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FULL PAPER

Characterization, Oxidative Stability and Antioxidant Potential of Linseed (*Linum usitatissimum L.*) and Chia (*Salvia hispanica L.*) Oils

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Abstract:

The aim of the present study was to assess the composition and oxidative stability of linseed and chia commercial oils, in addition to determining the kinetics of oxidation at temperatures of 100, 110, 120 and 130°C, as well as the quality parameters, acid value (AV), moisture and ash content. The data of oxidative stability index (OSI), moisture, acid value and ash content were acquired according to the methods: AOCS Cd 12b-92, EN ISO 8534 and AOAC, respectively. The fatty acid composition was assessed by gas chromatography coupled to flame ionization detector (FID). The antioxidant activity was assessed using the method of free radical scavenging of DPPH (2,2-diphenyl-1-picrylhydrazyl) and phenolic compounds using Folin-Ciocalteau reagent. The fatty acids identified in greater amount in the analyzed oils were the unsaturated acids linolenic, linoleic and oleic. Regarding the AV, linseed oil was more acid than chia oil. Chia oil offers better nutritional quality, resulting from the greater amount of unsaturations present in its composition, one of the factors that negatively affected its oxidative stability expressed as OSI. Regarding phenolic compounds and antioxidant potential, chia oil also showed better values, 319.12 mg g^{-1} and 149.57 µg mL^{-1}, respectively. Linseed oil showed better oxidative stability with activation energy (Ea) and acceleration factor Q10 of 82.12 kJ mol^{-1} and 1.92, respectively, determined by kinetic studies for oxidative degradation performed using Rancimat method.

Keywords: antioxidant activity; fatty acids; phenolic compounds; vegetable oils

1. Introduction

The current trend of consumers in acquiring food is associated with well-being and health, resulting in pressure on the food industry for food production with reduced amount of fat, sugar, cholesterol, salt and some additives [1,2], and distinguished by the content of Omega (ω), antioxidants, fiber, vitamins and other components that consuming public recognizes as a healthy contribution to human organism [3].

Edible vegetable oils, in addition to enhance absorption of fat-soluble vitamins and help in the production of hormones, have on their composition essential vitamins, including vitamin E composed of tocopherols and tocotrienols [4]. Although there is a wide range of vegetable oil sources worldwide, consumption is still dominated by palm, soy, canola and sunflower oil [5]. As a result, there is an increase in search for suitable and little explored plant species as sources of edible oils, such as linseed [6] and chia [7,8].

Seeds of linseed (*Linum usitatissimum* L.) and chia (*Salvia hispanica* L.) present high potential for production of edible oils, because they are sources of essential fatty acids such as linolenic acid (ω-3) and linoleic acid (ω-6), and natural antioxidants, with numerous health benefits

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including the prevention of diseases such as cancer, diabetes and Alzheimer's disease, regulate the cholesterol ratio, autoimmune disorders and are sources of iron, which make their oils promising research objects [6-11].

Assessments of physico-chemical characteristics and oxidative stability of linseed and chia oils are important for control and maintenance of quality in the food industry. Thus, the present study aims to assess the composition and oxidative stability of linseed and chia commercial oils, in addition to determining their kinetics of oxidation at temperatures of 100, 110, 120 and 130°C, as well as the quality parameters, acidity index (AI), moisture and ash content

2. Results and Discussion

2.1 Physico-chemical analyses

Table 1 shows the results of fatty acids profile of the vegetable oils of linseed and chia. The fatty acids identified in greater amount in linseed oil were linolenic acid (37.54%), oleic acid (23.26%), linoleic acid (24.63%) and palmitic acid (10.26%). Whereas chia oil presented linolenic acid (45.81%), linoleic acid (19.95%), palmitic acid (12.49%) and oleic acid (10.29%). Linolenic acid was the most abundant in both evaluated oils, however with higher content in chia oil than linseed oil, corroborating the literature data, which describe chia as the richest botanical source in linolenic acid [12]. The literature reports linolenic acid content of up to 57% for linseed [13] and 62.8% for chia [12].

Table 1. Fatty acids profile of edible vegetable oils of linseed (*Linum usitatissimum* L.) and chia (*Salvia hispanica* L.).

