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Shelf-life prediction of flaxseed oil blended with different antioxidants: Kinetic and thermodynamic study using Rancimat methodology

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Abstract

Omega-3 fatty acids are essential to health and have been associated with benefits like reduced inflammation, improved heart health, and protection of the brain against aging. Flaxseed oil is rich in healthy fatty acids. However, it can be oxidized in short periods due to its high content of polyunsaturated fatty acids. Blending this oil with suitable antioxidants is therefore highly recommended to extend its shelf life. Trolox, gallic acid, and caffeic acid were used as fortifying antioxidants in this study. The selected antioxidants were added to flaxseed oil in different concentrations (30, 60, and 90 ppm) and the blended samples were then evaluated for their thermo-oxidative stability (induction period, IP), oxidation kinetics (rate constant, k), oxidative stability enhancement, using Rancimat as accelerated shelf life test (ASLT) at 363, 373, and 383 °K. Prediction of shelf-life of all samples at room temperature (298 °K, IP25) were then measured. The Arrhenius equation and activated complex theory were used to estimate activation energies (Ea), activation enthalpies (ΔH), and entropies (ΔS), which varied from 89.34 to 104.31 kJ/mol, from 78.30 to 97.88 kJ/mol, and from −31.01 to −54.62 kJ/mol, respectively. Results showed that the kinetic parameters of lipid oxidation varied greatly among the type and concentrations of antioxidants used. Trolox-fortified oil had the highest IP at all tested temperatures and the longest predicted shelf life at ambient temperature (298 °K). Finally, results assessed the suitability of the Rancimat apparatus as a quick methodology for predicting the shelf life of edible oils.

Keywords: Rancimat, oxidation stability, kinetic and thermodynamic parameters, flaxseed oil, antioxidant

1. Introduction

The use of omega-3 fatty acids in functional foods is highly recommended due to their diverse health benefits and specifically for the heart and brain. However, these fatty acids are easily oxidized in the presence of oxygen, heat, light, and/or metal ions. As a result, the sensory qualities and consumer acceptance of the goods may be impaired [1, 2]. Moreover, earlier investigations using animal models have demonstrated the presence of genotoxic and cytotoxic substances in oxidative products [3]. Additionally, the presence of these reactive substances in diets has been suggested to be the possible major cause of several illnesses including diabetes, atherogenesis, neurodegenerative diseases, chronic inflammation, and some forms of cancer. Therefore, the use of synthetic antioxidants in

food formulations containing omega-3 fatty acids became so essential to keep their quality because of its impact on health as well as to meet consumer acceptability

Flaxseed oil is considered a good source of omega-3 fatty acids and it is thus very useful for the human health of heart and brain. However, it needs to be protected against oxidation via the use of suitable antioxidants. Choice of the most suitable antioxidant should be made based on the kinetics as well as the thermodynamics of the oxidation of flaxseed oil with and without antioxidants. Ghosh et al. (2019) studied the kinetics of lipid oxidation in omega fatty acids-rich blends of sunflower and sesame oils using Rancimat [4]. Rodríguez et al. (2020) determine the oxidative stability index (OSI) using the Rancimat method of

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chia sesame oil blends [5]. Elhussein et al. (2018) studied the Kinetic and thermodynamic oxidative stability of sesame oil extracted from seeds with different origins under accelerated conditions [6]. Yang and Chiang (2017) investigated the kinetic parameters of n-3 PUFA-rich oil during oxidation via Rancimat (at a temperature range of 70~100oC) [7].

Usually, accelerated shelf-life testing (ASLT) is carried out to assess a product's shelf-life [8], such as cosmetics, pharmaceuticals, and foods. The approach reduces the time needed to evaluate the shelf-life of a product by accelerating the quality depletion kinetics with increased storage temperature [9, 10]. ASLT is widely accepted because the temperature could be easily controlled and modified throughout the test. Moreover, the temperature dependence of quality index parameter decay can be easily modeled by mathematical thermodynamics like the Arrhenius equation [11]. The widely used Rancimat method, an international standard performed under accelerated storage conditions at high temperatures [12], is reliable, and reproducible, does not involve reagents

