J/mol K)
Arebiquina	−11.18	20.76	0.994	92.953874	−24.942763
Picholine	−11.84	22.01	0.997	98.441312	−14.549888
Picual	−11.59	21.13	0.994	96.362737	−21.866472
Koroneiki	−10.37	17.68	0.988	86.219291	−50.550807

4 Conclusion

The investigation showed that olive oils from different varieties where different regarding their chemical parameters such as acidity, peroxide value, fatty acid, tocopherol and phytosterol content and composition and physical parameters such as E_{232} and E_{270}. The measurement of the thermal stability as induction time by Rancimat at different temperatures revealed a strong dependency from the temperature, but also a strong influence of the variety was given. The Rancimat method was an accurate and effective method to investigate the kinetic data of lipid oxidation in olive oils at elevated temperature. The lowest value for the temperature coefficient was calculated for variety Koroneiki, while the highest value was found for variety Arbequina which may be influenced by the low content of linoleic acid prone to oxidation and the high content of antioxidant active compounds of oil from variety Koroneiki in comparison to olive oil from variety Arbequina. The oil from variety Koroneiki was the most stable oil in this investigation in comparison to oils from other varieties. The results of the kinetic calculations were also in good agreement with the fatty acid composition and the content of antioxidant active compounds. Thus, the calculation of different kinetic parameters such as activation energy or enthalpies (ΔH) and entropies (ΔS) could be important factors to access the thermal stability of olive oils.

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