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Kinetics of Lipid Oxidation in Ternary Mixtures of Grape, Sesame and Sunflower Oils by Rancimat Method

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ABSTRACT

The oil extracted from grape seed is a product with excellent antioxidant and nutritional characteristics, which can be used in the food industry. Due to these characteristics, this oil was incorporated, together with the sesame oil, in different proportions, into the sunflower oil, kept at a ratio of 50% in all blends. The pure oils and blends were evaluated for thermo-oxidative stability (oil stability index, OSI), phenolic compounds, DPPH, gas chromatography, oxidation kinetics, enthalpy and entropy, and shelf life (at 25 °C). The Arrhenius equation and activated complex theory were used to estimate the activation energies, enthalpies and entropy of activation, ranging from 80.66 to 90.37 kJ mol^{-1}, 77.84 to 86.61 kJ mol^{-1} and -129.46 at -104.37 J mol^{-1} K, respectively. The pure grape oil and the blends with the highest proportion of these added (B1 and B2) presented the best values in all parameters analyzed. Activation free energy values (ΔG), which ranged from 27.68 to 38.59 kJ mol^{-1}, unified and verified the results of the other kinetic parameters, which indicate a lower lipid oxidation rate for the B1 blend, with a higher proportion of grapes added.

Keywords: Antioxidant; Blends; Foods; Oxidation stability.

RESUMO

O óleo extraído da semente de uva é um produto com excelentes características antioxidantes e nutricionais, que pode ser utilizado na indústria alimentícia. Em virtude dessas características, este óleo foi incorporado, juntamente com o óleo de gergelim, em diferentes proporções, ao óleo de girassol, mantido na proporção de 50% em todas as blendas. Os óleos puros e blendas foram avaliados quanto à estabilidade termo-oxidativa (índice de estabilidade do óleo, OSI), compostos fenólicos, DPPH, cromatografia gasosa, cinética de oxidação, entalpia e entropia, e prazo de validade (a 25 ºC). A equação de Arrhenius e teoria do complexo ativado foram utilizados para estimar as energias de ativação, entalpias e entropias de ativação, que variaram de 80,66 a 90,37 kJ mol^{-1}, 77,84 a 86,61 k mol^{-1} e -129,46 a -104,37 J mol^{-1} K, respectivamente. O óleo de uva puro e as blendas com maior proporção deste adicionado (B1 e B2), apresentaram os melhores valores em todos os parâmetros analisados. Os valores de energia livre de ativação (ΔG), que variaram de 27,68 a 38,59 kJ mol^{-1}, unificou e constatou os resultados dos outros parâmetros cinéticos, que indicam menor taxa de oxidação lipídica para a blenda B1, com maior proporção de uva adicionada.

Palavras-chave: Antioxidante; Misturas; Alimentos; Estabilidade de oxidação

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1 INTRODUCTION

In Brazil, there is a wide range of vegetable oils with potential use in the food industry. Among these, we can mention the sunflower, soybean, linseed, sesame, grape oils, which have high nutritional value and health benefits (Curvelo *et al*. 2011; Kiralan *et al*. 2017; Srivastava *et al*. 2017).

Vegetable oils such as sesame and grape, are gaining more space in the food, cosmetics and/or pharmaceuticals areas. Sesame oil is known for its balanced proportion of omega 6 and omega 9 fatty acids, rich in bioactive lignins (sesamolin, sesamol, sesamin) and tocopherols, which also contribute to the excellent thermo-oxidative stability (Hemalatha, 2007; Mousumi *et al*. 2019). However, according to Ghosh *et al*. (2018), despite acting as natural antioxidants, the tocopherols degrade at high temperatures making the oil more susceptible to the oxidative process.

Grape oil is edible and obtained from seeds and/or bagasse present in wastes from juices and wines production, which contain high concentrations of phenolic compounds such as anthocyanins, which act as natural antioxidants, offering several benefits to human health (Gönül Akin *et al*. 2018), as well bioactives such as essential fatty acids, polyphenols, flavonoids, tannins and phytosterols (Lutterodt *et al*. 2011; Silva *et al*. 2008; Jokic *et al*. 2016). Brazil is one of the major producers of grapes and wines in the world, producing about 1.4 million tons (IBGE, 2018). However, the generation of by-products of this activity has also been rising. The main waste generated are grape bagasse (15% dry, 20-45% wet), grape stalk (2.5-7.5%) and wine lees (3.5-8.5%), which can be reused as raw material in different sectors of industry, such as food, and not disposed of in the environment, which could result in environmental pollution (FAO, 2013).

According to Upadhyay *et al*. (2017), lipid oxidation in edible fats and oils used in cooking (e.g. sunflower oil and soybean oil), rich in omega 6, makes these lipid materials do not meet the thermal stability requirements, resulting in rancidity. This is one of the most important reactions that can reduce their quality. Thus, blending vegetable oils with different properties, is one of the simplest and most effective methods to improve the nutritional properties and increase oxidative stability, thereby reducing, or even, making unnecessary the use of synthetic antioxidants in these blends (Upadhyay e Mishra, 2015).

