ATIVIDADE ANTIOXIDANTE DO EXTRATO DE SEMENTE DE UVA EM ÓLEO DE SOJA

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RESUMO: O óleo de soja (SO) tem baixa estabilidade oxidativa, sendo uma das principais causas de deterioração deste produto. Por outro lado, extratos de semente de uva (GSE) tem compostos bioativos com atividade antioxidante. Portanto, o objetivo deste trabalho foi investigar o efeito antioxidante de um novo GSE em SO, preparado com etanol, acetona e água. Amostras de SO com GSE ou/e BHT (butil-hidróxitolueno) ou sozinho foram submetidos a 60ºC / 12 dias e a degradação foi monitorada pelos índices de acidez, peróxido, dienos e trienos conjugados, TBARS, ácidos graxos (FA), DSC e ATR-FTIR. SO com GSE ou/e BHT não apresentaram diferença significativa

para dienos, trienos e peróxidos. GSE e GSE+BHT apresentou menor degradação de FA do SO. Grupos funcionais identificados por ATR-FTIR demonstraram similaridade entre SO com BHT e SO com GSE nas bandas em 2853, 1742 e 1160 cm-1. Outras bandas apresentaram mudanças muito pequenas com variações menores que 0,5%. Análises por DSC apresentaram que a presença de antioxidantes é significante. O tempo de vida de prateleira do SO foi de 894 dias para GSE+BHT, 44 dias para SO com BHT e 74 dias para GSE com GSE. Portanto, GSE pode substituir parte do BHT em SO para protegê-lo contra oxidação, estendendo sua vida de prateleira.
PALAVRAS-CHAVE: *Vitis labrusca*, estabilidade oxidativa, sub-produtos de vinho.

GRAPE SEED EXTRACT ANTIOXIDANT ACTIVITY IN SOYBEAN OIL

ABSTRACT: Soybean oil (SO) has low oxidative stability, being one of the main causes of the deterioration of this product. On the other hand, grape seed extracts (GSE) have bioactive compounds with antioxidant activity. So, the purpose of this work was to investigate the antioxidant effect of a new GSE in SO prepared with ethanol, acetone, and water. Samples of SO with GSE or/and BHT (hydroxytoluene butyl) or alone were submitted to 60°C/12 days and degradation was monitored by acidity, peroxide index, conjugated dienes and trienes, TBARS, fatty acids (FA), DSC and ATR-FTIR. SO with GSE or/and BHT presented no difference for dienes, trienes and peroxides. GSE and GSE+BHT showed lower SO degradation of FA. Identified functional groups at ATR-FTIR demonstrated similarity between SO with BHT and SO with GSE in bands at 2853, 1742, and 1160 cm-1. Other bands showed very small changes with variations lower than 0.5%. Analysis by DSC showed that the presence of antioxidants is significant. The SO shelf life was 894 days for GSE+BHT, 44 days for SO with BHT, and 74 days for SO with GSE. So, GSE can substitute part of BHT in SO to protect it against oxidation, extending its shelf life.
KEYWORDS: *Vitis labrusca*, oxidative stability, wine by-products.

INTRODUCTION

Agribusiness produces huge amounts of solid and liquid waste, which is the result of the transformation process into food consumption products but represents a growing problem with negative effects on the economy and environment (Lucarini et al., 2018). The by-products (skins and seeds) of the grape processing industry are potential sources of phenolic compounds and other bioactives (Lucarini et al., 2018). They can avoid the oxidation process in the human body or in food bases (Unusan, 2020). Grape seed extracts have been applied as natural antioxidant, preservative, fungicide in health foods and food packaging (Chen et al., 2020). The use of ethanolic grape seed extract has already been used to prevent soybean oil oxidation (Hegazy & Abdel-Maksoud, 2016). However, extracts prepared by a solvent mixture of ethanol, acetone and water has demonstrated higher antioxidant capacity than extracts prepared by single solvent or a binary mixture (Dalposso et al., 2022).

By the other way, the oxidation of oils and fats is the main deteriorating reaction occurring in food processing and storage (Umeda & Jorge, 2021). The chemical composition changes significantly during oil storage, affecting its shelf life and sensory properties (Ghanbari et al., 2018; Ghanbari et al., 2019). The use of synthetic antioxidants concerns consumers because of their security, what is increasing the demand for natural antioxidants. So, the use of grape seed extract with high antioxidant capacity in soybean oil can be a promising alternative to prevent lipid oxidation and develop a product with beneficial health characteristics.

To evaluate the oxidation of oils and fats, many techniques can be used (Fadda et al., 2022). Fourier Transform Infrared Spectroscopy (FTIR) is known for being a fast, non-destructive, solvent-free and easy technique and has been developed for several applications as in the analysis of edible oils and fats (Daoud et al., 2019). Differential exploratory calorimetry (DSC) has also proved to be a technique that can be applied for studies of auto-oxidation of natural or synthetic products (Ostrowska-Ligęza et al., 2010). In this way, FTIR and DSC together with another commonly used techniques can contribute to evaluate the efficiency of a natural antioxidant to avoid the oxidation of soybean oil.

So, this work aimed to investigate the antioxidative effect of a new grape seed extract (GSE), prepared by a mixture of ethanol, acetone and water, in soybean oil. Comparing between GSE and the synthetic antioxidant hydroxytoluene butyl (BHT), as well as its synergistic effect under accelerated conditions of thermal stress has been investigated. The oxidative degradation of soybean oil was monitored through analysis of acidity index and peroxides, conjugated dienes and trienes, substances reactive to thiobarbituric acid - TBARS, major fatty acids (FA) and response of functional groups of samples through infrared spectra (ATR-FTIR). Besides that, mathematical modeling to evaluate the kinetic data obtained by differential exploratory calorimetry (DSC) and to obtain the shelf life prediction was also performed.

