International Journal of Food Properties, 19:257–271, 2016

ISSN: 1094-2912 print/1532-2386 online
DOI: 10.1080/10942912.2015.1020438

Taylor & Francis
Taylor & Francis Group

Antioxidant Activity, Reaction Mechanisms, and Kinetics of *Matricaria recutita* Extract in Commercial Blended Oil Oxidation

Seyed Mohammad Bagher Hashemi[1], Mary Susan Brewer[2], Javad Safari[3], Masoud Nowroozi[4], Moosa Hamid Abadi Sherahi[1], Behrooz Sadeghi[1], and Moslem Ghafoori[1]

[1]*Food Science and Technology Department, College of Agriculture, Fasa University, Fasa, Iran*
[2]*Department of Food Science and Human Nutrition, University of Illinois, Urbana, Illinois, USA*
[3]*Food Science and Technology Department, College of Agriculture, Shiraz University, Shiraz, Iran*
[4]*Head of Nargess Oil Company, Shiraz, Iran*

Antioxidant activity, reaction mechanisms, and kinetics of *Matricaria recutita* crude extract (CE; total phenolics: 41 ± 2.5 mg/g, total flavonoids: 26 ± 1.4 mg/g, IC_{50}: 82.3 ± 2.8 µg/mL and reducing power: 10.45 ± 0.56 mmol Fe^{2+}/mass) in comparison to *tert*-Butylhydroquinone during oxidation of blended vegetable oil (sunflower, soybean, and palm oil) at 120, 130, and 140°C were studied. Good correlations existed between the Rancimat oil stability index and stability indices (induction period) calculated from peroxide value, conjugated diene value, and anisidine value with no significant differences in kinetic parameters calculated from them. The temperature acceleration (Q_{10}), activation energy (E_a), frequency factor (A), enthalpy (ΔH^{++}), entropy (ΔS^{++}), and free energy of activation (ΔG^{++}) for oils containing crude extract were lower than for oils containing *tert*-Butylhydroquinone (0.0025, 0.005, 0.01, and 0.02%). Values were independent of crude extract or *tert*-Butylhydroquinone concentration. For crude extract and *tert*-Butylhydroquinone, E_a and A were well correlated with ΔH^{++} and ΔS^{++} values, respectively, but correlation between E_a and Q_{10} for crude extract compared to *tert*-Butylhydroquinone was poor. Furthermore, the rate of Monounsaturated:Polyunsaturated fatty acids formation did not differ significantly between crude extract and *tert*-Butylhydroquinone, but concentrations of them did affect Monounsaturated:Polyunsaturated ratio. Based on the results obtained, crude extract decreased the rate of the oxidation reaction due to the decrease in the concentration of the activated complex and reduction in the rate at which the activated complex dissociated into oxidation products.

Keywords: Antioxidant activity, Blended oil, Kinetic parameters, *Matricaria recutita* extract, *tert*-Butylhydroquinone.

Received 3 December 2014; accepted 14 February 2015.
Address correspondence to Seyed Mohammad Bagher Hashemi, Food Science and Technology Department, College of Agriculture, Fasa University, Fasa, Iran. E-mail: hasshemii@yahoo.com
Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/ljfp.

INTRODUCTION

In order to prolong the storage life of vegetable oils, various synthetic antioxidants such as *tert*-Butylhydroquinone (TBHQ), butylated hydroxytoluene (BHT), and butylated hydroxyanisole (BHA) are currently used, but these substances have toxic and carcinogenic effects on the human health.[1] Therefore, the development of natural antioxidants has attracted considerable attention and is thought to be a preferable option. Crude extracts (CEs) of herbs and spices rich in phenolics are of increasing interest in the food industry because they retard oxidative degradation of lipids and improving the quality and nutritional value of food.[2,3]

Matricaria recutita, a member of Asteraceae family, is an endemic plant that is greatly distributed in Iran. Babooneh Shirazi is the Persian name of this plant which is used in traditional and herbal medicine (especially the dried flower heads of the plant) for its anti-inflammatory, antipeptic, sedative, antibacterial, and antifungal properties.[4,5] A good understanding of the kinetics of Babooneh Shirazi extract as an antioxidant in vegetable oils would improve our ability to formulate food products that maintain their existing oil quality and minimize the appearance of undesirable breakdown products. Kinetic data are necessary for predicting oxidative stability of vegetable oils under varying heat processing, storage, and distribution conditions.[6] Therefore, the aim of this research was to study antioxidant activity, reaction mechanisms, and kinetics of Babooneh Shirazi extract as an antioxidant using the Arrhenius equation in commercial blended vegetable oil.

MATERIAL AND METHODS

Materials

The dried flower heads of *M. recutita* were obtained from a local market. Fully refined and deodorized blended vegetable oil (sunflower oil [50%], soybean oil [40%], and palm oil [20%]) with no additives was purchased from Nargess Oil Company, Shiraz, Iran (Table 1). All chemical

TABLE 1
Initial characteristics of blended vegetable oil used in this study

Characteristic	
Fatty acids (mg/100 g; relative %)	
C12	0.16
C14	0.52
C16	28.33
C18	6.92
C18:1	28.52
C18:2	31.3
C18:3	4.21
Peroxide value (meq/kg oil)	1.6
Conjugated diene value (mmol/L)	1.8
Anisidine value	2.1
Acid value (mg KOH per g oil)	0.3
Refractive index	1.46240
Specific gravity	0.9055

All values are means of three determinations.

reagents were obtained from Merck (Darmstadt, Germany) and Sigma Chemical Company (Sigma-Aldrich GmbH, Sternheim, Germany).

Preparation of *M. recutita* Methanolic Extract

Babooneh Shirazi was ground into a fine powder in a mill (Mulinex Depose-Brevete S.G.C.G., France). Extraction of the powders was carried with methanol (1:20 w/v) by agitation in the dark at room temperature for 48 h. The extracted material was filtered and concentrated using rotary evaporator (Hahn Shin, Korea) at 45°C. The crude extract (CE) was then stored at –18°C until further use.

Total Phenolic Compounds

Total phenolic content of CE were determined using the FolinCiocalteu method.[7] Appropriately diluted sample (400 μL) was placed into a test tube. Diluted Folin-Ciocalteu's reagent (2000 μL) was added and mixed with vortex for 3 min. Sodium carbonate solution (1600 μL) was added and incubated in the dark at ambient temperature for 30 min. For preparation of the blank, distilled water (400 μL) was used instead of the sample. Absorbance of the samples was determined spectrophotometrically against the blank at 765 nm (UV/Visible Philips Cambridge, UK).

Total Flavonoids

Total flavonoids were determined using a colorimetric assay according to the methods described by Kim et al.[8] One milliliter of CE or a standard solution of quercetin (0–500 mg/L) was added to 4 mL of H_2O. At zero time, 0.3 mL of 50 g/L $NaNO_2$ was added. After 5 min, 0.3 mL of $AlCl_3$ (100 g/L) was added. After 6 min, 2 mL of 1 mol/L NaOH was added then the mixture was diluted with 2.4 mL of H_2O. Absorbance of the mixture was read at 510 nm against a water blank. Total flavonoids were expressed as milligrams of quercetin equivalent (mg/g).