Since lipid oxidation can lead to deterioration of the oil and its mixtures, to study the kinetics of this reaction is of utmost importance, before making any recommendation on their applications. This study is useful to assess the differences or similarities between oil properties, as well as make a prediction of oxidative stability at different temperature conditions (Farhoosh *et al*. 2008; Upadhyay e Mishra, 2016). Several accelerated oxidation methods such as Sylvester Test, Oxygen Active Method (AOM) or Swift test, schaal oven test, differential scanning calorimetry, among others, have been used to evaluate the resistance to oxidation of oils and fats. However, the Rancimat method is one of the most widely used due to its reproducibility and rapidity the analysis (Silva *et al*. 1998; Farhoosh *et al*. 2008; Mousumi *et al*. 2019).

Therefore, knowing that Brazil is one of the major producers of grapes and wines, grape oil produced from these waste was purchased, to be incorporated along with sesame oil, at different proportions, into sunflower oil, with the aim of presenting an alternative to this type of by-product. It is expected that the mixtures produced are richer in nutritional value than the pure oils, and that they do not require addition of any type of synthetic antioxidants. Thus, oils and blends were assessed regarding thermo-oxidative stability, being subjected to heating at four different temperatures, in the Rancimat test. In order to study the kinetics of lipid oxidation the Arrhenius equation and activated complex theory were used. Pure oils and blends were compared regarding shelf-life, using the oxidisability index (Cox) and fatty acid composition by gas chromatography.

2 MATERIAL AND METHODS

2.1. Materials

Oils: sunflower ($SO_{control}$) (Helianthus annuus L.), sesame ($SEO_{control}$) (Sesamum indicum L.) and grape ($GO_{control}$) (Vitis sp.) were acquired in a natural products company, located in São Paulo, SP. According to the fact sheet, the oils were extracted by cold pressing, followed by filtration and refining. None of the oils contained any kind of antioxidant.

2.2. Preparation of blends

The mixtures used were: Blend 1 (B1) (50% sunflower + 40% grape + 10% sesame); Blend 2 (B2) (50% sunflower + 30% grape + 20% sesame); Blend 3 (B3) (50% sunflower + 25% grape + 25% sesame); Blend 4 (B4) (50% sunflower + 20% grape + 30% sesame) and Blend 5 (B5) (50% sunflower + 10% grape + 40% sesame). The oils were mixed in 250 mL glass bekers and placed under stirring for 5 minutes for homogenization.

2.3. Phenolic compounds and antioxidant activity (DPPH)

For determination of phenolic compounds content, the samples were prepared at the concentration of 100 μg mL^{-1} of Folin-Ciocalteau reagent (98-99%, Sigma-Aldrich). An analytical curve (1; 5; 10; 15; 30; 40 μg) employing gallic acid was used as reference standard (Djeridane *et al*. 2006).

The antioxidant activity test with the free radical DPPH (2,2-diphenyl-1-picrylhydrazyl - 98-99%, Sigma-Aldrich) was performed with samples at concentrations of 10-1000 μg mL^{-1} (Kumaran & Karunakaran, 2006).

The results were expressed in relation to the percentage of inhibition, which was calculated using the following equation:

$$(\%\Delta 0) = 100 \text{ x} \frac{A0 - A}{A0} \qquad \text{(Eq. 1)}$$

where (% Δ0) is the percentage of elimination, A0 is the absorbance of the reference solution and A is the absorbance of sample after 30 min of reaction. With the data obtained, a linear regression was performed and the IC_{50} was calculated. Samples were prepared as triplicates for both analyses.

2.4. Assessment of thermal stability

2.4.1. Rancimat test

According to the official standard of the American Oil Chemists' Society (AOCS Official Method Cd 12b-92), in the Rancimat test the oil should be at a temperature of 373.15 K under constant air flow of 20 L h^{-1}. In addition to this official method temperature, the temperatures of 383.15, 393.15 and 403.15 K were added under saturated air (20 L h^{-1}) for the kinetic study. The analysis was performed using model 893, Metrohm, Switzerland and oxidation stability expressed as oil stability index (OSI) in hours. OSI values, which correspond to the time before rapid deterioration of the oil, were determined from the maximum of the second derivative of the conductivity curve versus time, by software StabNet (Metrohm) installed on the computer with the interface equipment.

2.5. Gas chromatography (GC-FID)

The fatty acid was esterified and profile analysis was performed employing a gas chromatograph Shimadzu GC 17, with flame ionization detector. Chromatographic separation was performed using a fused silica column Restek RTx-5MS (30 m long x 0.25 mm internal diameter, 0.25 µm film thickness), with injection volume of 1 µL of sample in split mode (1:20), with helium as carrier gas (5 mL min^{-1}), and heating rate beginning at 130 ºC for 1 minute, rising to 170 °C at 6.5 °C min^{-1}, from 170 to 215 °C at flow rate of 2.75 °C min^{-1} kept for 12 minutes, and finally from 215 to 230 ºC at 40 ºC min^{-1} The injector temperature was 220 °C. Identification of methyl esters of fatty acids was performed by comparison with the retention times of standard fatty acids: C6:0; C8:0; C10:0; C12:0; C14:0; C16:0; C16:1; C18:0; C18:1; C18:2; C18:3; C20:0; C20:1; C22:0; C22:1; C24:0 (98-99%, Sigma-Aldrich).