MATERIALS AND METHODS

Sample, reagents and standard

The grape seed of the cultivar Bordô (*Vitis labrusca*) was obtained from a local winery, vacuum packed and stored -20 °C under light protection. The edible refined soybean oil (4 L) was purchased from local stores. The soybean oil composition in 13 mL was of 0 g of carbohydrates, 0 g of proteins, 12 g of total fat and 1.2 mg of vitamin E.

The reagents were of analytical grade and standards DPPH, Trolox, ferrous sulphate, ABTS [2,2' - azinobis- (3-ethylbenzothiazolin-6-sulfonic acid)], ethylenediaminetetraacetic acid (EDTA), ferrozine, gallic acid, quercetin, trans- resveratrol, 1,1,3,3-tetraetoxypropane, nonadecanoic acid (C19:0), FAME MIX C4 - C24 189-19, from Sigma-Aldrich brand.

Grape seed extract (GSE) and oil samples

GSE was prepared according to previous study (Dalposso et al., 2022), that resulted in a great antioxidant activity. Seeds were grinding, sieved at 20 mesh and weighed in Erlenmeyers (10.0000 ± 0.0001g). The solvent mixture of ethanol, acetone and distilled water (48:14:38) (v/v) was added, the Erlenmeyers were sealed with parafilm and extracted at 45°C, protected from light, in orbital agitator, for 4 hours at 250 rpm. The extracts were filtered and their solvents were evaporated at 45°C under reduced pressure. After deep-frozen at -80 °C and freeze-dried (-50°C, 150 - 200mmHg), GSE was stored at - 18 °C. The antioxidant activity of GSE prepared is shown in Table 1.

Analysis	GSE
DPPH (μmol TE g^{-1} sample)	1300.00 ± 0.00
ABTS (μmol TE g^{-1} sample)	1054.00 ± 0.01
FRAP (μmol EFeSO4 g^{-1} sample)	5290.00 ± 0.06
Phenolic Compounds (mg GAE g^{-1} sample)	240.80 ± 0.04
Flavonoids (mg EQ g^{-1} sample)	14.60 ± 0.00
Chelating Ability (%)	60.89 ± 2.50
Trans – resveratrol (mg L^{-1})	1.74 ± 0.04

Analyses performed in triplicate.

Table 1: Antioxidant activity of grape seed extract (GSE)

GSE and BHT were diluted in ethyl alcohol 99.5 % (v/v) and added to soybean oil (SO) at a final concentration of 0.02 % (200 mg Kg-1). The percentage of added antioxidant was determined taking into account previous studies and according to the limit of 200 ppm, which appears in the current legislation (Brasil, 1998). The purpose of this study was to compare the synergism between a natural and a synthetic antioxidant. Four sets of samples were prepared in 15 mL tubes, 8.0000 ± 0.0001 g each, coated with aluminum foil and capped, in triplicate, being them: pure soybean oil, SO with BHT, SO with GSE, SO with GSE+BHT. All samples were incubated in an oven at 60 ± 5°C and analyzed over periods of 0, 3, 6, 9 and 12 days, consisting of the induced oxidation time, as in the Schaal test (Cottica et al., 2019). Analysis of acidity index, peroxide index, conjugated dienes and trienes, TBARS, FA, FTIR and DSC were performed.

Monitoring of oxidation indicators

For acidity index, samples were homogenized and weighed 2.0000 ± 0.0001 g in 125 mL erlenmeyer flask. 25 mL of alcohol-ether solution (2:1) was added, two drops of phenolphthalein and titrated with KOH 0.01 mol L-1 until the pink coloration was fixed for 30 seconds, according to the Instituto Adolfo Lutz (2008) with adaptations.

For peroxide index, samples were homogenized, weighed 2.0000 ± 0.0001 g in a 125 mL erlenmeyer flask, where 12 mL of 3:2 acetic-chloroformic acid solution was added and homogenized. 0.2 mL of potassium iodide (KI) saturated solution was added and let stand for 1 min under light. It was added 12 mL of distilled water and it was initiated a titration with 0.01 mol L-1 sodium thiosulfate until the yellow coloration disappeared. In sequence, 0.2 mL of starch indicator solution was added and titration with thiosulfate continued until the blue complex disappeared. Lastly, a white one was prepared and the analyses were performed in triplicate, according to Instituto Adolfo Lutz (2008) with adaptations.

The test for dienes and trienes brings clarity of the bonds broken during the alteration, even the alteration is significant throughout the study. The samples were homogenized and weighed about 20.0 ± 0.1 mg in a test tube and added 10 mL of iso-octane:isopropanol solution (2:1 v/v) and mixed for 30 seconds in vortex. The samples were centrifuged at 4500 rpm and the absorbance of the supernatant read in 232 nm (dienes) and 266 nm (trienes) in UV-VIS spectrophotometer (PG Instruments Ltda, Model T 80+). The concentration of dienes and trienes was calculated by the molar absorptivity of linoleic acid (ℰ = 26000) and its molar mass (280 g mol-1), and the values expressed in mg g-1 of lipids, according to Kiokias et al. (2006), with adaptations.

The monitoring of thiobarbituric acid reactive compounds - TBARS was performed according to Ke & Woyewoda (1979). The oxidized samples (0, 3, 6, 9 and 12 days) were homogenized, weighed about 25.0 ± 0.1 mg in tube and added 5 mL of 2-Tiobarbituric acid solution - TBA, composed of TBA solution (0.04 mol^{-1}), chloroform and Na2SO3 solution (0.3 mol L^{-1}) (12 mL:8 mL:1 mL), prepared 30 minutes in advance. Along with the oil, homogenization was performed for 15 seconds and the tubes were incubated in a water bath for 45 min at 95 °C, followed by cooling. Added 2.5 mL of trichloroacetic acid solution (0.28 mol L-1) was homogenized and centrifuged for 10 min at 2500 rpm. The absorbance of the aqueous phase at 538 nm was measured in a spectrophotometer (PG Instruments Ltda, Model T 80+) against the white. 1,1,3,3-tetraetoxypropane (0,1 mmol L-1) was used as analytical standard to construct the calibration curve (R2 = 0,9995) and to express the results.

