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Headspace GC/MS for identification of bioactive compounds of *Curcuma longa* L. leaf extract: Industrial application as antioxidant for soybean oil

Headspace GC/MS para identificação de compostos bioativos do extrato da folha de *Curcuma longa* L.: Aplicação industrial como antioxidante para óleo de soja

Izabel de Paula Duarte Alves[1], Zamira Abel de Jesus Maria[2*], Julião Pereira[2], Gardênia Martins de Sousa[2], Tatianne Ferreira de Oliveira[2]

[1]Universidade Federal de Goiás/UFG, Faculdade de Farmácia, Goiânia, GO, Brasil
[2]Universidade Federal de Goiás/UFG, Departamento de Engenharia de Alimentos, Faculdade de Agronomia, Goiânia, GO, Brasil
*Corresponding author: maria2345678@discente.ufg.br
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ABSTRACT

The use of natural antioxidants extracted from plants is an alternative to the application of synthetic antioxidants. In this study, we evaluated the oxidative stability of soybean oil after the addition of *Curcuma longa* L. leaf extracts compared to its oxidative stability with the synthetic antioxidant butylated hydroxytoluene (BHT Different concentrations (0.5%, 1.0%, and 1.5%) of ethanolic extract of *Curcuma longa* L. leaves were added to the oil, and the mixture was heated at 60 ±2 °C for 12 days. Several parameters of oxidative stability, including the peroxide index (PI), thiobarbituric acid reactive substances (TBARS), and conjugated dienes and trienes, were analyzed every three days. The results were promising, the oils to which the *Curcuma longa* L. leaf extract was added showed a reduction in all parameters, indicating oxidative deterioration under the influence of the concentration of the extract and the duration of treatment. The extract was less effective at low concentrations (0.5%), the parameters did not vary considerably. The PI was low in all treatments until the third day. The PI of the soybean oil treated with 1.5% extract was lower than that after treatment with the synthetic antioxidant and the blank treatment on days 6 to 12. The highest production of TBARS was observed in the blank treatment on days 6 to 12, and the lowest values of TBARS were recorded in the soybean oil treated with 1.5% extract. For the same concentration, the conjugated dienes varied from 2.05 to 8.6, and the trienes from 0.57 to 1.59.

Index terms: Natural antioxidants; oxidative stability; peroxide index; plants.

RESUMO

O uso de antioxidantes naturais de plantas tem sido uma abordagem alternativa aos antioxidantes sintéticos. O objetivo desse trabalho foi avaliar a estabilidade oxidativa do óleo de soja com adição de extrato de folhas de *Curcuma longa L.* em comparação com antioxidante sintético hidroxitolueno butilado (BHT). Ao óleo, adicionou-se o extrato etanólico de folhas de *Curcuma longa* L. nas seguintes concentrações 0.5%, 1.0%, 1.5%, submetidos a aquecimento a 60 °C ±2, por um período de 12 dias. Analises dos parâmetros da estabilidade oxidativa: índice de peróxido (IP), substâncias reativas ao ácido tiobarbitúrico (TBARS), dienos e trienos conjugados foram feitas a cada 3 dias. Os resultados foram promissores, os óleos com extrato de folha de *Curcuma longa L.* apresentou redução de todos os parâmetros indicativos de deterioração oxidativa, com influência da concentração e do tempo de estudo. A concentração 0,5% foi menos efetiva, os parâmetros não sofreram grandes variações. O IP foi menor em todos os tratamentos até o 3° dia, no óleo de soja com extrato de 1.5% o valore de índice de peroxido foi menor que o antioxidante sintético e o branco nos dias (6 a 12). Quanto ao TBARS a maior produção foi observada no branco nos dias (6 a 12) e os menores valores de TBARS foram evidenciados no óleo de soja com extrato 1.5%, para a mesma concentração os dienos conjugados variaram entre 2.05 a 8.6, e os trienos de 0.57 a 1.59.

Termos para indexação: Antioxidante natural; estabilidade oxidativa; índice de peroxido; plantas.

INTRODUCTION

Edible oils can deteriorate during storage, handling, or cooking due to lipid oxidation which is responsible for the loss of quality of oil, decrease in nutritional value, and development of undesirable flavors, which make it less acceptable to consumers. The extent of lipid oxidation depends on internal factors of oil, such as the degree of unsaturation and presence of antioxidant compounds or metals, such as copper and iron, and external factors, such as oxygen availability and temperature (Fadda et al., 2022).

Soybean oil is extracted from soybean seeds (*Glycine max*). It is considered to be the most common and healthy vegetable oil because the oil contains high quantities of polyunsaturated fatty acids, including linoleic (47%) and linolenic (26%) acids (Prabakaran et al., 2018). However, soybean oil has low oxidative stability and turns rancid easily during frying and storage due to its high content of polyunsaturated fatty acids (Olagunju; Adelakun; Olawoyin, 2022).

The oxidation of oil is currently reduced by using synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butylhydroquinone (TBHQ), and propyl gallate (PG). Due to the safety concerns associated with the use of synthetic antioxidants and to meet the increasing consumer demands for natural foods, various methods based on the application of natural antioxidants have been developed to decrease the oxidation of vegetable oils (Xu et al., 2021).

Natural antioxidants from plants might be used instead of synthetic antioxidants, and some studies have investigated their application in food (Alizadeh et al., 2019). For example, Franco et al. (2018) reported that the antioxidant showed a non-linear relationship with the concentration of peanut skin extract. They concluded that 750 mg/kg of peanut skin extract inhibited primary lipid oxidation and secondary oxidation of soybean oil. This inhibition was similar to that achieved with 200 mg/kg of BHT. Hence, peanut skin extract might be used as an effective natural antioxidant for maintaining the stability of soybean oil (Franco et al., 2018).

Curcuma longa L. or turmeric is a plant of Asian origin, and it might be used as a potential natural antioxidant. It is mainly grown for its rhizomes (Choudhary; Rahi, 2018), while the leaves are discarded during post-harvest operations. Information on the composition and antioxidant potential of these leaves is limited (Braga; Vieira; Oliveira, 2018).

Some studies have evaluated the antioxidant capacity of these leaves and found that these leaves have a high content of phenolic compounds (Braga; Vieira; Oliveira, 2018) and better antioxidant activity in vegetable oil than that exhibited by synthetic antioxidants (Modh nor et al., 2009).

Santos et al. (2021) evaluated the effect of *Curcuma longa* L. extract as a natural antioxidant in soybean oil Their findings showed that the extract of *Curcuma longa* L. has a good antioxidant capacity and a high content of total carotenoids, but the extract could not effectively prevent the lipid oxidation of soybean oil during storage for 20 days.

In this study, we identified the compounds present in the extracts of turmeric (*C. longa* L.) leaves and used them as an antioxidant agent in industrial crude soybean oil to evaluate the oxidative stability of soybean oil after adding the natural extract and compared its oxidative stability after treatment with the synthetic antioxidant butylated hydroxytoluene (BHT).

MATERIAL AND METHODS

Sample preparation

The leaves of *Curcuma longa* L. were collected between December 2020 and April 2021 at the Cooperative of Saffron Producers (Cooperaçafrão) (Mara Rosa, Goiás, Brazil) (14°00'10.9" E, 49°07'11.8" W). They were stored in nylon bags and refrigerated in the laboratory in the Department of Food Engineering at the School of Agronomy of the Federal University of Goiás (Goiânia, Goiás, Brazil) for 24 h.