The value of oxidisability index (COX) of the oils was calculated based on the percentage of unsaturated fatty acids C18, applying the equation:

$$\text{Cox} = \frac{1(18:1\%) + 10.3\,(18:2\%) + 21.6\,(18:3\%)}{100}$$

(Eq. 2)

2.6. Kinetics of oxidation

The kinetic constant of reaction (k) for lipid oxidation of oils was estimated as the inverse of OSI (k = 1/OSI, h^{-1}) (Upadhyay e Mishra, 2016). Using the Arrhenius equation (Eq. (3)), the values of activation energy (Ea, kJ mol^{-1}) and frequency factors (A, h^{-1}) for lipid oxidation in oils were calculated using:

$$\log(k) = \log(A) - (\frac{\text{Ea}}{RT})$$

(Eq. 3)

Where k is the kinetic constant (h^{-1}) and R is the ideal gas constant (8.314 J mol^{-1} K).

Using the activated complex theory, the values of enthalpy (ΔH) and entropy (ΔS) of activation for lipid oxidation of oils, were calculated using:

$$\log\left(\frac{k}{T}\right) = \log\left(\frac{k\text{B}}{h}\right) + \left(\frac{\Delta S}{R}\right) - \left(\frac{\Delta H}{RT}\right)$$

(Eq. 4)

where k_B is Boltzmann's constant (1.380×10^{-23} J K^{-1}) and h is the Planck constant (1.841×10^{-37} J h^{-1}). The ΔH and ΔS were calculated from the slope and intercept of the straight line (Eq. 4).

For a reaction at a certain temperature, the free energy of activation (ΔG KJ mol^{-1}), was obtained from the values of ΔH and ΔS by the equation:

$$\Delta\text{G} = \Delta\text{H} - \text{T}\Delta\text{S}$$

(Eq. 5)

2.7. Estimation of shelf-life

The shelf-life of oils was estimated by tracing the natural logarithm of OSI vs. absolute T (T) using Eq. (6). Semi-logarithmic graphs were extrapolated up to 25 °C to estimate the shelf-life (SL_{25}).

$$\log(OSI) = a\,(T) + b \qquad \text{(Eq. 6)}$$

where a and b are the slope and the linear coefficient of straight line (Eq. 6), respectively. The temperature coefficients (Tc, $°C^{-1}$) were determined from the slopes of logarithmic curves (OSI) versus T according to the equation obtained by linear regression (Eq. 6). The acceleration factor Q_{10}, indicating an increase in speed of oxidation reaction every 10 °C increase in temperature, was obtained by the equation (Eq. 7):

$$Q_{10} = 10^{-10Tc} \qquad \text{(Eq. 7)}$$

3 RESULTS AND DISCUSSION

3.1. OSI, phenolic compounds and DPPH

The lowest OSI values were found in $SO_{control}$, at the four temperatures tested. In blends, these values are relatively higher according to the increase in proportion of grape oil incorporated (Table 1). There was no significant difference between blends B1 and B2, in which the proportion of grape oil is greater, however, OSI values decreased in B5, whose proportion of grape oil is lower, being significant at the temperature of 373.15 K compared to the other four blends at the same temperature. At the temperature of 403.15 K, the highest OSI values were from $GO_{control}$ and B1.

The phenolic compounds showed potential action as antioxidants, and their values in sunflower, grape and sesame oils were 267.54 ± 1.03, 275.68 ± 0.81, and 173.59 ± 1.03 mg g^{-1}, respectively, being higher in grape oil. In blends, the values were proportional to the lower or greater amount of grape oil incorporated, being significantly higher in blends B1 and B2 compared to B5, with minor percentage of grape oil. There was no significant difference between blends B3, B4 and B5.

Table 1 - Values of oil stability index (OSI), phenolic compounds and DPPH of different samples

Oils	OSI (h)		Phenolic compounds (mg g^{-1})		DPPH IC$_{50}$ (µg mL^{-1})	
	373.15 K	383.15 K	393.15 K	403.15 K		
SO$_{control}$	11.24 ± 0.01^{d}	5.55 ± 0.01^{d}	2.46 ± 0.01^{d}	1.47 ± 0.01^{d}	267.54 ± 1.03^{b}	47.96 ± 0.07
GO$_{control}$	15.96 ± 0.03^{a}	7.54 ± 0.04^{a}	3.68 ± 0.02^{a}	1.82 ± 0.03^{a}	275.68 ± 0.81^{a}	43.71 ± 0.06
SEO$_{control}$	14.23 ± 0.14^{b}	6.71 ± 0.04^{b}	3.29 ± 0.03^{b}	1.64 ± 0.03^{b}	173.59 ± 1.03^{d}	49.02 ± 0.12
B1	12.65 ± 0.12^{c}	6.08 ± 0.01^{c}	2.99 ± 0.05^{c}	1.88 ± 0.04^{c}	183.54 ± 0.46^{c}	45.73 ± 0.07
B2	12.63 ± 0.15^{c}	6.02 ± 0.01^{c}	3.00 ± 0.04^{c}	1.57 ± 0.01^{c}	159.57 ± 0.33^{e}	46.21 ± 0.17
B3	12.62 ± 0.13^{c}	6.03 ± 0.08^{c}	3.02 ± 0.01^{c}	1.55 ± 0.02^{c}	152.80 ± 0.25^{f}	47.59 ± 0.51
B4	12.60 ± 0.10^{c}	6.03 ± 0.03^{c}	2.95 ± 0.01^{c}	1.53 ± 0.01^{c}	151.29 ± 0.15^{f}	49.17 ± 0.20
B5	11.52 ± 0.18^{d}	5.98 ± 0.10^{c}	2.94 ± 0.02^{c}	1.52 ± 0.01^{c}	149.97 ± 0.09^{f}	49.63 ± 0.16

Values of OSI and phenolic compounds. Mean and standard deviation followed by the same lowercase letters in columns do not differ from each other. Tukey test, $p < 0.05$.

