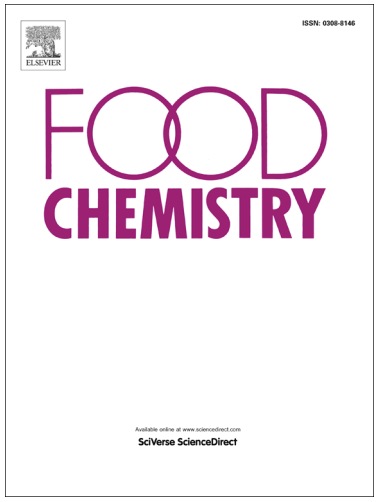

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Effect of tomato (*Solanum lycopersicum* L.) lycopene-rich extract on the kinetics of rancidity and shelf-life of linseed (*Linum usitatissimum* L.) oil

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ABSTRACT

The effect of tomato lycopene-rich extract (TLE) addition on shelf-life of linseed oil was evaluated. Linseed oil was extracted by cold pressing and TLE by supercritical CO_2. Linseed oils with and without TLE addition were characterized for moisture, colour, refractive index, fatty acid composition and antioxidants. Adding TLE to 80 mg lycopene/kg oil improved linseed oil stability, showing the same induction time at 110 °C (by Rancimat) of control linseed oil with 200 mg/kg butylhydroxytoluene. The increase of free fatty acid, peroxide value, *p*-anisidine value, K_{232} and K_{268} at 40, 50, and 60 °C until 90 days followed first-order kinetics. Rancidity rate augmented with temperature. TLE addition slowed oil degradation without changing the mechanism since the Arrhenius lines were parallel. Mean *Ea* were respectively 38.2, 24.7, 38.0, 38.2, 41.5 kJ/mol. TLE addition increased linseed oil shelf-life by 31% (Rancimat) and by 42% (stability kinetics during storage).

Key words: antioxidants, linseed oil, lycopene extract, oil hydrolysis, oxidation kinetics, Rancimat, shelf-life.

1. Introduction

Flax (*Linum usitatissium* L.) has been cropped by man for thousands of years (Tańska, Roszkowska, Skrajda, & Dąbrowski, 2016). Currently it is cultivated for a dual purpose: to obtain vegetable fiber from the stem and to extract oil from the seeds. According to FAOSTAT (2014), 686 498 t of linseed oil were produced worldwide; the largest producer is China (25%), followed by Belgium (19%), United States (17%), Germany (8 %), Ethiopia (7%) and India (7%).

Linseed or flaxseed oil has several industrial purposes, such as production of paint and floor covering (linoleum), additive in PVC plastics, coal-agglomerating agent, etc. The growing interest in the utilization of linseed oil as food is due to its high content of two polyunsaturated fatty acids (PUFA), α-linolenic acid (ALA; omega-3) and linoleic acid (LA; omega-6). The α-linolenic acid varies between 49-64% of total fatty acids and the linoleic between 15-17% (Shadyro, Sosnovskaya, & Edimecheva, 2017). Several studies have been carried out to test the benefits deriving from the consumption of PUFA, which contribute to prevent the onset of cardiovascular diseases, cancer and inflammation (Shadyro et al., 2017). However, the high degree of α-linolenic acid unsaturation implies a great susceptibility of linseed oil to oxidation during production and storage (Tańska et al., 2016), leading to significant deterioration of sensory characteristics and drastic reduction in nutritional value, thus limiting its access to the food and pharmaceutical markets.

The protection of vegetable oils, especially polyunsaturated oils, from oxidation is thus fundamental to guarantee an extended shelf-life. Numerous studies evaluated the effectiveness of natural and synthetic antioxidants to delay oxidative phenomena in vegetable oils (e.g. Wani, Sogi, Singh, & Götz, 2013), and in particular in linseed oil (Shadyro et al., 2017). Synthetic compounds such as butylhydroxyanisole (BHA), butylhydroxytoluene (BHT), and tert-butyl-hydroquinone (TBHQ) are widely used antioxidants due to their low cost, high stability and efficacy; a maximum amount of 200 mg/kg oil of any combination of these antioxidants is allowed (Codex Alimentarius, 2017a). However,

possible toxicological effects on human health have been observed and some countries (e.g. Japan, Romania, Sweden, and Australia) banned their utilization (Ghatak & Sen, 2017).

In recent years there is a steady growth in studies dealing with the improvement of oxidative stability of linseed oil through the addition of natural antioxidants. Michotte, Rogez, Chirinos, Mignolet, Campos, and Larondelle (2011) demonstrated that myrecitin was the phenolic compound that most reduced the production of primary oxidation products. Interestingly, Van Ruth, Shaker, and Morrissey (2001) decreased the amount of primary oxidation products by 30% and of secondary products by 99% with the addition of soybean methanol extracts.

Another promising antioxidant candidate is lycopene (Nour, Corbu, Rotaru, Karageorgou, & Lalas, 2018), an acyclic carotenoid abundant in tomatoes and tomato-based products, accounting for more than 85% of all lycopene sources in the diet, but present also in guava, cranberry, peaches, pink grapefruit, apricot, watermelon and papaya (Kaur & Kaur, 2015). Lycopene is a fat-soluble pigment able to quench damaging free radicals, especially the most aggressive reactive oxygen species (the singlet oxygen) and has antioxidant and antitumor properties (Kaur & Kaur, 2015).

Therefore, aim of this work was to investigate the kinetics of rancidity (hydrolysis, primary and secondary oxidation) as well as the shelf-life of linseed oil before and after the addition of tomato lycopene-rich extract (TLE).

2. Materials and methods

Seeds of brown flax variety were procured from the market of Ayacucho (Peru) and tomatoes cv. Perseo from the Universidad Nacional Agraria La Molina (Lima, Peru).

2.1. Linseed oil extraction

The flax seeds were dried on a metal tray at 50 °C for one hour as pretreatment and stored at 4 °C for 24 h in low-density polyethylene bags. The cold extraction of oil from flax seeds was carried out using a Komet CA 59 G single screw expeller (IBG Monforts Oekotec, Germany), at a speed of 24 RPM and with an 8 mm diameter nozzle. Before the extraction, the cake outlet nozzle was preheated to 60 °C for 30 min to prevent its obstruction. Some linseed oil was passed and discarded to obtain a continuous flow during the extraction. The effective extraction temperature of the oil was 43.7 °C. Afterwards, centrifugation (Centra CL2, Thermo Fisher Scientific, USA) was performed at 1421 *g* for 15 min to remove impurities from the oil. Linseed oil was stored at 4 °C under nitrogen atmosphere in the dark until enrichment and analysis.

