

## Research Article

### Antioxidant Effect of Red Pepper (*Capsicum frutescens*) Extract in Soybean Oil Under Accelerated Storage Test

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**Abstract:** This study aimed to evaluate the antioxidant effect of pepper extract in soybean oil under storage. The following treatments were subjected to an accelerated storage test (60°C/20 days): Soybean oil, Soybean oil + 100 mg/kg of TBHQ, Soybean oil + 200 mg/kg of TBHQ, Soybean oil + 100 mg/kg of BHA, Soybean oil + 200 mg/kg of BHA, Soybean oil + 100 mg/kg of phenolic compounds from the extract and Soybean oil + 200 mg/kg of phenolic compounds from the extract. Extract<sub>200</sub> proved to be effective in protecting soybean oil against peroxide formation and conjugated dienoic acids after 20 days of storage. Furthermore, Extract<sub>200</sub> showed no mass gain until the end of the accelerated storage test and also retained high amount of tocopherols. Finally, the pepper extracts added to soybean oil, especially Extract<sub>200</sub>, were capable of reducing the changes undergone by soybean oil during the process of storage, thus being able to replace synthetic antioxidants.

**Keywords:** Antioxidant activity, malagueta extract, peppers, phenolics compounds, soybean oil

## INTRODUCTION

Approximately 80% of produced vegetable oils are applied in foods. They are used in salads, fried foods, mayonnaise and margarine, whereas the other 20% are used in industrial applications for the production of detergents, cosmetics, lubricants, paints, varnishes and plastics (Ribeiro *et al.*, 2005).

Soybean oil is composed of, on average, 59.8% of polyunsaturated fatty acids (linoleic and linolenic acids), 23.6% of monounsaturated fatty acids (oleic acid) and 16.6% of saturated fatty acids (palmitic and stearic acids). However, intense heat leads to degradation and nutrient losses in these oils (Casal *et al.*, 2010; Rodrigues *et al.*, 2012). In order to delay oxidative processes, some antioxidants are added to foods. Yet, their efficiency is questionable due to the occurrence of oxidative and thermal reactions (Marmesat *et al.*, 2010).

Antioxidants, as the name suggests, inhibit the development of oxidative rancidity in foods containing oils and fats, especially meat, dairy, vegetable oils and fried foods (Politeo *et al.*, 2007). Antioxidants in foods may be natural or synthetic. Synthetic antioxidants, such as BHA, BHT and TBHQ, are relatively inexpensive, colorless, tasteless and odorless and are

widely used by industries. However, they present solubility problems and some contribute to the development of off-flavor, besides being highly toxic. Therefore, their use is limited in many countries, which makes the search for natural antioxidants that can partially or totally replace them increase (Pokorný, 2007).

The Solanaceae family comprises 150 genera and 3000 species, including peppers. These peppers, along with *Capsicum frutescens* pepper, are often consumed fresh, although they are also used in the form of paste, dried and canned. They are rich sources of phenolic compounds, carotenoids, vitamin A and ascorbic acid, although the levels of these compounds vary according to the degree of ripeness (Davis *et al.*, 2007).

Malagueta pepper extract presents considerable content of phenolic compounds, when compared to other extracts and, thus, can be used as natural antioxidant to be applied in vegetable oils (Andreo and Jorge, 2007).

Considering the interest in reducing the changes that occur in oil during the heating process, as well as the incentive to reduce or replace synthetic antioxidants, it is necessary to study the antioxidant activity of Malagueta pepper extract added to soybean oil under accelerated storage in oven. It is also

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important to compare its effect with the synthetic antioxidants, used by the food industry in order to delay oxidative changes.

## MATERIALS AND METHODS

**Pepper extracts:** Ripe fruits of Malagueta (*C. frutescens*), bought locally (São José do Rio Preto, São Paulo, Brazil) in March/2016, were used *in natura*. After collection, whole and fully ripe fruits were selected, washed in running water and dried at room temperature. The hydroalcoholic extract (ethanol 96°GL) was prepared according to the methodology of Costa *et al.* (2010). The fruits (20 g) were kept under intense agitation with a hydroalcoholic solution (200 mL) at room temperature, for 30 min. Then, the mixture was centrifuged at 3.000 rpm for 10 min and the supernatant was filtered. After that, the solvent used was removed, under reduced pressure at 45°C. The extracts obtained were stored at -32°C and inertized for 50-56 h. Then, they were dried in a lyophilizer (Model L101; Liotop, São Carlos, São Paulo, Brazil) for 26 hours and stored at -18°C until the moment of analysis.