Fatty Acids	Vegetable Oils	
	Linseed (%)	Chia (%)
Capric acid **C10:0**	0.03±0.01	nd
Lauric acido **C12:0**	0.14±0.06	nd
Myristic acid **C14:0**	0.14±0.02	0.09±0.00
Pentadecylic **C15:0**	0.05±0.01	0.05±0.00
Palmitic acid **C16:0**	10.26±1.56	12.49±0.45
Margaric acid **C17:0**	0.06±0.00	0.07±0.00
Steraric acid **C18:0**	7.96±1.06	5.84±0.34
Oleic acid **C18:1**	23.26±3.00	10.29±0.69
Linoleic acid **C18:2**	19.95±3.02	24.63±1.04
Linolenic acid **C18:3**	37.54±5.93	45.81±1.30
Beenic acid **C22:0**	0.19±0.00	0.12±0.00
Erucic acid **C22:1**	0.13±0.00	0.08±0.00
Arachidonic acid **C20:4**	0.21±0.01	0.40±0.04
SFA	18.83±2.72	18.66±0.79
MUFA	23.39±3.00	10.37±0.69
PUFA	57.70±8.96	70.84±2.38
PUFA/SFA	3.06	3.79
ω-6/ω-3	0.53	0.54
Cox value	10.40±1.63	12.53±0.25

nd: not detected; SFA: sum of saturated fatty acids; MUFA: sum of monounsaturated fatty acids; PUFA: sum of polyunsaturated fatty acids. ω-6: Linoleic acid; ω-3: Linolenic acid. Results expressed as mean ± S.D. of the three replicates.

The proportions between polyunsaturated and saturated fatty acids (PUFA/SFA) greater than 0.45 are recommended for daily human intake [14]. Thus, the results regarding linseed (3.06) and chia (3.79) oils indicate that these oils have high nutritional quality [15]. In addition, the high ratio between omega 6 and 3 (ω-6/ω-3), formed from linoleic acid and linolenic acid, respectively, is a risk factor for cancer and coronary heart disease. The recommendation is a ratio lower than 4 [15] and the data obtained in this study were 0.53 and 0.54 for linseed and chia oils, respectively.

Based on oxidation rate of fatty acids that compose a vegetable oil it is possible to determine its oxidisability value (Cox) [16]. The Cox value obtained for linseed oil (10.40) was lower than that of chia oil (12.53). Oils with higher value of Cox are more prone to oxidation [17], therefore chia oil are more prone to oxidation than linseed oil.

The results of physico-chemical analyses of

vegetable oils of linseed and chia are presented in Table 2.

Table 2. Physico-chemical characterization of edible vegetable oils of linseed (*Linum usitatissimum* L.) and chia (*Salvia hispanica* L.).

Properties	Vegetable Oils	
	Linseed	Chia
AV (mg KOH g^{-1})	6.82±0.01	3.68±0.01
Moisture (%)	0.09±0.00	0.08±0.00
Ash (%)	0.02±0.00	0.01±0.00
OSI (h)	2.45±0.04	1.24±0.01
DPPH IC_{50} (μg mL^{-1})	160.48±0.50	149.57±0.28
PC (mg g^{-1})	244.65±1.06	319.12±0.12

AV: Acid value; OSI: Oxidative stability; PC: Phenolic compounds. PC (mg g^{-1}). Results expressed as mean ± S.D. of the three replicates.

The AV is one of the important parameters used to demonstrate vegetable oil quality [18]. Linseed and chia oils presented AV of 6.82 and 3.68 mg KOH g^{-1}, moisture index of 0.09 and 0.08% and ash content of 0.02 and 0.01%, respectively. In this study, the acidity value found for linseed oil is above the standards established as maximum by ANVISA [19] and by the Codex Alimentarius [20], which determine maximum values of 4.00 mg KOH g^{-1} for cold-pressed oils. It is noteworthy that the study in question was carried out on commercial oils. The storage of linseed oil in specific may not have adequate control because it is stored in clear glass and the conditions of exposure (shelves) in supermarkets may not be ideal. According to Henrique and Pivaro [6], linseed oil must be protected from light and kept in dark containers to avoid possible changes in its characteristics.

Regarding oxidative stability (OSI) determined at 110°C, linseed oil presented 2.45 h, while chia oil 1.24 h (Figure 1). In studies by Epaminondas et al. [21], linseed oil presented OSI value of 2.17 h, similar to that achieved in this study. Regarding chia oil, studies showed OSI values of 1.41 h [22] and 1.49 h [23], both results similar to that observed in the present study.