2,2-diphenyl-1-picrylhydrazyl Assay

The antioxidant activity of CE was evaluated by monitoring their ability to quench the stable free radical DPPH using the method described by Choi et al.[9] with brief modifications. Various ethanol dilutions of CE (15, 45, 65, 85, 105, 125, 155, and 185 [μg/mL]) were mixed with 1.0 mL of a 0.3 mM DPPH ethanol solution. Ethanol (1.0 mL) plus CE solution was used as a blank. Absorbance was determined at 517 nm after 30 min of reaction time at room temperature. The CE concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against CE concentration. BHT was used as a control and all tests were carried out in triplicate.

Ferric Reducing-Antioxidant Power (FRAP) Assay

The FRAP was determined using the method of Benzie and Strain.[10] Briefly, 900 mL FRAP reagent was mixed with 90 mL distilled water then warmed to 37°C in a water bath. The control reading of the reagent was determined at 595 nm. Subsequently, 30 mL of sample solution (100 mg in 10 mL of n-hexane) was added and absorbance was determined at 595 nm against the control solution. A standard curve was prepared using various concentrations (200–2000 mmol/L) of $FeSO_4.7H_2O$. The results are expressed in mmol Fe^{2+} per unit mass.

Preparation of Oil Samples

CE (0.025, 0.05, 0.1, and 0.2%) and TBHQ (0.0025, 0.005, 0.01, and 0.02%) were added separately to blended vegetable oil in open glass bottles (25 mL). Samples were then held in a laboratory oven at 120, 130, and 140°C with air circulation in the dark. At each sampling time, samples were removed from storage, at random, and subjected to chemical analysis.

Determination of Fatty Acid by Gas Chromatography–Flame Ionization Detector

Fatty acid composition of oil samples was determined by gas chromatography (GC) and was reported in relative area percentages. Fatty acids were transesterified into their corresponding fatty acid methyl esters (FAMEs) by vigorous shaking of oil in hexane (0.3 g in 7 mL) with 2 mL of 7 M methanolic KOH at 50°C for 10 min. The FAMEs were determined using an HP-5890 chromatograph (Agilent, Palo Alto, CA, USA) equipped with a CP 88 (Varian 3400, USA) capillary column of fused silica (120 m in length × 0.25 mm in internal diameter, 0.25 μm film thickness) using a flame ionization detector (FID). Helium was used as the carrier gas at a flow rate of 0.8 mL/min. The oven temperature gradient was 5°C each 5 min from 160 to 200°C; temperatures of the injector and the detector were 210 and 300°C, respectively.[11] Heptadecanoic acid was added to the oils as an internal standard for quantification before esterification.

Rancimat Test

The OSI of each oil sample (3 g) was determined using a Rancimat instrument (Metrohm model 734, Switzerland) at temperatures of 120, 130, and 140°C and at an air flow rate of 15 L/h.[12]

Measurement of Peroxide Value (PV), Conjugated Diene Value (CDV), and Anisidine Value (AnV)

PV was measured by treating a of sample oil solution (5 ± 0.05 g) in 30 mL acetic acid-chloroform with 0.5 mL saturated potassium iodide solution then titrated with 0.1 N sodium thiosulphate. In order to determine AnV, the absorbance of a solution of sample oil (0.5 – 4 ± 0.001 g) in 25 mL isooctane, treated with 1 mL *p*-anisidine reagent was read at 350 nm using solvent with using *p*-anisidine reagent as a blank in the reference cuvette.[13] CDV was measured spectrophotometrically (UV/Visible Philips Cambridge, UK) at 234 nm. The oil samples were diluted with hexane (HPLC grade). An extinction coefficient of 29,000 mol/L was utilized to quantify the concentration of conjugated dienes formed during oxidation.[14]

Kinetic Data Analyses

Temperature coefficients (T_C,°C^{-1}) were calculated from the slopes of the following linear regression equation:

$$\log \text{OSI or IP} = aT + b \tag{1}$$

where, T is temperature (°C), and a and b are the slope and intercept of the equation, respectively. Also, the Q_{10} number, which indicates the increase in reaction rate due to a 10°C rise in temperature, was calculated using the equation derived from the Eq. (1):

$$Q_{10} = 10^{-10Tc} \tag{2}$$

The effect of temperature on the rates of oxidation was evaluated by means of the Arrhenius equation:

$$\log k = \log A - \left(\frac{Ea}{2.303RT}\right) \tag{3}$$

where, k (h^{-1})is the reaction rate constant, R is the molar gas constant (8.3143 J/mol K), T is the absolute temperature (K), E_a is the activation energy (kJ/mol), and A (h^{-1}) is the pre-exponential factor. Enthalpies (ΔH^{++}) and entropies (ΔS^{++}) of activation were determined by regressing log k/T versus the inverse of temperature (T, K) via the equation derived from the activated complex theory:

$$\log\left(\frac{k}{T}\right) = \log\left(\frac{kB}{h}\right) + (\Delta S^{++}/2.303R) - (\Delta H^{++}/2.303RT) \tag{4}$$

where, k_B is the Boltzmann constant (1.380658×10^{-23} J/K, the ratio between R and Avogadro's number, 6.022×10^{23} mol^{-1}) and h is Planck's constant ($6.6260755 \times 10^{-34}$Js). From the slopes and intercepts of the lines, the values of $\Delta H^{++}$and ΔS^{++}were calculated. Finally, for a reaction at a given temperature, the free energy of activation (ΔG^{++}) can be written in terms of $\Delta H^{++}$and $\Delta S^{++}$by the equation:[15,16]

$$\Delta G^{++} = \Delta H^{++} - T\Delta S^{++} \tag{5}$$

Statistical Analyses

The data were analyzed using one-way analysis of variance (ANOVA), and significant differences between groups were determined using the Duncan's multiple range test. All statistical analyses were performed using SPSS (SPSS 16.0 for Windows; SPSS Inc., Chicago, IL, USA). Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Extract Characterization

Growing interest in natural antioxidants as well as other beneficial compounds from bioresource materials has encouraged the study of additional herbs and spices. Therefore, phenolic compounds as molecules most closely associated with antioxidant properties of various plant extracts were part of our study. Our results showed that total phenolics content in the CE was 41 ± 2.5 mg/g. Phenolic compounds play a significant role in the food industry and in human health. They are considered to be primary or chain breaking antioxidants in free radical chain reactions, converting lipid radicals to more stable products, thus extending the shelf life of vegetable oils.[17] Results also showed total flavonoids content in the CE was 26 ± 1.4 mg/g. Flavonoids are plant secondary metabolites distributed in the plant kingdom. It has been demonstrated that flavonoid compounds in various aqueous extracts have very strong antioxidant and free radical scavenging activities.[18] Composition of herb extracts can differ according to climate, soil composition, plant organ, genetic modifications, age, vegetative cycle and storing conditions.[19]

Antioxidant Activity of CE Assessed by DPPH and FRAP Method

The hydrogen atom or electron donating ability of some of the corresponding pure compounds were determined using the bleaching of a purple-colored methanol solution of DPPH.[20] In the present study, the free radical scavenging activity of CE was determined to be 82.3 ± 2.8 µg/mL, whereas IC_{50} value of BHT was 18.9 ± 0.8µg/mL. In the DPPH assay, CE exhibited antioxidant activity which may be associated with phenolic and flavonoid contents.

