



Effect of *Curcuma longa* L. extract as a natural antioxidant in soybean oil

Efeito do extrato de *Curcuma longa* L. como antioxidante natural em óleo de soja

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ABSTRACT: This work aimed to evaluate the antioxidant potential of *Curcuma longa* L. extract in the oxidative stability of soybean oil. For this, the treatments Control (soybean oil), TBHQ₁₀₀ (soybean oil with 100 mg of TBHQ/kg), Extract₁₀₀ (soybean oil with 100 mg of β -carotene extract/kg) and TBHQ₅₀+Extract₅₀ soybean oil with 50 mg of TBHQ/kg of soybean oil + 50 mg of β -carotene extract/kg) were submitted to the accelerated oven test (60 °C/20 days). The extract presented 51.17% antioxidant activity by the DPPH \cdot and 98.74 μ M Trolox.g⁻¹ by FRAP, in addition to 457.9 μ g β -carotene.g⁻¹. Although all treatments showed low values of peroxide index (0.55-0.65 meq.kg⁻¹) at 20 days, Control presented greater oxidative degradation, 46.15 meq.kg⁻¹. TBHQ₁₀₀ and TBHQ₅₀+Extract₅₀ had the lowest values of *p*-anisidine. Even though TBHQ₁₀₀ showed higher oxidative stability (8.25 h), TBHQ₅₀+Extract₅₀ (6.41 h) showed greater stability than Control (3.86 h). All treatments presented high concentrations of the γ -tocopherol isomer. The synthetic antioxidant has proved to be more efficient in preventing the oxidation reaction than the natural antioxidant since it has a high degree of purity. However, TBHQ₅₀+Extract₅₀ presented satisfactory results, being the second best treatment able to reduce the soybean oil alteration, allowing the application to reduce the concentration of the synthetic antioxidant.

KEYWORDS: Antioxidant activity, Carotenoids, Tocopherols.

RESUMO: O trabalho teve como objetivo avaliar o potencial antioxidante do extrato de *Curcuma longa* L. na estabilidade oxidativa do óleo de soja. Para isso, os tratamentos Controle (óleo de soja), TBHQ₁₀₀ (óleo de soja com 100 mg de TBHQ/kg), Extrato₁₀₀ (óleo de soja com 100 mg de β -caroteno de extrato/kg) e TBHQ₅₀+Extrato₅₀ (óleo de soja com 50 mg de TBHQ/kg de óleo de soja + 50 mg de β -caroteno de extrato/kg) foram submetidos ao teste acelerado em estufa (60 °C/20 dias). O extrato apresentou 51,17% de atividade antioxidante pelo método DPPH \cdot e 98,74 μ M Trolox.g⁻¹ pelo FRAP, além de 457,9 μ g β -caroteno.g⁻¹. Embora, inicialmente, todos os tratamentos tenham apresentado baixos valores de índice de peróxidos (0,55-0,65 meq.kg⁻¹), em 20 dias, o Controle mostrou maior degradação oxidativa, 46,15 meq.kg⁻¹. O TBHQ₁₀₀ e TBHQ₅₀+Extrato₅₀ apresentaram os menores valores de *p*-anisidina. Apesar do TBHQ₁₀₀ ter apresentado maior estabilidade oxidativa (8,25 h), o TBHQ₅₀+Extrato₅₀ (6,41 h) mostrou maior estabilidade que o Controle (3,86 h). Todos os tratamentos apresentaram elevadas concentrações do isômero γ -tocoferol. O antioxidante sintético provou ser mais eficiente na prevenção da reação de oxidação do que o antioxidante natural, visto que possui elevado grau de pureza. Entretanto, o TBHQ₅₀+Extrato₅₀ apresentou resultados satisfatórios, sendo o segundo melhor tratamento capaz de reduzir as alterações do óleo de soja, possibilitando a aplicação para a redução da concentração do antioxidante sintético.

PALAVRAS-CHAVE: Atividade antioxidante, Carotenoides, Tocoferóis.