The fatty acid methyl esters (FAME) of oxidized samples were prepared in triplicate according to Hartman and Lago (1973), using the nonandecanoic acid methyl ester (C19:0) (1.031 mg mL-1) as internal standard. Chromatographic analysis was performed in Perkin Elmer (Clarus 680) gas chromatograph, coupled with flame ionization detector (CG-FID) and Elite-Wax fused silica capillary column (60 m long, 0.25 mm internal diameter and 0.25 µm film coating). The column was programmed at an initial temperature of 140 °C to 4°C min-1 to 180 °C for 10 min with a second ramp of 10 °C to 240 °C maintained for 12 min. The H2 drag gas flow rate was 1.20 mL min^{-1}, the flame composed of synthetic air and hydrogen at a ratio of 400:40 mL min-1, with an injection volume of 1 μL and the sample split ratio (Split) was 1:100, with injector temperature of 220 °C and detector at 245 °C. The

peak areas were determined by TotalChrom 6.3.2 software and identified by comparison of the retention time with pattern mix containing fatty acid methyl esters (FAME MIX C4 - C24 189- 19, Sigma-Aldrich) and comparison with FAME esters from avocado, flaxseed, canola, and grape oil through their majority FAs, following individual analysis and by pattern addition (spiking). The quantification of the FA was done according to equation 1. The results are expressed in mg g-1.

$$C_x = (A_x \cdot M_{19:0} \cdot FCT) / (A1_{9:0} \cdot M_A \cdot F_{CEA}) \quad (1)$$

Being:

C_x = concentration of fatty acid x in mg g^{-1} lipid.

A_x = area of the methyl esters corresponding to fatty acid X.

$M_{19:0}$ = mass of the internal standard (mg).

F_{CT} = theoretical correction factor: C16:0 (1.0546), C18:0 (1.0347), C18:1 (1.0207), C18:2 (1.0277) and C18:3 (1.0137).

$A_{19:0}$ = area of the internal standard.

M_A = mass in grams.

F_{CEA} = conversion factor from FAME (fatty acid methyl ester) to FA (fatty acid): C16:0 (1.0547), C18:0 (1.0493), C18:1 (1.0500), C18:2 (1.0496) and C18:3 (1.0504).

The mid-infrared spectra were obtained in FTIR Spectrum 65 (Perkin Elmer) spectrophotometer with total attenuated reflectance accessory (ATR) in transmittance mode. Spectral range used was 4000 to 620 cm-1, with resolution of 4 cm-1 and 40 scans Before the acquisition, each spectrum was cleaned with cotton and isopropyl alcohol and the background signal was corrected, 200 µL of the samples were added to the ATR module crystal and the spectra were obtained in triplicate, using the medium spectrum for the comparative analysis of the treatments, according to Nurwahidah et al. (2019) with adaptations.

The oxidative induction time (OIT) analysis was performed in a simultaneous thermal analyzer (STA 6000 PerkinElmer), calibrated with indium, using 10 microliters of sample in an open platinum crucible. The isotherms were performed in 383, 393, 403 and 413 K, using oxidizing atmosphere (O2 - White Martins S.A., 99.99 % purity) with 50 cm^3 min^{-1} flow. The calorimetric curve data were used to predict the useful life of the samples not oxidized at 20 °C, calculated by linear regression of log t0 versus T in Kelvin. The parameters of Arrhenius, E_a (Activation energy) and A (pre-exponential factor), were obtained by the linear regression of the log plotted by ln (k) vs. 1/T adapted from Aktar & Adal (2019) and the Arrhenius equation (2):

$$ln\,(k) = ln\,A - \frac{E_a}{RT} \quad (2)$$

Being:

k = Constant reaction rate or oxidation time.

A = Pre-exponential factor or frequency factor (h-1).

E_a = Activation energy (kJ mol^{-1}).

R = Molar gas constant (8.314510 J K-1 mol-1)

Statistical analysis

The results were structured in a split-plot factorial design with the samples evaluating the presence of GSE, BHT and their combination in the first level and time (0, 3, 6, 9 and 12 days), being the second level divided into subplots, evaluated by the Tukey test and by analysis of variance (ANOVA) with a 5% significance level, through the Statistica program, version 10.

RESULTS AND DISCUSSION

The GSE of this study presented higher antioxidant activity (Table 1) than the one from previous study of extraction optimization from *Vitis labrusca* seeds (Dalposso et al., 2022). The bioactive compounds responsible for the antioxidant activity of GSE (Lucarini et al., 2018) can act as protectors against soybean oil oxidation during its storage. Regarding that, some indicators of oxidation of soybean oil was investigated.

The cumulative evaluation of each oxidation parameter (Table 2) was determined by the Δt (difference between the time of greatest accumulation (Time 9) and the initial time). Soybean oil samples with GSE presented higher value for acidity index. For the dienes parameters, the oil with BHT and with GSE+BHT showed no significant difference ($P>0.05$), being the two treatments with better response. For conjugated trienes there was no significant difference between the 4 treatments. The three samples supplemented also did not present significant difference between them for the formation of peroxides, with an equivalent capacity for protection between the both of them. The data presented by Kehili et al. (2018) pointed out that to obtain an equivalence between the two applied compounds (BHT and oleoresin), it was necessary to work with a concentration of 1000 μg g-1, which corresponds to 0.2% versus 0.02 % for BHT, which is 10x higher than the concentration applied in this research for GSE.

The time and temperature relation (Table 2) started to influence significantly from the third point of analysis (Time 6). Significant difference between treatments is present in Time 9, where the oil with GSE presented higher acidity index (0.35 mg KOH g-1), followed by the oil sample with GSE+BHT (0.31 mg KOH g-1), without significant difference between them. For the oil with BHT in Time 9 it is evident the action of BHT by the reduction of the acidity index until the end of 12 days. An increase in acidity was also reported by Guo et al. (2016) in sample supplemented with rosemary extract.