FRAP test is a simple, reproducible and rapid procedure that measures the ability of antioxidative compounds to reduce Fe^{3+} to Fe^{2+}, as a measure of total antioxidant capacity.[21] In this study, reducing power of CE was 10.45 ± 0.56 mmol Fe^{2+}/mass indicating that the reducing power of CE of metal ions may play a key role in its antioxidant activity. The CE demonstrated chelating effects on Fe^{2+}, suggesting that it can sequester Fe ions or minimize the concentration of the metal in the Fenton reaction. Since ferrous ions are considered to be one of the most pro-oxidative molecules in food systems, higher chelating effects of the CE would be valuable.[22]

Antioxidant Activity, Reaction Mechanisms, and Kinetics of CE in Blended Vegetable Oil

The physicochemical characteristics of blended vegetable oil are shown in Table 1. Fatty acid composition is expressed as mg/100 g of oil and relative percentage of total fatty acids. Vegetable oil contained mainly linoleic (31.1%), followed by oleic (28.52%), palmitic (28.33%), stearic (6.92%), and linolenic (4.21%) acids, which, in total, constituted 99.08% of the fatty acids. The ratio of Monounsaturated:Polyunsaturated (MUFA:PUFA) as a measure of the tendency of vegetable oil to undergo autoxidation[23] was 0.808. The higher ratios indicate greater oxidative stability of vegetable oils. Oxidation level was low as shown by the initial PV, CDV, and AnV. PV and CDV measure hydroperoxide and conjugated diene products in oil, respectively. AnV measures the level of carbonyl compounds as secondary oxidation products.

The OSI and IP of the oil samples at 120–140°C are shown in Table 2. Evolution of the primary and secondary oxidation compounds during the oxidation of blended oil at 120–140°C provided kinetic curves consisting of two characteristic phases: A low slope linear stage known as the initiation phase, followed by a second linear stage but higher in slope value known as the propagation phase. The induction periods were calculated at the intersection point of the two extrapolated parts of the exponential oxidation curves. Although the IP was lower than the OSI measured by the Rancimat test, a good correlation between IP and OSI was observed. Lacoste and Lagardere[24] reported similar results for evolution of some quality parameters during oil oxidation using the Rancimat test. A good correlation was found between IP and the Rancimat test for olive oils at 100–130°C.[25] Difference between IP and OSI may be explained by the fact that the Rancimat test measures the tertiary oxidation products for which their tangible formation takes

TABLE 2
Monounsaturated:Polyunsaturated fatty acids (MUFA:PUFA) ratio and formation rate (a*) of it in blended oil samples at 120–140°C

Samples	*120°C*	*130°C*	*140°C*	*a*	R^2
TBHQ-25**	0.936^{bA}	1.088^{bB}	1.260^{bC}	0.015^{b}	0.998
TBHQ-50	0.911^{cA}	1.008^{cB}	1.118^{cC}	0.01^{c}	0.998
TBHQ-100	0.857^{dA}	0.935^{dB}	1.016^{dC}	0.007^{d}	0.999
TBHQ-200	0.856^{eA}	0.918^{eB}	0.985^{eC}	0.005^{e}	0.999
Extract-250	0.936^{bA}	1.087^{bB}	1.254^{bC}	0.015^{b}	0.999
Extract-500	0.911^{cA}	1.008^{cB}	1.112^{cC}	0.01^{c}	0.999
Extract-1000	0.858^{dA}	0.934^{dB}	1.000^{dC}	0.007^{d}	0.998
Extract-2000	0.856^{eA}	0.918^{eB}	0.973^{eC}	0.005^{e}	0.999
Control	1.003^{aA}	1.169^{aB}	1.343^{aC}	0.017^{a}	0.999

All values are means of three determinations with coefficient of variations (CV = SD/mean×100) <6%;

Means within a column with the same lowercase letters are not significantly different at $P<0.05$ and means within a row with the same uppercase letters are not significantly different at $P<0.05$;

*MUFA:PUFA = aT + b; a: slope (formation rate) and T: temperature (°C);

**Antioxidant concentration (ppm).

times longer than those required to form primary and secondary oxidation compounds during the kinetically step-by-step oxidation of lipid systems.[25] As can be seen in Table 2, OSI and IP in all oil samples decreased significantly as temperature increased. Stability of oil samples containing CE or TBHQ was significantly higher than the control. Moreover, oil stability increased as the concentration of CE or TBHQ increased.

The ratio of MUFA:PUFA varied from 0.856 to 1.343 during storage at 120, 130, and 140°C (Table 3). With an increase in storage temperature, this ratio increases slowly due to the conversion of PUFA to MUFA. The ratios of both linoleic and linolenic to palmitic acids, are applicable indicators of the level of deterioration. Talpur et al.[26] reported a reduction in both ratios during heating and frying of canola oil. The decrease in unsaturation may be ascribed to the obliteration of double bonds by oxidation and subsequent polymerization.[27] In this study, the obtained MUFA: PUFA ratio in control samples was greater than that in oil samples supplemented with CE or TBHQ. Although the type of antioxidant had no significant effect on MUFA:PUFA ratio, antioxidant concentration did significantly affected it (Fig. 1). Effect of CE or TBHQ concentrations on

TABLE 3
The oil stability index (OSI, h) and induction periods (IP, h) of the blended oil samples at 120–140°C