The leaves were selected based on their color (green) and integrity. They were washed with drinking water and sanitized by treating them with sodium hypochlorite solution (0.1 mL L^{-1}) for 15 min. The stems were removed, and the leaves were cut into squares of approximately 2 cm. Then, they were placed on perforated stainless steel trays and subjected to convective drying at 40 °C (Braga; Vieira; oliveira, 2018) in an air circulation oven (Tecnal. TE-394/3, Piracicaba, Brazil) for 72 h (final humidity was 10.29 ±0.07 g/100 g on dry basis). After drying, they were packed in vacuum plastic bags and stored in cool and dark well-aerated conditions at room temperature until analysis.

Obtaining extracts of Curcuma longa L. leaves

The quantification of the total phenolic content (TPC) and antioxidants was performed using ethanol/water (70%:30%), following the methods described by Michiels et al. (2012). The leaves were mixed with 1 g of the dehydrated sample and 20 mL of each extractive solution and stirred in a magnetic mixer (Tecnal. TE-089, Piracicaba, Brazil) at room temperature (25 °C ±2 °C) for 1 h. The extracts were filtered, and the solid residue was scraped from the filter and extracted again with 20 mL of the solvent. The volume of the extract was measured to 50 mL before being stored in amber flasks at –20 °C.

Analyses of the total phenolic content and antioxidant capacity

The TPC was determined for each of the extracts prepared according to item 2.2 (extracts). After the spectrophotometric analysis, the result of the TPC was expressed as mg of gallic acid equivalent (GAE) per 100 g of the dry matter of the sample. The antioxidant capacity was measured by analyzing the DPPH and FRAP. The

analysis of DPPH was performed according to Chan, Lim, and Lim (2007) with modifications (1 g of dried leaves was mixed with 20 mL of the solvent). The radical scavenging activity was calculated as IC50 and expressed as Trolox equivalent antioxidant capacity (TE) in mg of Trolox per 100 g of the material as follows Equation 1:

$$TE = \frac{IC_{50}(\text{Trolox})}{IC_{50}(\text{sample})}.10^5 \quad (1)$$

Here,
TE: mg TE.$100^{-1.}$ g^{-1}
IC_{50}: the amount of substance required for a 50% reduction in the concentration of DPPH ion. The iron-reducing power (FRAP) was evaluated using the method suggested by Pulido, Bravo, and Saura-Calixto (2000) with modifications (tripled quantities, maintaining proportions).

The result was expressed in µMol of $FeSO_4$ per 100 g of the material

Conditions maintained for the HRGCMS-Headspace analysis

The analyses were performed using a gas chromatograph coupled to a mass spectrometer (Shimadzu Nexis GC2030) equipped with a capillary column (SH-Stabilwax-ms; 30 m, 250 µm id, 0.25 µm). The samples were previously heated via headspace at 80 °C for 30 min, and 2.5 mL of the sample was injected into the GC.

The split mode was used with a ratio of 10:1 and an equilibrium time of 3 min. The oven temperature program was as follows: the initial temperature was 40 °C, which was maintained for 1 min. The heat was increased by 5 °C min^{-1} to 160 °C, and then, it was increased by 10 °C min^{-1} to 250 °C and maintained for 15 min. The total analysis time was 49 min. Helium 5.0 was used as the carrier gas, with a pressure of 4.7 psi, a flow rate of 0.79 mL min^{-1}, and a linear velocity of 32.4 cm s^{-1}. The temperature of the injector, interface, and ion source was maintained at 250 °C. The mass spectrometer operated in the scan mode recorded ions in the range from 25 to 500 m/z with a scan time of 150 ms.

Accelerated storage test

The accelerated oven test was performed following the methodology proposed by Mohd Nor et al. (2009). Refined, bleached, and degummed soybean oil was used without the addition of synthetic antioxidants. The ethanolic extract of leaves of *C. longa* L. was first added to 100 g of oil in 150 mL transparent glass beakers. The beakers were heated in an oven (Quimis, Q-317B232, Brazil) for 12 days. Aliquots were removed every three days to evaluate the parameters of oxidative stability. A blank (oil without BHT) and positive control (ethanolic extract and oil containing BHT at 0.02 g/100 g) were also evaluated for each temperature studied (60 °C) under the same experimental conditions.

Evaluation of oxidative stability

Peroxide index (PI)

The PI was evaluated according to American Oil Chemists' Society (American Oil Chemists' Society - AOCS, 2003). This method required a mixture of 1 g of soybean oil with 50 mL of 3:2 (v/v) acetic acid/iso-octane solution and 0.5 mL of a freshly saturated potassium iodide (KI) solution. The sample was titrated with 0.01 mol/L sodium thiosulfate solution ($Na_2S_2O_3$) till the blue color, formed by the addition of 0.5 mL of 1% starch solution as an indicator, disappeared.

The test was performed in duplicate along with a control test. The calculation was performed according to Equation 2.

$$PI = \frac{(S-B).N.1000}{P\,A} \quad (2)$$

Here,
PI represents the peroxide index in mEq
S represents the quantity (mL) of 0.01 mol/L sodium thiosulfate solution used as the titrant in the titration of the samples,
B represents the quantity (mL) of 0.01 mol/L sodium thiosulfate solution used as the titrant in the titration of the control,
N represents the concentration of the sodium thiosulfate solution in mol/L, and
PA represents the weight of the sample in g.

The result was expressed in milliequivalent (mEq) peroxide per kg of the sample analyzed.

Thiobarbituric acid reactive substances (TBARS)

The TBARS assay was performed according to the method described by Tarladgis et al. (1960), in which 10 g of the sample was previously mixed with 50 mL of distilled water. The mixture was distilled in a Kjeldahl distillation flask, and 50 mL of the distillate was collected at the end. In a test tube, 5 mL of the distillate and 5 mL of TBA solution (0.5766 g of thiobarbituric

acid diluted in 20 mL of distilled water and 180 mL of acetic acid P.A.) were added and stirred. The absorbance of the sample was measured using a spectrophotometer (Rayleigh model UV 800, Beijing, China) at 538 nm. The calculation was performed by multiplying the absorbance reading by 7.8 (experimental value) and was expressed in mg of malonaldehyde (MDA) per 1,000 g of the sample analyzed.

Dienes (CD) and triple conjugates (CT)

The CD and CT were analyzed according to the AOCS method (1990). First, 10 mL of isooctane P.A. was added to the sample (0.01 g). Then, the absorbance was measured at 233 nm (dienes) and 270 nm (trienes) using a quartz cuvette. The dilutions were performed to obtain absorbance values between 0.1 and 0.8. The calculation was performed according to Equation 3.

$$\frac{A\lambda}{cl} = K\lambda \quad (3)$$

Here,
$K\lambda$: $E1\%$
1 cm indicates specific extinction at wavelength,
$A\lambda$ represents the absorbance measured at a specific wavelength,
c represents the concentration of the solution in g/100 mL, and
l represents the light path of the cuvette in cm.

Statistical analysis

The data were analyzed by performing ANOVA and Tukey's test ($p < 0.05$). The STATISTICA 7.0 program (StatSoft Inc., Tulsa, Oklahoma, USA) was used for conducting the analysis, and the significance level was considered to be 95% ($p < 0.05$).

RESULTS AND DISCUSSION

Identification of the compounds present in the sample by GC/MS

The results of the characterization and identification of the compounds present in the leaves of *Curcuma longa* L., as determined by the GC/MS analysis, are presented in Figure 1 and Table 1.