2.2. Extraction of the lycopene-rich extract from tomatoes

The tomatoes were washed, cut (2.5 cm) and dried at 60 °C for 24 h using a pilot heated air flow system. The dried tomatoes were then ground with an analytical mill (A 11 Basic, IKA, USA) and sieved, to obtain particles with a max diameter of 0.71 mm. The dried tomato powder was then introduced interleaved with glass beads into the extraction cell of a multi-solvent equipment (2802 0000, Top Industrie, France) using a volume reducer with a capacity of 100 mL and under the conditions suggested by Baysal, Ersus, and Starmans (2000), slightly modified and adapted to our equipment, as follows: supercritical CO_2 flow, 40 g/min; temperature, 55 °C; pressure, 30000 kPa; time, 6 h; co-solvent, ethanol 8% (vol) and co-solvent flow, 3.5 mL/min. The ethanol extract was passed through a rotavapor (Laborota 4003, Heidolph, Germany) at a temperature of 35 °C to remove the solvent, and finally dried with nitrogen. The TLE was stored at -20 °C under nitrogen in the dark until use and analysis.

2.2. Preparation of enriched oil

The lycopene concentration in the TLE was determined before the formulation of the enriched linseed oil. A concentrated enriched linseed oil was prepared with the addition of TLE. Dilutions of 40, 80 and 120 mg lycopene/kg oil were prepared from the concentrated oil. The lycopene content in the TLE and oils was determined by HPLC, according to the method proposed by Gupta, Sreelakshmi and Sharma (2015).

2.3. Chemical and physical determinations

Moisture, proteins, lipids and ash of flax seed and dried tomato powder were performed following the methods reported by FAO (1986). Carbohydrates content was determined by difference. The oil moisture was measured following the method AOCS Ca 2d-25 (AOCS, 1998). The refractive index was determined with a temperature-controlled Abbe refractometer (Carl Zeiss Jena, Germany) following the method 921.08 (AOAC, 2016). The colour was measured in triplicate using a Tristimulus colorimeter, Chroma Meter CR-400 (Konica Minolta, Japan) using the standard-white reflector plate and illuminant C. The analysis led to the determination of the coordinates values of L^* (luminosity), a^* (red-green) and b^* (yellow-blue).

The oxidation stability index (OSI), also known as induction time, was evaluated using a Rancimat equipment (743 Rancimat Metrohm Co., Switzerland) following Official Method Cd 12b-92 (AOCS, 1998) at 90, 100 and 110 °C with an air flow of 20 L/h. Shelf-life at 25 °C was extrapolated.

To evaluate the degree of lipid hydrolysis, free fatty acids (FFA, % oleic acid) were determined according to the official method Ca 5a-40 (AOCS, 1998). As indices of primary oxidation, peroxide value (PV, milliequivalents oxygen/kg oil) and specific extinction at 232 nm (K_{232}) were tested following methods AOCS Cd 8-53 (AOCS, 1998) and ISO 3656 (ISO, 2011), respectively. The secondary oxidation was assessed by measuring the *p*-anisidine value (*p*-AV) (IUPAC method 2.504, IUPAC, 1987) and the specific extinction at 268 nm (K_{268}) (ISO 3656 method, ISO, 2011).

The fatty acids (FA) composition was determined as fatty acid methyl esters (FAME) by gas chromatography after transesterification of the oils with 2 N KOH in methanol, according to IUPAC Standard Method 2.302 (IUPAC, 1987) The chromatographic analysis was done using an Autosystem XL instrument (Perkin Elmer, USA). The capillary column was a 30 m x 0.25 mm, d_f 0.25 μm, SUPELCOWAX® 10 (Supelco, USA). The operative conditions were: carrier, H_2 at 68.9 kPa; oven temperature, 90 °C for 2 min, increased by 30 °C/min to 180 °C, kept at 180 °C for 10 min, increased by 1 °C/min to 220 °C, increased by 2 °C/min to 230 °C, kept at 230 °C for 5 min; injection temperature 250 °C; flame ionization detector temperature, 270 °C.

Tocopherols were determined according to IUPAC Standard Method 2.432 (IUPAC, 1987). Oil solutions in hexane:isopropyl alcohol (90:10 v/v) at concentrations of 10 mg/mL and 100 mg/mL were prepared, filtered through a 0.2 μm PTFE filter, and immediately analyzed by NP-HPLC as detailed in Brandolini, Hidalgo, Gabriele, and Heun (2015).

The lycopene quantification in the TLE obtained from the supercritical extraction and the linseed oil samples enriched with TLE were carried out following the HPLC method proposed by Gupta et al. (2015), with minor modifications. 30 mg of TLE or linseed oil with TLE were dissolved in 25 mL of methanol:tert-methyl butyl ether (60:40) and diluted 5/25. The samples were filtered (0.45 μm PTFE filters; Whatman, UK) and 20 μL were injected in a 1260 infinity LC System (Agilent, Germany) with a 3 μm 4.6 x 150 mm Acclaim™ C30 column (Thermo Fisher Scientific, USA) at 20 °C and a diode array detector (Agilent 1260 DAD, Germany) at 472 nm. Gradient elution was performed using methanol/water (98:2, v/v) (A), methanol/water (95:5, v/v) (B) and tert-methyl butyl ether (C) mobile phases at flow rate 1.0 mL/min, following the linear gradient profile: 0-2 min from 80% A, 20% C to 60% A, 40% C; 2-12 min linear change to 0% A, 0% B, 100% C; 12-13 to initial conditions (80% A, 20% C) followed by a final re-equilibration time of 7 min. The quantification of lutein, zeaxanthin and β-cryptoxanthin was instead performed on the oil solutions in hexane:isopropyl alcohol (90:10 v/v) at a

concentration of 100 mg/mL. The mixes were filtered through a 0.2 μm PTFE filter, and analyzed immediately by NP-HPLC as described in Brandolini et al. (2015).

For total polyphenol content (TPC) determination, 1.5 g oil was weighed in a test tube, then 2 mL of hexane was added and vortexed. Subsequently, the mixture was transferred to a 50 mL separating funnel and a total volume of 5 mL of 60% methanol, split into 1.5, 1.5 and 2 mL, was added. The methanolic phase was retrieved in a 10 mL tube to measure the recovered volume. TPC in samples extracted with methanol was assessed with the Folin-Ciocalteu method as described by Yilmaz, Brandolini, and Hidalgo (2015) using a Du-62 Beckman spectrophotometer (Beckman Coulter, Nyon, VD, Switzerland). The TPC, in mg gallic acid equivalent (GAE)/kg, was computed from a reference curve obtained from six gallic acid (Sigma-Aldrich, St. Louis, MO, USA) concentrations (range: 0-250 mg/L).