**Vegetable oil and antioxidants:** Refined soybean oil was used without the addition of synthetic antioxidants (TBHQ and citric acid), provided by Cargill Agricola S/A (Mairinque, São Paulo, Brazil). Furthermore, the synthetic antioxidants tert-butylhydroquinone (TBHQ) and Butylated Hydroxyanisole (BHA) were purchased in their commercial form, with 97% purity (Sigma-Aldrich, St. Louis, MO, USA).

**Accelerated storage test:** Synthetic antioxidants and pepper extract were added to soybean oil, according to Table 1. Concentrations of 100 and 200 mg/kg of synthetic antioxidants TBHQ and BHA, respectively, were defined in the treatments, since those are the maximum amounts permitted in Brazil (Brazil, 2005). All treatments were subjected to an accelerated storage test in an oven at 60°C (Model 035; Marconi, Piracicaba, São Paulo, Brazil), with 100 mL beakers containing 60 mL of sample with surface/volume ratio of 0.3/cm. All samples, at different time intervals (0, 5, 10, 15 and 20 days), were collected and inertized with nitrogen gas and stored at approximately -18°C until the moment of analysis.

**Determination of peroxide value, conjugated dienoic acids and oxidative stability in the treatments:** The analyses of peroxide value and conjugated dienoic acids in oils were performed according to the official methods of American Oil Chemists' Society (AOCS, 2009). The oxidative stability was determined according to the official methods of American Oil Chemists' Society (AOCS, 2009) as well, by employing Rancimat instrument (model 743; Metrohm brand,

Table 1: Antioxidants added to the soybean oil

Abbreviations	Treatments
Control	Soybean oil
TBHQ <sub>100</sub>	Soybean oil + 100 mg/kg TBHQ
TBHQ <sub>200</sub>	Soybean oil + 200 mg/kg TBHQ
BHA <sub>100</sub>	Soybean oil + 100 mg/kg BHA
BHA <sub>200</sub>	Soybean oil + 200 mg/kg BHA
Extract <sub>100</sub>	Soybean oil + 100 mg/kg of phenolic compounds from the extract
Extract <sub>200</sub>	Soybean oil + 200 mg/kg of phenolic compounds from the extract

Herisau, Switzerland). The determination occurred at 110°C and with airflow rate of 20 L/h. A curve of electrical conductivity versus time was plotted over the reaction and test course and the induction period was determined in hours.

**Mass gain in the treatments:** For mass gain analysis, 2.0 g of each sample were placed in glass Petri dishes, which were kept in an oven (Model 035; Marconi, Piracicaba, São Paulo, Brazil) at 60°C. The oxidation rate, in terms of mass gain, was recorded at 24 h intervals for 30 days. The stability index is defined as the time required for a 0.5% mass gain of oil and the mass gain was calculated as percentage of the original weight (Iqbal and Bhangar, 2007). The weighing machine used was model AS200; Ohaus (number of digit 0.0001 g and precision 0.0025 g).

**Determination of tocopherols in the treatments:** Tocopherol composition in oils was determined by the official methods of American Oil Chemists' Society (AOCS, 2009). The analysis was performed by high-performance liquid chromatography (model Pro Star 210; Varian, Santa Clara, CA, USA) with fluorescence detection under the following conditions: silica column 4.6×250 mm with 5 μm pore; as mobile phase, the mixture of *n*-hexane and isopropanol (99.5:0.5 v/v); 1.2 mL/min flow, excitation wavelength of 290 nm and emission wavelength of 330 nm. The quantification of each isomer was performed by external standardization based on peak areas, using standards of  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -tocopherol (Sigma-Aldrich, St. Louis, MO, USA), whose results were expressed in mg/kg.