Analysis	Sample	Exposure time at 60 ± 5 °C (days)					
		0	3	6	9	12	Time Variation (Δt = t9 – t0)
Acidity Index (mgKOH g^{-1})	Soybean oil	0.18 ± 0.01^{aA}	0.20 ± 0.02^{aA}	0.23 ± 0.01^{abA}	0.26 ± 0.01^{bA}	0.29 ± 0.03^{bA}	0.08 ± 0.01^{a}
	BHT	0.18 ± 0.01^{aA}	0.20 ± 0.03^{abA}	0.23 ± 0.04^{abA}	0.24 ± 0.01^{abA}	0.25 ± 0.00^{bA}	0.06 ± 0.02^{a}
	GSE	0.19 ± 0.02^{aA}	0.20 ± 0.03^{aA}	0.24 ± 0.03^{aA}	0.35 ± 0.04^{bB}	0.25 ± 0.00^{abA}	0.16 ± 0.04^{b}
	GSE+BHT	0.19 ± 0.02^{aA}	0.19 ± 0.00^{aA}	0.24 ± 0.03^{abA}	0.31 ± 0.04^{bAB}	0.28 ± 0.01^{bA}	0.12 ± 0.03^{ab}
Peroxide Index (mEqO2Kg^{-1})	Soybean oil	1.49 ± 0.02^{aA}	2.76 ± 0.14^{aA}	9.33 ± 0.54^{abA}	39.35 ± 1.05^{cA}	35.62 ± 2.90^{cC}	37.85 ± 1.04^{a}
	BHT	1.48 ± 0.02^{aA}	2.99 ± 0.02^{aA}	8.65 ± 0.41^{bA}	30.40 ± 2.94^{dBC}	24.11 ± 1.72^{cA}	28.90 ± 2.94^{b}
	GSE	1.50 ± 0.01^{abA}	3.20 ± 0.32^{abcA}	7.92 ± 0.71^{bcA}	34.24 ± 0.60^{dB}	30.80 ± 0.82^{dB}	32.73 ± 0.60^{b}
	GSE+BHT	1.48 ± 0.01^{abA}	2.46 ± 0.05^{abcA}	6.17 ± 0.33^{bcA}	29.23 ± 2.43^{cD}	25.16 ± 1.10^{dA}	27.73 ± 2.43^{b}
Conjugated Dienes (mg g^{-1})	Soybean oil	3.46 ± 0.23^{aA}	4.19 ± 0.25^{abA}	5.27 ± 0.31^{bA}	9.65 ± 0.23^{dA}	6.87 ± 0.48^{cA}	6.15 ± 0.23^{a}
	BHT	3.32 ± 0.49^{aA}	3.58 ± 0.11^{aA}	7.60 ± 0.45^{cB}	6.48 ± 0.29^{bcB}	5.80 ± 0.12^{bA}	3.18 ± 0.30^{bc}
	GSE	3.30 ± 0.12^{aA}	3.60 ± 0.14^{aA}	6.77 ± 0.01^{bB}	7.35 ± 0.64^{bB}	6.73 ± 0.08^{bA}	4.05 ± 0.64^{b}
	GSE+BHT	3.88 ± 0.44^{aA}	4.08 ± 0.19^{aA}	6.10 ± 0.52^{bB}	6.48 ± 0.12^{bB}	6.52 ± 0.50^{bA}	2.58 ± 0.12^{c}
Conjugated Trienes (mg g^{-1})	Soybean oil	1.81 ± 0.10^{aA}	2.32 ± 0.18^{abA}	2.43 ± 0.12^{bA}	3.11 ± 0.31^{cA}	2.32 ± 0.20^{abA}	1.31 ± 0.31^{a}
	BHT	2.07 ± 0.10^{aA}	2.08 ± 0.03^{aA}	3.15 ± 0.30^{bBC}	2.99 ± 0.30^{bAB}	2.12 ± 0.10^{aA}	0.90 ± 0.27^{a}
	GSE	1.99 ± 0.04^{aA}	2.14 ± 0.10^{aA}	3.72 ± 0.04^{cB}	2.80 ± 0.22^{bAB}	2.14 ± 0.03^{aA}	0.76 ± 0.29^{a}
	GSE+BHT	1.98 ± 0.24^{abA}	2.31 ± 0.10^{abcA}	2.74 ± 0.01^{bcAC}	2.52 ± 0.13^{bcB}	2.13 ± 0.20^{abA}	0.52 ± 0.13^{a}
TBARS (μM TBARS g^{-1} Oil)	Soybean oil	ND	ND	0.04 ± 0.01^{aA}	0.15 ± 0.03^{bA}	0.10 ± 0.00^{abA}	0.15 ± 0.03^{a}
	BHT	ND	ND	0.03 ± 0.01^{aA}	0.04 ± 0.01^{abB}	0.10 ± 0.01^{bA}	0.04 ± 0.01^{c}
	GSE	ND	ND	0.03 ± 0.01^{aA}	0.06 ± 0.02^{aBC}	0.14 ± 0.00^{bA}	0.06 ± 0.02^{bc}
	GSE+BHT	ND	ND	0.02 ± 0.01^{aA}	0.12 ± 0.01^{bAC}	0.14 ± 0.01^{bA}	0.12 ± 0.01^{ab}

Averages ± standard deviation followed by equal lowercase superscript letters (in the same line), upper case (in the same column) do not differ significantly by Tukey's test (P>0,05). GSE: grape seed extract. BHT: Synthetic butylated hydroxitoluene. ND: Not detected. Analysis performed in triplicate. Δt: Values of mean ± standard deviation obtained through the difference between Time 9 and Time 0 in triplicate, followed by equal lowercase superscript letters (in the same column) do not differ significantly by Tukey's test (P>0,05), for the respective analyses.