Several peaks were identified in the leaves of *Curcuma longa* L. with 42 peaks detected, which corresponded to different compounds. The main compound was eucalyptol (42.82%). Eucalyptol, also known as 1,8-cineol, is a terpene oxide found in the essential oils of plants (Andrade-Neto et al., 1994). It is often used as a flavoring agent for food products (Al-Sereiti; Abu-Amer; Sem, 1999). This compound can be found in various types of plants, such as eucalyptus, rosemary, and saffron. Eucalyptol has various pharmacological activities, such as antibacterial, antifungal, anti-inflammatory, and bronchodilator. It can also be used for treating people with respiratory diseases, such as rhinosinusitis, bronchitis, asthma, and chronic obstructive pulmonary disease (COPD) (Połeć et al., 2019).

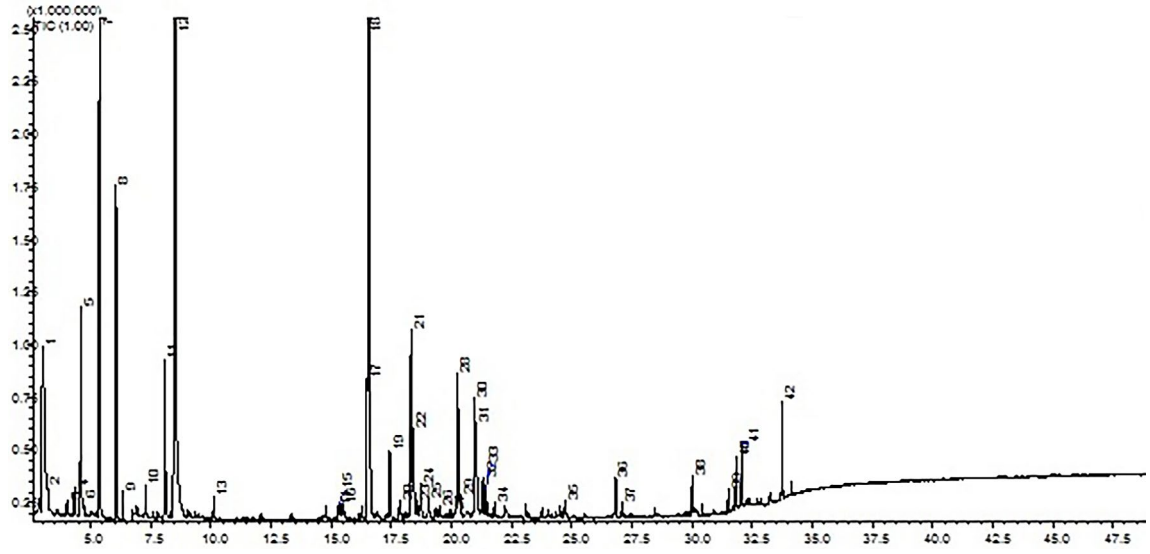

Figure 1: Chromatographic profile of the active constituents of the leaves of *Curcuma longa L.*

Table 1: List of the active constituents of *C. longa* L. leaves.

No.	Name	Molecular Formula	Retention Time (RT)	Relative content (%)	MS Similarity (%)
1	Methyl Alcohol	CH4O	3.027	5.33	97
2	Butanal, 3-methyl-	C5H10O	3.192	0.47	86
3	2,3-Butanedione	C4H6O2	4.017	0.26	86
4	Tricyclo [2.2.1.0(2,6)]heptane, 1,3,3-trimethyl-	C10H16	4.336	0.42	94
5	(1R)-2,6,6-Trimethylbicyclo [3.1.1]hept-2-ene	C10H16	4.587	2.99	96
6	Bicyclo [3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)-	C10H16	4.689	0.27	80
7	Camphene	C10H16	5.353	5.32	95
8	Bicyclo [3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	C10H16	6.048	3.29	95
9	Bicyclo [3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	C10H16	6.324	0.25	96
10	β.-Myrcene	C10H16	7.276	0.33	96
11	D-Limonene	C10H16	8.079	2.11	94
12	Eucalyptol	C10H18O	8.514	42.82	95
13	o-Cymene	C10H14	10.107	0.26	96
14	Acetic acid	C2H4O2	15.286	0.20	78
15	Cyclohexene, 4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(1-methylethyl)-, (3R-trans)-	C15H24	15.371	0.19	90
16	trans-Linalool oxide (furanoid)	C10H18O2	15.496	0.27	91
17	(-)-.beta.-Bourbonene	C15H24	16.480	1.66	90
18	(+)-2-Bornanone	C10H16O	16.552	14.09	96
19	Linalool	C10H18O	17.430	1.04	97
20	(S,1Z,6Z)-8-Isopropyl-1-methyl-5-methylenecyclodeca-1,6-diene	C15H24	17.848	0.32	89
21	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]-	C15H24	18.308	3.13	96
22	Caryophyllene	C15H24	18.418	1.60	95
23	Bicyclo [2.2.1]heptan-2-ol, 2,3,3-trimethyl-	C10H18O	18.583	0.28	92
24	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-	C10H18O	18.724	0.57	95
25	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,7.beta.,8a.alpha.)]-	C15H24	19.045	0.27	91
26	(S,1Z,6Z)-8-Isopropyl-1-methyl-5-methylenecyclodeca-1,6-diene	C15H24	19.500	0.27	93
27	Bicyclo [3.1.1]heptan-3-ol, 6,6-dimethyl-2-methylene-, [1S-(1.alpha.,3.alpha.,5.alpha.)]-	C10H16O	19.961	0.16	95
28	Isoborneol	C10H18O	20.254	2.76	97
29	Cyclohexanemethanol, .alpha.,.alpha.-dimethyl-4-methylene-	C10H18O	20.374	0.36	92

Continue...

Table 1: Continuation.

No.	Name	Molecular Formula	Retention Time (RT)	Relative content (%)	MS Similarity (%)
30	L-.alpha.-Terpineol	C10H18O	20.960	1.71	93
31	Bicyclo [2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)-	C10H18O	21.024	1.53	87
32	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha.,7.alpha.,8a. beta.)]-	C15H24	21.306	0.54	97
33	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2.alpha.,4a. alpha.,8a.beta.)]-	C15H24	21.430	0.38	93
34	(-)-Carvone	C10H140	21.796	0.23	92
35	Benzofuran, 6-ethenyl-4,5,6,7-tetrahydro-3,6-dimethyl-5-isopropenyl-, trans-	C15H20O	24.730	0.39	95
36	Caryophyllene oxide	C15H24O	26.829	0.65	91
37	Isospathulenol	C15H24O	27.087	0.19	81
38	Epicurzerenone	C15H18O2	30.002	0.52	94
39	Curcumenol	C15H2202	31.510	0.24	82
40	Cyclodeca [b]furan-2(3H)-one, 3a,4,5,8,9,11a-hexahydro-3,6,10-trimethyl-, [3S-(3R*,3aR*,6E,10E,11aR*)]-	C15H2202	31.828	0.54	73
41	Cyclohexene, 4-pentyl-1-(4-propylcyclohexyl)-	C20H36	32.070	0.68	72
42	Curcumenol	C15H2202	33.744	1.09	94

The second major compound identified in the leaves of *C. longa* L. was (+)-2-bornanone (14.09%), also known as D-camphor. It is a terpenoid group, extracted from plants such as lavender, turmeric, and those in the Lauraceae family. This compound has antimicrobial activity against all studied microorganisms except *Escherichia coli* ATCC 25922 (Canli et al., 2019).