**Statistical analysis:** The results obtained from the analytical measures were subjected to analyses of variance in the completely randomized design, in factorial design (Gacula Jr. *et al.*, 1984). Analyses of variance and Tukey test for the averages at 5% were performed through the program ESTAT - System for Statistical Analyses, version 2.0 (UNESP, Jaboticabal, São Paulo, Brazil).

## RESULTS AND DISCUSSION

**Peroxide value, conjugated dienoic acids and oxidative stability:** The results of the peroxide value, conjugated dienoic acids and oxidative stability of the treatments are shown in Fig. 1.

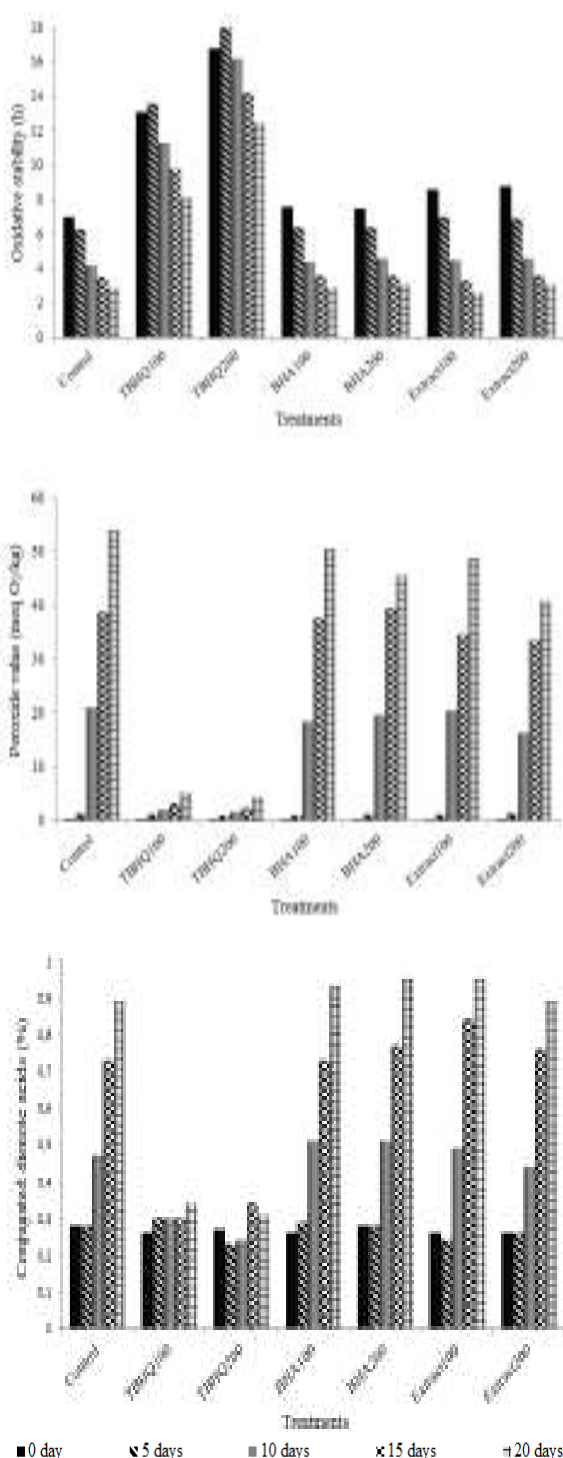


Fig. 1: Peroxide value, conjugated dienoic acids and oxidative stability in soybean oil with the addition of antioxidants during accelerated storage at 60°C

It is observed that, in all treatments, the peroxide value had a large increase as of 10 days of storage, except in TBHQ<sub>100</sub> and TBHQ<sub>200</sub>, which kept low values up to 20 days of heating. Extract<sub>100</sub> and Extract<sub>200</sub> showed significant differences in values only

at 10 and 20 days of storage. Extract<sub>200</sub> presented lower rate of peroxides when compared with Control, BHA<sub>100</sub>, BHA<sub>200</sub> and Extract<sub>100</sub>. At the end of 20 days, it was observed that the best treatments for oil protection against the formation of the primary oxidation compounds were TBHQ<sub>100</sub> and TBHQ<sub>200</sub>, with 90 and 92% protection, respectively, followed by Extract<sub>200</sub> with 24% protection, all calculated in comparison to the Control.