To measure the antioxidant activity, the 2-2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation scavenging capacity test was performed on hydrophilic (HF) and lipophilic (LF) fractions (Prior, Wu, & Schaich, 2005) as follows: exactly 2 g of oil was weighed, and 2 mL of n-hexane was added, vortexing until complete dissolution. Subsequently, 2 mL of 80:20 methanol-water was added and vortexed. The mixture was centrifuged at 987 *g* for ten minutes. Finally, the methanolic phase (located in the lower part of the centrifuge tube) and the n-hexane phase (found in the upper part of the tube) were separated, and their volumes measured. For the antioxidant capacity determination, the oil samples were diluted with 1:4 n-hexane, while the TLE was diluted 1:200 with n-hexane. Hydrophilic and lipophilic fractions (30 μL) were allowed to react with 3 mL of a diluted ABTS radical cation solution in ethanol (absorbance: 0.70 ± 0.02 AU at 734 nm). The mixture absorbance was measured at 734 nm using a spectrophotometer model V650 (Jasco, Japan) after 30 min at 30 °C, using ethanol as blank. Antioxidant activity was evaluated as percentage of absorbance decrease (inhibition percentage). A reference curve was built with 11 concentrations (from 0.1 to 2.0 mM) of Trolox

(Sigma-Aldrich St. Louis, MO, USA) in methanol. The results were expressed as μmol of Trolox equivalent (TE)/g.

2.4. Kinetic analysis of lipid rancidity

The kinetic of lipid rancidity was performed following the evolution of FFA, PV, K_{232}, *p*-AV and K_{268} of the oils stored at 40, 50 and 60 °C for 40, 60 and 90 days, respectively. Kinetics of order zero: $(a)\ C_{index} = C_{index0} + kt$ and of order one: $(b)\ lnC_{index} = lnC_{index0} + kt$ were used to fit the data (**Fig. 1)**. C_{index0} is the initial value of each oxidation index at time 0, C_{index} is the value of the oxidation index after a certain time at a given temperature (T), and k is the rate constant of the reaction, computed using the slope of the straight line between lipid oxidation index and time (t).

The resulting *k* values from first order model (ln k) versus 1/T were plotted and the activation energy was obtained for lipid oxidation using the Arrhenius model: $(c)\ ln\ k = \frac{E_a}{RT} + lnA_{index}$

where *Ea* is the activation energy, R is the universal constant of gases (8.314 J/mol °K), T is the absolute temperature (°K), and A_{index} is the frequency factor. From the inclination of the Arrhenius graph, the *Ea* and the *z* value ($z = 2.303 \times R \times T^2/Ea$), i.e. the temperature range that determines a ten-fold increase of the reaction rate were calculated; T^2 is the mean absolute temperature square of the considered temperature range.

Finally, the shelf-life of the oils at 25 °C was estimated for each index by determining the *k* at 25 °C from the Arrhenius graph and by computing the time required, using the first-order kinetics, to reach the maximum values reported by Codex Alimentarius (2017a) for vegetable oils not covered by individual standards (2% oleic acid for FFA and 15 meq O_2/kg for PV), the limits reported by Codex Alimentarius (2017b) for virgin olive oils (2.6 for K_{232} and 0.25 for K_{268}) and the limit of 2 for *p*-AV reported by Choo, Birch, and Dufour (2007).

2.5. Statistical analysis

The results were processed by one-way analysis of variance (ANOVA); when significant differences were detected, Fisher's least significant difference (LSD) at $p \leq 0.05$ was computed. Two-sample comparisons were performed by t-test. All the analyses were performed using the Statistical Program STATGRAPHICS® plus version 4 (Statpoint Technologies, Inc., USA). The average values and standard deviation were calculated using Excel 2007 (Microsoft, USA).

3. Results and discussion

3.1. Chemical composition of flax seeds and dried tomato

Moisture, protein, lipid, ash and carbohydrate content of flax seeds (7.38 ± 0.09, 17.24 ± 0.02, 42.12 ± 0.47, 3.32 ± 0.01, and 29.94 ± 0.53 g/100 g, respectively) were similar to the data (7.7, 20.0, 41.0, 3.4, and 29.0 g/100 g, respectively) reported by Morris (2007) for brown flaxseed varieties from Canada.

The moisture, protein, lipid and ash concentrations of dried tomato were 4.81 ± 0.39, 12.13 ± 0.06 1.29 ± 0.27, and 10.73 ± 0.48 g/100 g, similar to the results reported for tomato dried at 68 °C for 24 h by Surendar, Shere, and Shere (2018).

3.2. Extraction of linseed oil

To better preserve quality and stability of the oil, the temperature during the cold extraction process was always lower than 50 °C; oil yield was 33.38% ± 0.98 (w/w), slightly lower than those achieved with supercritical CO_2 (36.49%), accelerated extraction with solvent (42.40%) or with hexane (41.90%) in Egyptian linseed by Khattab and Zeitoun (2013); the same authors noted that, although the yield

with supercritical CO_2 was lower than that obtained with other methods, the oil was richer in PUFA, phenolic compounds and lignans.

3.3. Extraction and quantification of lycopene

The lycopene content in the oleoresin extract from tomatoes through supercritical CO_2 extraction was 5.34 ± 0.04 g lycopene/100 g. The extraction yield was 219.95 ± 1.76 μg lycopene/g fresh tomato. Cadoni, De Giorgi, Medda, and Poma (1999) using a different tomato variety, lower temperature (40 °C) and pressure (27600 kPa), and chloroform as co-solvent, obtained an inferior extraction (149.2 μg lycopene/g); however, after GC/MS analysis of the extracts detected chloroform traces, a new extraction at higher temperature and without co-solvent yielded much better values (644.1 μg lycopene/g). Additionally, comparing six common tomato cultivars and six high-lycopene cultivars from Italy, Lenucci et al. (2010) on average found 99 μg lycopene/g vs. 196 μg lycopene/g in common and in high-lycopene cultivars, respectively; the extraction conditions were 65-70 °C, 45 000 kPa, CO_2 flow from 18 to 20 kg/h, and 3 h extraction.

3.4. Oxidative stability by Rancimat

Table 1 shows the induction times at 110 °C measured by Rancimat for linseed oil before and after the addition of TLE (40, 80, or 120 mg lycopene/kg oil) or BHT (200 mg/kg oil), as control. The induction time of linseed oil increased with lycopene concentration. The same trend was reported by Li, Liu, Sun, Du, and Xing (2011) after supplementing soybean oil with 100 to 400 mg lycopene/kg. However, a certain lycopene prooxidant activity was observed by Henry, Catignani, and Schwartz (1998): the addition of 100 mg lycopene/kg of safflower oil increased the OSI, but higher concentrations (500, 1000 and 2000 mg/kg) decreased the OSI significantly. The initial test performed at 110 °C showed that the addition of TLE to 80 mg lycopene/kg oil allowed an increase in oxidative

stability similar to that obtained with BHT, thus all subsequent tests were carried out with this enrichment level. The tests performed at 90 and 100 °C confirmed a higher induction time at this level of enrichment than in the pure linseed oil. Furthermore, the shelf-life of linseed oil at 25 °C increased by 31%. Increasing induction times were also observed by Nour et al. (2018) after the addition of carotenoids, extracted from dry tomato waste, in unrefined corn oil, refined sunflower oil and peanut oil.