Table 2: Indicators of soybean oil oxidation

Lipid hydroperoxides are the main oxidation products of the early stage of oxidation (Ghanbari et al., 2019) and can decompose into volatile (aldehydes) and non-volatile (dienes, trienes and free acids) secondary products, which deteriorate the oil quality (Umeda et al., 2021). Pure soybean oil sample presented the highest value, being the maximum point in Time 9 of 39.4 mEq O2 Kg^{-1}, consequently, with significant difference in relation to the other samples (Table 2). At Time 6 and Time 9, the oil with BHT and oil with GSE+BHT, presented a similarity in hydroperoxide content in relation to dienes and trienes (Time 6 and Time 9), indicating that the action of BHT earlier than GSE. The oil with GSE+BHT and with BHT had the lowest peroxide levels during the study, with no significant difference ($P>0.05$) between them. At Time 12 there was a decrease in peroxide values in all samples. Yang et al. (2016) obtained peroxide indexes in the first time equal to this study, however, over 24 days of monitoring, the values were lower, with 23.72 mEq kg-1, in the final time for pure soybean oil and with better protection by rosemary extract (0.04 %) compared to BHT+BHA (0.02 %). Extra virgin olive oil presented higher peroxide levels in the beginning storage time at room temperature for filtered and unfiltered samples (8.94 and 11.89 72 mEq O2 kg^{-1} oil, respectively) and reached values around of 18.00 mEq O2 kg^{-1} oil for both after 12 months of storage (Ghanbari et al., 2019).

The results obtained for conjugated dienes, present a significant difference ($P^{>}0,05$) from Time 6 (Table 2). The soybean oil presented conjugated dienes at Time 9 (9.6 mg g-1) and with a drop in Time 12 to 6.9 mg g-1. The sample that presented the highest antioxidant capacity throughout the study time was the soybean oil with GSE+BHT which had a considerable increase in Time 6 (6.1 ± 0.5 mg g-1), remaining without significant difference until the end of the study. The samples containing the antioxidants showed no significant difference, demonstrating that the capacity of protection of both antioxidants and their joint action has no statistical difference ($P>0.05$). Kehili et al. (2018) also reported a significant increase in dienes followed by a significant drop in the last time. The sample with the highest content of conjugated trienes was the oil with GSE (3.7 mg g-1), followed by BHT (3.1 mg g-1), showing a significant difference ($P<0.05$) in relation to the samples of pure oil and oil with GSE+BHT. The data indicates that GSE+BHT has a greater capacity of protection against production of conjugated trienes, and the reduction observed in these levels can also be attributed to the decomposition of secondary compounds (ketone), where oxidation can destroy the double bond between carbon and oxygen.

In Time 6, the amount of reactive substances to thiobarbituric acid was low, while in Time 9, the values were higher and presented a significant difference between times and samples, with a drop in Time 12 for the pure soybean oil sample (Table 2). Time is a significant variable in TBARS formation. Malonaldehyde-type compounds are highly reactive. Thus, they present results only from products that happen temporarily in the oxidation stages. The smallest development of TBARS until time 9 was for BHT soybean oil and GSE soybean oil, with no significant difference ($P>0.05$) between both samples. However, the joint action

of the antioxidant compounds acted as antagonist, and the values approached the pure soybean oil sample. Zhang et al. (2018) evaluated the effect of synthetic antioxidants and natural phenolic compounds on the oxidative stability of pecan oil. They observed that the synthetic compounds (BHT, BHA and TBHQ) were more efficient in protecting against TBARS.

The soybean oil used for the study presented as the main FA the C16:0 (10.31 ± 0.07%), C18:0 (3.67 ± 0.11%), C18:1 (25.45 ± 0.30%), C18:2 (52.53 ± 0.20 %) and C18:3 (6.48 ± 0.10 %). Figure 1 shows the results for the major fatty acid content during the period of forced oxidation for the samples.

Time 12 was the one that presented FA results with higher significance ($P<0.05$). The lowest degradation was observed for C16:0 and C18:0, which is related to the absence of unsaturation since the carbon-hydrogen bonds present higher energies and differ from the transfer of atoms by a peroxyl radical (ROO.), which occurs readily in unsaturated fatty acids. The oil with BHT remained stable until Time 9, showing a significant drop in the content of the major FA in Time 12, showing less protection against oil with GSE and with GSE+BHT, and having no significant difference from pure oil. The sample with GSE, showed a gradual decomposition from Time 0 to Time 12, respectively of 287.03, 260.15, 245.19 mg g-1 for C18:1. Similar results to this work were also obtained by Liu et al. (2018), when evaluating the degradation of soybean oil from 0 to 50 h of frying at 180 ± 5 °C. Multari et al. (2019) evaluated new types of oils (Hempseed, Lupine and Oats) and conventional oils (Rapeseed, Soybean and Sunflower) by the method of frying at 180 °C. For soybean oil, the authors reported a significant increase in oleic acid from 18.8 to 20.9 % at T20 min, followed by stabilization at T60 min. At the same time, they observed a drop in linoleic acid and α-linolenic 52.9 - 50.9 % and 8.89 - 8.00 %, respectively.