Relating the PUFA content in oils of linseed and chia, 57.70% and 70.84% to OSI values, 2.45 h and 1.24 h, and Cox of 10.40% and 12.53%, respectively, it is possible to infer that the higher the PUFA content and Cox percentage, the lower the OSI value, i.e. higher PUFAs content and Cox result in less oxidative stability and consequent shorter shelf life of the oil [17, 24].

The determination of antioxidant activity in relation to the reduction of a radical, such as DPPH (2,2-diphenyl-1- picrylhydrazyl), is one of the methods that can be employed in the assessment of antioxidant potential of an extract or specific substance [25-27]. In this type of analysis, the result can be expressed as IC_{50}, which is the required concentration of antioxidant to reduce in 50% the DPPH radical and the lower the IC_{50}, the higher the antioxidant activity of the material analyzed [28]. Regarding the IC_{50} found in this study, it was possible to observe that chia oil presented better antioxidant activity compared to linseed oil, because it took 149.57 μg mL^{-1} of chia oil to reduce 50% of the DPPH, while for linseed oil 160.48 μg mL^{-1} were required to reduce the same proportion of radical and therefore a lower antioxidant activity.

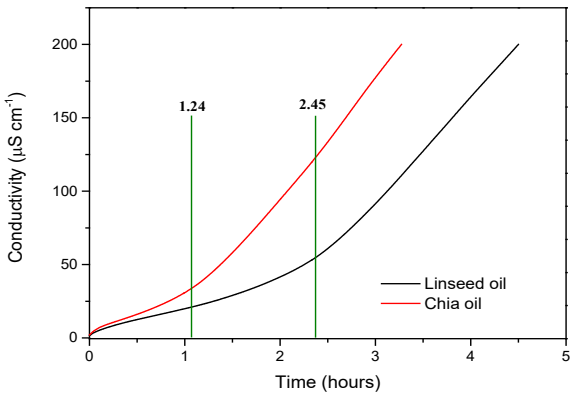

Figure 1. Curves of conductivity versus time for determination of oxidative stability index at 110°C for samples of linseed (*Linum usitatissimum* L.) and chia (*Salvia hispanica* L.) oils.

Phenolic compounds also have antioxidant activity in fats and oils, due to their oxidation-

reduction properties that may play an important role in absorbing and neutralizing free radicals, chelating triplet and singlet oxygen or decomposing peroxides [29]. Chia oil showed higher phenolic compounds content, 319.12 mg g^{-1} compared to linseed oil, which featured 244.65 mg g^{-1} (Table 2). The results obtained in this study indicate that there is a correlation between the highest phenolic compounds content and the greatest antioxidant activity.

2.2. Kinetic studies of oxidative degradation

Analyzing OSI values determined at different temperatures (100, 110, 120 and 130°C) for linseed and chia oils, it was found that these values tend to duplicate with the decrease of temperature in 10°C, as evidenced in Table 3 and Figure 2, corroborating the literature data [23].

Table 3. Determination of oxidative stability index (OSI) at different temperatures of edible vegetable oils of linseed (*Linum usitatissimum* L.) and chia (*Salvia hispanica* L.).

Vegetable Oils	Temperatures (°C)			
	100	110	120	130
Linseed	5.36±0.12	2.45±0.04	1.20±0.02	0.77±0.01
Chia	2.75±0.05	1.24±0.01	0.75±0.01	0.43±0.01

Results expressed as mean ± S.D. of the three replicates.