	OSI			*IP_{PV}*			*IP_{CDV}*			*IP_{AnV}*		
Samples	*120°C*	*130°C*	*140°C*	*120°C*	*130°C*	*140°C*	*120°C*	*130°C*	*140°C*	*120°C*	*130°C*	*140°C*
TBHQ-25*	3.64^{dA}	1.99^{dA}	1.08^{gA}	3.09^{dB}	1.71^{dB}	0.95^{gB}	3.13^{dB}	1.73^{dB}	0.96^{gB}	3.15^{dB}	1.76^{dB}	0.97^{gB}
TBHQ-50	3.92^{cA}	2.18^{cA}	1.2^{fA}	3.42^{cB}	1.91^{cB}	1.07^{fB}	3.46^{cB}	1.92^{cB}	1.09^{fB}	3.49^{cB}	1.94^{cB}	1.11^{fB}
TBHQ-100	5.13^{bA}	2.79^{bA}	1.56^{dA}	4.32^{bB}	2.43^{bB}	1.37^{dB}	4.38^{bB}	2.45^{bB}	1.39^{dB}	4.4^{bB}	2.45^{bB}	1.4^{dB}
TBHQ-200	6.35^{aA}	3.47^{aA}	1.87^{bA}	5.28^{aB}	2.98^{aB}	1.69^{bB}	5.34^{aB}	3.01^{aB}	1.7^{bB}	5.37^{aB}	3.03^{aB}	1.72^{bB}
Extract-250	3.67^{dA}	2.02^{dA}	1.28^{dA}	3.08^{dB}	1.73^{dB}	1.06^{dB}	3.1^{dB}	1.75^{dB}	1.09^{dB}	3.13^{dB}	1.77^{dB}	1.1^{dB}
Extract-500	3.98^{cA}	2.15^{cA}	1.37^{eA}	3.43^{cB}	1.92^{cB}	1.22^{eB}	3.46^{cB}	1.93^{cB}	1.25^{eB}	3.47^{cB}	1.93^{cB}	1.26^{eB}
Extract-1000	5.16^{bA}	2.77^{bA}	1.78^{cA}	4.31^{bB}	2.42^{bB}	1.56^{cB}	4.38^{bB}	2.45^{bB}	1.58^{cB}	4.4^{bB}	2.46^{bB}	1.6^{cB}
Extract-2000	6.4^{aA}	3.57^{aA}	2.31^{aA}	5.29^{aB}	3.00^{aB}	1.89^{aB}	5.36^{aB}	3.04^{aB}	1.92^{aB}	5.39^{aB}	3.06^{aB}	1.94^{aB}
Control	2.53^{eA}	1.05^{eA}	0.39^{hA}	1.81^{eB}	0.78^{eB}	0.30^{hB}	1.86^{eB}	0.79^{eB}	0.31^{hB}	1.87^{eB}	0.81^{eB}	0.32^{hB}

All values are means of three determinations with coefficient of variations (CV = SD/mean × 100) <6%; Means within a column with the same lowercase letters are not significantly different at $p < 0.05$. For the same temperatures, means within a row with the same uppercase letters are not significantly different at $p < 0.05$; PV: peroxide value (meq O2 per kg oil), CDV: conjugated diene value (mmol/L) and AnV: anisidine value; *Antioxidant concentration (ppm).

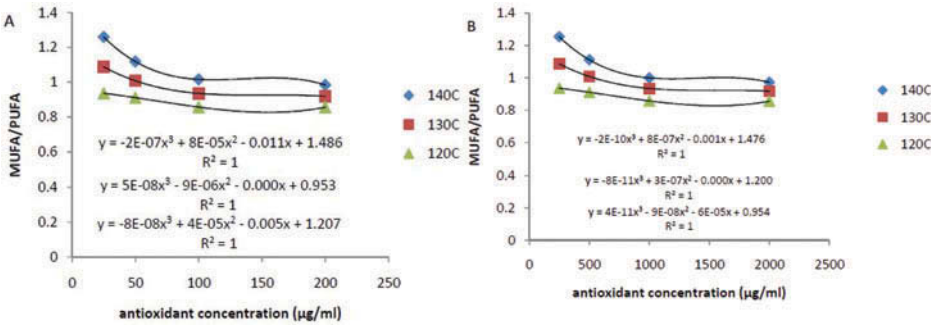

FIGURE 1 Relationship between Monounsaturated:Polyunsaturated fatty acids (MUFA:PUFA) ratio and antioxidant concentration for the oxidation of blended oil samples at 120–140 °C. TBHQ (A), Crude extract (B).

the MUFA:PUFA ratio is polynomial ($R^2 = 1$) and complicated. It indicates each concentration of antioxidant has a different rate of MUFA:PUFA formation and an increase in the concentration of antioxidant causes decrease in the rate of MUFA:PUFA formation.

The semi-logarithmic relationship between OSI or IP and temperature showed a linear dependency in all oil samples with a good correlation coefficient ($R^2 \geq 0.99$; Table 4). The T_C and Q_{10} numbers, which show the degree of temperature effect on the oxidation rate of lipid systems, are considered to be the quantities representative of vegetable oils.[28] The T_C calculated in terms of OSI, IP_{PV}, IP_{CDV}, and IP_{AnV} ranged from –0.0223 to –0.0433°C^{-1}, from –0.0233 to –0.0387°C^{-1}, from –0.0223 to –0.0383 and from –0.0227 to –0.0387°C^{-1}, respectively. These Q_{10} values ranged from 1.67 to 2.71, from 1.67 to 2.44, from 1.67 to 2.42, and from 1.67 to 2.44, respectively. Mancebo-Campos et al.[29] reported the Q_{10} factor of virgin olive oil obtained from PV was 2.1. The Q_{10} value of the control was significantly higher than those of samples containing CE or TBHQ. Therefore, the temperature dependence of lipid oxidation decreased among the blended oils in the presence of CE or TBHQ. The temperature dependence of lipid oxidation in the presence of CE was lower than that of TBHQ. In other words, CE was more active than TBHQ at high temperatures. Moreover, various concentrations of CE or TBHQ did not significantly affect temperature dependence of lipid oxidation. This indicates Q_{10} and T_C are independent of antioxidant concentration, but the type of antioxidant does affect them.

Employing E_a, which is the least amount of energy that must be overcome in order for a chemical reaction to occur, is another way to state the dependence of the rate of lipid oxidation on temperature.[30] The E_a of the oil sample oxidation varied significantly from 68.34 to 126.09 kJ/mol (Table 5). Vaidya and Eun[31] reported that, based on PV and CDV, activation energies of walnut oil oxidation were 75.87 and 83.84 (kJ/mol), respectively. Results showed that with few exceptions, the same Arrhenius equation parameters calculated from OSI and IP for the oxidation of each blended oil sample were statistically similar. The activation energy of control samples was significantly higher than for oil samples containing CE or TBHQ. That for samples with TBHQ was significantly higher than that for oils with CE. Moreover, it can be observed the activation energies of blended oil samples containing various concentrations of CE (or TBHQ) did not differ significantly. A higher activation energy implies that a smaller temperature change is required to induce a certain change in the rate of oxidation.[32] In other words, the chemical reactions with high activation energies are temperature sensitive and reactions with low activation energies are temperature insensitive. Therefore, the reaction temperature change has a significant effect on the chemical reaction rates in temperature sensitive reactions. Since the addition of CE or TBHQ decreased the activation energy of blended oil oxidation, addition of CE or TBHQ to blended oil decreased the temperature dependency of the oxidation reaction. Oil samples supplemented with CE have a lower temperature dependency with regard to oxidation compared to samples with TBHQ because they have lower activation energy. Oils containing 0.02% TBHQ is more susceptible to oxidative degradation ($E_{a\ OSI}$ = 82.47 kJ/mol) at higher temperature than oils containing 0.02% CE ($E_{a\ OSI}$ = 68.84 kJ/mol). Although oil samples containing TBHQ showed good correlation coefficient between activation energy and Q_{10}, blended oils containing CE demonstrated poor correlation between the two (Fig. 2) indicating that CE has a different mechanism and temperature dependency with respect to lipid oxidation compared to TBHQ and Q_{10} affects activation energy differently. According to the Arrhenius equation, the frequency factor is considered to be the other most important kinetic parameter affecting the rate of the reaction. This factor showed a pattern similar to that of the activation energy for the blended oil samples; however, minor changes in activation energy values resulted in substantial changes in frequency factors (Fig. 2). This indicates a higher contribution of the frequency factor than of activation energy in terms of the rate of lipid oxidation or the oxidative stability of the blended oils.[16,33] The frequency factor for oxidation of the blended oil samples varied significantly over a wide range (from 0.22×10^9 h^{-1} to 0.22×10^{17} h^{-1}). The frequency factor of samples containing TBHQ was significantly higher than that of samples containing CE. The loss of rotational freedom in the transition state due to presence of CE or TBHQ can lead to low frequency