3.5. Oils characterization

Table 2 shows the results of oils characterization. The moisture content of linseed oil and linseed oil with TLE (80 mg lycopene/kg oil) were similar to the ranges reported by Tańska et al. (2016) in commercial linseed oils from Poland (0.13 to 0.75 g/100 g) and by Choo et al. (2007) in New Zealand commercial linseed oils (0.45 to 1.08 g/100 g), and indicated their good quality; high moisture can be detrimental as it contributes to hydrolysis and generates free fatty acids (Choo et al., 2007). The refractive index was unchanged after TLE addition and showed levels very similar to those reported by Kasote, Badhe, and Hegde (2013) in cold-pressed linseed oil. The color coordinates were L^* = 36.23, a^* = -1.40 and b^* = 24.43 for linseed oil. Choo et al. (2007), in their study of oils from seven different varieties of flax seed from New Zealand, reported significantly higher values for all coordinates, i.e. L^* = 63.71, a^* = 9.56 and b^* = 99.80. Tańska et al. (2016), after visual analysis, described the color of linseed oil from light yellow to yellow. The addition of TLE resulted in a significant change in CIELAB coordinates for all parameters (**Table 2**), as the oils with TLE became less luminous (20.97), redder (5.21) and less yellow (4.70).

With regards to the oil stability indices, the levels of free fatty acids for both oils (natural and lycopene-enriched) were lower than those (0.89, 0.96 and 0.94% oleic acid) found by Khattab and Zeitoun (2013) in Egyptian linseed oil obtained by three different extraction methods (Soxhlet using

hexane, supercritical CO_2 and accelerated solvent, respectively). The PV of both oils were lower than the limit (15 meq O_2/kg oil) established by the Codex Alimentarius (2017a) for vegetable oils obtained by cold pressing. The *p*-anisidine values, K_{232} and K_{268}, were inferior to those reported by Choo et al. (2007), which found values between 0.36 and 0.74 for *p*-AV, about 1.7 - 2.5 for K_{232} and about 0.2 - 0.4 for K_{270}, in oils from seven different varieties of flax seed from New Zealand. No significant differences were observed between the two oil formulations for stability indices, with the exception of *p*-anisidine value.

3.6. Fatty acids profile

Table 3 shows the fatty acid content of linseed oil and of enriched linseed oil (80 mg lycopene/kg). TLE supplementation did not determine significant differences in the acidic profile, with the exception of linoleic acid, whose variation was minimal. Polyunsaturated fatty acids (α-linolenic and linoleic) were predominant in both oils, followed by monounsaturated fatty acids (oleic and vaccenic), while saturated fatty acids (palmitic, stearic and arachic) were scarce. Our fatty acid composition was very similar to the results reported by Shadyro et al. (2017) for linseed oil produced in Belarus and by Anastasiu, Chira, Banu, Ionescu, Stan, and Rosca (2016) in oil from different flax cultivars from Romania. Interestingly, the data reported by Tańska et al. (2016) for commercial linseed oils from the Polish market are lower for polyunsaturated acids, while are superior for palmitic, stearic and oleic acids. Kasote et al. (2013) found lower ALA contents i.e. 46.03% after single press, 47.15% of ALA after double press, and 46.10% after triple press. In fact, Choo et al. (2007) pointed out that an increase in α-linolenic acid was mirrored by a decrease in oleic acid, and the other way around. Differences in oil composition are influenced by genotype, environment and growth conditions. Anastasiu et al. (2016) suggested a strong influence of temperatures and precipitation levels on fatty acids content: in cooler climates the unsaturated acids percentage is greater. The fatty acids composition depends also on

the extraction process: Khattab and Zeitoun (2013) reported higher unsaturated fatty acids concentrations in linseed oil extracted with supercritical CO_2 compared to that obtained by solvent extraction or accelerated extraction with solvent, although the yields were lower.

3.7. Evaluation of antioxidant compounds and antioxidant capacity

Table 3 shows tocopherols, carotenoids, total polyphenol content (TPC) and antioxidant capacity (ABTS method) for both oils: γ-tocopherol was the most abundant homologue in linseed oil (57.15 ± 2.51 mg/100 g), followed by δ-tocopherol (0.78 ± 0.11 mg/100 g), β-tocopherol (0.59 ± 0.00 mg/100 g) and α-tocopherol (0.35 ± 0.00 mg/100 g). The same proportions were found by Khattab and Zeitoun (2013): γ-tocopherol corresponded to 92% of total tocopherols and α-tocopherol was the least abundant. Our contents were similar to those reported by Shadyro et al. (2017; 55.53 ± 2.66 mg/100 g of γ-tocopherol, 0.80 ± 0.03 mg/100 g of δ-tocopherol and 0.78 ± 0.03 mg/100 g of α-tocopherol). Khattab and Zeitoun (2013) instead found lower concentrations of (β+γ)-tocopherol (32.20 mg/100 g) and higher levels of α-tocopherol (0.63 mg/100 g) and δ-tocopherol (1.84 mg/100 g). Choo et al. (2007), in oils from seven flax varieties from New Zealand, reported a much lower content of γ-tocopherol (10.56-15.00 mg/100 g) but did not detect β- and δ-tocopherol, while α-tocopherol ranged between 0 and 0.55 mg/100 g, except for one organic linseed oil (9.11 mg/100 g); the same authors identified another important lipophilic antioxidant compound, plastochromanol-8 (3.42-5.53 mg/100 g). According to Obranović et al. (2015) the large variations in tocopherol content may be due to cropping environment and variety, without forgetting storage time of the already-packaged linseed oil. In fact, storage time and heating decrease the content of tocopherols and vitamin E, promoting fat auto-oxidation (Nykter, Kymäläine, Gates, & Sjöberg, 2006). The quantity of tocopherols in linseed oil does not depend on the extraction method: Khattab and Zeitoun (2013) did not find significant differences in tocopherols content of linseed oil obtained by different extraction techniques (supercritical CO_2,

solvent extraction and accelerated solvent extraction), because the lipophilic tocopherols are easily extracted together with the oil, independently from the extraction medium.