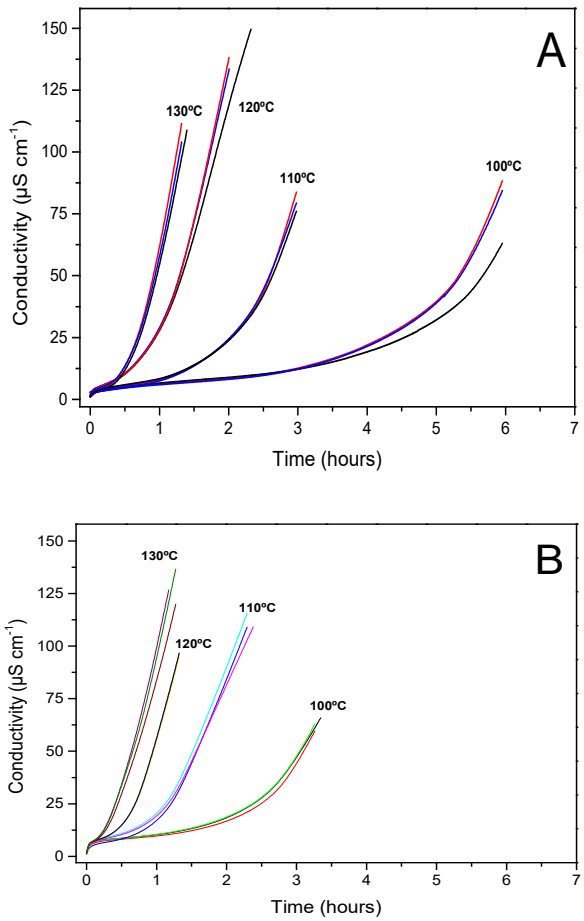

Figure 2. Curves of conductivity versus time for determination of oxidative stability index (OSI) at different temperatures for samples of oils of: **A.** linseed (*Linum usitatissimum* L.) and **B.** chia (*Salvia hispanica* L.).

The values of oxidative stability expressed as OSI of the present study showed that the speed of chemical reactions of degradation tend to duplicate with the increase in temperature of 10°C, as evidenced in studies by Villanueva et al. [23], Farhoosh et al. [30] and Santos et al. [31].

The data regarding activation energy (E_a) of the linseed and chia oils were calculated from the slopes (angular coefficient) of the lines for log *k* graphically represented as a function of the inverse of absolute temperature, using the Arrhenius equation. Using the temperature coefficients (T_C, °C^{-1}), determined from the angular coefficients of the straight line equation obtained by linear regression for the graphs of log OSI vs. temperature, the acceleration factor Q10 was acquired. The linear regressions for determination of E_a and Q10 using the OSI values at different temperatures (100, 110, 120 and 130°C) resulted in straight line graphs with good linear correlation, whose data of R^2 and equation can be seen in Figure 3.

Table 4 presents the determined values of E_a and Q10 number, along with shelf life, obtained by extrapolation of Arrhenius at 25°C, of each oil studied.

E_a is the minimum energy required to perform a chemical reaction. For linseed and chia oils analyzed, the E_a was 82.12 and 75.37 kJ mol^{-1}, respectively. The values mentioned above

showed that linseed oil is more resistant to oxidation in relation to chia oil requiring greater amount of energy to start the process of oxidative degradation. Symoniuk et al. [32] using the pressure differential scanning calorimetry (PDSC) technique in studies with different samples of cold-pressed linseed oil at temperatures from 90 to 140°C obtained E_a values ranging from 93.14 to 94.53 kJ mol^{-1}. Ixtaina et al. [33] and Guitto et al. [34] by Differential Scanning Calorimetry (DSC) and P-DSC techniques, respectively, in studies with chia oil at temperatures from 10 to 350°C obtained values of E_a equal to 69.5 and 71.95 kJ mol^{-1}. Considering variations due to the techniques used (PDSC, DSC or Rancimat) and chemical composition of each oil in relation to the production site, it can be concluded that the values of E_a of linseed and chia oils determined by this study are consistent with data from the literature.

Table 4. Arrhenius parameters, Q10 number and shelf life at 25°C for lipid oxidation of edible vegetable oils of linseed (*Linum usitatissimum* L.) and chia (*Salvia hispanica* L.).

Parameters	Vegetable Oils	
	Linseed	Chia
E_a (kJ mol^{-1})	82.12	75.37
Q10	1.92	1.81
Shelf life (days)*	161	59

*Shelf life calculated by Arrhenius parameter.

Farhoosh et al. [30] showed that E_a values are influenced by the amount of unsaturated bonds present in vegetable oils. Correlating the amount of unsaturations present in the composition of the oils studied with E_a values and shelf life, it can be concluded that the greater the amount of unsaturated fatty acids, the lower the values of E_a, more sensitive to oxidative degradation the oil will be and consequently the lower its expiration date. Relating the values of PUFA (57.70 and 70.84%) and Cox (10.40 and 12.53%) obtained for linseed and chia oils respectively, with their respective shelf life (161 and 59 days), it can be concluded that smaller values of PUFA and Cox lead to a greater expiration date for linseed oil. There have been no studies on the shelf life of linseed and chia oils based on the Arrhenius equation by the Rancimat method.