INTRODUÇÃO

Oxidative stability of edible oils is an important indicator of nutritional quality, since lipid oxidation may occur during processing and storage (TAGHVAEI; JAFARI, 2015; WALALLAWITA et al., 2016). The oxidation is responsible for the reduction of shelf life, the production of unpleasant odors and flavors, and the formation of degradation compounds, which may cause harmful effects to human health (WANG et al., 2011; MARTÍNEZ-YUSTA; GUILLÉN, 2014).

In order to prevent or delay the actions caused by free radicals and oxidizing compounds, antioxidants are used in vegetable oils (SHAHIDI; AMBIGAIPALAN, 2015). Due to the stability, availability and low cost, synthetic antioxidants, such as butylhydroxy-toluene (BHT), butylhydroxy-anisole (BHA), tert-butyl-hydroquinone (TBHQ), and propyl-gallate (PG) are used by the food industry (GORJI et al., 2016). However, these have been associated with possible carcinogenic effects (MI et al., 2016; MASKAN, HORUZ, 2017). Thus, interest has grown in the application of natural antioxidants in vegetable oils, which can be obtained from extracts of fruits, vegetables, spices and herbs such as pepper, rosemary, among others (CASAROTTI; JORGE, 2014; KIOKIAS; VARZAKAS, 2014; BEDDOU et al., 2015; JORGE et al. 2016).

Curcuma longa L. is a plant native to Asia but cultivated in tropical and subtropical regions of the world (ZHANG et al., 2017). In Brazil, *Curcuma longa* L. has little economic expression. Production is almost entirely to the national industries and food dyes (MUNIZ, 2011). Cultivation is carried out in beds, the root or rhizome being the most commonly used part. It is harvested when the plant begins to dry and its rhizomes have intense yellow pigments. It is widely used as a seasoning, flavoring, coloring and food condiment in the form of dry powder, after processing (GILDA et al., 2010; GOUNDER; LINGAMALLU, 2012; BANIK et al., 2017). In addition, it has therapeutic properties, which include insecticidal, anti-inflammatory, antiviral, anticancer, antimicrobial, antifungal and antioxidant actions (PRIYA et al., 2012; AVANÇO et al., 2017; ZHANG et al., 2017).

Curcuminoids, which mainly include curcumin, diferoloylmethane, demethoxycurcumin and bismetoxicurcumin (AHAMEFULA et al., 2014), are the compounds responsible for the characteristic yellow coloration of the *Curcuma* species (SHIRSATH et al., 2017). Curcumin, a phenolic carotenoid, is the biologically active

constituent comprising from 0.3 to 5.4% of *Curcuma longa* L. *in natura* (AKRAM et al., 2010).

Although there are reports of the presence of antioxidant compounds in *Curcuma longa* L., the effects of its application on vegetable oil are still unknown. Therefore, the present work had as objective to evaluate the antioxidant potential of the hydroalcoholic extract of *Curcuma longa* L. in soybean oil under oven accelerated storage.

MATERIAL AND METHODS

Extract

The organic rhizomes of *Curcuma longa* L. were obtained *in natura*, in Adamantina, São Paulo, Brazil (latitude: 21° 41 '07 "S, longitude: 51° 04' 21" W). About 200 g of rhizomes were washed in distilled water to remove the soils, dried on a paper towel and sliced manually. After, the rhizomes were frozen at -35 °C/24 h and lyophilized for 48 h (Model L101, Liobrás, São Carlos, Brazil).

To obtain the extract, 16 g of lyophilized material were added with 160 mL of ethanol-water solution (95:5, v/v), according to the methodology of Costa et al. (2010), with modifications. The mixture was intensely stirred for 30 sec, resting every 2 min until complete 30 min stirring. The mixture was then centrifuged for 5 min at 1077 x g, the supernatant was separated and the solvent was rotoevaporated under reduced pressure at 40 °C. Subsequently, the extract was lyophilized for 48 h.

Synthetic antioxidant

The synthetic antioxidant tert-butyl hydroquinone (TBHQ) in its commercial form, with a purity of 97% (Sigma-Aldrich, St. Louis, Mo. USA) was used for comparison with the extract.