consumption, and its measurements can be easily automated [13].
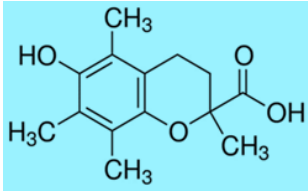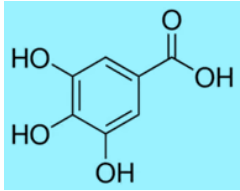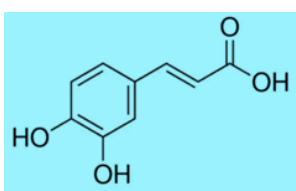
The thermal vulnerability of dietary lipids (e.g., fats and oils) rich in omega (ω) fatty acids is a problem in the food industry. **So far, the goal of this study** is to evaluate and compare the activity of trolox, gallic acid, and caffeic acid antioxidants for stabilizing flaxseed oil by testing their ability to extend the shelf-life of flaxseed oil and to examine the suitability of the Rancimat method to predict the shelf life of the oil on basis of oxidation kinetics and thermodynamic properties.

2. Materials and methods

2.1. Materials

Cold-pressed flaxseed oil (FO) was provided by the oil extraction unit, National Research Centre, Egypt. Three types of synthetic antioxidants that are commonly used in the edible oil industry in Egypt were used in this study including trolox, gallic acid, and caffeic acid. All were purchased from Sigma-Aldrich. Formulae and chemical structures of these antioxidants are listed in Table 1.

Table 1. Chemical structure, formula, and molar mass of used synthetic antioxidants

Antioxidant	Trolox	Gallic acid	Caffeic acid
Chemical structure			
Synonym	(S)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid	3,4,5-Trihydroxybenzoic acid	3,4-Dihydroxycinnamic acid
Formula	$C_{14}H_{18}O_4$	$C_7H_6O_5$	$C_9H_8O_4$
Molar mass	250.29 g/mol	170.12 g/mol	180.16 g/mol

2.1.1. Fortification of oil with different antioxidants
Trolox (T), gallic acid (G), and caffeic acid (C) at three different concentrations being 30, 60, or 90 ppm were introduced individually in ethanol to the

oil and then was purged with nitrogen gas to evaporate the solvent [14-15].

2.2. Methods

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2.2.1. Oxidative stability index using the Rancimat method

The Rancimat method is an automatic assay for the measurement of the ability of oils and fats to resist oxidation under relatively high temperatures. It requires no repeated analytical determinations and, hence, no organic solvents are needed for titrations. This technique is based on monitoring the increase in conductivity of deionized water after passing dry air bubbled into a heated oil sample. Such dry air carries the volatile acids (mainly short-chain acids, C1–C3) produced from oil oxidation [16]. This time is taken to induce a sudden increase in conductivity usually termed induction period (IP) and represents the oxidative stability index (OSI) of this oil. OSI correlates well with stability under various conditions of lipid oxidation as well as with other sensory and analytical methods [17]. Many studies have revealed that the Rancimat test can be used to determine and estimate the kinetic parameters of oils [18-19]. The determination of such kinetic parameters is valuable for predicting the oxidative stability and hence shelf life of oils under various storage conditions.

The oxidative stability of flaxseed oil with no additives as well as samples of this oil containing antioxidants was assessed at three different temperatures (90°C, 100°C, and 110°C). Antioxidants were added to flaxseed oil in various concentrations of 30, 60, and 90 ppm. Professional Rancimat 892 equipment (Metrohm AG, CH-9100, Herisau, Switzerland) was used to assess the oxidative stability at an airflow rate equal to 20 L/h. The automatically recorded induction periods (IP, in hours) of the samples were used as the breakpoints for the curves that were plotted.

2.2.2. Enhancement percentage of induction periods of fortified flaxseed oil

Enhancement of IP was estimated using equation (Eq.1).

Enhancement % = (IP $_{Ant}$-IP $_{Con}$)/(IP $_{Con}$) X 100 (1)

Where IP$_{Ant}$ and IP$_{Con}$ denoted induction periods of oil with antioxidants and control oil without antioxidants, respectively.