According to Labuza [35], Q10 number is associated with the ratio between kinetic constants of reaction at temperatures differing by 10°C, i.e., the increase of shelf life of a product resulting from the reduction of temperature in 10°C. For linseed and chia oils, Q10 numbers were 1.92 and 1.81, respectively. These results indicate that the influence on shelf life with a reduction in temperature of 10° C is higher in linseed oil than chia oil. In the study by Symoniuk et al. [32] using PDSC technique with cold-pressed linseed oils at temperatures from 90 to 140°C, the mean value obtained for Q10 number was 2.12. To the best of our knowledge, there are no studies of Q10 number for chia oils. According to Farhoosh et al. [30], in kinetic studies of oxidative degradation, using the Rancimat method with canola, soy, sunflower, corn and olive oils the Q10 number determined were 2.13, 2.18, 2.15, 2.10 and 2.08, respectively. Data from the literature indicate that the Q10 number might vary between 1.5 and 2.5 for vegetable oils and olive oil [35].

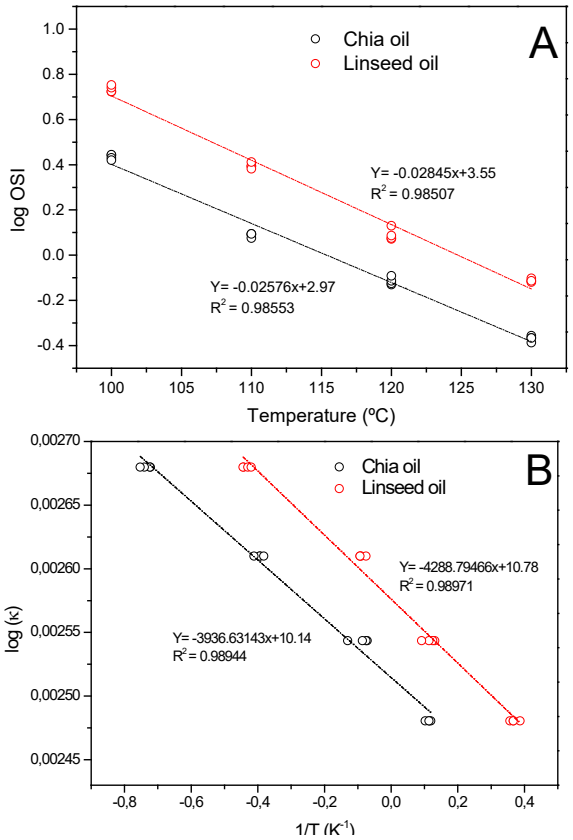

Figure 3. Curves and linear regression data of: **A.** log OSI in function of temperature for determination of acceleration factor Q10 and **B.** log *k* in function of reciprocal of the absolute temperature (1/T) for determination of activation energy (E_a) of edible vegetable oils of linseed (*Linum usitatissimum* L.) and chia (*Salvia hispanica* L.).

3. Material and Methods

3.1. Samples

Chia and linseed extra-virgin cold-pressed oils [36] were purchased commercially in the municipality of Dourados-MS, stored in a cool and dry place at room temperature in absence of light.

3.2. Fatty acids

The fatty acid profile was determined by transesterification of 10 mg of biomass with 0.2 mL of chloroform: methanol (2:1 v/v) and 0.3 mL hydrochloric acid 0.6 mol L^{-1} in methanol heated at 85°C using a dri-block heater for 1 hour [37]. Then, it was performed the extraction of fatty acid methyl esters in 1 mL hexane and subsequent analysis by gas chromatography [38]. Methyl esters were identified using a gas chromatograph, GC-MS (Agilent Technologies, California, EUA), coupled to flame ionization detector (FID), with fused silica capillary column (100 m x 250 µm x 0.2 µm, Supelco SP). The operating parameters were optimized as follows: injector and detector temperature of 260°C, oven temperature of 140°C for 5 minutes, reaching 240°C at a rate of 4°C/min, with total time of analysis of 48 minutes, using helium as carrier gas at flow rate of 1.2 mL min^{-1} and injection volume of 10 µL. For identification, the retention times of fatty acids were compared to those of standard methyl esters (Sigma-Aldrich, St. Louis, MO, EUA). The retention times and peak area percentage were automatically calculated by the Software ChemStation.