Since *Codex Alimentarius Commission* (Codex, 2009) establishes peroxide index of 10 meq O<sub>2</sub>/kg for refined oils, it is possible to verify that all treatments were effective up to 5 days, although such index remained only in TBHQ<sub>100</sub> and TBHQ<sub>200</sub> treatments throughout the storage period.

Zhang *et al.* (2010) evaluated the stability of sunflower oil using rosemary extract and other antioxidants (BHA, BHT and TBHQ) at a concentration of 200 mg/kg. The formation of peroxide was inhibited in 85, 25, 41 and 93%, respectively, demonstrating that the synthetic antioxidant TBHQ is more efficient. In a study by Andreo and Jorge (2007), it was observed that ginger ethanol extract, TBHQ and the mixture (TBHQ and ginger extract), once added to soybean oil, reduced peroxide formation by 57, 90 and 92%, respectively, after 12 days of accelerated storage at 60°C, suggesting that ginger extract enhances the protective action of TBHQ.

Monitoring the formation of conjugated dienoic acids in oils also provides an indication of the changes that occur during the oxidative process, since its increase is proportional to the absorption of oxygen and the peroxide formation during the initial stages of oxidation (McClements and Decker, 2007). Thus, there was a gradual and progressive increase over time for the treatments Control, BHA<sub>100</sub>, BHA<sub>200</sub>, Extract<sub>100</sub> and Extract<sub>200</sub>. It was also noticed that Extract<sub>200</sub> showed lower formation of conjugated dienes than Extract<sub>100</sub> as of 10 days of storage.

Luzia and Jorge (2009) studied the content of conjugated dienes in soybean oil with the addition of lemon extract (2.400 mg/kg) subjected to accelerated storage at 60°C, for 12 days. At the end of the process, lemon extract showed decrease of only 7% in conjugated diene formation, while lemon extract, mixed with the synthetic antioxidant TBHQ (2.400 mg/kg of lemon extract + 50 mg/kg TBHQ), reduced the formation of these undesirable compounds by 49%. This demonstrates the efficacy of synergism between the two types of antioxidants.

As for the oxidative stability, there was a decrease in all treatments as the number of heating days increased, but the stability was higher during 20 days of heating in treatments TBHQ<sub>100</sub> and TBHQ<sub>200</sub>. Extract<sub>100</sub> and Extract<sub>200</sub> did not differ significantly throughout the storage period, showing that the increase in the concentration had no influence on stability. In

addition, the natural antioxidant extracted from Malagueta pepper presented the same efficiency as Control, BHA<sub>100</sub> and BHA<sub>200</sub> within 20 days of storage.

**Mass gain:** The analysis of mass gain is usually employed to estimate the amount of oxygen added to the unsaturated content of lipid molecules and formation of hydroperoxides during oxidation. The results are shown in Fig. 2.

Control (Soybean Oil), TBHQ<sub>100</sub> (Soybean oil + 100 mg/kg of TBHQ), TBHQ<sub>200</sub> (Soybean oil + 200 mg/kg of TBHQ), BHA<sub>100</sub> (Soybean oil + 100 mg/kg of BHA), BHA<sub>200</sub> (Soybean oil + 200 mg/kg of BHA), EXTRACT<sub>100</sub> (Soybean oil + 100 mg/kg of phenolics compounds from the extract), EXTRACT<sub>200</sub> (Soybean oil + 200 mg/kg from phenolics compounds of the extract).

Initially, the mass gain was not significant. However, there was a sudden increase between 15 and 20 days. In 20 days of storage, TBHQ<sub>200</sub> treatment stood out with high mass gain, differing significantly from the other treatments. After 20 days, there was a decline in mass gain in all treatments. Such decline is due to the fact that the peroxides formed during the accelerated storage period, can be degraded into low molecular weight compounds, including volatile substances