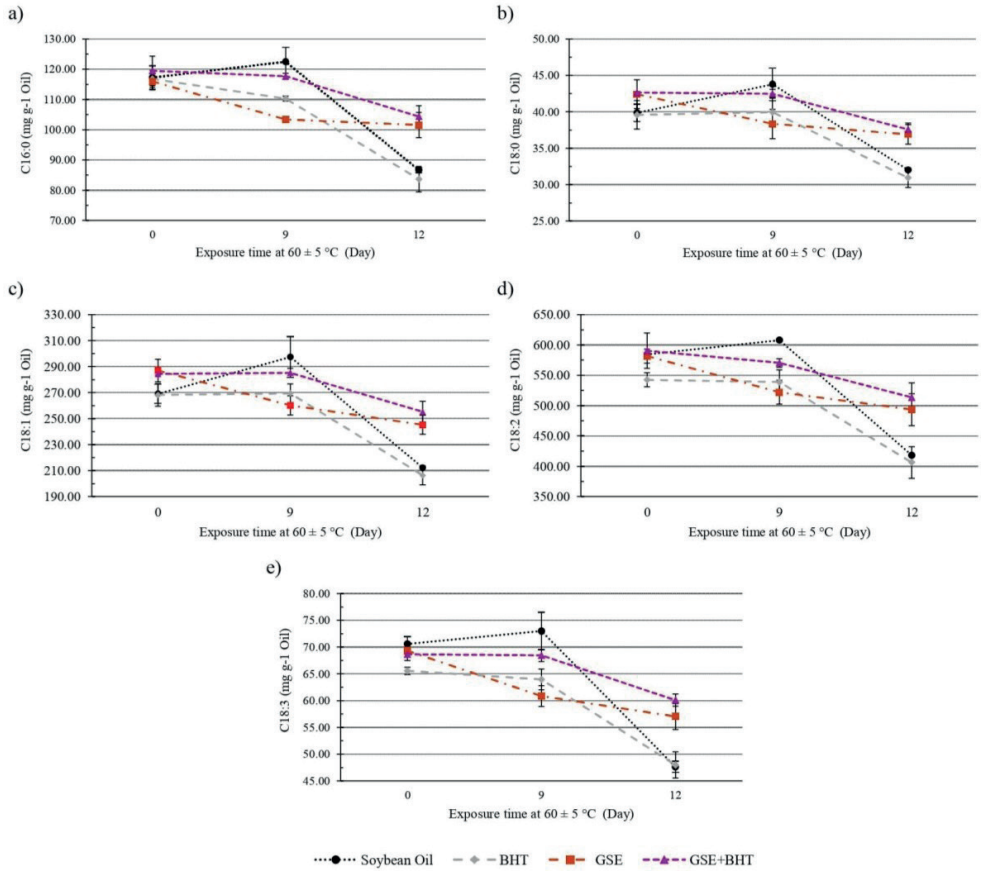


Figure 1. Main fatty acids of soybean oil (mean ± standard deviation) before and after oxidation at 60 ± 5 °C (GSE = grape seed extract).

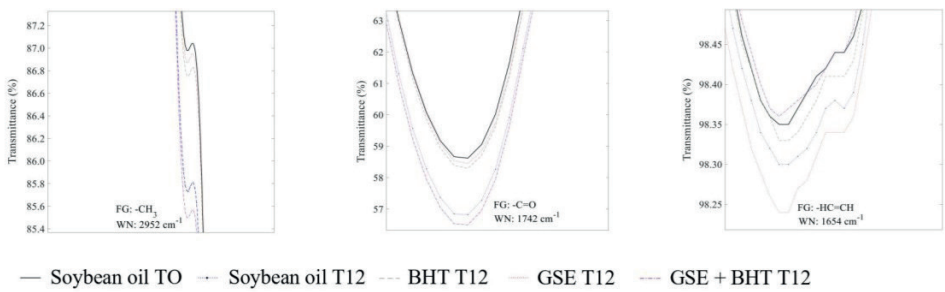
Similar data are presented in Figure 1 c) AG-C18:1, where it can be observed that in pure soybean oil, there is a significant increase ($P<0.05$) in Time 9, followed by a significant drop in Time 12. Linoleic and linolenic acids are prone to oxidation, as they contain two and three double bonds, respectively. The oleic acid is more stable because it contains only one instauration. The saturation process of double bonds during lipid oxidation converts linolenic acid to linoleic acid, from linoleic acid to oleic acid, and from oleic acid to stearic acid, which is usually negligible. The antioxidants evaluated in this study demonstrated the delay of oxidation (Figure 1: d, e) of FA C18:2 and C18:3, which, due to the presence of unsaturation, are more prone to oxidation.

Yang et al (2016) structured the evaluation of rosemary extract with some changes to this study (temperature of 62 °C for 24 direct days) and did not evaluate the synergism between the extract (0.04%) and synthetic antioxidants (BHT+BHA - 0.02 %). They found that FA C14:0, C16:0, C18:0, and C18:1 presented an increase in composition for all treatments and a significant decrease for C18:2 (41.47 %) and C18:3 (5.7 %), with rosemary

extract has been more efficient in oxidative protection. Compared with the results obtained in this work, the oxidation time was a factor that influenced the increase in saturated FA and the decrease in concentration of C18:2 (19.86 %) and C18:3 (50.72 %). It was observed on Figure 1 that soybean oil enriched with GSE and in joint action with BHT were more efficient than BHT in protecting against the degradation of the majority FA after 12 days of accelerated oxidation.

The spectra for the 4 treatments (non-oxidized and oxidized) were obtained (Figure 2). The absorption bands were identified and the functional groups responsible for the absorption, according to Nurwahidah et al. (2019), as follows: 3009 cm-1 (*cis* stretch =CH), 2923 and 2853 cm-1 (symmetric and asymmetric stretch of -CH2), 1742 cm^{-1} (stretch -C=O) ester, 1654 cm^{-1} (cis stretch -CH=CH), 1464 cm^{-1} (flexion -CH2), 1377 cm^{-1} (flexion -CH3), 1237 cm^{-1} (stretch -CO), 1160 cm^{-1} (stretch -CO); bending -CH2), 1098 cm^{-1} (stretch -CO), 1033 cm-1 (stretch -CO), 968 cm-1 (-HC=CH (*trans*), 914 cm-1 (-HC=CH (*cis*)), 722 cm-1 (cis -CH=CH curved out of plane). A non-characteristic band for soybean oil was identified in 968 cm-1 (-HC=CH *trans*), which is related to out- of-plane deformation of hydrogen bound in the *trans* configuration in lipids obtained from plants. In soybean oil *trans* fatty acids do not occur naturally and their formation occurs in the oil deodorization stage when high temperatures are employed.