The tocopherols, present in high amounts in sunflower oil, are well known for acting as natural antioxidants, however, degrade rapidly under high temperatures (Ghosh *et al*. 2018), which might explain the lowest OSI values at the four different temperatures compared to GO$_{control}$ and SEO$_{control}$.

The highest values of OSI in GO$_{control}$ and its rise in blends compared to SO$_{control}$, in which the proportion of 50% was fixed, can be explained by the presence of minor compounds in grape oil such as vitamin E, phytosterols and phenolic compounds, indicated by some authors as important antioxidants, especially at high concentration of γ-tocotrienol isomer of vitamin E, rarely found in other vegetable oils (Fernandes *et al*. 2013; Madawala *et al*. 2012; Mathaus, 2008). Ionizable hydrogens in its structure are donated to free radicals formed in the beginning of the oxidative process, stopping the oxidation mechanism (Ramalho & Jorge, 2006).

Assessing the performance of sesame oil, despite being rich in bioactive compounds (sesamolin, sesamol, sesamin), which also contribute to excellent thermo-oxidative stability (Hemalatha, 2007), and presenting high values of OSI compared to SO$_{control}$, by increasing its ratio (40%) in blend B5 the OSI value rose to 11.52 ± 0.18 at the temperature of 373.15 K, with no significant difference between both. This can be explained by the presence of tocopherols in sunflower oil, which as previously mentioned, degrades rapidlly at high temperatures; due to the reduction in 10% of the amount of grape oil incorporated, as well as the lower value of phenolic compounds in $SEO_{control}$, 173.59 ± 1.03, compared to $SO_{control}$ and $GO_{control}$.

Regarding DPPH, all samples showed good antioxidant activity, but the lowest values were observed in $GO_{control}$ and B1, 43.71 ± 0.02 ($\mu g\ mL^{-1}$) and 45.73 ± 0.01 ($\mu g\ mL^{-1}$), respectively. Similar to those reported by Fernandes *et al*. (2013) studying different grape varieties. The lower the IC_{50}, the greater the ability to capture free radicals formed in the beginning of the oxidative process.

Sunil *et al*. (2015) investigated the mixture of sunflower oil with peanut, palm, refined rice bran, sesame and coconut oils, containing natural antioxidants and observed that these mixtures indicated greater stability to oxidation. In other studies such as Abdel-Razek & Hassanein (2011), the mixtures analyzed by the Rancimat method were with sunflower, soybean and olive oils. These mixtures showed OSI between 9.67 and 16.3 (h) in binary mixtures, which are similar to those found in this study.

3.2. Fatty acids composition

The initial characteristics of the vegetable oils studied are summarized in Table 2. $GO_{control}$ showed higher percentage of saturated fatty acids (SFA), with significant difference compared to $SEO_{control}$. Regarding SFA% of blends, there was no significant difference between $GO_{control}$, $SO_{control}$ and B1, but there was significant difference between $GO_{control}$ and B2, B3, B4, and B5. Due to high levels of oleic acid (18:1), the sunflower oil, sesame oil, blends B4 and B5 showed the highest percentages of mono-unsaturated fatty acids (MUFA), being statistically significant in all samples, except between $SO_{control}$ and B4. The MUFA% in sunflower oil and B4 were similar, however, much higher than in grape oil, B1, B2 and B3. There was no significant difference between the percentages of polyunsaturated fatty acids (PUFA) of grape oil, B1, B2 and B3, however, significantly higher than the sunflower and sesame oils, B4 and B5.

From the information described above, there was no significant difference between samples regarding PUFA/SFA.

It is important to highlight that the thermal stability of MUFAs is greater than PUFAs (Ramadan, 2013). The proportion of MUFA/PUFA is a measure of the tendency of oils to suffer auto-oxidation. Values of PUFAs (omega 3 and omega 6) were balanced between grape oil, B1, B2 and B3, which indicates greater protection of these fatty acids omega against thermal variations, as reported by Devarajan *et al*. (2016). According to these same authors, a balanced diet in MUFA/PUFA provides beneficial effect, decreasing the risk of degenerative diseases, especially heart disease and diabetes. The Agência Nacional de Vigilância Sanitária (ANVISA) and American Heart Association recommend consuming edible fats with ratio SFA/MUFA of 1:1 (Abdel-razek *et al*. 2011), therefore, the mixtures B1 and B2 are close to the recommended.

Regarding COX values, calculated based on the percentage of C:18 fatty acids, there was no significant difference between sesame oil and blend B5, with the lowest values, indicating they are less prone to oxidation, however, lower content of natural antioxidants (phenolic compounds) 173.59 ± 1.03 mg g^{-1} and 149.97 ± 0.09 mg g^{-1}, respectively. Despite the other oils and blends present COX values just above those found in sesame and blend B5, these are lower than the observed by Farhoosh *et al*. (2009) in soybean oil, 6.81, indicating that the oils and blends of this study are less prone the oxidation than soybean oil, the most commercialized in Brazil.