TABLE 4
Temperature coefficient (T_C, °C^{-1}) and Q_{10} number calculated based on oil stability index (OSI, h) and induction periods (IP, h) of the blended oil samples at 120–140°C

	OSI			*IP_{PV}*			*IP_{CDV}*			*IP_{AnV}*		
Samples	T_C	Q_{10}	R^2	T_C	Q_{10}	R^2	T_C	Q_{10}	R^2	T_C	Q_{10}	R^2
TBHQ-25*	-0.0263^{bA}	1.83^{bA}	1	-0.0247^{bA}	1.77^{bA}	0.998	-0.0253^{bA}	1.79^{bA}	1	-0.0253^{bA}	1.79^{bA}	1
TBHQ-50	-0.0253^{bA}	1.79^{bA}	1	-0.0247^{b}	1.77^{bA}	1	-0.0253^{bA}	1.79^{bA}	0.999	-0.0243^{bA}	1.75^{bA}	0.999
TBHQ-100	-0.0253^{bA}	1.79^{bA}	0.999	-0.0243^{bcA}	1.75^{bcA}	1	-0.0243^{bA}	1.75^{bA}	0.999	-0.0247^{bA}	1.77^{bA}	0.999
TBHQ-200	-0.0263^{bA}	1.83^{bA}	1	-0.0243^{bcB}	1.75^{bcB}	1	-0.0243^{bB}	1.75^{bB}	1	-0.0243^{bB}	1.75^{bB}	1
Extract-250	-0.0223^{cA}	1.67^{cA}	0.994	-0.0233^{cdA}	1.71^{cdA}	1	-0.0223^{cA}	1.67^{cA}	0.997	-0.0227^{cA}	1.69^{cA}	0.997
Extract-500	-0.0223^{cA}	1.71^{cA}	0.992	-0.0223^{dA}	1.67^{dA}	0.997	-0.0223^{cA}	1.67^{cA}	0.992	-0.0227^{cA}	1.69^{cA}	0.991
Extract-1000	-0.0233^{cA}	1.71^{cA}	0.99	-0.0223^{dA}	1.67^{dA}	0.994	-0.0223^{cA}	1.67^{cA}	0.993	-0.0227^{cA}	1.69^{cA}	0.992
Extract-2000	-0.0223^{cA}	1.67^{cA}	0.993	-0.0223^{dA}	1.67^{dA}	0.993	-0.0223^{cA}	1.67^{cA}	0.996	-0.0223^{cA}	1.67^{cA}	0.996
Control	-0.0433^{aA}	2.71^{aA}	0.998	-0.0387^{aB}	2.44^{aB}	0.996	-0.0383^{aB}	2.42^{aB}	0.999	-0.0387^{aB}	2.44^{aB}	0.999

Means within a column with the same lowercase letters are not significantly different at $p < 0.05$. For the same parameters, means within a row with the same uppercase letters are not significantly different at $p < 0.05$; PV: peroxide value (meq O2 per kg oil), CDV: conjugated diene value (mmol/L) and AnV: anisidine value; *Antioxidant concentration (ppm).

TABLE 5
Frequency factor (A, h^{-1}) and activation energy (E_a, kJ/mol) calculated based on oil stability index (OSI, h) and induction periods (IP, h) of the blended oil samples at 120–140°C

	OSI			IP_{PV}			IP_{CDV}			IP_{AnV}		
Samples	*logA*	E_a	R^2	*logA*	E_a	R^2	*logA*	E_a	R^2	*logA*	E_a	R^2
TBHQ-25*	10.33^{bA}	81.97^{bA}	0.999	10.08^{bA}	79.6^{bA}	0.999	10.1^{bA}	79.73^{bA}	0.999	10.05^{bA}	79.46^{bA}	0.999
TBHQ-50	10.01^{bA}	79.86^{bA}	0.999	9.88^{bA}	78.37^{bA}	0.999	9.82^{bA}	77.93^{bA}	1	9.73^{bA}	77.3^{bA}	1
TBHQ-100	9.96^{bA}	80.32^{bA}	1	9.65^{bcA}	77.41^{bA}	0.999	9.65^{bA}	77.43^{bA}	0.999	9.62^{bA}	77.26^{bA}	1
TBHQ-200	10.15^{bA}	82.47^{bA}	0.999	9.5^{bcA}	76.92^{bA}	0.999	9.53^{bcA}	77.22^{bA}	0.999	9.48^{bcA}	76.82^{bA}	0.999
Extract-250	8.9^{cA}	71.13^{cA}	0.996	9.1^{cdA}	72.11^{cA}	0.999	8.89^{cdA}	70.58^{cA}	0.998	8.89^{cdA}	70.6^{cA}	0.998
Extract-500	8.98^{cA}	72.03^{cA}	0.994	8.76^{dA}	69.87^{cA}	0.996	8.61^{dA}	68.78^{cA}	0.995	8.56^{dA}	68.43^{cA}	0.994
Extract-1000	8.85^{cA}	71.9^{cA}	0.993	8.49^{dA}	68.59^{cA}	0.996	8.52^{dA}	68.87^{cA}	0.995	8.45^{dA}	68.34^{cA}	0.994
Extract-2000	8.35^{cA}	68.84^{cA}	0.995	8.52^{dA}	69.51^{cA}	0.998	8.49^{dA}	69.31^{cA}	0.997	8.44^{dA}	69.01^{cA}	0.997
Control	16.34^{aA}	126.09^{aA}	0.997	15.84^{aA}	121.22^{aA}	0.997	15.78^{aA}	120.84^{aA}	0.998	15.54^{aA}	119.06^{aA}	0.998

Means within a column with the same lowercase letters are not significantly different at $p < 0.05$. For the same parameters, means within a row with the same uppercase letters are not significantly different at $p < 0.05$; PV: peroxide value (meq O2 per kg oil), CDV: conjugated diene value (mmol/L) and AnV: anisidine value; *Antioxidant concentration (ppm).