After the period of induction to oxidation, the obtained spectra showed no deformation, only reduction or increase in transmittance. All bands identified for Time 12 of pure soybean oil showed a reduction in transmittance when compared to Time 0 of pure soybean oil, indicating that there was an increase in absorbance. During the oxidation of unsaturated vegetables oils occurs formation of hydroperoxide, those decomposition results in formation of aldehydes, that absorbs at 1742 cm-1. Decomposition of hydroperoxides also results in increase of methyl groups, due to formation of hydrocarbons, that absorbs at 2952 cm-1. Soybean oil with BHT and soybean oil with GSE did not show the same level of increase in the bands when compared to pure soybean oil after 12 days of oxidation, showing that both have efficiency in protection.



FG: Functional group; WN: Wavenumber; T0: Sample without undergoing forced oxidation; T12: Sample oxidized for 12 days at 60 ± 5 ºC.

Figure 2. Individual analysis of identified bands and functional groups (GSE = grape seed extract).

Xu et al. (2016) evaluated the oxidative stability of peanut oil at 7-day intervals for 42 days of samples exposed to light and ambient temperature. In each analyzed period they observed an increase in intensity in regions 3471 cm-1, 1706 cm-1, 968 cm-1 and 914 cm-1 of transmittance. The authors attributed this increase due to the formation of hydroperoxides, free fatty acids (FFA) and also observed a reduction in intensity in the region of 722 cm-1. Daoud et al. (2019) [10] evaluated oil emulsion spectra in water before and after oxidation and identified deformation of the spectra as well as the appearance of bands in other regions. These initiation deformations indicate the presence of alkyl and peroxyl radicals, and these reactive radicals abstract hydrogen atoms from other unsaturated FA, forming hydroperoxides, and decompose into secondary products (ketones, aldehydes, ethers and alkanes). The fact that the band in the 1654 cm-1 region presents low reduction in transmittance may be related to the formation of other compounds, such as dienes and conjugated trienes. Oyman et al. (2003) obtained data of reduction of these regions, being related to the conversion of *cis* -C=C non-conjugated into C=C conjugated *cis-trans* or *trans- trans*. The formation of dienes and trienes was significant mainly for soybean oil, but, as well as peroxides, there was no reduction or degradation in other compounds in Time 12.

Data obtained in global analysis (Table 2), showed that there was a small significant difference ($P>0.05$) between treatments with antioxidants at certain times. However, when observing the results of FA (Figure 1), it is observed that soybean oil with BHT showed a significant degradation when compared with soybean oil with GSE and oil with GSE+BHT for all the majority of FA evaluated after 12 days of oxidation. On the other hand, in the FTIR analysis, the sample with GSE+BHT showed an increase in absorbance for all identified bands except 1654 cm-1 (-HC=CH), being very similar to the non-oxidized soybean oil, which may be the effect of the joint application of GSE and BHT. Another point to be considered is the formation of TBARS, in which the sample with GSE+BHT showed a formation similar to the negative control ($P>0.05$) with significant difference when comparing the samples with BHT and GSE. The degradation of TBARS changes the carbonyl band (1742 cm-1). Thus, by FTIR analysis, the addition of GSE+BHT in soybean oil presented an antagonistic action in the protection against oxidation, while the use of GSE separate indicates to be as efficient as BHT.

Several published and recognized articles worked with DSC, and based on an antioxidant limit due to legislation, the main interest of this study was to evaluate its stability against forced temperature conditions, thus performing the prediction. The determination of the oxidative stability by OIT was performed in oil samples. Data, obtained from the DSC curves, made possible to obtain a logarithmic relation represented by ln OIT *vs*. 1/T. This provides a linear relation of T versus log t0 (where T is temperature and t0 is time), which is represented by the semi-logarithmic graph (Figure 3). This graph, adjusted by means of the straight equation, allows the application of the Arrhenius equation. From equations (T = b - a (log t0)) of the straights obtained (Figure 3) by the semi- logarithmic regression for

the 4 treatments, the forecast obtained for shelf life at 20 °C in days (Tab. 3), in descending order, was: SO with GSE+BHT > pure SO > SO with GSE > SO with BHT. The shelf life of soybean oil with GSE+BHT was 894 days.

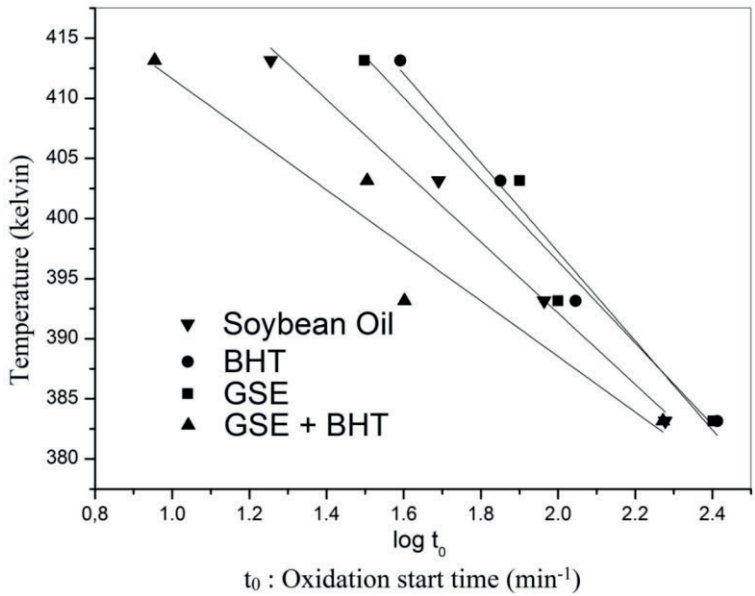


Figure 3. Calorimetric curve for the 4 treatments represented (GSE = grape seed extract).

Yang et al. (2016) obtained the induction period at 120 °C per rancimat for soybean, cotton seed and rice oils, supplemented with rosemary extract and BHA+BHT and noted that the synthetic antioxidants for all oils were superior over the control, with lower performance for rosemary extract. Hegazy & Abdel- Maksoud (2016), in their study using ethanolic GSE (200 ppm), obtained an expiration of 14 months, while in our study, using a solvent mixture to prepare GSE, the shelf life was of almost 30 months, matching GSE + BHT in SO. Applying the Arrhenius equation to the straight line equation (Table 3), the activation energy data and the pre-exponential factor of the evaluated reactions were obtained.