The acidity value (AV), measured from the free fatty acids (FFA) content in vegetable oils is considered one of the main parameters of quality and state of degradation. In this study, the acidity values are below those established by Agência Nacional de Vigilância Sanitária (ANVISA) and Codex Alimentarius, which set maximum acidity value of 0.6 mg KOH g^{-1} for cold pressed and refined oils.

Table 2 - Initial characteristics and fatty acids composition of samples of vegetable oils and their mixtures

Fatty acids (%)	SO	GO	SEO	B1	B2	B3	B4	B5
C6:0	-	0.4	-	0.2	0.2	0.1	0.1	0.1
C8:0	-	0.8	-	0.4	0.2	0.2	0.2	0.1
C10:0	-	0.5	-	0.3	0.3	0.3	0.3	0.1
C12:0	0.1	0.1	-	0.1	0.1	0.1	0.1	0.1
C14:0	0.2	0.2	0.1	0.2	0.2	0.2	0.2	0.2
C16:0	7.0	8.5	8.2	8.3	8.2	8.1	7.8	7.8
C16:1	0.2	0.2	0.1	0.2	0.2	0.2	0.2	0.2
C18:0	7.0	4.9	5.1	4.7	4.7	4.7	4.7	4.9
C18:1	25.8	14.7	37.5	18.6	19.8	21.8	26.3	29.1
C18:2	57.1	58.2	46.8	58.4	57.9	57.5	55.1	52.4
C18:3	0.3	1.3	0.3	1.3	1.2	1.2	0.9	0.7
C20:0	0.3	0.3	0.4	0.3	0.2	0.2	0.2	0.2
C20:1	0.4	0.1	0.3	0.1	0.1	0.1	0.1	0.1
C22:0	0.5	0.3	0.3	0.3	0.2	0.2	0.2	0.2
C22:1	0.3	-	-	-	-	-	-	-
C24:0	0.2	0.3	0.3	0.3	0.2	0.2	0.2	0.2
SFA	15.3^{a}	16.3^{a}	14.4^{b}	15.1^{a}	14.5^{b}	14.3^{b}	14.2^{b}	13.9^{b}
MUFA	26.7^{b}	15.0^{h}	37.9^{a}	18.9^{g}	20.1^{f}	22.1^{e}	26.6b^{d}	29.4^{c}
PUFA	57.4^{b}	59.5^{a}	47.1^{c}	59.7^{a}	59.1^{a}	58.7^{a}	56.0^{d}	53.1^{e}
SFA/MUFA	0.57^{c}	1.08^{a}	0.37^{e}	0.79^{b}	0.72^{b}	0.64^{c}	0.53^{d}	0.47^{d}
MUFA/PUFA	0.46	0.25	0.80	0.31	0.34	0.37	0.47	0.56
PUFA/SFA	3.75^{a}	3.65^{a}	3.27^{a}	3.95^{a}	4.07^{a}	4.10^{a}	3.94^{a}	3.82^{a}
Acidity value AV (mg KOH g^{-1})	0.17	0.08	0.47	0.08	0.22	0.15	0.14	0.14
Acidity in oleic acid (%)	0.08	0.04	0.23	0.04	0.11	0.07	0.07	0.07
COX	6.20^{b}	6.42^{a}	5.26^{c}	6.48^{a}	6.42^{a}	6.39^{a}	6.13^{b}	5.83^{c}