2.2.3. Kinetics and thermodynamic parameters of oxidation stability

As an accelerated shelf-life test, the approach to the assessment of thermal oxidative stability involved measurement of the induction period (IP), as an indicator of quality loss, at 363, 373 and 383°K for the control sample (flaxseed oil without fortification) and oil samples fortified with different antioxidants. Thence, a mathematical equation that best fit it was applied. For most food products, loss of quality over time could be modeled by measuring the kinetic variation of a specific quality index at a constant temperature [8], using Eq.2:

$$dA /dT = k An \quad (2)$$

where k is the specific reaction rate constant; t is the time; A is the measurable quantity that expresses the quality index in our case is IP; and n is the reaction order.

Taking into our consideration that A is represented by the IP in this work, the values of the kinetic rate constant of the oxidation of flaxseed oil samples have been estimated as the inverse of the induction period,

$$K = 1/IP \quad (3) \quad [20]$$

2.3. Shelf-life prediction

In the present work, according to [16], flaxseed oil either alone (control) or fortified with different antioxidants was examined with Rancimat assay at 363, 373, and 383 °K, and the recorded shelf lives have been extrapolated to forecast shelf life at 298 °K.

3. Results and Discussion

3.1. Oxidative stability index using the Rancimat method

As shown in Table 2, IP (in h) of flaxseed oil (control sample without any additives) as well as oil samples fortified with trolox (T), gallic acid (G), and caffeic acid (C) at three different concentrations being 30, 60, or 90 ppm and heated at 363,373 and 383°K are presented. In general, there was a general increase in induction periods for all fortified samples compared to the control flaxseed oil. These substances can function as antioxidants in a variety of ways, including as reducing agents, hydrogen donors, scavengers of free radicals, and quenchers of singlet oxygen [21]. As the concentration of antioxidants increased, the induction time was nearly halved with every 10-degree rise in temperature for all tested samples. In the same context, Almoselhy [22] determined the oxidative stability of oils with and without butylated hydroxyanisole as an antioxidant by the Rancimat method. Pei et al. evaluated the effects of seven kinds of antioxidants against lipid oxidation by monitoring Rancimat induction time [23].

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Table 2. Rancimat induction periods (IP) of flaxseed oil with and without fortified antioxidants at 363, 373, and 383 °K.

Samples	IP (h)		
	363 °K	373 °K	383 °K
*Control	6.17	3.21	1.43
T (30 ppm)	17.38	8.4	4.25
T (60 ppm)	30.46	12.7	6.4
T (90 ppm)	38.34	13.33	7.91
G (30 ppm)	21.2	10.97	4.4
G (60 ppm)	26.2	12.65	5.41
G (90 ppm)	28.68	13.58	6.41
C (30 ppm)	16.61	7.37	2.89
C (60 ppm)	19.55	9.62	3.46
C (90 ppm)	21.11	10.22	4.16

*Control = Flaxseed without fortification; T =Trolox, G =gallic acid, and C = caffeic acid.

3.1.1. Enhancement percentage of induction periods of fortified flaxseed oil

The percentage enhancement of IP of all fortified samples ranged between 102.1 to 521.39 (Table 3).

Trolox-fortified oil presented the highest enhancement percentage followed by gallic and caffeic acids as shown in Table 3.

Table 3. Enhancement percentages of the period (IP) of flaxseed oil after fortification with antioxidants at 363, 373, and 383 °K.

Samples	Stability Enhancement %		
	363 °K	373 °K	383 °K
T(30 ppm)	181.69	161.68	197.20
T (60 ppm)	393.68	295.64	347.55
T (90 ppm)	521.39	315.26	453.15
G (30 ppm)	243.60	241.74	207.69
G (60 ppm)	324.64	294.08	278.32
G (90 ppm)	364.83	323.05	348.25
C (30 ppm)	169.21	129.60	102.10
C (60 ppm)	216.86	199.69	141.96
C (90 ppm)	242.14	218.38	190.91