Methyl alcohol was the third compound identified in the leaves of *C. longa* L. (5.33%). It is a volatile, odorous, toxic compound that affects the central nervous system. The plant generates methanol in the cells despite the demethylation reaction of macromolecules, such as DNA, RNA, and proteins (Dorokhov; Sheshukova; Komarova, 2018). Estimates from food derivatives show ethanol and methanol in humans at 7.9 mg/kg in beans, lentils, and peas (National Toxicology Program, 2003). However, Gürler et al. (2022) found that the methanol content was 150 mg/kg for vegetables such as tomatoes, eggplant, and pickles and 130–390 mg/kg for legumes, such as beans, maize, and peas. These high levels might be due to food processing. The fourth major compound found in *C. longa* L. leaves was Camphene, also known as 2.2-dimethyl-3-methylidenobicyclo [2.2.1] heptane. It belongs to the monoterpene hydrocarbon group and is one of the major compounds in essential oils.

Characterization of the ethanol extract and water

The results of the characterization of *C. longa* L. leaf extract are presented in Table 2.

Ethanol/water is considered to be the ideal combination for industrial food processing as it is safe and food compatible. The mixture of organic solvents and water is more efficient than the mono-solvent system since glycosylated phenolic compounds are more soluble in water. In the combined ethanol/water solvents, besides showing a selective behavior in the extraction of glycosylated compounds, ethanol can also extract non-glycosylated compounds due to the high availability of free electrons (Pedro et al., 2018).

Phenolic compounds present in *C. longa* L. leaves can protect against oxidative damage caused by free radicals. The content of phenolic compounds found in *C. longa* L. leaves was 2, 422.04 mg EAG.100 g^{-1}, which was higher

than the content reported by Johnson et al. (2008), who determined the content of phenolic compounds in different medicinal plants obtained for the extract curcuma powder. This difference occurred probably because the authors used the turmeric rhizome. The total phenolic content (TPC) of *C. longa* L. leaves was higher than that of goji berry *Lycium barbarum* L. in ethanolic extract (1,351.45 mg of EAG.100 g^{-1}) (Pedro et al., 2018). Phenolic compounds are the major constituents of plant materials and contribute to their antioxidant capacity. Plants, fruits, and their extracts that reflect concentrations of phenolic compounds are thus considered to be good sources of antioxidants for inhibiting the oxidation of foods (Pennington; Fisher, 2009).

The DPPH assay involves a fast electron transfer reaction (SET) and a slow hydrogen atom transfer reaction (HAT), mainly in hydrogen-accepting solvents, such as methanol and ethanol (Huang; Boxin; Prior, 2005). The DPPH value of *C. longa* L. leaves (1,847.08 mg TE.100 g^{-1}) was lower than that of basil leaves (*Ocimum basilicum* L. Lamiaceae; 4,505.10 mg TE. 100 g^{-1}) (Złotek et al., 2016), and goji berry (*Lycium barbarum* L.; Solanaceae; 583 mg TE. 100 g^{-1}) (Pedro et al., 2018).

The antioxidant capacity of ferric ion reduction in *C. longa* L. leaves was 217.99 µMol of $FeSO_4$.100 g^{-1}. Somogyi et al. (2015) found the following FRAP values for 7% w/w concentration of herbs immersed in vegetable oils: 500, 580, and 600 µMol of $FeSO_4$.100 g^{-1} for dried leaves of sage (*Salvia officinalis* L., Labiatae) and 300, 310, and 380 µMol of $FeSO_4$.100 g^{-1} for dried leaves of oregano (*Origanum vulgare* L., Lamiaceae). These values were higher than those found in this study. Differences in antioxidant activity values, regardless of the method used, might occur due to the solvents used for extraction and/or factors such as variety, the cultivation site, fertilization, and climate, among others (Santos et al., 2021).

Balboa et al. (2014) studied seaweed extracts, which are natural and inexpensive sources bioactive compounds, and obtained good antioxidant capacity responses *in vitro*, measured as the DPPH radical eliminating activity (IC50 of 0. 297 mg mL^{-1}) and FRAP (574.59 µMol of $FeSO_4$), by adopting environmentally friendly processes and using water and ethanol, as they are non-toxic solvents.

Regarding solvent toxicity, ethanol and water are safer solvents than other organic solvents and, therefore, are recommended for use in food (Balboa et al., 2014). Therefore, the ethanol-water extract is suitable for application as an antioxidant agent in industrial soybean oils.

Evaluation of oxidative stability

After applying the *C. longa* L. leaf extract in crude soybean oil, the oil turned dark green because of the base color of the extract, which is green because of the presence of chlorophyll. When the oil was heated in the accelerated oven test, it turned dark, which was also reported by Mohd Nor et al. (2009), who mixed palm oil with ethanolic extract of *C. longa* L. leaves and conducted an accelerated oxidation test on the mixture, and then, they fried the mixture at 180 °C. The authors reported that the coloration of the oil was not considerably affected by the frying time for the blank and BHT samples, but the oil with the added extract of *C. longa* L. darkened significantly due to the presence of pigments and phenolic compounds, and their decomposition products formed during heating.

For evaluating the parameters of oxidative stability, peroxides were considered to be the first compounds formed during oxidation and were relatively stable, provided that the sample was not in an advanced oxidative state (Shahidi; Ambigaipalan, 2015). Their content decreased after prolonged heating (Mohd Nor et al., 2009), and they underwent rearrangements generating conjugated dienes, which were not stable after 30 days of storage.

These underwent decomposition to secondary oxidation products, such as aldehydes, which can be measured more accurately after long-term storage (Matumoto-Pintro et al., 2017).

Peroxide index (PI)

The peroxide indices of industrial soybean oil subjected to a storage acceleration test for 12 days are presented in Table 3 and Figure 2.

Table 2: Characterization of the ethanol/water extract of *C. longa* L. leaves.

Solvent	TPC mg GAE (100 g^{-1} dry matter)	DPPH (mg TE 100 g^{-1})	FRAP (µMol $FeSO_4$ 100 g^{-1})
E/W	2422.04	1847.08	217.99

***** The data are expressed as the average of the triplicate experiment. Legend: EAG: Gallic acid equivalent, TE: Trolox equivalent antioxidant capacity, E: ethanol, W: water, TPC: total phenolic content, DPPH: Sequestering capacity of the 2-diphenyl-1-picryl hydrazyl radical, and FRAP: Ferric Ion Reducing Antioxidant Power.

Table 3: Peroxide index (PI) (mEq peroxide kg^{-1}) of soybean oil that was subjected to the accelerated storage test for 12 days.

Concentration (%)	Peroxide index				
	Time (days)				
	0	3	6	9	12
0.5%	1.7219[a]	3.4811[b]	13.1892[c]	19.1918[d]	70.1865[e]
1.0%	1.9912[a]	3.4575[b]	4.4519[c]	11.1386[d]	65.1163[e]
1.5%	1.8242[a]	4.6945[b]	3.4666[c]	9.3543[d]	31.4712[e]
Blank	1.7349[a]	3.7299[b]	29.1885[c]	45.2020[d]	103.2724[e]
Control (BHT)	1.2273[a]	1.7219[b]	16.2080[c]	28.8447[d]	85.7655[e]

Values with different small letters along the row are significantly different ($p < 0.05$).