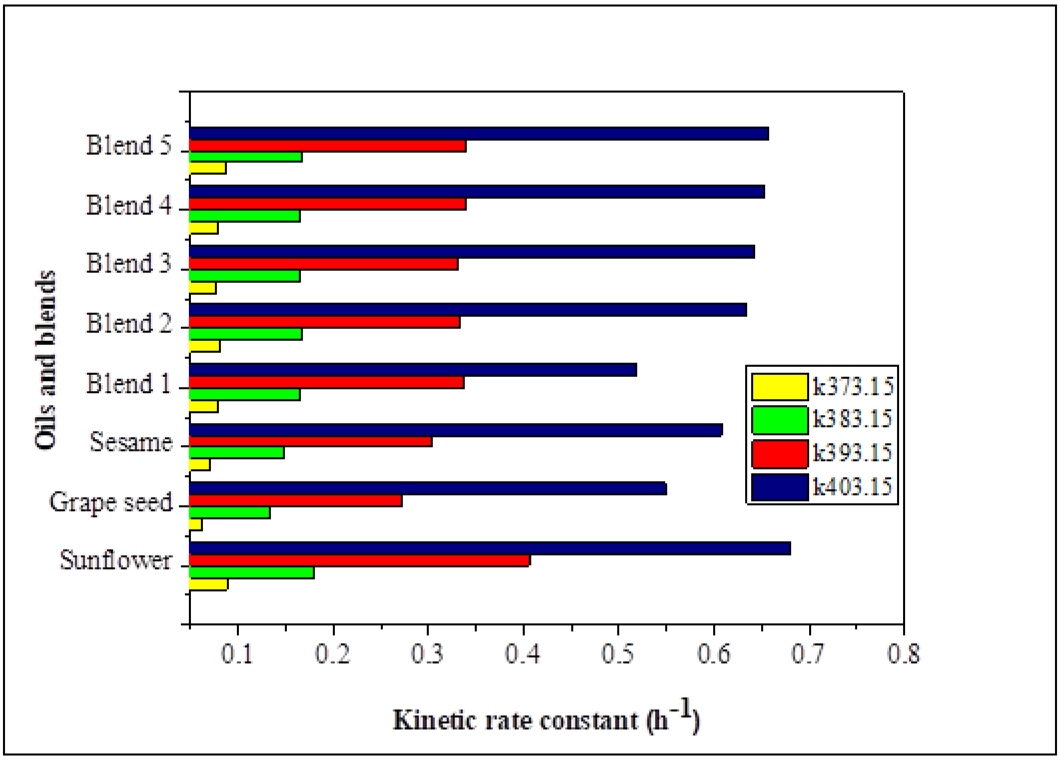
3.3. Kinetic study

A growing trend in k was observed in function of the employed temperatures (373.15 - 403.15 K) ($p < 0.05$) (Figure 1). High values of k indicate rapid lipid oxidation and, based on these values, the speed at which oxidation occurred in $SO_{control}$ was higher than in all other oils and blends at all temperatures. These observations can be associated with the presence of tocopherols in the chemical composition of $SO_{control}$, which degrade more rapidly when subjected to high temperatures (Ghosh *et al*. 2018).

Among the oils, the lipid oxidation rate is: $SO_{control}$ > $SEO_{control}$ > $GO_{control}$. Between blends, B5 showed higher speed on oxidation rate, at the temperatures of 373.15 and 403.15 K. On the other hand, blend B1, at the highest temperature (403.15

K), showed the lowest lipid oxidation rate, indicating that the proportion of oils used in B1 led to a thermal superiority. This result reflects the highest amount of phenolic compounds of B1 (183.54 mg g^{-1}), which strongly act as natural antioxidants, and DPPH (45.73 µ g mL^{-1}), since the lower the IC$_{50}$, the greater the ability to capture free radicals formed in the beginning of the oxidative process. Studies such as those of Farhoosh *et al*. (2008) with canola, soybean, sunflower, corn and olive oils, and Farhoosh *et al*. (2009) with canola and soybean oils, also indicated the influence of the amount of phenolic compounds on oxidation stability. The soybean oil from the above-mentioned study, showed high levels of tocopherols, however, the high amount of phenolic compounds in canola oil (124.0 mg g^{-1}) led to a greater thermal stability.

Figure 1 - Kinetic constant (k) of lipid oxidation of oils and blends, obtained by the Rancimat method.



The other kinetic parameters of lipid oxidation, such as Ea which is the least amount of energy that must be overcome in order for a chemical reaction to occur (Hashemi *et al*. 2016), is affected by the degree of poli-unsaturation present in vegetable oils. According to Adhvaryu *et al*. (2000), high contents of PUFA (linoleic acid and linolenic acid) decrease the value of Ea and a high content of oleic acid increases this value. This statement explain the Ea values found in B1 and B2, 80.66 kJ mol^{-1} and 86.39 kJ mol^{-1}, respectively. The energy of activation ranged from 80.66 to 90.37 kJ mol^{-1} (Table 3), which correlated well with previously reported values for some oils by Farhoosh *et al*. (2008) (86.86 kJ mol^{-1} for olive oil, 88.14 kJ mol^{-1} for corn oil and 90.44 kJ mol^{-1} for sunflower oil) and Farhoosh *et al*. (2014) (94.25 kJ mol^{-1} for semi-virgin oil).

Lower values of Ea indicate greater propensity to lipid oxidation, i.e. the higher the activation energy, the more stable to oxidation will be the oil when subjected to high temperatures (Galvan *et al*., 2013; Hashemi *et al*., 2016). Therefore, the effect of temperature on oxidation rate was greater in blends B1, B2, B4 and B5, 80.66, 86.39, 87.99 and 87.26 kJ mol^{-1}, respectively, with no significant difference between B3, B4 and B5. For pure oils, the effect of temperature on oxidation rate is: $SO_{control}$ > $GO_{control}$ > $SEO_{control}$, with no significant difference between $GO_{control}$ and $SEO_{control}$.

According to Farhoosh & Hoseini-Yazdi (2014), both Ea and A, obtained by the Arrhenius equation, can be used as parameters of oxidative stability. Values of A ranged from 1.36 x 10^{12} to 9.91 x 10^{12}. Ghosh *et al*. (2018) report that small changes in Ea resulted in considerable changes in A, which happened in this study. This means that oxidation, at different temperatures, affected more the values of A than the Ea on the lipid oxidation rate of oils and blends studied, especially for $SO_{control}$ (9.91 x 10^{12}) and B5 (6.42 x 10^{12}).

The effect of temperature on oxidation rate of vegetable oils was also evidenced by the numbers of Q_{10}. In general, a larger number of Q_{10} indicates that a lower temperature change was necessary to induce certain change in oxidation rate. Table 3 shows that the number of Q10 ranged from 1.91 (B1) to 2.09 ($SO_{control}$), while Tc ranged from -2.78 x 10^{-2} (B1) to -3.22 x 10^{-2} ($SO_{control}$), indicating a slower oxidation rate for B1, followed by B2, B3, B4 and B5, $GO_{control}$, $SE_{control}$ and $SO_{control}$, in ascending order of lipid oxidation rate. It should be emphasized that although the values of Ea for blend B1 are smaller, indicating greater propensity to oxidation, the Q10 indicated slow oxidation rate, justifying the other parameters and confirming the action of the natural antioxidants, present in higher amounts in this blend compared to the others.