No significant differences for total tocopherols content were found between linseed oil (58.88 ± 2.61 mg/100 g) and linseed oil enriched with TLE (58.91 ± 1.94 mg/100 g); however, α-tocopherol increased from 0.35 ± 0.00 to 1.56 ± 0.02 mg/100 g since it is the predominant form of vitamin E present in tomatoes (Hidalgo, Di Prima, Fongaro, Cappa, & Lucisano, 2017). This increase should not have a significant impact because α-tocopherol represented only 2.6% of the overall tocopherol concentration. Additionally, contrasting results about the antioxidant activity effect of the different homologues on vegetable oils are described (Seppanen, Song, & Saari Csallany, 2010), while Shadyro et al. (2017) reported that the addition of α-tocopherol (200, 500 and 1000 mg/kg oil) did not improve the oxidative stability of linseed oil.

Linseed oil showed relatively small amounts of β-cryptoxanthin, lutein, and zeaxanthin (total carotenoid content: 0.61 mg/100g). A similar concentration (0.7 mg/100 g) was reported by Tuberoso, Kowalczyk, Sarritzu, and Cabras (2007), while Tańska et al. (2016) found higher values (1.21-2.95 mg/100 g) in commercial linseed oil from Poland. According to Tańska et al. (2016), lutein is the most abundant compound. The addition of TLE only led to significant changes in the concentration of lycopene.

Linseed oil presented a total polyphenol content of 56.39 ± 2.73 mg GAE/kg, not significantly different from the oil enriched with TLE (55.16 ± 1.76 mg GAE/kg; **Table 3)**. Kasote et al. (2013), analyzing the effect of single, double and triple press oil extraction on quality of Indian linseed oil, observed TPC contents of 10 mg GAE/100 g after a single press, 25 mg GAE/100 g after a double press and about 24 mg GAE/100 g after a triple press, always lower than our data. Velickovska, Brühl, Mitrev, Mirhosseini, and Matthäus (2015) reported instead a higher content (72.54 mg GAE/kg) in cold-pressed linseed oil from Macedonia. Khattab and Zeitoun (2013) stated that linseed oil has a

relatively low content of phenolic compounds, because these molecules are not directly extracted during cold pressing or solvent extraction but remain in the defatted residue. The same authors reported that linseed oil extracted with supercritical CO_2 and ethanol as co-solvent showed higher phenolic compounds content, because the polar solvent facilitated its extraction from the matrix. TPC is also influenced by seed development, for example Herchi et al. (2011) reported a decrease in linseed oil (from 140.71 to 14.23 mg caffeic acid/kg) during seed maturation. Among the phenolic compounds, Khattab and Zeitoun (2013) identified *p*-hydroxybenzoic, trans-sinapic and trans-*p*-coumaric acids, while Tuberoso et al. (2007) identified only vanillic acid.

The antioxidant capacity (ABTS method; **Table 3**) was always higher in the lipophilic fraction than in the hydrophilic one; in fact, the hydrophilic antioxidant capacity of the oils is mainly due to the phenolic compounds while the lipophilic antioxidant capacity is mostly related to the tocopherols. Tuberoso et al. (2007) found a 1.01 mmol TE/L antioxidant capacity (DPPH method) in linseed oil: 0.91 mmol TE/L were due to the lipophilic fraction and 0.19 mmol TE/L to the hydrophilic fraction, giving a LF/HF ratio (4.79) higher than ours (3.25). The TLE had an antioxidant capacity of 14.46 ± 0.03 (μmol TE/g). With its addition to linseed oil, a significant increase of both hydrophilic and lipophilic fraction was recorded, leading to an overall variation from 2.48 ± 0.08 to 3.39 ± 0.01 μmol TE/g in total antioxidant capacity. Lenucci et al. (2010) mentioned a synergistic antioxidant and health positive effects of lycopene with other natural compounds such as α-tocopherol.

3.8. Rancidity kinetics of oils

Fig. 1 shows the kinetics of FFA, PV, K_{232}, *p*-AV and K_{268} increase in pure oil and in lycopene extract-enriched oil during storage at 40 °C, 50 °C and 60 °C. FFA is an index of oil hydrolysis; PV and K_{232}, which indicate the presence of conjugated dienes, are markers of oil primary oxidation; while *p*-AV and K_{268} are indices of secondary oxidation. The *p*-AV is correlated to the presence of ketones,

while K_{268} of conjugated trienes. All the indices increased during storage as a function of time and temperature, but at 40 °C PV seemed to reach a plateau at the end of the trial; thus, the interpolation of PV was performed excluding the last points of storage. The first-order kinetics best described the oxidation mechanisms that occurred in the oil during storage (r^2 = 0.88-0.99). For all the parameters considered, the rate constant k increased with the temperature, while TLE addition determined their decrease at all the temperatures. For pure linseed oil, the FFA k constant varied between 0.0073 $days^{-1}$ (40 °C) and 0.0168 $days^{-1}$ (60 °C), for PV between 0.0494 $days^{-1}$ (40 °C) and 0.0873 $days^{-1}$ (60 °C), for K_{232} between 0.0084 $days^{-1}$ (40 °C) and 0.0201 $days^{-1}$ (60 °C), for *p*-AV between 0.0300 $days^{-1}$ (40 °C) and 0.0682 $days^{-1}$ (60 °C) and for K_{268} between 0.0100 $days^{-1}$ (40 °C) and 0.0259 $days^{-1}$ (60 °C). The same trend was evident in the enriched oil (80 mg lycopene/kg oil), but the rate constants had slightly lower values: 0.0050-0.0127 $days^{-1}$ for FFA, 0.0395-0.0701 $days^{-1}$ for PV, 0.0057-0.0137 $days^{-1}$ for K_{232}, 0.0183-0.0469 $days^{-1}$ for *p*-AV, and 0.0077-0.0201 $days^{-1}$ for K_{268}. Thus, the addition of TLE to the oil determined a decrease in the reaction rate for all the parameters considered. The rate constant of the reaction was highest for PV formation at all the temperatures in both oils, suggesting that the high degree of unsaturation of linseed oil favors the formation of hydroperoxides more than other deterioration indices. Nevertheless, hydroperoxides are also the most unstable compounds leading to secondary oxidation products, thus reaching the above-mentioned plateau. Li et al. (2011) reported lower PV in soybean oil enriched with 100, 200 and 400 mg lycopene/kg oil than in pure oil during storage at 60 °C for 15 days; Montesano, Cossignani, D'Arco, Simonetti, and Damiani (2006) reported lower PV in extra virgin olive oil with 5 and 10 mg lycopene/kg oil compared to oil without antioxidant stored for 37 weeks in amber bottles and at room temperature. Shadyro et al. (2017) assessed the oxidative stability of linseed oil for twelve months at room temperature after enrichment with ascorbyl palmitate (1% v/v) and bean and soya beans (0.8% v/v); according to the authors, the addition of antioxidants, both natural and synthetic, led to a lower accumulation of acid value, PV, and p-AV