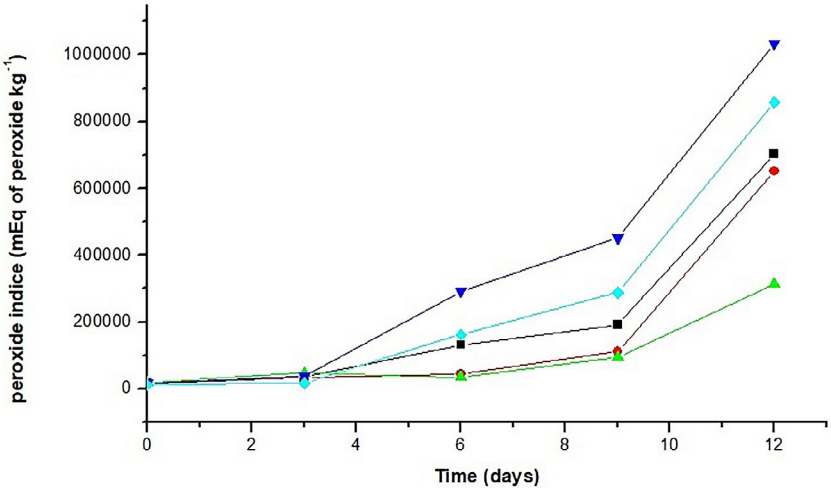

Figure 2: Kinetics of the evolution of the peroxide index (mEq peroxide. kg^{-1}) as a function of time, C1 (■) –0.5%; C2 (●)- 1.0%; C3 (▲)-1.5%; Blank (►)-0.0%; Control BHT (♦) –0.02% at 60 °C.

The peroxide index (PI) is a quality parameter that measures the concentration of peroxides and hydroperoxides formed in the initial stage of lipid oxidation. The number of peroxides present in vegetable oils indicates their level of oxidation, and consequently, their tendency to become rancid (Marina; Wan Rosli; Noorhidayah, 2013).

Our results showed that the PI was lower in all the experiments from day 0 to day 3, and the peroxide index in soybean oil with 1.5% extract was lower than the synthetic antioxidant BHT from days 6 to 12; this parameter increased by about 21.38% on day 6. In the blank sample, the PI on day 12 was about 83.04% higher than the PI of the synthetic antioxidant BHT (Table 3).

The use of 0.5% extract on day 9 presented a higher PI (about 58.03%) compared to the PI of soybean oil containing 1.0% extracts (Table 3). The storage time significantly influenced the formation of peroxides. This phenomenon was observed when analyzing the soybean oil treated with 0.5% extract, in which the PI parameter increased over the study period. This significant increase in the peroxide index was characterized by the propagation phase of the lipid oxidation reaction, where peroxide formation occurred. The peroxide index is the most common parameter to detect fat rancidity; the lower the PI, the lower the oxidation rate of the sample and the higher its oxidative stability. In this study, *C. longa* L. leaf extracts promoted the oxidative stability of soybean oil.

Our findings can also be compared to those of Qiu, Jacobsenb and Sørensen (2018), who evaluated the effect of adding rosemary (*Rosmarinus officinalis* L, Lamiaceae) extract on the oxidative stability of fish oil-enriched cow's

milk and soy milk. The authors reported that the PI values increased considerably from days 3 to 9 in the samples without rosemary extracts, indicating that the lipids were significantly oxidized and that the addition of rosemary extract to the oil-containing samples significantly decreased the PI value from days 3 to 12. Additionally, the extract with the highest concentration decreased the PI most effectively.

Another study showed that the peroxide indices of microencapsulated fish oil treated with 0.15% rosemary extract were lower (3.08 mEq peroxide kg^{-1} oil) than those in the fish oil of the control group treated with commercial antioxidant BHT (4.25 mEq peroxide kg^{-1} of oil) (Yeşilsu; Özyurt, 2019). In this study, for the oil samples containing 1.50% *C. longa* L. leaf extract, the PI was also lower (9.3545 mEq peroxide kg^{-1} oil) than that for the oil samples containing BHT (28.84 mEq peroxide kg^{-1} oil) on day 9 of the study (Table 3).

Alves et al. (2021) conducted a preliminary study and found that 1% ethanolic extract of *Curcuma longa* L. leaves inhibited peroxide formation more effectively than synthetic antioxidant BHT at 60 °C, mainly on days 6 and 9. In this study, the inhibitory effect of peroxides was greater when 1.5% extracts were used on days 6 and 9 relative to the inhibitory effects of control BHT and the other treatments.

According to international standardization, the maximum permissible amount of peroxides for oils is 10 mEq m of active oxygen kg^{-1} (Food and Agriculture Organization - FAO, 1999). We found that the industrial soybean oil with 1.5% *C. longa* L. leaf extracts showed an average PI of 9.3543 mEq of active oxygen kg^{-1} of oil on day 9, while in the oil with synthetic antioxidant BHT, this value was 28.8447 mEq of active oxygen kg^{-1} of oil, within the same duration (Table 3). These results indicated that the extract of *C. longa* L. leaves maintained the PI within the maximum allowed recommendation, while the synthetic antioxidant could not maintain the value within the permissible limit.

Mohd Nor et al. (2009) found that in the lower concentration extracts, the peroxide values decreased after prolonged heating, indicating their conversion into secondary oxidation products, such as ketones, aldehydes, hydrocarbons, and epoxides. In this study, peroxide values increased over time in soybean oil with 0.5% extract, indicating that higher concentrations of *C. longa* L. leaf extract promoted the reduction of the peroxide index.

Thiobarbituric Acid Reactive Substances (TBARS)

The results of TBARS are presented in Table 4 and Figure 3.

During oxidation, hydroperoxides are formed, but they can also break down into secondary compounds, such as aldehydes, ketones, and alcohols, among others. Therefore, to better understand lipid oxidation during storage, the secondary compounds had to be determined (Jacobsen, 2015; Qiu; Jacobsenb; Sørensen, 2018).

Our results showed significant differences in the duration of the study and the formation of thiobarbituric acid reactive substances. Soybean oil with 0.5% extract of *Curcuma longa* L. leaves showed a similar response to the synthetic antioxidant BHT on the third day, and from the ninth day, we recorded a 92% increase in the TABRS, which was confirmed in soybean oil with a low concentration of the extract (0.5%). The TBARS values decreased approximately by 48.51% in the soybean oil with 1.5% extract on day 6 relative to the TBARS values in the synthetic antioxidant BHT. The TBARS in the soybean oil with 1.5% extract was lower than the TBARS in the other treatments throughout the evaluation period. The TBARS value of the blank sample was 75.47% higher than that in the soybean oil with 1.0% *Curcuma longa* L. leaf extract on day 12 of the study (Table 3). Generally, higher TBARS production occurred in the extract with a lower concentration of *C. longa* L. leaves and in the blank samples.

Table 4: Thiobarbituric acid reactive substances (TBARS) (mg of malonaldehyde kg^{-1}) in soybean oil subjected to accelerated storage test for 12 days.

Concentration (%)	TBARS				
	Time (days)				
	0	3	6	9	12
0.5%	0.1677^{a}	0.3237^{b}	0.2574^{c}	0.4485^{d}	1.1973^{a}
1.0%	0.1521^{a}	0.2652^{b}	0.2691^{c}	0.351^{d}	1.092^{e}
1.5%	0.1014^{a}	0.1053^{b}	0.1911^{b}	0.2418^{c}	0.7176^{d}
Blank	0.234^{a}	0.3198^{b}	0.6708^{c}	0.6669^{d}	1.4469^{e}
Control (BHT)	0.2028^{a}	0.3393^{b}	0.3939^{b}	0.4875^{c}	1.0335^{d}

Values with different small letters along the row are significantly different ($p < 0.05$).