3.1.2. Kinetics and thermodynamic parameters of oxidation stability

The kinetic rate constants are represented in Figure1. (A, B, C). As shown in Fig. 1 the rate constant of oxidation of all samples increased with the increase of temperature either for the control sample or other samples fortified with antioxidants. The decreasing order was Con >Caf> G ≥ T. Also, as the antioxidant concentration increased there were pronounced retardation of oxidation rates.
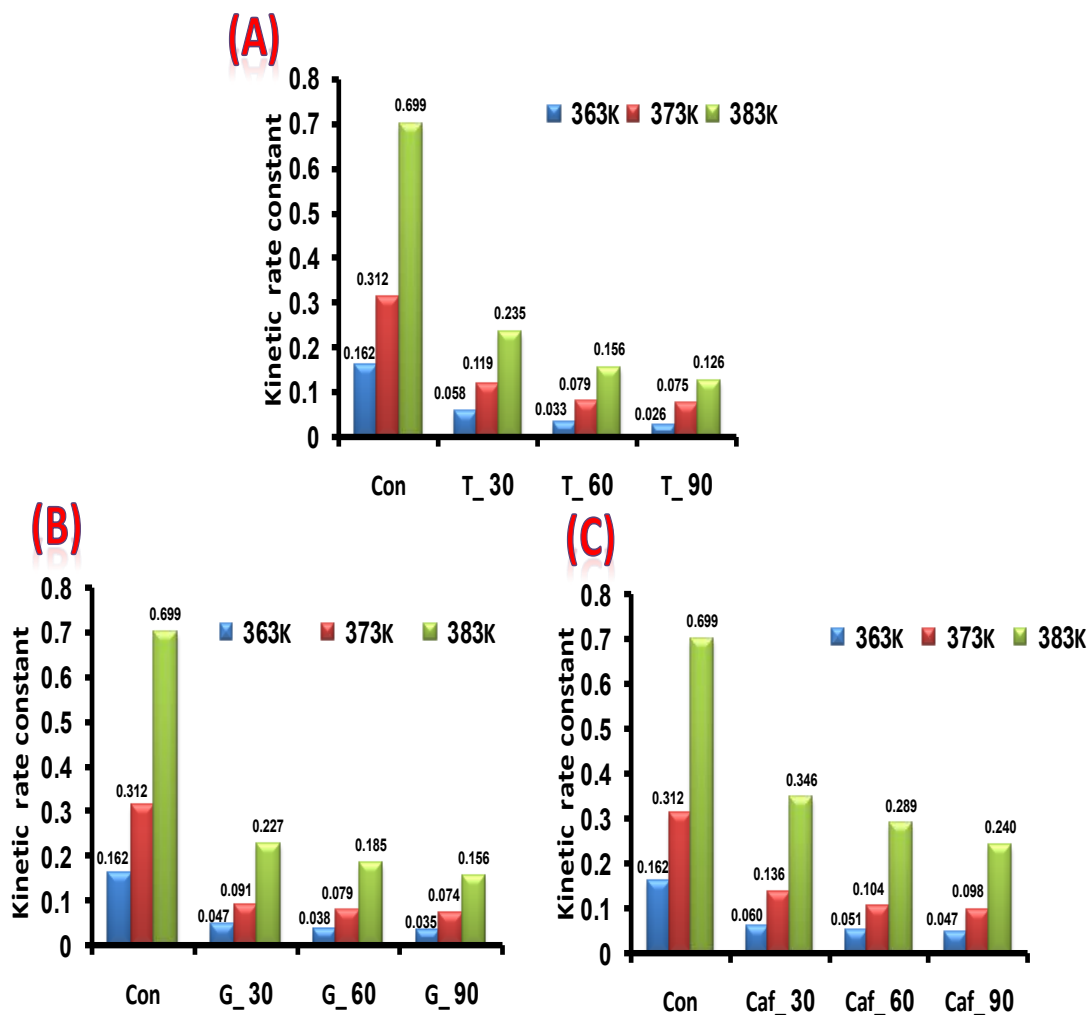
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Figure 1. Effect of antioxidants concentration (30, 60, 90 ppm) and temperature (363, 373 and 383 ºk) on the kinetic rate constants of flaxseed oil with and without fortification; (A) trolox (T), (B) gallic acid (G), and (C) caffeic acid (C).

Since the majority of food quality decay is strongly influenced by temperature, the dependence of their kinetics on system temperature can be described effectively by Arrhenius' Equation (4):

$$k = A e^{-Ea/RT} \quad (4)$$

Taking the natural logarithm of Arrhenius equation yields equation (5):

$$\ln(k) = \ln(A) - (Ea / RT) \quad (5)$$

where Ea is the activation energy (kJ/mol) which is the minimum energy needed for triggering the

reaction, R is the gas universal constant (8.318 kJ/mol), and T is the absolute temperature (°K).