during storage. Kehili, Choura, Zammel, Allouche, and Sayadi (2018) studied the effect of oleoresin tomato peel on the oxidative stability of olive and sunflower oil for 19 weeks at 50 °C, using BHT as a control (200 mg/kg) and found that they had a protective effect on primary oxidation, strongly correlated to the presence of lycopene. The sunflower oil used to extract lycopene from tomato wastes showed a better oxidative stability than the pure oil during storage at 60 °C for 21 days (Rahimi & Mikani, 2019). Zhang, Yang, Zu, Chen, Wang, and Liu (2010) observed the protective effects of carnosic acid addition on sunflower oil during 21 days at 60 °C, and reported significant lower FFA, PV and p-AV than those of the sunflower oil control as well as of oil enriched (200 mg/kg) with synthetic antioxidants (BHT and BHA). Similarly, essential rosemary and peppermint oils delayed FFA, PV, conjugated dienes and trienes formation in pistachio oil stored at 60 °C for 80 days (Esmaeili, Goli, Shirvani, & Shakerardakani, 2018).

The temperature also influenced oil stability: the highest values for the different indices were reached more rapidly with the increase in temperature (**Fig. 1**): it is therefore essential to store oil at low temperatures to prevent the oxidation, which cause quality deterioration and acceptability decrease of the product. The addition of TLE lowered the maximum values reached for the different stability parameters, increasing the stability of the oil and, consequently, the shelf-life of the product.

Fig. 2 reports the Arrhenius plots for all the oxidation indices, with r^2 ranging from 0.94 to 1.00. According to the Arrhenius equation, the frequency factor (A), i.e. the intercept of Arrhenius plot, is one of the main kinetic parametre affecting the rate of the oxidation. The frequency factor of linseed oil was higher than the frequency factor of linseed oil with TLE (**Fig. 2**). Furthermore, for each oxidation parameter the lines that describe the dependence of ln k from 1/T can be considered parallel because the activation energy varies only slightly before and after enrichment and, consequently, the angular coefficients (-Ea/R) of the lines are similar.

Table 4 shows the activation energy (*Ea*) and *z* values calculated from the slopes of the Arrhenius lines (**Fig. 2)** and the shelf-life at 25 °C calculated from the first-order kinetics, reported in **Fig. 1**. A similar activation energy was generally observed for the indices in the oil without and TLE enrichment. Therefore, the addition of TLE did not change the oxidation mechanism, but acted like a scavenger, slowing the phenomenon. On average *Ea* and *z* value were equal to 24.7 kJ/mol and 81.0 °C for the peroxide value, while they ranged from 35.9 to 41.7 kJ/mol and from 47.9 to 56.1 °C for the other four rancidity indices. In general, for all indices *Ea* was relatively low, i.e. less sensitive to temperature than reactions with high *Ea;* this implies that major changes in temperature are required to modify reaction rates (Tan, Che Man, Selamat, & Yusoff, 2001). The slightly lower *Ea* of PV may be related to its higher k constant at each temperature; the high reaction rate makes the oil less sensitive to the temperature change. Solak, Turan, Kurhan, Erge, and Karabulut (2018) reported a value of 29.95 kJ/mol for PV at 80-140 °C and 47.49 kJ/mol for *p*-anisidine at 80-180 °C in hazelnut refined oil. Wang, Hwang, Yoon, and Choe (2010), analyzing the autoxidation of perilla and corn oil between 20 and 80 °C, found instead higher *Ea* values (100.23 kJ/mol and 65.94 kJ/mol) for hydroperoxides formation in perilla and corn oils, respectively.

The shelf-life of the oils at 25 °C was calculated considering as acceptable maximum values 2% oleic acid for FFA, 15 meq O_2/kg for PV, 2.6 for K_{232}, 2 for *p*-AV and 0.25 for K_{268}. The shelf-life of the oil enriched with TLE increased for all the parameters considered; the highest percentage rise was for FFA (542.93 days, i.e. 61.1%), followed by *p*-AV (259.78 days, 56.7%), K_{232} (225.47, 43.5% days), PV (165.6 days, 24.1%) and K_{268} (246.71, 21.9%). Therefore, the average shelf-life increase was 42%. The absolute times were higher than those obtained by the Rancimat method (Table 1). In linseed oils, the high temperatures of the Rancimat method may affect the induction times as a consequence of polymerization, with the liberation of volatile compounds and the possible formation of a dried film

that may limit air access to the sample (Symoniuk, Ratusz, & Krygier, 2016). This phenomenon may thus lead to an underestimation of the shelf-life.

4. Conclusions

The presence of 80 mg lycopene/kg linseed oil led to the same induction time at 110 °C (by Rancimat) obtained using 200 mg/kg of the antioxidant artificial BHT. Significant differences were found between lycopene extract-enriched and pure linseed oil for color. TLE addition significantly increased linseed oil antioxidant capacity (ABTS test) from 2.48 to 3.33 μmol TE/g. During storage, the kinetics of formation of all rancidity parameters were first-order. The rate constant k increased with the temperature, while the addition of TLE determined its decrease at all temperatures. The lines that describe the dependence of the natural logarithm of k with respect to the inverse of the temperature (Arrhenius) were parallel; thus, the addition of TLE did not change the oxidation mechanism but, acting as a scavenger, slowed the phenomenon. In conclusion, the addition of TLE increased the shelf-life of linseed oil by 31% (Rancimat test) and by 42% (stability kinetics during storage).

Conflict of interests

The authors declare no conflict of interests

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Captions to Figures

Fig. 1. Free fatty acids (FFA), peroxide value, K_{232}, *p*-anisidine value and K_{268} during the storage of linseed oil (white symbols) and tomato lycopene extract enriched oil (80 mg lycopene/kg oil; black symbols) at 40 °C (Δ; ▲), 50 °C (□; ■), and 60 °C (○; ●). Curves represent the exponential interpolation (first-order kinetics) while error bars, the standard deviation.

Fig. 2. Arrhenius plots for free fatty acids (FFA), peroxide value, K_{232}, *p*-anisidine value and K_{268} related to the storage of linseed oil (○) and tomato lycopene extract enriched oil (80 mg lycopene/kg oil; ●).

Table 1. Induction time (h ± standard deviation) and shelf-life at 25 °C of different oils, by Rancimat.