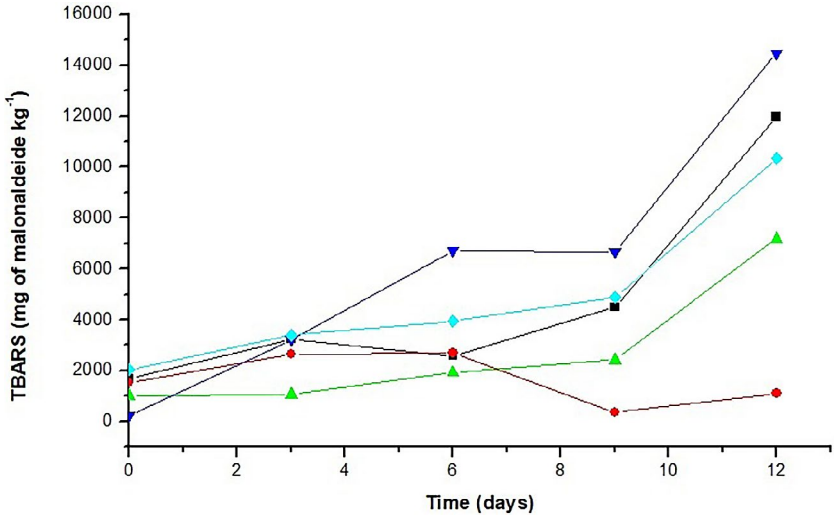

Figure 3: Kinetic evolution of Thiobarbituric Acid Reactive Substances (TBARS) as a function of time. Experimental conditions: C1 (■) 0.5%; C2 (●) 1.0%; C3 (▲) 1.5%; Blank (►) 0.0%; Control BHT (♦) 0.02% at 60 °C.

Our results showed that the highest formation of secondary oxidation compounds was on day 12 of the experiment. In general, the TBARS value increases during the food storage period, but the increase has not been standardized (Asnaashari; Tajik; Khodaparast, 2015; Zhang et al., 2017).

Zhang et al. (2018) reported that phenolic compounds, mainly caffeic acid, improved the oxidative stability of pecan oil and showed better response than BHT, as determined by the reduction of the TBARS value within 20 days of storage at 60 °C and other assays.

Juntachote et al. (2007) reported that adding dried galangal powder (0.05, 0.10, and 0.15%) and its ethanolic extract (0.17, 0.43, and 0.51%) to pork stews during storage at 51 °C for 14 days significantly reduced the TBARS values (greater inhibition was observed with 0.51% ethanolic extract). In this study, 1.5% *C. longa* L. leaf extract showed higher inhibitory activity than synthetic antioxidant BHT in preserving soybean oil, which was inferred from the decrease in the TBARS and peroxide index values.

Alves et al. (2021) reported that the TBARS values in soybean oil with 1.5% extract at 70 °C were lower than that in soybean oil with synthetic antioxidant BHT throughout the study period. The lower TBARS values in the 1.50% extract were also shown by the results obtained in this study. From day 9, a considerable increase in this parameter occurred in all treatments (Table 4).

Conjugated dienes (CD) and trienes (CT)

The formation of hydroperoxides from unsaturated fatty acids is usually accompanied by the formation of conjugated dienes and trienes through the rearrangement of double bonds (Barriuso; Astiasarán; Ansorena, 2013). The methylene groups adjacent to double bonds are more likely to lose a hydrogen atom, leaving an unpaired electron on this carbon atom. This molecule reaches its most stable form by rearranging and forming conjugated dienes (Pingret; Fabiano-Tixier; Chemat, 2013). The results of conjugated dienes and trienes are presented in Table 5 and Figures 4 and 5.

The results showed that in the oil with 0.5% extract, the CD values were 38.98% lower than the control BHT on the third day, and subsequently, an increase of 27.60% in the values of conjugated dienes was observed on day 9 relative to the values in soybean oil in which the extract was not added. The value of conjugated dienes was 4.63% lower in soybean oil with 1.5% extract on day 6 of the study relative to the value in the presence of the synthetic antioxidant BHT (Table 5). Higher levels of conjugated trienes were formed throughout the study period in the soybean oil samples in which the extract of *C. longa* L. was not added, while on day 6, the CT values were 5.08% lower for soybean oil containing 1.5% extract than the CT values for soybean oil containing the synthetic antioxidant (Table 5).

Higher CD and CT were recorded on day 12 of the study in all treatments (Table 5). The conjugated diene values were higher than the conjugated triene values in all treatments.

Zamuz et al. (2018) reported that adding different concentrations of chestnut (*Castanea sativa*; Fagaceae) leaf extracts to soybean oil affected all indices of lipid oxidation, including those of conjugated dienes.

Table 5: Conjugated dienes and trienes (CD/CT) in soybean oil subjected to accelerated storage test for 12 days.

Conjugated Dienes					
Concentration (%)	Time (days)				
	0	3	6	9	12
0.5%	4.3944^{a}	1.9865^{b}	1.2596^{b}	6.9102^{c}	10.8607^{d}
1.0%	3.8094^{a}	0.7234^{b}	0.5665^{c}	3.0093^{d}	12.0694^{e}
1.5%	2.0584^{a}	0.9325^{b}	0.2946^{a}	0.9077^{c}	8.6473^{d}
Blank	5.6591^{a}	5.4182^{a}	11.4173^{b}	1.9073^{c}	15.6597^{d}
Control (BHT)	5.5523^{a}	5.0960^{a}	6.3554^{b}	11.3211^{c}	12.9167^{d}
Conjugated Trienes					
Concentration (%)	Time (days)				
	0	3	6	9	12
0.5%	1.5581^{a}	0.6914^{b}	0.6677^{b}	1.5986^{c}	1.6291^{c}
1.0%	1.2376^{a}	0.5623^{b}	0.5665^{b}	1.0426^{a}	1.7694^{c}
1.5%	0.5756^{a}	0.3153^{b}	0.0902^{c}	0.6552^{a}	1.5925^{d}
Blank	1.8591^{a}	1.7500^{a}	1.9252^{a}	1.9073^{a}	1.6528^{a}
Control (BHT)	1.9186^{a}	1.7483^{a}	1.7727^{a}	1.8862^{a}	0.7541^{b}

Values with different small letters along the row are significantly different ($p < 0.05$).