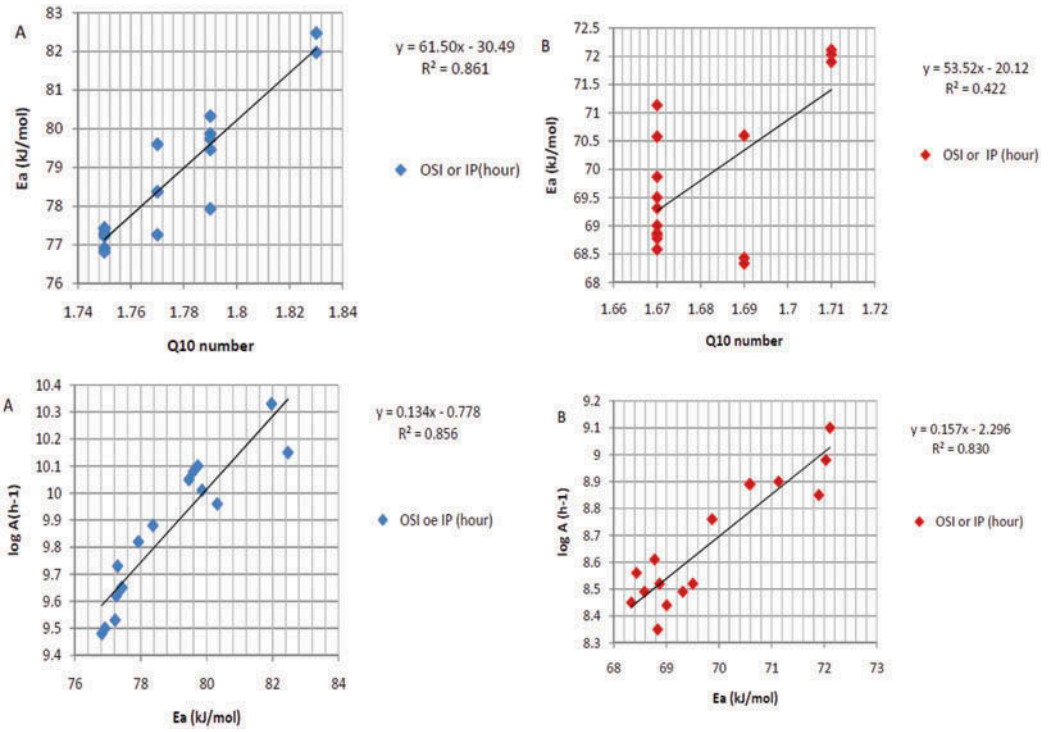

FIGURE 2 Relationship between activation energy (E_a) and Q_{10} number, frequency factor (A) and activation energy (E_a) for the oxidation of blended oil samples at 120–140 °C. TBHQ (A), Crude extract (B).

factors. Moreover, concentration of free radicals in blended oils supplemented with CE was lower in comparison to oil samples with added TBHQ. Therefore, frequency factors of oils containing CE were lower than oils containing TBHQ. It can be concluded that the decrease of frequency factor in the presence of CE may be due to the combination of hydrogen donating ability of CE and the loss of rotational freedom in the transition state.

The estimated ΔS^{++} and ΔH^{++} for lipid oxidation in all oil samples are summarized in Table 6. The high correlation of determination ($R^2 > 0.992$) showed adequate fit and characterization of the temperature dependence of lipid oxidation using the activated complex theory. The ΔH^{++} and ΔS^{++} values calculated from OSI showed no obvious differences from those calculated using IP ranging from 64.99 to 122.74 kJ/mol and from –95.91 to 57.08 J/mol K, respectively. The positive sign of ΔH^{++} shows the endothermic nature of the activated complex formation. Tan et al.[32] reported enthalpy was greater for highly unsaturated oils than for oils with lesser amounts of unsaturated fatty acids. In this study, enthalpy of oils containing CE was lower than that of oils containing TBHQ indicating that concentration of the activated complex in oil with CE was lower than in oil with TBHQ, so the rate of oxidation reaction was slower. The significantly greater negative entropy value for oils containing CE indicates fewer numbers of species in the activated complex state.[32] Consequently, the activated complex for lipid oxidation in oils containing CE is less probable and, therefore, the rate is slower. Cho[16] reported that the decrease in entropy of activated complex formation in the presence of antioxidants decreases the frequency factor. Figure 3 demonstrates very good linear relationships with high correlation coefficients between the E_a and ΔH^{++} and the A and ΔS^{++} for the oxidation of blended oil samples. As mentioned above, frequency factor undergoes greater changes than activation energy during lipid oxidation in the presence of

TABLE 6
Enthalpy (ΔH^{++}, kJ/mol) and entropy (ΔS^{++}, J/mol K) calculated based on oil stability index (OSI, h) and induction periods (IP, h) of the blended oil samples at 120–140°C

	OSI			*IP_{PV}*			*IP_{CDV}*			*IP_{AnV}*		
Samples	ΔH^{++}	ΔS^{++}	R^2	ΔH^{++}	ΔS^{++}	R^2	ΔH^{++}	ΔS^{++}	R^2	ΔH^{++}	ΔS^{++}	R^2
TBHQ-25*	78.62^{bA}	-58.00^{b}	0.999	76.23^{bA}	-62.70^{bA}	0.999	76.38^{bA}	-62.40^{bA}	0.999	76.11^{bA}	-63.17^{bA}	0.999
TBHQ-50	76.51^{bA}	-63.93^{bA}	0.999	75.04^{bA}	-66.56^{bA}	0.999	74.6^{bA}	-67.76^{bA}	1	73.95^{bA}	-69.49^{bA}	1
TBHQ-100	76.97^{bA}	-64.89^{bA}	1	74.14^{bcA}	-70.83^{bcA}	0.999	74.08^{bA}	-71.02^{bcA}	0.999	73.93^{bA}	-71.4^{bA}	1
TBHQ-200	79.12^{bA}	-61.35^{bA}	0.999	73.51^{bA}	-74.06^{bcA}	0.999	73.87^{bA}	-73.32^{bcA}	0.999	73.47^{bA}	-74.27^{bcA}	0.999
Extract-250	67.7^{cA}	-85.57^{cA}	0.995	68.66^{cA}	-81.82^{cdA}	0.998	67.23^{cA}	-85.57^{cdA}	0.998	67.25^{cA}	-85.6^{cdA}	0.998
Extract-500	68.68^{cA}	-83.75^{cA}	0.993	66.46^{cA}	-88.25^{dA}	0.996	65.43^{cA}	-90.93^{dA}	0.994	65.08^{cA}	-91.89^{dA}	0.993
Extract-1000	68.55^{cA}	-86.24^{cA}	0.992	65.29^{cA}	-93.12^{dA}	0.995	65.52^{cA}	-92.66^{dA}	0.995	64.99^{cA}	-94^{dA}	0.994
Extract-2000	65.48^{cA}	-95.91^{cA}	0.994	66.14^{cA}	-92.68^{dA}	0.997	65.96^{cA}	-93.23^{dA}	0.997	65.66^{cA}	-94.09^{dA}	0.997
Control	122.74^{aA}	57.08^{aA}	0.997	117.85^{aA}	47.31^{aA}	0.997	117.49^{aA}	46.36^{aA}	0.998	115.71^{aA}	41.76^{a}	0.997

Means within a column with the same lowercase letters are not significantly different at $p < 0.05$. For the same parameters, means within a row with the same uppercase letters are not significantly different at $p < 0.05$; PV: peroxide value (meq O2 per kg oil), CDV: conjugated diene value (mmol/L) and AnV: anisidine value; *Antioxidant concentration (ppm).