Q_{10} values were lower than those found in the study of Farhoosh e Hoseini-Yazdi (2014) on different samples of olive oil, which ranged from 2.12 to 2.35, and similar to those found by Reidarpour e Farhoosh (2018) on a mixture of olive oil with sunflower oil (2.05). The value of 2.01 for blend B5 corroborates with the statement of Ghosh *et al*. (2018) on the rapid degradation of tocopherols under high temperatures, which is the case of sesame oil, present in higher amounts in this blend.

The enthalpy and entropy thermodynamic parameters (ΔH and ΔS) for lipid oxidation are also shown in Table 3. According to the activated complex theory, reagent molecules, before being subjected to reaction, must form a complex that is in thermodynamic equilibrium with the molecules of the reagents. Lower values of ΔH and ΔS represent slower oxidation rates (Farhoosh & Hoseini-Yazdi, 2014). ΔH and ΔS of all samples ranged from 77.84 (B1) to 86.61 kJ mol^{-1} ($SEO_{control}$) and from -129.46 J mol^{-1} K (B1) to -104.37 ($GO_{control}$), respectively. The positive sign of ΔH reflects the endothermic nature of the formation of activated complex, showing that the reaction rate increases with temperature. Negative values of ΔS show the formation of an activated complex, which is desirable because it reflects greater stability to oxidation (Upadhyay e Mishra, 2016) and its lower values, as observed in B1, indicate that a smaller amount of molecules is involved in the activated complex, resulting in less potential to oxidation reaction and therefore a slower rate.

The study of Farhoosh and collaborators (2008) on pure olive oil, reported the value ΔS of (-116.66 J mol^{-1} K), close to that found in B1. On the other hand, in the study by Ghosh *et al*. (2018) in binary mixtures of sunflower oil with sesame oil, the values of ΔS varied from -35.58 to -4.81 J mol^{-1} K, which leads us to conclude that the values found in the ternary mixtures, in our study, showed a slower lipid oxidation rate.

Table 3 - Arrhenius parameter, enthalpies (ΔH) and entropies (ΔS) of activation and shelf-life (OSI_{25}) of oils and blends by Rancimat

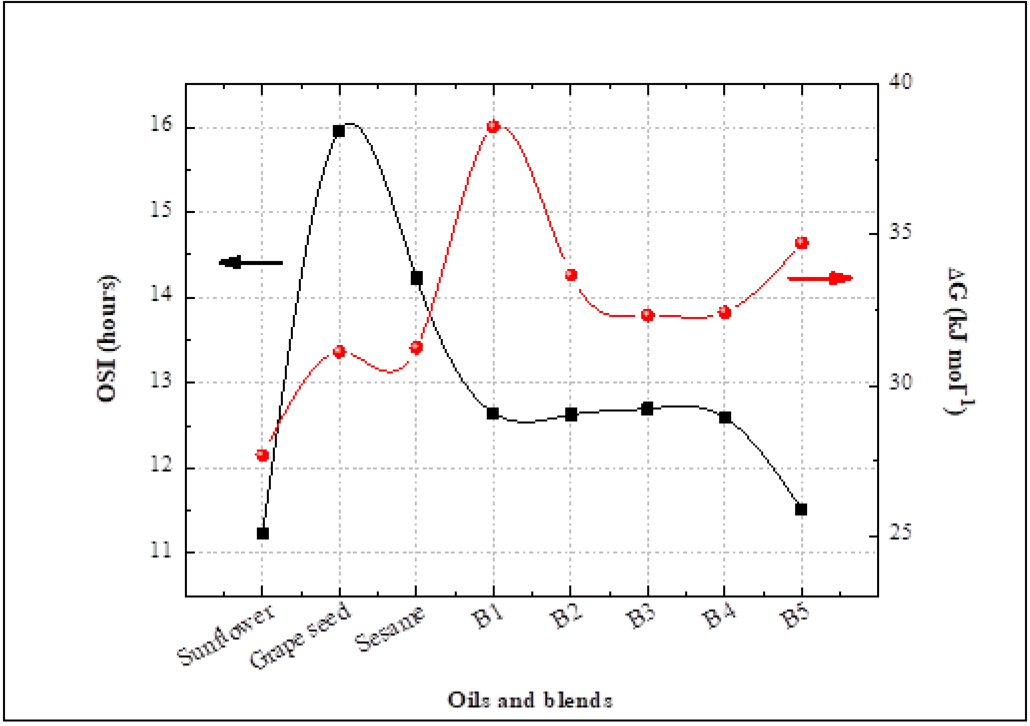
Oils SO:GO:SEO	log(k) = log(A) − (Ea/RT)			log(k/T) = log(k_B/h) + (ΔS/R) − (ΔH/RT)			log(OSI) = a (T) + b		
	A x 10^{12} (h^{-1})	Ea (kJ mol^{-1})	R^2	ΔH (kJ mol^{-1})	ΔS (J mol^{-1} K)	R^2	Tc x 10^{-2} (K^{-1})	Q_{10}	OSI_{25} (months)
$SO_{control}$	9.91^a	86.58^a	0.98	83.36^c	-92.79^a	0.99	-3.22^d	2.09^a	2.4
$GO_{control}$	2.77^b	89.83^c	0.99	87.15^e	-104.37^b	0.99	-3.14^c	2.04^a	4.68
$SEO_{control}$	2.69^b	90.37^c	0.99	86.61^e	-104.82^b	0.99	-3.12^c	2.05^a	4.08
B1	1.36^c	80.66^d	0.99	77.84^a	-129.46^f	0.99	-2.78^a	1.91^d	3.24
B2	1.58^c	86.39^a	0.98	83.17^c	-112.92^d	0.99	-3.00^b	1.99^{bc}	2.88
B3	1.70^c	88.15^b	0.99	84.93^d	-108.44^c	0.98	-3.06^b	1.99^{bc}	2.88
B4	1.63^c	87.99^b	0.99	84.76^d	-108.74^c	0.99	-3.05^b	2.00^b	2.58
B5	2.60^b	87.26^b	0.98	81.66^b	-116.51^e	0.99	-2.94^a	2.01^{bc}	2.52