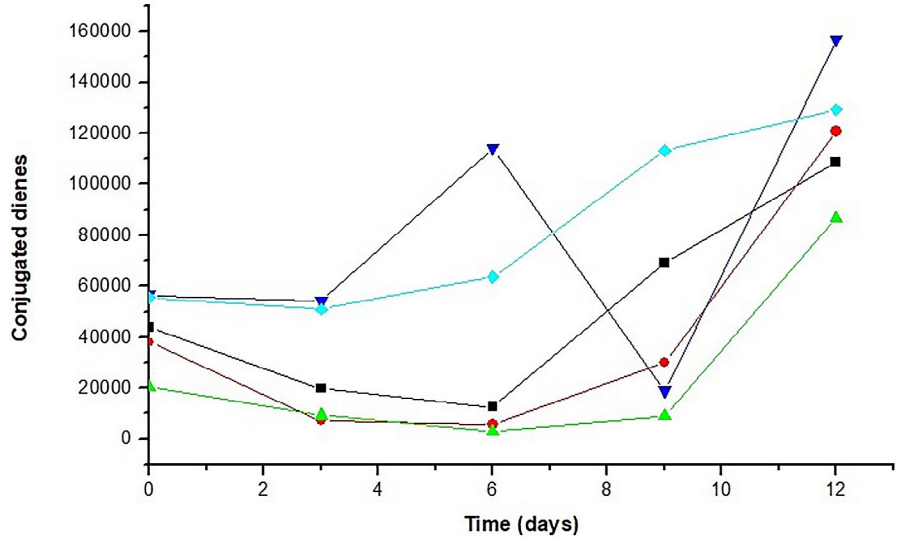

Figure 4: Kinetic evolution of conjugated dienes as a function of time under the following experimental conditions: C1 (■) 0.5%; C2 (●) 1.0%; C3 (▲) 1.5%; Blank (►) 0.0%; control BHT (♦) 0.02% at 60 °C.

Wang et al. (2018) found that after adding natural antioxidants from coriander (*Coriandrum sativum* L., Apiaceae) leaves, the value of the extinction coefficients of conjugated dienes and trienes decreased at the end of accelerated storage to 5.5 and 1.7, respectively. In our study, the values were lower than those reported in the above-mentioned study, with the lowest CT values observed on day 3 for the 1.0% extract (0.56) and on day 9 for the 1.5% extract (0.09).

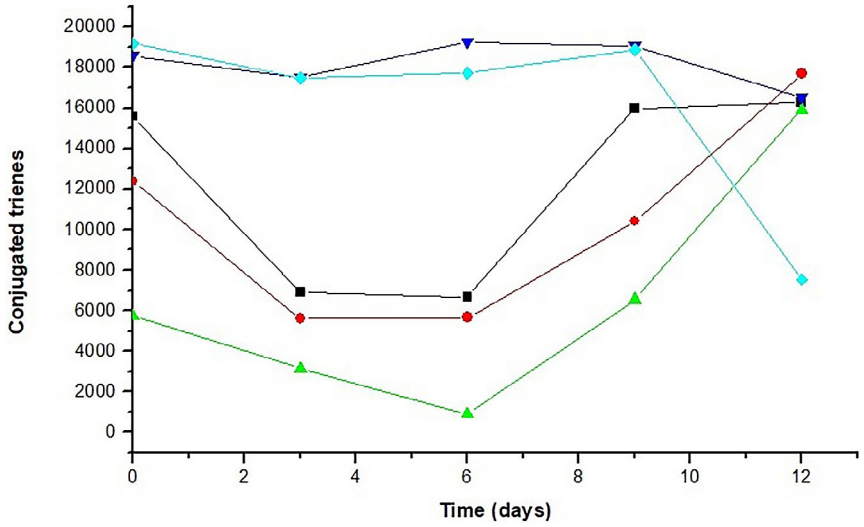

Figure 5: Kinetics of the evolution of conjugate trienes as a function of time under the following experimental conditions: C1 (■) 0.5%; C2 (●) 1.0%; C3 (▲) 1.5%; Blank (►) 0.0%; control BHT (♦) 0.02% at 60 °C.

Baştürk et al. (2018) reported that the CD values increased almost linearly (1.6 to 7.1) in an accelerated greenhouse test conducted for six weeks, while the CT values varied during the evaluated period (0.23 to 2.18). In this study, the CD and CT values varied between 2.05 and 8.64 and between 0.57 and 1.59, respectively, for the 1.5% extract (Tables 4 and 5). Higher concentrations (1.0% and 1.5%) of leaf extracts of *C. longa* L. had a greater influence on the inhibition of CT formation. Berghofer et al. (2007) found that basil ethanolic extract decreased the formation of conjugated dienes during the storage of pork meat, showing that natural extracts with polar characteristics were effective in preventing lipid oxidation. In this study, the application of *Curcuma longa* L. leaf extract significantly decreased the formation of CT compared to the formation of CD in soybean oil.

Our findings showed that varying the concentration of *C. longa* L. leaf extract significantly inhibited the formation of primary and secondary oxidation compounds, while time was the limiting factor that influenced the increase in the formation of these compounds throughout the study.

CONCLUSIONS

The leaves of *C. longa* L. had a high content of total phenolics. Our findings showed that the extracts decreased the values of IP, TBARS, CD, and CT in crude industrial soybean oil when the extract was added to the oil relative to the corresponding values in the oil samples in which antioxidants were not added. The extracts prevented the formation of oxidative compounds more effectively than the synthetic antioxidant BHT.

AUTHOR CONTRIBUTION

Conceptual Idea: Oliveira, T. F.; Alves, I. P. D.; Data collection: Alves, I. P. D., Methodology: Oliveira, T. F.; Alves, I. P. D., Data analysis and interpretation: Pereira, J.; Jesus Maria, Z. A., Writing and editing: Alves, I. P. D.; Martins, G.; Oliveira, T. F.; Jesus Maria, Z. A.

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REFERENCES

ALIZADEH, L. et al. Effects of tocopherol, rosemary essential oil and *Ferulago angulata* extract on oxidative stability of mayonnaise during its shelf life: A comparative study. Food Chemistry, 285:46-52, 2019.

AL-SEREITI, M. R.; ABU-AMER, K. M.; SEN, P. Pharmacology of rosemary (*Rosmarinus officinales* Linn.) and its therapeutic potentials. Indian Journal of Experimental Biology, 37:124-130, 1999.

ALVES, I. P. D. et al. The effects of *Curcuma longa* L. leaf extracts on the prevention of oxidation in soybean oil. Acta Scientiarum Polonorum Technologia Alimentaria, 20(3):325-336, 2021.

ANDRADE-NETO, M. et al. Volatile constituents of *Psidium pohlianum* Berg, and *Psidium guyanensis*. Journal of Essential Oil Research, 6(3):299-300, 1994.

AMERICAN OIL CHEMISTS' SOCIETY - AOCS. Official methods and recommended practices of the AOCS. Cd 8b-90 (4th ed.). Champaign: American Oil Chemists' Society. p. 1-3, 2003.

AMERICAN OIL CHEMISTS' SOCIETY - AOCS. Official methods and recommended practices of American Oil Chemists' Society. A.C.S. USA: American Oil Chemists' Society. 1990.1200p.

ASNAASHARI, M.; TAJIK, R.; KHODAPARAST, M. H. H. Antioxidant activity of raspberry (*Rubus fruticosus*) leaves extract and its effect on oxidative stability of sunflower oil. Journal of Food Science and Technology, 52:5180-5187, 2015.

BALBOA, E. et al. Potential of antioxidant extracts produced by aqueous processing of renewable resources for the formulation of cosmetics. Industrial Crops and Products, 58:104-110, 2014.

BARRIUSO, B.; ASTIASARÁN, I.; ANSORENA, D. A review of analytical methods measuring lipid oxidation status in foods: A challenging task. European Food Research and Technology, 236:1-15, 2013.

BAŞTÜRK, A. et al. Effects of some herbal extracts on oxidative stability of corn oil under accelerated oxidation conditions in comparison with some commonly used antioxidants. LWT-Food Science and Technology, 89:358-364, 2018.

BERGHOFER, E. et al. Antioxidative effect of added dried Holy basil and its ethanolic extracts on susceptibility of cooked ground pork to lipid oxidation. Food Chemistry, 100(1):129-135, 2007.