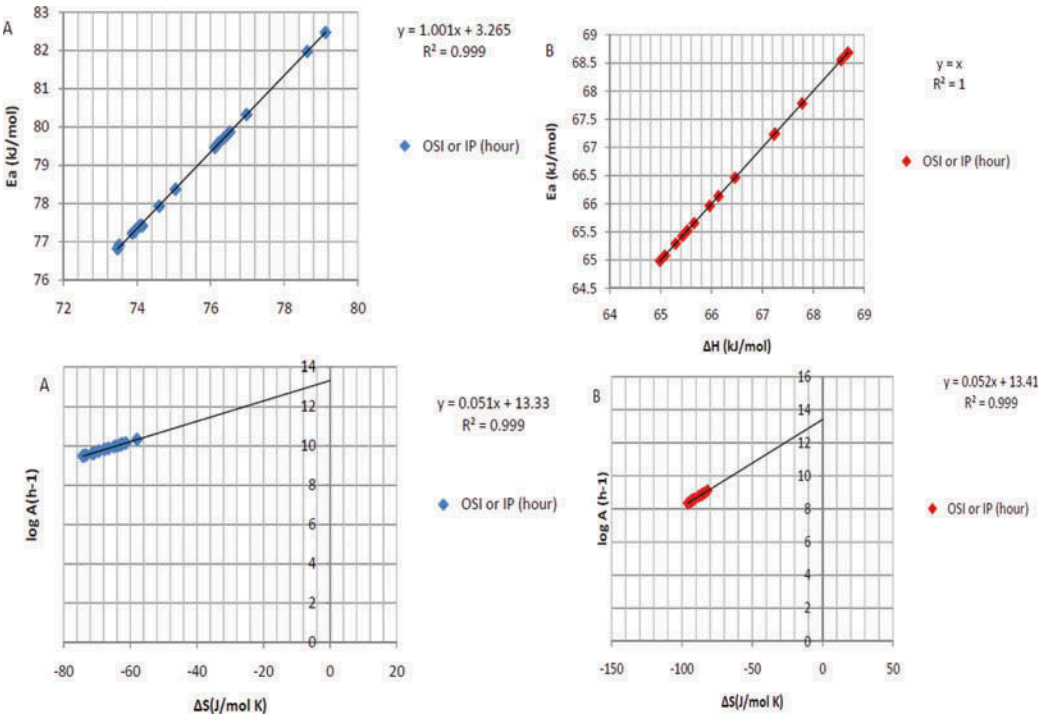

FIGURE 3 Relationship between enthalpy (ΔH^{++}) value of the activated complex formation and activation energy (E_a), entropy (ΔS^{++}) value of the activated complex formation and frequency factor (A) for the oxidation of blended oil samples at 120–140 °C. TBHQ (A), Crude extract (B).

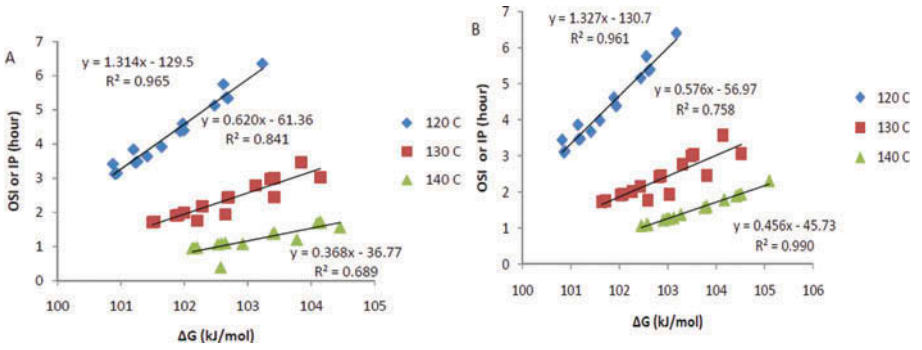

FIGURE 4 Relationship between free energy of activation (ΔG^{++}) value of the activated complex formation and the oil stability index (OSI) or induction periods (IP) for the oxidation of blended oil samples at 120-140 °C. TBHQ (A), Crude extract (B).

antioxidants. Therefore, CE or TBHQ influenced entropy (or A) more than enthalpy (or E_a) in the reaction rates of blended oil. In other words, the $\Delta G^{++}$of the activated complexes formed during the oxidation of blended oil samples was affected more by entropy than by enthalpy. Figure 4 shows the oxidative stability of the blended oils as a function of $\Delta G^{++}$of the activated complexes formation at 120

to 140°C. Linear equations could well explain the relationship between OSI or IP and ΔG^{++} as the oxidative stability of the blended oils rose constantly. The ΔG^{++} increased as temperature increased because of the endothermic nature of the activated complex formation during oxidation of the blended oils and also reduced disordering of the reactants in the activated complexes.[34] Oils containing CE have higher ΔG^{++} values than oils containing TBHQ, indicating slower oxidation reaction rates at a constant temperature. It can be concluded CE decreased the oxidation reaction rate due to the decrease in the activated complex concentration and reduction of the rate at which the activated complex dissociates into products.

CONCLUSION

The addition of CE to blended vegetable oil decreased the Q_{10} number, activation energy, frequency factor, enthalpy, entropy, and free energy of activation to minimize the reaction rate of lipid oxidation. These values were independent of antioxidant concentration. Blended oils containing TBHQ showed higher temperature sensitivities than those containing CE, a fact that should be considered when using them in high temperature processes. Our results also indicate that MUFA: PUFA ratio depends on the antioxidant concentration, but is independent of antioxidant type.