The Cox values of the oils were calculated based on the percentage of unsaturated C18 fatty acids, applying the formula [16]:

$$Cox = (1\ (18{:}1\%) + 10.3\ (18{:}2\%) + 21.6\ (18{:}3\%))/100 \quad \text{Equation 1}$$

3.3. Acid Value, moisture and ash content

Acid Value (AV) (expressed as mg KOH g^{-1} of sample) was determined using Potentiometric Titler Titrino Plus 848 (Metrohm, Switzerland), in accordance with the American Oil Chemists' Society (AOCS) recommendations [39].

Moisture percentage was determined by the EN ISO 8534 and ash content in accordance with AOCS [39].

3.4. Oxidative stability

Analyses of oxidative stability for determination of oxidative stability index (OSI) and shelf life of the oils were performed in accordance with standard method AOCS Cd 12b-92 from 3.00 g ± 0.01 g of sample in reaction tubes that were placed in heating blocks of the equipment for analysis of Oxidative Stability (Metrohm, Switzerland), model PROFESSIONAL RANCIMAT 893. Samples were analyzed under constant air flow of 20 L h^{-1}, at temperatures of 100, 110, 120 e 130°C, and their respective volatile products were collected in tubes containing 50 mL distilled and deionized water until reaching 200 μS cm^{-1} conductivity. The products of volatile oxidation were absorbed by the water, resulting in increased conductivity. Water conductivity was monitored in order to determine the OSI, acquired for the time of maximum value of the 2nd derivative of conductivity curve in function of time by the software StabNet.

3.5. Antioxidant activity and phenolic compounds

The antioxidant activity test with the free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) was performed at concentrations of 10, 50, 100, 200, 500 µg/mL. The results were expressed in relation to the percentage of inhibition, which was calculated using the following equation [40]:

$$\%\Delta 0 = 100 \times (A0 - A)/A0 \quad \text{Equation 2}$$

The samples were prepared at a concentration of 100 µg/mL for analysis of phenolic compounds content. For the analysis, it was employed the Folin-Ciocalteau reagent [41]. To calculate the content of phenolic compounds an analytic curve (10, 50, 100, 200, 400 µg/mL) employing gallic acid was used as standard. The analysis was performed in triplicate. The results were expressed as mg of gallic acid per g of sample.

3.6. Analysis of kinetic data from the oxidative stability

The temperature coefficients (Tc, $°C^{-1}$) were determined from the slopes of the log curves (OSI) versus T according to the equation obtained by linear regression [42]:

$$logOSI = aT + b$$ Equation 3

The acceleration factor Q10, which indicates an increase in speed of oxidation reaction when the temperature rises 10°C, was obtained by the equation [42]:

$$Q10 = 10e^{-10Tc}$$ Equation 4

The activation energy (E_a) was calculated from the slopes of lines drawn by linear regression for the graphs of log *k* in function of the inverse or reciprocal of absolute temperature, by the Arrhenius equation [42]:

$$\log k = logA - (\frac{Ea}{2.303RT})$$ Equation 5

4. Conclusions

The observed differences in characterization of vegetable oils analyzed were favorable to chia oil, which presented more than 70.84% of PUFAs, highest percentage of the unsaturated fatty acids linolenic acid (45.81%) and linoleic acid (24.63%) and AV within the standards established by ANVISA and Codex Alimentarius. The high percentage of unsaturated compounds is important from a nutritional point of view to maintain good health, but in this study negatively affected OSI values (1.24 h) at 110ºC and Cox (12.53%), which might indicate greater trend to oxidative degradation implying shorter time for consumption of this oil. In contrast, the linseed oil showed greater oxidative stability with OSI of 2.45 h and Cox of 10.40%. By extrapolation using the Arrhenius equation, in the kinetic study of analyzed oils, the greatest value of shelf life was estimated for the linseed oil with 161 days. In the assessment of antioxidant potential, determined by the phenolic compounds content and DPPH value, chia oil presented the best values, 319.12 mg g^{-1} and 149.57 µg mL^{-1}, respectively.

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