Means followed by the same lowercase letters in columns, for each kinetic parameter, do not differ. Tukey test, $p < 0.05$.

Regarding the free energy of activation (ΔG kJ mol^{-1}), the higher its value the slower the reaction speed and the greater the stability to oxidation at certain temperature. As the official standard for oils (AOCS Official Method Cd 12b-92) establishes the value of 373.15 K, in the Rancimat, a graph of ΔG and OSI values was performed at constant temperature of 373.15 K (Figure 2).

The ΔG of the activated complexes, formed during samples oxidation, showed that the best stability values of blend B1 was better explained by ΔS than by ΔH. According to Adhvaryu *et al*. (2000), the Ea or ΔH change more in function of variations in oleic structure, with major changes in A, than in the presence of antioxidants. According to Farhoosh e Hoseini-Yazdi (2014), antioxidants decrease formation of the activated complex that affects the ΔS (-61.73 J mol^{-1} K on blend 1), because they provide hydrogen, available in their structure, to free radicals formed during the oxidation process. Therefore, the oxidative stability of oils and blends is more affected by the presence to a greater or lesser amount of tocopherols and/or phenolic compounds than by fatty acids composition.

Figure 2 - Values of free energy of activation and OSI (h) of oils and blends at constant temperature of 373.15 K.



3.4. Shelf-life

Estimating the shelf-life of oils at room temperature (25 °C) can be time consuming. Farhoosh (2007), Farhoosh & Hoseini-Yazdi (2013) and Ghosh (2018) reported that lipid oxidation rates vary exponentially with temperature, as well as their expiration date, which can be foreseen, within hours, using Rancimat and extrapolating the values of OSI to temperatures (e.g. 25 °C). The blends showed higher OSI_{25} than $SO_{control}$ and followed the trend observed in the kinetic parameters (Table 3), mainly blend B1, whose proportion of grape oil and results of phenolic compounds were higher (Table 1).

Shelf-life of blend B5 (2.52 months), whose proportion of sesame oil is higher, was similar to $SO_{control}$ (2.4 months). These values in B5 can be assigned to the least amount of phenolic compounds in $SEO_{control}$ (173.59 mg g^{-1}) compared to $GO_{control}$ (275.68 mg g^{-1}). Despite blends B4 and B5 present shelf-life values similar to that of $SO_{control}$, all blends indicate that oil mixtures were more stable and better than sunflower oil ($SO_{control}$) alone, that is, less prone to oxidation.

Kochhar & Henry (2009) extrapolated the OSI values for different culinary vegetable oils, however, at the temperature of 20 °C and using in the Rancimat only the temperatures of 100, 110 and 120 °C. For pure oils of almond and grape, the shelf-life were 3.1 and 3.2 months, respectively, similar to those found for blends B2 and B3, and smaller than those found for $GO_{control}$ and $SEO_{control}$ (4.68 and 4.08 months, respectively). For walnut oil, the authors reported shelf-life of 0.6 months, much smaller than any value of our study. It is important to highlight the authors did not mention whether the studied oils had antioxidant additives or not, in addition, they extrapolated the values at a lower temperature (20 °C), indicating that the shelf-life of this study are greater, since they were extrapolated at 25 ºC.

4 CONCLUSION

The blends prepared with different vegetable oils, provided more stable mixtures than the oils alone, especially compared to sunflower oil. Using the Rancimat test, it was possible to compare the stability to oxidation of different samples and conclude that the grape oil and blends with higher proportion of this oil (B1 and B2), showed the best values in all parameters analyzed. It is interesting to observe that data regarding free energy and formation of activated complex unified the other kinetic parameters, which indicated lower lipid oxidation rate in blend B1, with higher proportion of grape oil; and that monitoring lipid oxidation does not show clear data using only OSI (h) values or only Ea values, but the unification of multiple parameters as shown in this study does. The kinetic studies demonstrated that oxidative stability in blends is more affected by the presence, to a greater or lesser amount of natural antioxidants, such as phenolic compounds, than by the fatty acids composition. Grape oil, extracted from seeds, is a viable alternative that can be used to increase the stability to oxidation, which results in more stable mixtures, without requiring the addition of antioxidants, and rich in nutrients.

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