REFERENCES

1. Labrador, V.; Fernandez Freire, P.; Perez Martin, J.M.; Hazen, M.J. Cytotoxicity of Butylated Hydroxyanisole in Vero Cells. Cell Biology and Toxicology **2006**, *23*, 189–199.
2. Lagouri, V.; Prasianaki, D.; Krystalli, F. Antioxidant Properties and Phenolic Composition of Greek Propolis Extracts. International Journal of Food Properties **2013**, *17*, 511–522.
3. Rawat, S.; Andola, H.; Giri, L.; Dhyani, P.; Jugran, A.; Bhatt, I.D.; Rawal, R.S. Assessment of Nutritional and Antioxidant Potential of Selected Vitality Strengthening Himalayan Medicinal Plants. International Journal of Food Properties **2014**, *17*(3), 703–712. DOI: 0.1080/10942912.2012.654563.
4. Avallone, R.; Zanoli, P.; Puia, G.; Kleinschnitz, M.; Schreier, P.; Baraldi, M. Pharmacological Profile of Apigenin, a Flavonoid Isolated from Matricaria Chamomilla. Biochemical Pharmacology **2000**, *59*, 1387–1394.
5. Zanoli, P.; Avallone, R.; Baraldi, M. Behavioral Characterization of the Flavonoids Apigenin and Chrysin. Fitoterapia **2000**, *71*, S117–S123.
6. Erkan, N.; Ayranci, G.; Ayranci, E. A Kinetic Study of Oxidation Development in Sunflower Oil under Microwave Heating: Effect of Natural Antioxidants. Food Research International **2009**, *42*, 1171–1177.
7. Kahkonen, M.P.; Hopia, A.I.; Vuorela, H.J. Antioxidant Activity of Plant Extracts Containing Phenolic Compounds. Journal of Agriculture and Food Chemistry **1999**, *47*, 3954–3962.
8. Kim, D.; Jeong, S.; Lee, C. Antioxidant Capacity of Phenolic Phytochemicals from Various Cultivars of Plums. Food Chemistry **2003**, *81*, 321–326.
9. Choi, C.W.; Kim, S.C.; Hwang, S.S.; Choi, B.K.; Ahn, H.J.; Lee, M.Y.; Paerk, S.H.; Kim, S.K. Antioxidant Activity and Free Radical Scavenging Capacity Between Korean Medicinal Plants and Flavonoids by Assay-Guided Comparison. Plant Science **2002**, *153*, 1161–1168.
10. Benzie, I.F.F.; Strain, J.J. The Ferric Reducing Ability of Plasma (FRAP) As a Measure of "Antioxidant Power:" The FRAP Assay. Analytical Biochemistry **1996**, *239*, 70–76.
11. Sherazi, S.T.H.; Kandhro, A.; Mahesar, S.A.; Bhanger, M.I.; Bhanger, M.I. Application of Transmission FT-IR Spectroscopy for the Trans Fat Determination in the Industrially Processed Edible Oils. Food Chemistry **2009**, *114*, 323–327.
12. Firestone, D. *Official Methods and Recommended Practices of the American Oil Chemists' Society*; American Oil Chemists' Society: Champaign, IL, 1993.
13. American Oil Chemist's Society. *Official Methods and Recommended Practices of the American Oil Chemist's Society.* American Oil Chemist's Society: S Champaign, IL, 1998.
14. Shimada, Y.; Roos, Y.; Karel, M. Oxidation of Methyl-Linoleate Encapsulated in Amorphous Lactose Based Food Model. Journal of Agriculture and Food Chemistry **1991**, *39*, 637–641.

15. Heberger, K.; Kemeny, S.; Vidoczy, T. On the Errors of the Arrhenius Parameters and Estimated Rate Constant Values. International Journal of Chemical Kinetics **1987**, *19*, 171–181.
16. Cho, H.Y. Reaction Mechanisms and Kinetics of Antioxidant Using Arrhenius Equation in Soybean Oil Oxidation. Journal of Food Science and Nutrition **1997**, *2*, 6–10.
17. Schaich, K.M. Lipid Oxidation: Theoretical Aspects. In *Bailey's Industrial Oil and Fat Products*; Shahidi, F.; Eds.; A Wiley-Interscience Publication, John Wiley and Sons: New York, NY, **2005**; 269–299 pp.
18. Vinson, J.A.; Dabbagh, Y.A.; Serry, M.M.; Jang, J. Plant Flavonoids, Especially Tea Flavonoids, Are Powerful Antioxidants Using An In Vitro Oxidation Model for Heart Disease. Journal of Agriculture and Food Chemistry **1995**, *43*, 2800–2802.
19. Proestos, C.; Boziaris, I.S.; Nychas, G.J.E.; Komaiti, M. Analysis of Flavonoids and Phenolic Acids in Greek Aromatic Plants: Investigation of Their Antioxidant Capacity and Antimicrobial Activity. Food Chemistry **2006**, *95*, 664–671.
20. Burits, M.; Bucar, F. Antioxidant Activity of Nigella Sativa Essential Oil. Phytotherapy Research **2000**, *14*, 323–328.
21. Prior, R.L.; Cao, R.L. In Vivo Total Antioxidant Capacity: Comparison of Different Analytical Methods. Free Radical Biology and Medicine **1999**, *27*, 1173–1181.
22. Einafshar, S.; Poorazrang, H.; Farhoosh, R.; Seiedi, S.M. Antioxidant Activity of the Essential Oil and Methanolic Extract of Cumin Seed (*Cuminum cyminum*). European Journal of Lipid Science and Technology **2012**, *114*, 168–174.
23. Beltran, G.; Rio, C.D.; Sanchez, S.; Martinez, L. Influence of Harvest Date and Crop Yield on the Fatty Acid Composition of Virgin Olive Oils from Cv. Picual. Journal of Agriculture and Food Chemistry **2004**, *52*, 3434–3440.
24. Lacoste, F.; Lagardere, L. Quality Parameter Evolution During Biodiesel Oxidation Using Rancimat Test. European Journal of Lipid Science and Technology **2003**, *105*, 149–155.
25. Farhoosh, R.; Hoseini-Yazdi, S.Z. Evolution of Oxidative Values during Kinetic Studies on Olive Oil Oxidation in the Rancimat Test. Journal of American Oil Chemist Society **2013**, *91*, 281–293.
26. Talpur, M.Y.; Sherazi, S.T.H.; Mahesar, S.A.; Naz, S.; Kara, H. Impact of Frying on Key Fatty Acid Ratios of Canola Oil. European Journal of Lipid Science and Technology **2012**, *114*, 222–228.
27. Choe, E.; Min, D.B.J. Chemistry of Deep-Fat Frying Oils. Journal of Food Science **2007**, *72*, 77–86.
28. Frankel, N. *Lipid Oxidation*; The Oily Press: Dundee, UK, 1998.
29. Mancebo-Campos, V.; Fregapane, G.; Salvador, M.D. Kinetic Study for the Development of An Accelerated Oxidative Stability Test to Estimate Virgin Olive Oil Potential Shelf Life. European Journal of Lipid Science and Technology **2008**, *110*, 969–976.
30. Labuza, T.P. Application of Chemical Kinetics to Deterioration of Foods. Journal of Chemical Education **1984**, *61*, 348–358.
31. Vaidya, B.; Eun, J.B. Effect of Temperature on Oxidation Kinetics of Walnut and Grape Seed Oil. Food Science and Biotechnology **2013**, *22*, 273–279.
32. Tan, C.P.; Che Man, Y.B.; Selamat, J.; Yusoff, M.S.A. Application of Arrhenius Kinetics to Evaluate Oxidative Stability in Vegetable Oils by Isothermal Differential Scanning Calorimetry. Journal of American Oil Chemist Society **2001**, *78*, 1133–1138.
33. Uri, N. Physico-Chemical Aspects of Autoxidation. In *Autoxidation and Antioxidants*; Lundberg, W.O.; Ed.; Interscience Publishers: New York, NY, **1961**; 55–106 pp.
34. Kondepudi, D. *Introduction to Modern Thermodynamics*; Wiley: Chichester, UK, 2008; 516 pp.