Figure 2 (A, B, C) shows the relation between ln (k) and 1/T for all oil samples. The values of the activation energy (Ea), which is the minimum energy necessary

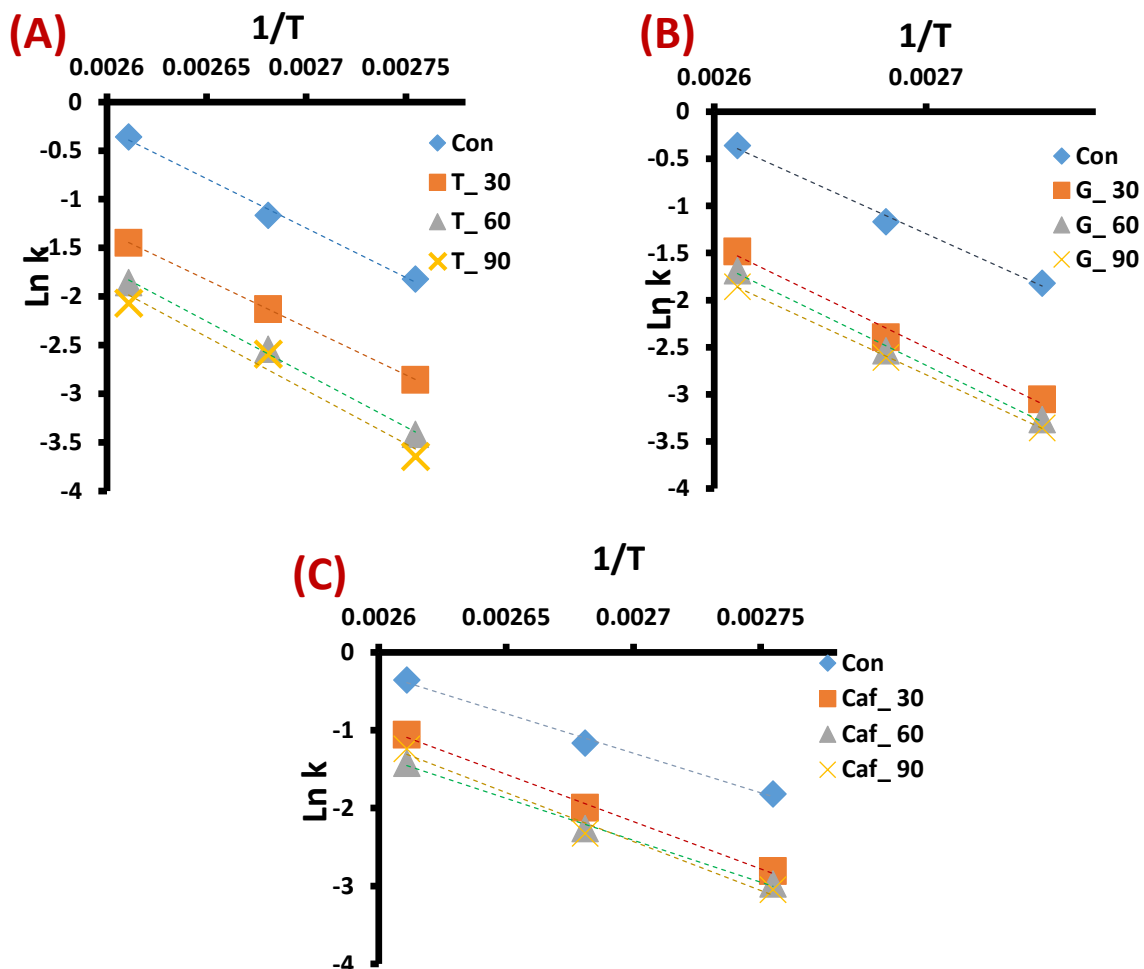to trigger the reaction [8], could be predicted from these figures.

Figure 2. Effect of antioxidants concentration and temperature on the kinetic rate constants of flaxseed oil with and without fortification

As shown in Table 3 the different formulations were characterized by different Ea values. The Ea calculated for the control sample was 81.39 which came in sequence with the general lipid oxidation reactions (24–240 kJ/mol) [24-25]. The Ea of all fortified samples were higher than that of the control one, reflecting as expected, their high antioxidative effects.