Accelerated storage in oven

The lyophilized extract was resuspended in ethanol at the ratio 1:20 (extract: ethanol, w/w) for application in soybean oil, without the addition of a synthetic antioxidant and citric acid, purchased locally. The following treatments were formulated: Control (soybean oil), TBHQ100 (soybean oil with 100 mg TBHQ/kg), Extract 100 (soybean oil with 100 mg β -carotene extract/kg) and TBHQ₅₀+Extract₅₀ soybean oil with 50 mg of TBHQ/kg of soybean oil + 50 mg of β -carotene extract/kg).

The treatments were homogenized in Ultrasonic cleaner (model 740D, Odontobrás, Ribeirão Preto, Brazil), for 5 min, placed in beakers with a surface/volume ratio of 0.3/cm and submitted to accelerated storage in an oven at 60 °C for 20 days according to the methodology of Dutton (1978), with modifications. Samples were collected at different time intervals (0, 10, 20 days), inert with nitrogen gas and stored at -18 °C.

Extract analysis

The rhizome *in natura* was submitted to moisture analysis, according to the method of AOCS (2009). The yield of the extract was calculated and expressed as a percentage (%).

The DPPH method was performed according to Mensor et al. (2001) in a spectrophotometer (UV mini model 1240, Shimadzu, Kyoto, Japan) and the results were expressed as a percentage of antioxidant activity. The amount of sample required to decrease the initial concentration of DPPH by 50% (EC₅₀) was obtained by linear regression. FRAP was performed according to the method of Szydłowska-Czerniak et al. (2008), expressed in μ M Trolox.g⁻¹.

To obtain the total carotenoid content, an ethanolic solution with a concentration of 1000 μ g.mL⁻¹ of *Curcuma longa* L. extract was prepared and the absorbance was read in a spectrophotometer (UV mini model 1240, Shimadzu, Kyoto, Japan) at 450 nm, being expressed in μ g of β -carotene.g⁻¹ extract (RODRIGUEZ-AMAYA, 1999).

Oil analysis

Peroxide, *p*-anisidine, total oxidation value and oxidative stability were determined by the official methodology of the American Oil Chemist's Society (AOCS, 2009).

The tocopherols profile was performed according to the AOCS (2009) method using high-efficiency liquid chromatograph (Model 210, Varian Inc., Walnut Creek, USA) equipped with a fluorescence detector. Under the conditions of analysis: silica column of 250 x 4.6 mm with a pore size of 5 μ , flow of 1.2 mL.min⁻¹, excitation wavelength at 290 nm and emission at 330 nm and as mobile phase, the mixture of 99.5% n-hexane and 0.5% isopropanol. The identification of the tocopherol isomers (α , γ - and δ -tocopherol) was performed in comparison to the 95% purity standards retention time (Supelco, Bellefonte, USA). They were quantified by external standardization and the levels of tocopherols expressed in mg.kg⁻¹.

Statistical analysis

The work was carried out in a completely randomized design, with results using an analysis of variance (ANOVA) and Tukey's test at the 5% probability level, using the software STATISTICA, version 7.0. (STATSOFT INC, 2004).

RESULTS AND DISCUSSION

Extract characterization

The rhizome *in natura* presented a moisture content of 78.6% (Table 1), unlike other studies that demonstrated values of 82-91.5% (BRAGA et al., 2003; HIRUN et al., 2014). This variation can occur due to cultivation, planting site, agricultural practices, use of fertilizers and degree of maturation of rhizomes. The extraction yield with the hydroethanol solution was 9.4%.

Table 1. *Curcuma longa* L. extract characterization.

Rhizome	
Moisture (%)	78.6
Extract	
Yield (%)	9.4
DPPH• (%)	51.2
EC ₅₀ (mg.mL ⁻¹)	38.1
FRAP (μM Trolox.g ⁻¹)	98.7
Total carotenoids (μg β-carotene.g ⁻¹)	457.9

By the DPPH method, the extract showed antioxidant activity of 51.2%, close to that found by Gilda et al. (2010) in a methanol extract of *Curcuma longa* L. On the other hand, the value presented in this study is lower when compared to the Nahak and Sahu (2011) study that found 67.69-74.61% of antioxidant activity in extract ethanol in various concentrations. The EC₅₀ result showed a coefficient of determination (R²) of 0.9924. In relation to the antioxidant activity by the FRAP method, it was found an amount higher than the result obtained by Carelsen et al. (2010), which was 10.2 μM Trolox.g⁻¹. Such contrasts in the value of antioxidant activity, regardless of the method used, may occur due to solvents used for extraction and/or factors such as variety, cropping site, fertilization, climate, among others.