Concerning treatments with extract, there was no significant increase in mass gain during storage, which shows potential antioxidant activity of Malagueta pepper extract. Iqbal and Bhanger (2007) studied mass gain of sunflower oil with addition of garlic extract (250, 500 and 1000 mg/kg) subjected to accelerated storage test in an oven at 65°C for 14 days. They found that garlic extract at 1000 mg/kg was more effective in protecting the oil, followed by BHT. Sunflower oil without antioxidants, unlike soybean oil, showed mass

gain in the first days of storage. This shows that sunflower oil is more susceptible to oxidation because its unsaturation (89%) is higher than in soybean oil (83.4%). Da Silva and Jorge (2014) found that protection against soybean oil oxidation was best evidenced by sun mushroom extract (3500 mg/kg), which showed no significant mass increase until up to 15 days, followed by TBHQ (100 mg/kg) and Shiitake extract (3500 mg/kg).

**Tocopherols:** The retention of tocopherols in soybean oil is one way to evaluate the efficiency of pepper extracts added to oil during accelerated storage and compare them with synthetic antioxidants (Table 2). It was possible to observe a decrease in the amounts of all tocopherol isomers as heating time increased. At the end of 20 days of storage, high levels of  $\alpha$ -,  $\gamma$ - and  $\delta$ -tocopherols were found in TBHQ<sub>100</sub> and TBHQ<sub>200</sub>. Among the treatments with the addition of extract, Extract<sub>200</sub> showed 35.1, 86.8 and 93.3% retention of  $\alpha$ -,  $\gamma$ - and  $\delta$ -tocopherols, respectively, at 20 days of storage.

Regarding the levels of total tocopherols, it was observed that the protective effect of all treatments was reduced during storage. At the end of 20 days, the oil showed 432.2 mg/kg of total tocopherols in TBHQ<sub>100</sub>, 422.2 mg/kg in TBHQ<sub>200</sub>, 357.5 mg/kg in Extract<sub>200</sub>, 334.7 mg/kg in Extract<sub>100</sub>, 333.0 mg/kg in BHA<sub>200</sub>, 323.7 mg/kg in BHA<sub>100</sub> and 322.2 mg/kg in Control.

## CONCLUSION

Malagueta pepper extract has high amounts of compounds considered beneficial for health, as well as for granting antioxidant properties *in vitro*. At the end

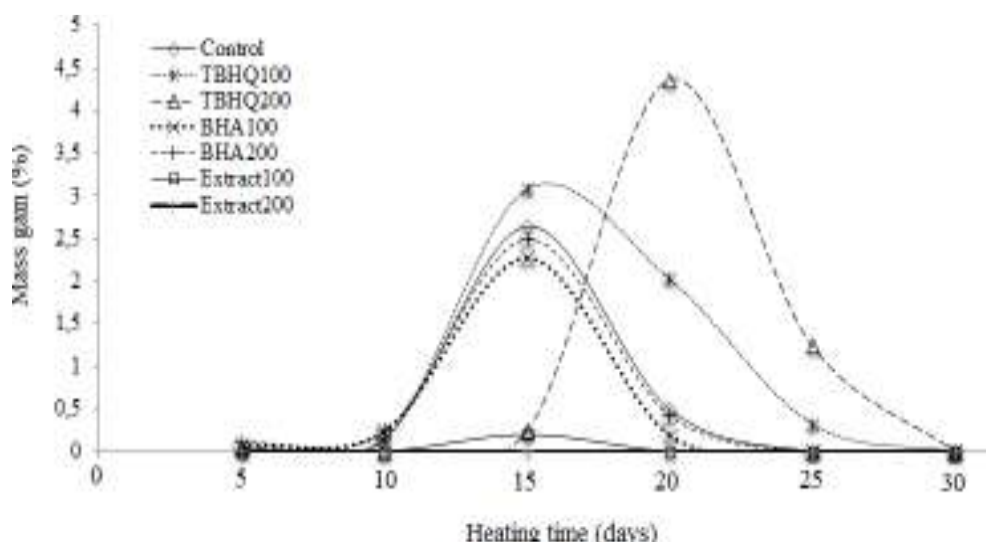


Fig. 2: Mass gain of treatments subjected to accelerated storage

Table 2: Tocopherols in soybean oil with the addition of antioxidants during accelerated storage at 60°C