Sample	Straight equation	Coefficient	Prediction of Shelf Life	ln (k) = a (1/T) + b				
	y = b - a (x)	*R^2*	*Days*	*a*	*b*	*R^2*	*AOIT (h-1)*	*E_a hJ mol^{-1})*
Soybean Oil	T = 451.4 -29.63 ($logt_0$)	0.9848	148.15	-12313.21	30.92	0.9982	2.60 x 1013	102.38
BHT	T = 471.3 -37.02 ($logt_0$)	0.9761	44.10	-9875.47	24.38	0.9988	3.59 x 1010	82.11
GSE	T = 464.5 -34.02 ($logt_0$)	0.9337	73.81	-10728.97	26.57	0.9995	3.38 x 1011	89.21
GSE + BHT	T = 434.7 -23.11 ($logt_0$)	0.9037	894.43	-15794.01	40.15	0.9995	2.64 x 1017	131.32

T: Temperature in kelvin; t_0: Oxidation start time (min^{-1}); a: Line slope; b: Line interception; A_{OIT}: pre-exponential factor or frequency factor; E_a: activation energy; GSE = grape seed extract.

Table 3: Shelf life forecast at 20 °C (in days) obtained by the linear regression equation and Arrhenius parameters

The activation energy (Ea) for the treatments varied significantly, being in the same decreasing order of shelf life. Ea is another way of expressing dependence on the rate of polyunsaturation (linoleic and linolenic acids) present in vegetable oils, that is, lower Ea more oleic acid. However, in this study the composition of fatty acids is the same for oils, varying only the antioxidants. Gülmez & Şahin (2019), analyzed hazelnut oil and activation energy data ranging from 86.30 to 106.34 kJ mol-1, according to the added phytochemical. Chemical reactions with high values of activation energy are temperature sensitive, and reactions with low activation energy are less temperature sensitive. The sample of soybean oil with GSE+BHT showed a higher dependence on temperature, consequently its reduction to 20 °C allows a higher oxidative stability when compared to other treatments. Ragnarsson & Labuza (1977) point out that the concentration of antioxidants is inversely proportional to oxidation, and that the Ea is higher in the presence of antioxidants, that is, the oxidation inhibitors decrease the oxidation rate and increase Ea. In moreover, the addition of antioxidants, oxygen pressure and other factors can alter the mechanism.

Therefore, inhibitors partially decrease the oxidation rate by increasing the overall Ea. Farhoosh et at. (2008) evaluated different oils and found data for frequency factor (AOIT) 28.03 x 1013 h^{-1} and Ea of 92.42 kJ mol^{-1} for pure soybean oil, obtaining an oxidative stability index at 20 °C of approximately 179 days, a little higher than this work, where they worked with variation of the amount of samples and heating rate. All these factors have their importance in varying degrees for the oxidation of lipids by triplet ($^3O2$) and singlet ($^1O2$) oxygen. The temperature affects the singlet oxygen oxidation very little, but has a significant effect on the triplet oxygen oxidation, which requires high Ea. Polyunsaturated fatty acids (PUFA) are more susceptible to triplet oxygen oxidation than monounsaturated fatty acids (MUFA), since the Ea is lower than that of MUFA. Considering Arrhenius' equation, frequency factor A together with Ea are considered the main kinetic parameters that affect the reaction rate.

Data obtained by Farhoosh and Hoseini-Yazdi (2014) showed a similar pattern of the *A* factor, comparing Ea for the oils studied, however, the kinetic parameters should not be used individually. The data obtained in this work for Factor *A* present similarity to the researched literature. As Ea increased, the increase in factor *A* was equivalent, the inverse also. A higher value of factor *A* means a higher probability of successful collisions that cause chemical changes. Unlike Ea, the frequency factor has a relationship with the composition of the sample under study. Ostrowska-Ligeza et al. (2010) analyzed 4 types of olive oil by DSC and rancimat, also observed that as Ea increased the *A* factor also increased significantly. The authors reported a possible irregularity in the olive oil, which means words, change in its composition.

Kinetic parameters of olive oils were also determined by Gharby et al. (2016, 2021). These authors found strong dependency with the temperature of oil from different olive varieties and higher oxidation induction time was recorded in virgin olive oil as compared to refined olive oil. In this work, the *A* factor and log t0 presented a positive correlation (R^2= 1.0000 and R^2= 0.9989, respectively) with Ea, demonstrating that the factors that most influenced the results by the DSC analysis were the presence of GSE and BHT, and the presence of antioxidants together has a significant response at storage temperature.

CONCLUSION

Edible soybean oil (SO), purchased at local market, was enriched with Bordô grape seed extract (*Vitis labrusca*) (GSE). This oil shows little or no significant difference with SO enriched with BHT and or with both antioxidants at its maximum point (Time 9) of products oxidation accumulation for acidity, peroxide, conjugated dienes, trienes and TBARS analysis. Those indicate that the natural antioxidant is equivalent to the synthetic for these parameters.

Regarding the preservation of FA, after 12 days of accelerated oxidation SO enriched with GSE and in joint action with BHT, was more efficient than BHT alone.

For the analysis of ATR-FTIR, good similarity was observed between the oil with BHT and oil with GSE at bands 2923, 2853, 1742 and 1160 cm-1, while the antioxidants GSE+BHT added simultaneously to the SO, presented an antagonistic effect, since all the bands diminished their transmittance, showing a very similar response to the oxidized pure SO.

Similarly, data obtained from DSC analysis showed that GSE+BHT oil was more temperature-dependent, but with a predicted shelf life at 20 °C higher than other treatments (894 days), while BHT and GSE soybean oil were less temperature-dependent.

Therefore, the GSE used in this work presented itself as promising to be used as a natural antioxidant in SO, demonstrating in the various tests performed an equivalent or superior response to BHT. This implies an application for this bioactive agro-industrial residue, besides a possible substitution to the use of the synthetic antioxidant.

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