The extract presented low amount of carotenoids, 457.9 μg β-carotene/g, when compared to a study by Jorge et al. (2016) who investigated the amount of carotenoids in peppers and obtained from 1632.3-2140.5 μg β-carotene.g⁻¹. However, it is considered a high amount when compared to other vegetable sources such as carrot (88.36 μg β-carotene.g⁻¹), spinach (55.97 μg β-carotene.g⁻¹) and chicory (73.1 μg β-carotene.g⁻¹) (FERNÁNDEZ-GARCÍA et al., 2012; SAINI et al., 2015).

Oil's physicochemical properties

At first, the treatments presented similar peroxide indices, however, during the storage period, there was a significant increase ($p < 0.05$) in all treatments (Table 2).

Table 2. Effect of treatments x storage times on the oxidation compounds.

Analysis	Treatments	Storage times (days)		
		0	10	20
Peroxides (meq.kg ⁻¹)	Control	0.65 ± 0.02 ^{cA}	18.50 ± 0.40 ^{bA}	46.15 ± 0.01 ^{aA}
	TBHQ ₁₀₀	0.59 ± 0.01 ^{cA}	4.30 ± 0.02 ^{bC}	7.55 ± 0.11 ^{aD}
	Extract ₁₀₀	0.55 ± 0.02 ^{cA}	14.90 ± 0.02 ^{bB}	44.70 ± 0.02 ^{aB}
	TBHQ ₅₀ +Extract ₅₀	0.59 ± 0.01 ^{cA}	4.75 ± 0.01 ^{bC}	12.95 ± 0.01 ^{aC}
<i>p</i> -anisidine	Control	2.49 ± 0.16 ^{cA}	5.30 ± 0.01 ^{bA}	8.09 ± 0.68 ^{aA}
	TBHQ ₁₀₀	2.82 ± 0.09 ^{aA}	2.98 ± 0.05 ^{aB}	3.36 ± 0.21 ^{aC}
	Extract ₁₀₀	2.61 ± 0.09 ^{bA}	3.27 ± 0.01 ^{bB}	6.49 ± 0.07 ^{aB}
	TBHQ ₅₀ +Extract ₅₀	2.80 ± 0.03 ^{aA}	2.37 ± 0.19 ^{aB}	2.99 ± 0.01 ^{aC}
Totox	Control	3.79 ± 0.29 ^{cA}	42.30 ± 0.03 ^{bA}	100.39 ± 4.41 ^{aA}
	TBHQ ₁₀₀	4.01 ± 0.01 ^{cA}	11.58 ± 0.01 ^{bC}	18.46 ± 0.77 ^{aD}
	Extract ₁₀₀	3.70 ± 0.18 ^{cA}	33.07 ± 0.01 ^{bB}	95.89 ± 0.01 ^{aB}
	TBHQ ₅₀ +Extract ₅₀	3.99 ± 0.03 ^{cA}	11.87 ± 0.33 ^{bC}	28.89 ± 0.01 ^{aC}

Means ± standard deviations followed by the same lowercase letters in the lines do not differ by the Tukey test ($p > 0.05$). Means ± standard deviations followed by the same capital letters in the columns do not differ by the Tukey test ($p > 0.05$).

In 20 days of storage, the Control had a higher peroxide index, 46.15 meq.kg⁻¹. The treatments with addition of extract had lower peroxide indices than Control in 20 days of storage, however, TBHQ₅₀+Extract₅₀ showed greater protection against lipid oxidation of soybean oil, 12.95 meq.kg⁻¹, due to the synergistic effect of extract with TBHQ. The *Codex Alimentarius Commission* (2009) maximum limit of 10 meq.kg⁻¹ of peroxide index for refined vegetable oils. TBHQ₁₀₀ was the only treatment that remained below this limit throughout the storage period, however, TBHQ₅₀+Extract₅₀ remained below this limit up to 10 days of storage.