Tocopherol	Heating times (days)				
	0	5	10	15	20
<b>α-</b>					
Control	69.1±0.1 <sup>aC</sup>	65.5±0.2 <sup>bD</sup>	57.3±0.4 <sup>cE</sup>	33.2±0.2 <sup>dD</sup>	15.4±0.3 <sup>eD</sup>
TBHQ <sub>100</sub>	82.0±0.1 <sup>aA</sup>	68.2±0.2 <sup>bB</sup>	67.6±0.4 <sup>bcA</sup>	66.8±0.5 <sup>cA</sup>	63.6±0.0 <sup>dA</sup>
TBHQ <sub>200</sub>	66.3±0.4 <sup>aE</sup>	60.3±0.3 <sup>cE</sup>	60.1±0.1 <sup>cB</sup>	62.7±0.1 <sup>bb</sup>	56.3±0.3 <sup>dB</sup>
BHA <sub>100</sub>	67.3±0.3 <sup>aDE</sup>	67.1±0.1 <sup>aC</sup>	55.1±0.1 <sup>bd</sup>	29.4±0.5 <sup>cE</sup>	13.5±0.3 <sup>dF</sup>
BHA <sub>200</sub>	71.0±0.2 <sup>aB</sup>	69.4±0.4 <sup>bA</sup>	55.4±0.5 <sup>cD</sup>	30.1±0.7 <sup>dE</sup>	14.4±0.1 <sup>eEF</sup>
Extract <sub>100</sub>	68.4±0.6 <sup>aC</sup>	68.4±0.6 <sup>aB</sup>	55.0±0.6 <sup>bd</sup>	33.3±0.5 <sup>cD</sup>	15.2±0.2 <sup>dDE</sup>
Extract <sub>200</sub>	68.3±0.2 <sup>aCD</sup>	66.3±0.2 <sup>bCD</sup>	60.1±0.3 <sup>cB</sup>	41.5±0.0 <sup>dC</sup>	24.0±0.3 <sup>cC</sup>
<b>γ-</b>					
Control	310.7±0.3 <sup>aC</sup>	308.1±0.0 <sup>bD</sup>	293.4±0.4 <sup>cF</sup>	270.2±0.2 <sup>dG</sup>	243.6±0.4 <sup>eF</sup>
TBHQ <sub>100</sub>	362.7±0.5 <sup>aA</sup>	356.6±0.3 <sup>bA</sup>	336.3±0.4 <sup>cA</sup>	323.6±0.4 <sup>dA</sup>	300.0±0.1 <sup>eA</sup>
TBHQ <sub>200</sub>	335.9±0.8 <sup>aB</sup>	321.6±0.4 <sup>cB</sup>	327.7±0.2 <sup>bb</sup>	312.7±0.3 <sup>dB</sup>	299.6±0.4 <sup>cA</sup>
BHA <sub>100</sub>	311.3±0.3 <sup>aC</sup>	310.2±0.2 <sup>bc</sup>	305.7±0.2 <sup>cd</sup>	276.6±0.3 <sup>dF</sup>	246.5±0.1 <sup>eE</sup>
BHA <sub>200</sub>	311.5±0.3 <sup>aC</sup>	310.4±0.4 <sup>bc</sup>	308.9±0.1 <sup>cC</sup>	283.5±0.2 <sup>dD</sup>	251.5±0.8 <sup>cD</sup>
Extract <sub>100</sub>	311.4±0.3 <sup>aC</sup>	299.9±0.9 <sup>bE</sup>	299.9±1.0 <sup>bE</sup>	279.7±0.2 <sup>cE</sup>	254.6±0.7 <sup>dC</sup>
Extract <sub>200</sub>	308.7±0.2 <sup>aD</sup>	308.7±0.1 <sup>aD</sup>	300.0±0.2 <sup>bE</sup>	290.2±0.2 <sup>cC</sup>	268.0±0.6 <sup>dB</sup>
<b>δ-</b>					
Control	70.1±0.1 <sup>aCD</sup>	69.4±0.3 <sup>aB</sup>	67.4±0.3 <sup>bcD</sup>	68.3±0.4 <sup>bb</sup>	63.2±0.1 <sup>dD</sup>
TBHQ <sub>100</sub>	83.3±0.1 <sup>aA</sup>	70.5±0.4 <sup>bA</sup>	70.2±0.1 <sup>aBA</sup>	68.5±0.6 <sup>cB</sup>	68.5±0.3 <sup>cA</sup>
TBHQ <sub>200</sub>	69.2±0.5 <sup>bE</sup>	68.7±0.4 <sup>bBC</sup>	67.2±0.1 <sup>cD</sup>	70.5±0.7 <sup>aA</sup>	66.3±0.2 <sup>dB</sup>