	90 °C	100 °C	110 °C	Shelf life (days)
Linseed oil	11.24±0.02[b]	4.07±0.01[b]	2.18±0.05[d]	90.60[b]
Linseed oil + BHT* (200 mg/kg)			2.94±0.03[b]	
Linseed oil + TLE** (40 mg lycopene/kg)			2.65±0.04[c]	
Linseed oil + TLE** (80 mg lycopene/kg)	14.72±0.06[a]	6.40±0.05[a]	2.91±0.07[b]	118.76[a]
Linseed oil + TLE** (120 mg lycopene/kg)			3.06±0.04[a]	

Different letters in the same column or row indicate significant differences among induction times ($p<0.05$) following LSD multiple range test; * butylhydroxytoluene; **tomato lycopene extract

Table 2. Characterisation (mean ± standard deviation) of linseed oil without and with addition of tomato lycopene extract (TLE) at a concentration of 80 mg lycopene/kg oil.

	Linseed oil	Linseed oil + TLE
Moisture	0.52 ± 0.02 [a]	0.61 ± 0.01 [b]
Refractive index	1.481 ± 0.000 [a]	1.480 ± 0.000 [b]
Color coordinates:		
*L**	36.23 ± 0.35 [a]	20.97 ± 0.01 [b]
*a**	-1.40 ± 0.26 [a]	5.21 ± 0.05 [b]
*b**	24.43 ± 0.78 [a]	4.70 ± 0.02 [b]
Free fatty acids (% oleic acid)	0.61 ± 0.01 [a]	0.63 ± 0.01 [a]
Peroxide value (meq O_2/kg)	0.29 ± 0.02 [a]	0.31 ± 0.06 [a]
p-Anisidine value	0.16 ± 0.04 [a]	0.23 ± 0.08 [b]
K_{232}	1.39 ± 0.03 [a]	1.42 ± 0.03 [a]
K_{268}	0.10 ± 0.02 [a]	0.11 ± 0.01 [a]

Different letters in the same row indicate a significant difference among means ($p<0.05$) following t-test.

Table 3. Fatty acid composition, tocopherols, carotenoids, total polyphenol content (TPC) and antioxidant capacity of linseed oil without and with addition of tomato lycopene extract (TLE) at a concentration of 80 mg lycopene/kg oil.

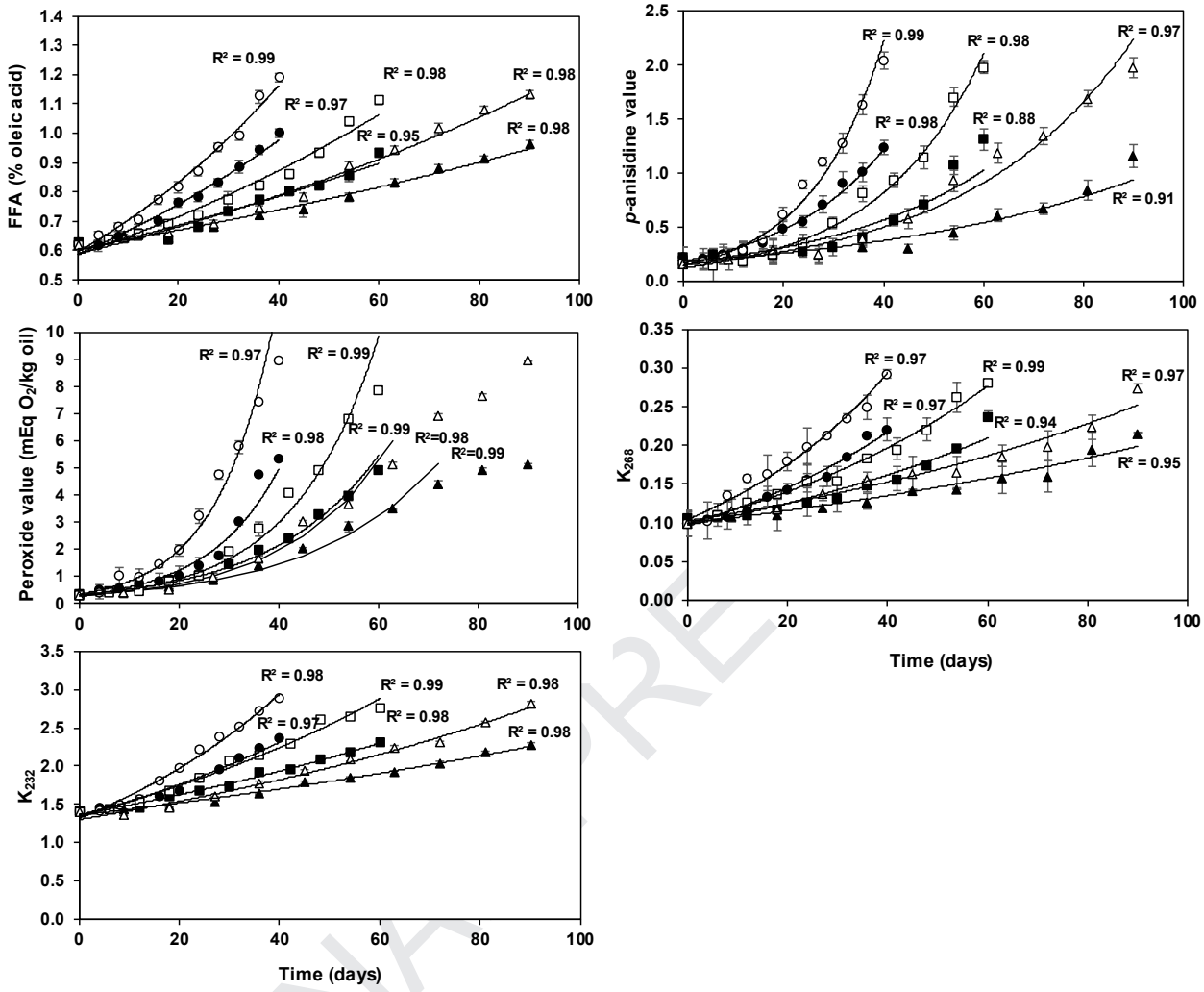
	Linseed oil	Linseed oil + TLE
Fatty acids (%)		
C 16:0 (Palmitic)	4.70 ± 0.06 [a]	4.81 ± 0.04 [a]
C 18:0 (Stearic)	4.14 ± 0.01 [a]	4.10 ± 0.03 [a]
C 18:1 (ω9) (Oleic)	19.12 ± 0.14 [a]	19.26 ± 0.01 [a]
C 18:1 (ω7) (Vaccenic)	0.59 ± 0.10 [a]	0.66 ± 0.00 [a]
C 18:2 (ω6) (Linoleic)	15.40 ± 0.03 [a]	15.74 ± 0.00 [b]
C 18:3 (ω3) (α-linolenic)	55.90 ± 0.25 [a]	55.29 ± 0.01 [a]
C 20:0 (Arachic)	0.15 ± 0.01 [a]	0.14 ± 0.00 [a]
SFA	8.99 ± 0.05 [a]	9.05 ± 0.01 [a]
MUFA	19.71 ± 0.24 [a]	19.92 ± 0.01 [a]
PUFA	71.30 ± 0.28 [a]	71.03 ± 0.01 [a]
α-tocopherol (mg/100 g)	0.35 ± 0.00 [a]	1.56 ± 0.02 [b]
β-tocopherol (mg/100 g)	0.59 ± 0.00 [a]	0.60 ± 0.03 [a]
γ-tocopherol (mg/100 g)	57.15 ± 2.51 [a]	56.08 ± 2.01 [a]
δ-tocopherol (mg/100 g)	0.78 ± 0.11 [a]	0.68 ± 0.03 [a]
β–cryptoxanthin (mg/100 g)	0.03 ± 0.00	0.04 ± 0.00
Lutein (mg/100 g)	0.54 ± 0.01	0.50 ± 0.03
Zeaxanthin (mg/100 g)	0.04 ± 0.00	0.05 ± 0.00
Lycopene (mg/100 g)	nd	8.00 ± 0.00
TPC (mg GAE/kg)	56.39 ± 2.73 [a]	55.16 ± 1.76 [a]
ABTS (μmol TE/g)		
Hydrophilic	0.57 ± 0.04 [a]	0.66 ± 0.01 [b]