During storage, the formation of by-products exhibited behavior similar to that of the primers. For the Control, the index of *p*-anisidine had a gradual increase with the course of storage, and in 20 days it reached 8.09.

The *p*-anisidine values of TBHQ₁₀₀ and TBHQ₅₀+Extract₅₀ remained constant and did not differ statistically with the storage time, proving that the presence of TBHQ alone or associated with the extract is efficient against lipid oxidation. Thus, due to the synergistic effect, it is possible to reduce the amount of TBHQ in soybean oil.

Zhang et al. (2018), studying the effect of natural polyphenol under the oxidative stability of pecan nut oil, found superior effect of synthetic antioxidants (TBHQ, BHT and BHA) under the polyphenols studied. According to Allen and Hamilton (1983), refined oils can be considered of good quality, since they have *p*-anisidine values below 10, and, as observed in this study, even though there was an increase in *p*-anisidine indices in Control and Extract₁₀₀ at the end of the storage period, they did not exceed this limit.

Regarding the totox value, the treatments presented a progressive increase during the storage; however, in 20 days, TBHQ showed lower degradation, 18.46, followed by TBHQ₅₀+Extract₅₀ (28,89).

Among the treatments, TBHQ₁₀₀ presented greater stability (8.25 h), followed by TBHQ₅₀+Extract₅₀ (6.41 h), demonstrating the synergistic effect between TBHQ and extract (Figure 1a). During the course of storage, a decrease in the oxidative stability of the treatments occurred, probably due to the formation of degradation compounds (Figure 1b).

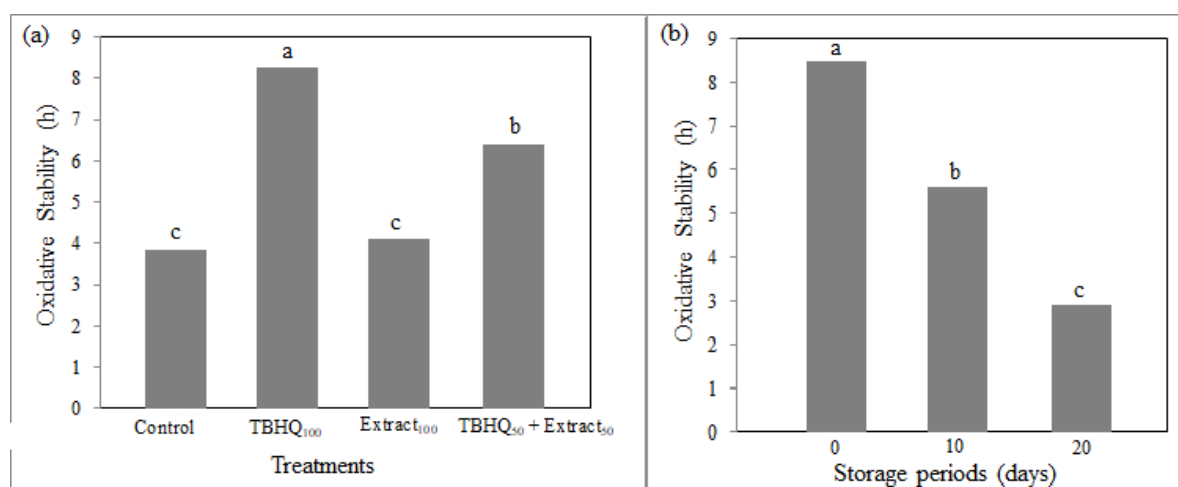


Figure 1. Oxidative stability: treatments (a) and storage times (b).

At the initial time, the Extract₁₀₀ presented higher concentration of α -tocopherol. However, with the storage time, there was a degradation of this isomer, and the TBHQ₅₀+Extract₅₀ showed the highest retention of α -tocopherol, possibly due to the synergistic effect with TBHQ (Table 3).