BHA <sub>100</sub>	69.4±0.1 <sup>aDE</sup>	69.3±0.1 <sup>aB</sup>	68.2±0.1 <sup>bb</sup>	68.0±0.9 <sup>bb</sup>	63.7±0.2 <sup>cD</sup>
BHA <sub>200</sub>	72.2±0.1 <sup>aB</sup>	71.2±0.1 <sup>aA</sup>	69.9±0.3 <sup>cA</sup>	66.9±0.5 <sup>dC</sup>	67.0±0.3 <sup>dB</sup>
Extract <sub>100</sub>	70.5±0.4 <sup>aC</sup>	68.7±0.2 <sup>bBC</sup>	68.0±0.4 <sup>bcC</sup>	65.6±0.4 <sup>cD</sup>	64.8±0.3 <sup>dC</sup>
Extract <sub>200</sub>	70.1±0.1 <sup>aCD</sup>	68.3±0.3 <sup>bc</sup>	67.3±0.2 <sup>cCD</sup>	67.0±0.1 <sup>cC</sup>	65.4±0.1 <sup>cC</sup>
<b>Total</b>					
Control	449.9±0.4 <sup>aD</sup>	443.0±0.2 <sup>bD</sup>	418.1±1.0 <sup>cF</sup>	371.7±0.6 <sup>dG</sup>	322.2±0.3 <sup>eF</sup>
TBHQ <sub>100</sub>	528.0±1.5 <sup>aA</sup>	495.3±0.6 <sup>bA</sup>	474.2±0.0 <sup>cA</sup>	458.9±0.1 <sup>dA</sup>	432.2±0.3 <sup>cA</sup>
TBHQ <sub>200</sub>	471.5±1.1 <sup>aB</sup>	450.5±0.2 <sup>bB</sup>	455.0±0.3 <sup>bb</sup>	445.8±0.9 <sup>dB</sup>	422.2±0.7 <sup>eB</sup>
BHA <sub>100</sub>	448.0±0.5 <sup>aE</sup>	446.6±0.2 <sup>aC</sup>	429.0±0.2 <sup>bd</sup>	374.0±0.0 <sup>cF</sup>	323.7±0.2 <sup>dF</sup>
BHA <sub>200</sub>	454.6±0.5 <sup>aC</sup>	451.0±0.8 <sup>bb</sup>	434.2±0.4 <sup>cC</sup>	380.4±1.1 <sup>dD</sup>	333.0±0.5 <sup>eE</sup>
Extract <sub>100</sub>	450.3±0.7 <sup>aD</sup>	437.0±1.1 <sup>bE</sup>	422.9±1.1 <sup>cE</sup>	378.6±0.3 <sup>dE</sup>	334.7±0.9 <sup>dD</sup>
Extract <sub>200</sub>	447.1±0.3 <sup>aE</sup>	443.3±0.3 <sup>bD</sup>	427.4±0.1 <sup>cD</sup>	398.8±0.2 <sup>dC</sup>	357.5±0.3 <sup>cC</sup>

The results represent the mean±standard deviation of triplicate analyzes. a, b ... means followed by same letters in the lines do not differ by Tukey test (p>0.05). A, B ... means followed by the same letters in columns do not differ by Tukey test (p>0.05)

of the accelerated storage test, the best treatments for oil protection against the formation of primary oxidation compounds were TBHQ<sub>100</sub> and TBHQ<sub>200</sub>, followed by Extract<sub>200</sub>. Thus, Malagueta pepper natural extracts can be applied to vegetable oils as a way of reducing degradation caused by lipid oxidation and the use of synthetic antioxidants.

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#### CONFLICTS OF INTEREST

There are no conflicts of interest.

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