Using the activated complex theory, the activation enthalpies (ΔH) and entropies (ΔS) of lipid oxidation were calculated using the following equation:

$$Ln\ (k/T) = ln\ (k_B/h) + (\Delta S/R) - (\Delta H/RT) \qquad (6)$$

where k_B is the Boltzmann constant (1.380×10^{-23} J/K) and h is the Planck's constant (6.63×10^{-34} J s).

Figure 3 (A, B, C) shows the relation between ln (k/T) and 1/T for all oil samples. The values of the ΔH and ΔS could be predicted from these figures. The ΔH and ΔS were calculated from the slope and intercept of Eq. (6). Table 4 shows that the enthalpy ranged from 78.30 to 97.88 and have positive values, thus the reaction is endothermic. This endothermic nature of the lipid oxidation system was in agreement with [8] and [6]. The calculated entropy ranged between -31.01 and -54.68 J/K mol. As shown in Table 4, the positive

sign of ΔH and the negative sign of the ΔS for two driving forces for oil oxidation are to be done.
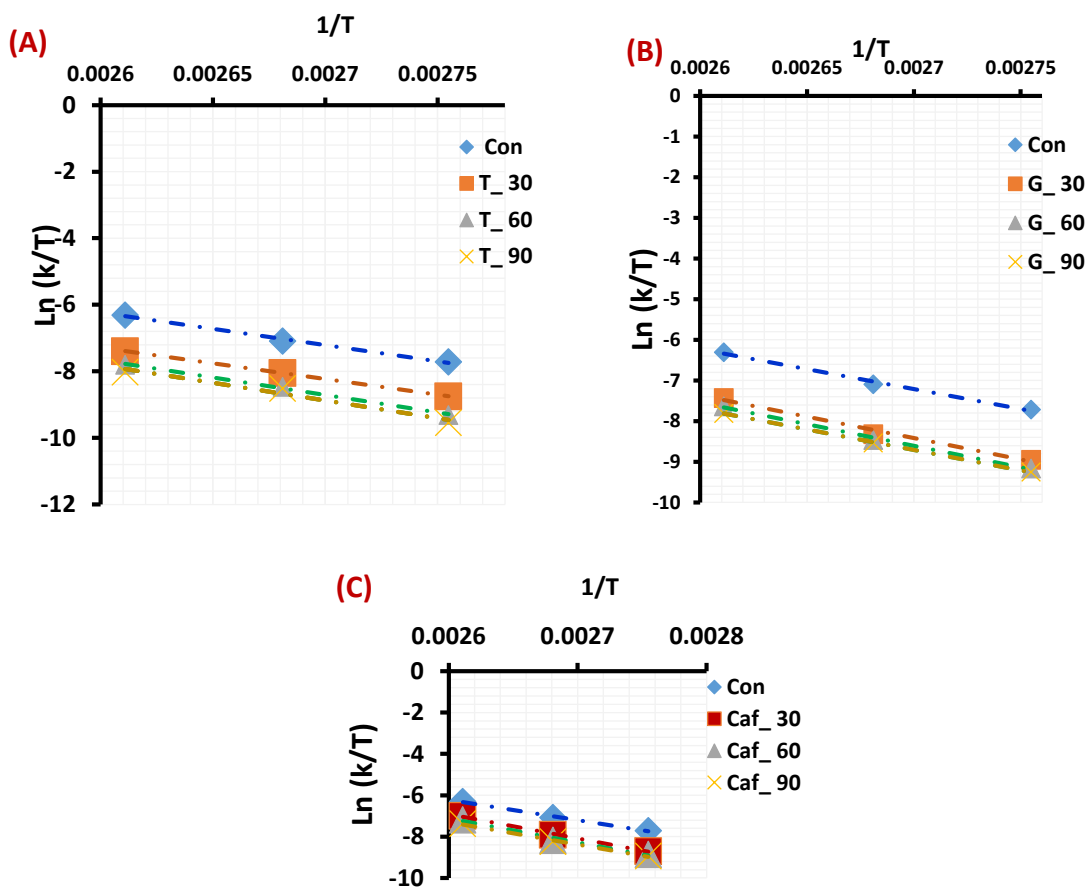

Figure 3: Semi-logarithmic relationship between ln (k/T) and temperature values (1/T) for lipid oxidation of the prepared samples.