Table 3. Influence of treatments x storage times on the composition of tocopherols.

Tocopherols (mg.kg ⁻¹)	Treatments	Storage times (days)	
		0	20
α-tocopherol	Control	49.34 ± 0.16 ^{aB}	19.05 ± 0.01 ^{bC}
	TBHQ ₁₀₀	44.70 ± 0.36 ^{bC}	47.42 ± 0.05 ^{aA}
	Extract ₁₀₀	65.90 ± 0.59 ^{aA}	29.87 ± 0.02 ^{bB}
	TBHQ ₅₀ +Extract ₅₀	48.61 ± 0.03 ^{aB}	47.54 ± 0.16 ^{bA}
γ-tocopherol	Control	191.31 ± 0.35 ^{bC}	215.17 ± 1.16 ^{aA}
	TBHQ ₁₀₀	215.31 ± 0.61 ^{aB}	213.33 ± 0.03 ^{bB}
	Extract ₁₀₀	260.22 ± 0.25 ^{aA}	211.41 ± 0.01 ^{bC}
	TBHQ ₅₀ +Extract ₅₀	183.62 ± 0.11 ^{aD}	178.63 ± 0.05 ^{bD}
δ-tocopherol	Control	57.61 ± 0.01 ^{bC}	66.41 ± 0.56 ^{aA}
	TBHQ ₁₀₀	61.26 ± 1.05 ^{aB}	62.12 ± 0.61 ^{aB}
	Extract ₁₀₀	75.84 ± 0.33 ^{aA}	65.57 ± 0.09 ^{bA}
	TBHQ ₅₀ +Extract ₅₀	57.13 ± 0.05 ^{aC}	57.70 ± 0.05 ^{aC}
Total	Control	298.25 ± 0.01 ^{aC}	300.63 ± 3.98 ^{aC}
	TBHQ ₁₀₀	321.26 ± 0.13 ^{aB}	322.87 ± 1.37 ^{aA}
	Extract ₁₀₀	401.95 ± 3.38 ^{aA}	306.85 ± 0.01 ^{bB}
	TBHQ ₅₀ +Extract ₅₀	289.35 ± 0.07 ^{aB}	283.87 ± 0.16 ^{bD}

Means ± standard deviations followed by the same lowercase letters in the lines do not differ by the Tukey test ($p > 0.05$). Mean ± standard deviations followed by the same capital letters in the columns do not differ by the Tukey test ($p > 0.05$).

Among the isomers, γ-tocopherol was found in greater quantity, highlighting Extract₁₀₀ with 260.22 mg.kg⁻¹. However, during storage, TBHQ₁₀₀ and TBHQ₅₀+Extract₅₀ presented higher retention, 99.08 and 97.28%, respectively. Regarding the δ-tocopherol isomer, although the Extract₁₀₀ showed a higher amount at the beginning, TBHQ₁₀₀ and TBHQ₅₀+Extract₅₀ stood out, as they remained constant throughout the storage since Extract₁₀₀ showed a loss of 13.5%.

TBHQ₁₀₀, although not showing higher total tocopherols, in the beginning, remained constant up to 20 days of storage. On the other hand, Extract₁₀₀, which showed a higher amount initially (401.95 mg.kg⁻¹), after 20 days of storage retained only 76%. On the other hand, TBHQ₅₀+Extract₅₀ showed high retention (98%) at 20 days of storage, possibly due to the synergism between TBHQ and extract.

CONCLUSION

The *Curcuma longa* L. extract has a good antioxidant capacity and a high content of total carotenoids, however, it did not demonstrate great protective effects against the lipid oxidation of soybean oil during storage conditions. However, the TBHQ₅₀+Extract₅₀, had similar efficacy to TBHQ₁₀₀, since there were no statistical differences in the analysis of *p*-anisidine and levels of α -tocopherol in 20 days of storage. Thus, under the conditions studied, it becomes possible to partially replace the TBHQ with this extract in soybean oil. This substitution can result in a higher economic value of the oil, but it is a new option for the use of *Curcuma longa* L., and consequently, enriches the soybean oil, making it healthier.

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