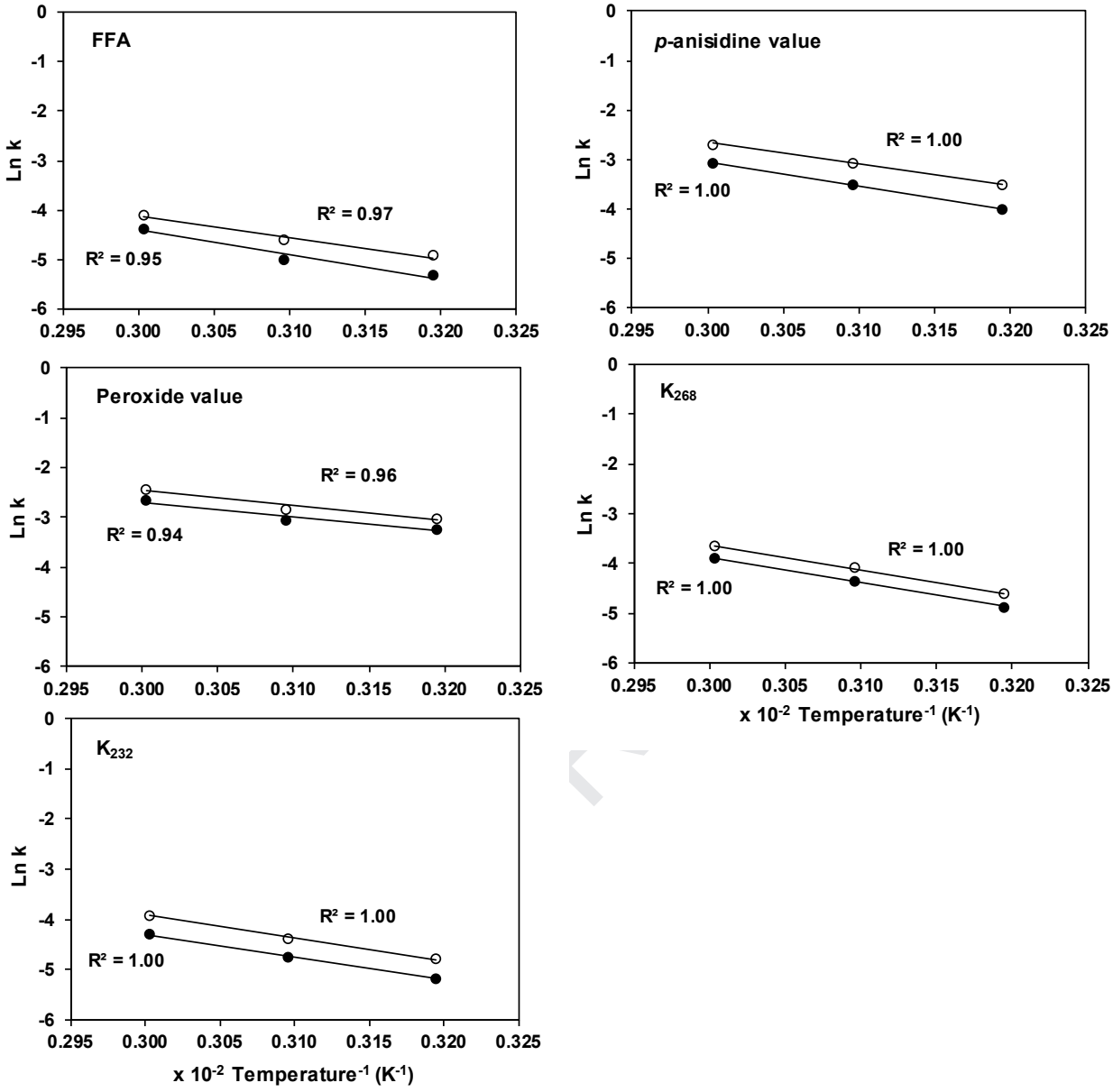
Lipophilic	1.85 ± 0.05 [a]	2.67 ± 0.03 [b]
Total antioxidant capacity	2.48 ± 0.08 [a]	3.39 ± 0.01 [b]

Different letters in the same row indicate a significant difference among means ($p<0.05$) following t-test; not detectable.

Table 4. Activation energy (Ea), z-value and shelf-life at 25 °C (days), for free fatty acids (FFA), peroxide value (PV), K_{232}, *p*-anisidine value (*p*-AV) and K_{268} in linseed oil without and with addition of tomato lycopene extract (TLE) at a concentration of 80 mg lycopene/kg oil.

	Ea (kJ/mol)		z (°C)		Shelf-life (days)	
	Linseed	Linseed + TLE	Linseed	Linseed + TLE	Linseed	Linseed + TLE
FFA	35.9	40.5	55.6	49.3	337.1	542.9
PV	24.5	24.8	81.4	80.6	132.7	165.4
K_{232}	37.8	38.2	52.9	52.4	157.1	225.5
p-AV	35.6	40.8	56.1	49.0	165.8	259.8
K_{268}	41.2	41.7	48.4	47.9	202.4	246.7





Highlights

The effect of tomato lycopene extract (TLE) on linseed oil shelf life was evaluated

80 mg lycopene/kg oil showed the same induction time at 110 °C of 200 mg BHT/kg oil

TLE addition slowed oil degradation without changing the mechanism

Rancidity followed first order kinetics during storage at 40-60 °C until 90 days

TLE increased oil shelf-life at 25 °C by 31% (Rancimat) and 42% (kinetics)