Table 4: Values of estimated activation energy (Ea), enthalpy (ΔH) as well as the values of entropy (ΔS).

Sample	Ea KJ/mol	ΔH KJ/mol	ΔS J/mol K
Control	81.39	78.30	-54.62
T (30 ppm)	89.34	96.80	-49.88
T (60 ppm)	100.98	97.88	-37.99
T (90 ppm)	104.31	90.66	-51.55
G (30 ppm)	86.57	83.47	-43.81
G (60 ppm)	90.72	87.62	-31.01
G (90 ppm)	91.09	87.99	-31.59
C (30 ppm)	84.40	81.29	-37.99
C (60 ppm)	90.24	87.14	-34.75
C (90 ppm)	91.48	88.38	-32.76

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3.2. Shelf-life prediction

The pressure of food safety authorities to maintain markets free from deteriorated or rancid food commodities, it is common in the food industry (especially for oils and fats) to use accelerated shelf life test (ASLT), for products with a long shelf-life (≥ 6 months) at ambient temperature (298 °K). According to [14], the rates of lipid oxidation vary exponentially with temperature, and Rancimat could forecast shelf life within hours for lower temperatures (such as 298 OK) as IP298.

Results in Table 5 indicates that the shelf-life of all fortified samples was, as expected, longer than that of flaxseed oil alone (control sample) which recorded the least shelf life (0.18 year) whereas Trolox fortified flaxseed oil registered the highest shelf life (1.67 years) at 90 ppm concentration. The general decreasing order of shelf life of all samples was T ≥ G > C > Con. This order of sample's shelf life went in a parallel way to results obtained for reaction rate constant (Trolox samples recorded the lowest rate, Fig. 1) as well as results of activation energy (Trolox fortified flaxseed oil had the highest Ea, Table 4).

It is apparent that fortification, and especially the addition of antioxidants greatly enhances the oxidation stability and shelf life of oils with high polyunsaturated fatty acids such as flaxseed oil [24]. This technique has been employed recently by researchers to predict the oil's shelf life. Ghosh et al. (2018) used the Rancimat test to predict the shelf life of blends of sunflower and sesame oil with various ratios [27]. The shelf life of avocado oil at 25°C was predicted by Aktar and Adal (2019) using the Rancimat test [28].

Table 5: Forecasting of shelf life (IP_{293}) of flaxseed oil samples with and without antioxidant fortification at 298 °K by extrapolation of IP data obtained by Rancimat at 363, 373, and383 °K.

Samples	Hours	Years
Control	1614	0.18
T (30 ppm)	10076	1.15
T (60 ppm)	12833	1.46
T (90 ppm)	14685	1.67
G (30 ppm)	9550	1.09
G (60 ppm)	11061	1.26
G (90 ppm)	14292	1.63
C (30 ppm)	8549	0.97
C (60 ppm)	10556	1.20
C (90 ppm)	8462	0.96

4. Conclusions

The present work is a comparative study that was carried out to investigate the effect of trolox, gallic acid, and caffeic acid to retard the oxidation of flaxseed oil and to predict the shelf life of polyunsaturated rich oil like flaxseed oil using an accelerated shelf life test (ASLT). Although ASLT does not completely resembles the actual circumstances for shelf storage conditions, the pressure of new and innovative product development that invade markets makes it common in the food industry to use ASLT, especially for ambient stable products with a long shelf-life (≥ 6 months). ASLT represented by Rancimat assay was used to minimize the time taken until the induction period (IP) is reached. Then the oxidation kinetics and thermodynamic parameters were calculated by applying the Arrhenius equation and activated complex theory. It was apparent that all antioxidants used have succeeded to extend the IP and hence the shelf life of flaxseed oil with Trolox being superior to all tested antioxidants. Kinetics and thermodynamic parameters revealed that the examined oil oxidation

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reaction was a nonspontaneous endothermic process that was affected greatly by antioxidant type and concentration as well as the applied temperature.

5.   Funding:

6.   Conflicts of Interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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