BRAGA, M. C.; VIEIRA, E. C. S.; OLIVEIRA, T. F. *Curcuma longa* L. leaves: Characterization (bioactive and antinutritional compounds) for use in human food in Brazil. Food Chemistry, 265:308-315, 2018.

CANLI, K. et al. *In vitro* antimicrobial activity screening of ethanol extract of lavandula stoechas and investigation of its biochemical composition. Advances in Pharmacological Sciences, Article ID 3201458, 2019.

CHAN, E. W. C.; LIM, Y. Y.; LIM, T. Y. Total phenolic content and antioxidant activity of leaves and rhizomes of some ginger species in Peninsular Malaysia. Gardens' Bulletin Singapore, 59(1-2):47-56, 2007.

CHOUDHARY, A. K.; RAHI, S. Organic cultivation of high yielding turmeric (*Curcuma longa* L.) cultivars: A viable alternative to enhance rhizome productivity, profitability, quality and resource-use efficiency in monkey-menace areas of north-western Himalayas. Industrial Crops and Products, 124:495-504, 2018.

DOROKHOV, Y. L.; SHESHUKOVA, E. V.; KOMAROVA, T. V. Methanol in plant life. Frontiers in Plant Science, 9:1623, 2018.

FADDA, A. et al. Innovative and sustainable technologies to enhance the oxidative enhance the oxidative stability of vegetable oils. Sustainability, 14(2):849, 2022.

FAO – Food and Agriculture Organization of the United Nations, 1999. Codex standard for edible fats and oils not covered by individual standards (CODEX STAN 19--1981. Rev. 2 – 1999). Available in: <http://www.fao.org/fao-who-codexalimentarius/en/> Access in: April 12, 2023.

FRANCO, D. et al. Optimization of antioxidants extraction from peanut skin to prevent oxidative processes during soybean oil storage. LWT-Food Science and Technology, 88:1-8, 2018.

GÜRLER, M. et al. Estimates of non-alcoholic food-derived ethano and methanol exposure in humans. Journal of Analytical Toxicology, 46(2):200-211, 2022.

HUANG, D.; BOXIN, O. U.; PRIOR, R. L. The chemistry behind antioxidant capacity assays. Journal of Agricultural and Food Chemistry, 53(6):1841-1856, 2005.

JACOBSEN, C. Some strategies for the stabilization of long chain n-3 PUFA-enriched foods: A review. European Journal of Lipid Science and Technology, 117(11):1853-1866, 2015.

JOHNSON, C. E. et al. Comparative assement of total phenolic in selected medicinal plants. Nigerian journal of Natural Products and Medicine, 12:40-42, 2008.

JUNTACHOTE, T. et al. The effect of dried galangal powder and its ethanolic extracts on oxidative stability in cooked ground pork. LWT-Food Science and Technology, 40(2):324-330, 2007.

MARINA, A. M.; WAN ROSLI, W. I.; NOORHIDAYAH, M. Quantitative analysis of peroxide value in virgin coconut oil by ATRFTIR spectroscopy. The Open Conference Proceedings Journal, 4:53-56, 2013.

MATUMOTO-PINTRO, P. T. et al. Effects of storage time and temperature on lipid oxidation of egg powders enriched with natural antioxidants. Food Chemistry, 228:463-468, 2017.

MICHIELS, J. A. et al. Extraction conditions can greatly influence antioxidant capacity assays in plant food matrices. Food Chemistry, 130(4):986-993, 2012.

MOHD NOR, F. et al. Antioxidative properties of *Curcuma longa* leaf extractin accelerated oxidation and deep-frying studies. Journal of the American Oil Chemists' Society, 86:141-147, 2009.

NATIONAL TOXICOLOGY PROGRAM. NTP-CERHR monograph on the potential human reproductive and developmental effects of bisphenol a. North Carolina: US Department of Health and Human Services. 2003. 321p.

OLAGUNJU, A. I.; ADELAKUN, S. O.; OLAWOYIN, S. M. The effect of rice bran extract on the quality indices, physicochemical properties and oxidative stability of soybean oil blended with various oils. Measurement: Food, 6:2772-2759, 2022.

SANTOS, L. R. et al. Effect of *Curcuma longa* L. extract as a natural antioxidant in soybean oil. Diversitas Journal, 6(2):1966-1979, 2021.

SOMOGYI, L. et al. Effect of immersed herbs on the oxidative stability of edible oils. Periodica Polytechnica Chemical Engineering, 59(4):272-276, 2015.

PEDRO, A. C. et al. Bioactive compounds of organic goji berry (*Lycium barbarum* L.) prevents oxidative deterioration of soybean oil. Industrial Crops and Products, 112:90-97, 2018.

PENNINGTON, J. A. T.; FISHER, R. A. Classification of fruits and vegetables. Journal of Food Composition and Analysis, 22:23-31, 2009.

PINGRET, D.; FABIANO-TIXIER, A. S.; CHEMAT, F. Degradation during application of ultrasound in food processing: A review. Food Control, 31(2):593-606, 2013.

POŁEĆ, K. et al. The influence of terpinen-4-ol and eucalyptol: The essential oil components-on fungi and plant sterol monolayers. Biochimica et Biophysica Acta (BBA) - Biomembranes. 1861(6):1093-1102, 2019.

PULIDO, R.; BRAVO, L.; SAURA-CALIXTO, F. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. Journal of Agricultural and Food Chemistry, 48(8):3396-3402, 2000.

PRABAKARAN, M. et al. Changes in soybean (*Glycine max* L.) flour fatty-acid content based on storage temperature and duration. Molecules, 23(10):2713, 2018.

QIU, X.; JACOBSEN, C.; SØRENSEN, A. D. M. The effect of rosemary (*Rosmarinus officinalis* L.) extract on the oxidative stability of lipids in cow and soy milk enriched with fish oil. Food Chemistry, 263:119-126, 2018.

SHAHIDI, F.; AMBIGAIPALAN, P. Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects: A review. Journal of Functional Foods, 18(10): 820-897, 2015.

TARLADGIS, B. G. et al. A distillation method for the quantitative determination of malonaldehyde in rancid foods. Journal of the American Oil Chemists Society, 37:44-48, 1960.

WANG, L. et al. Quickly verifying the antioxidant contribution of the individual composition in natural antioxidants by HPLC-free radical scavenging. LWT-Food Science and Technology, 96:461-468, 2018.

XU, X. et al. Synthetic phenolic antioxidants: Metabolism, hazards and mechanism of action. Food Chemistry, 353:129488, 2021.

YEŞILSU, A. F.; ÖZYURT, G. Oxidative stability of microencapsulated fish oil with rosemary, thyme and laurel extracts: A kinetic assessment. Journal of Food Engineering, 240(14):171-182, 2019.

ZHANG, L. et al. Composition and bioactivity assessment of essential oils of *Curcuma longa* L. collected in China. Industrial Crops and Products, 109:60-73, 2017.

ZHANG, Y. et al. Effect of natural polyphenol on the oxidative stability of pecan oil. Food and Chemical Toxicology, 119:489-495, 2018.

ZŁOTEK, U. et al. The effect of different solvents and number of extraction steps on the polyphenol content and antioxidant capacity of basil leaves (*Ocimum basilicum* L.) extracts. Saudi Journal of Biological Sciences, 23(5):628-633, 2016.

ZAMUZ, S. et al. Stored under MAP: Evaluation of their impact on physicochemical properties, lipid oxidation, antioxidant, and antimicrobial potential. Food Research International, 112:263-273, 2018.