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Oxidative stability of soybean and corn oils enriched with *Pluchea quitoc* hydroalcoholic extract

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SUMMARY: Soybean and corn oils are among the most popular vegetable oils, and are ingredients which are widely used in cooking and in the food industry. These oils contain many unsaturated fatty acids such as oleic, linoleic and linolenic acids, which makes them easily oxidized by oxygen. Extensive efforts are being made to prevent or minimize vegetable oil oxidation through the development of antioxidants. Phenolic antioxidants which are present in some extracts can be used as food additives to prevent lipid oxidation. In this study chromatographic analyses (HPLC and GC) of the *Pluchea quitoc* hydroalcoholic extract were performed. The content of phenolic compounds by the Folin-Ciocalteau method and the antioxidant properties against radicals 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) were also evaluated. The effect of samples prepared with soybean and corn oils enriched with *Pluchea quitoc* hydroalcoholic extract was determined and compared with samples of these oils which were free of antioxidants and with samples containing the synthetic antioxidant BHT. The results showed potential for application of the extract. A high content of phenolic compounds (314 milligrams of gallic acid equivalents (GAE)/g of extract) and good IC50 values were detected for the inhibition of the radicals DPPH and ABTS (13.2 $\mu g \cdot mL^{-1}$ and 5.6 $\mu g \cdot mL^{-1}$). In the evaluation of the oxidative stability of the oils enriched with this extract, it was found that at 1% concentration it was possible to obtain values of induction period (IP) close to the samples with added BHT.

KEYWORDS: *Hydroalcoholic extract; Induction period (IP); Lipid oxidation; Pluchea quitoc*

RESUMEN: ***Estabilidad oxidativa de aceites de soja y maíz enriquecidos con extracto hidroalcohólico de*** **Pluchea quitoc.** Los aceites de soja y maíz se encuentran entre los aceites vegetales más populares, ingredientes ampliamente utilizados en la cocina y también en la industria alimentaria. Estos aceites contienen muchos ácidos grasos insaturados como los ácidos oleico, linoleico y linolénico que se oxidan fácilmente con el oxígeno. Se están realizando grandes esfuerzos para prevenir o minimizar la oxidación de los aceites vegetales mediante el desarrollo de antioxidantes. Los antioxidantes fenólicos presentes en algunos extractos se pueden utilizar como aditivos alimentarios para prevenir la oxidación de lípidos. En este estudio se realizó la obtención y análisis cromatográficos (HPLC y GC) del extracto hidroalcohólico de *Pluchea quitoc*. El contenido de compuestos fenólicos se evaluó por el método de Folin-Ciocalteau y las propiedades antioxidantes frente a radicales DPPH y ABTS. Se determinó el efecto de muestras preparadas con aceites de soja y maíz enriquecidas con extracto hidroalcohólico de *Pluchea quitoc* y se comparó con muestras de estos aceites libres de antioxidantes y con el antioxidante sintético BHT. Los resultados mostraron potencial para la aplicación del extracto. Se detectó un alto contenido de compuestos fenólicos (314 mg GAE) y buenos valores de IC50 para la inhibición de los radicales DPPH y ABTS (13.2 $\mu g \cdot mL^{-1}$ y 5.6 $\mu g \cdot mL^{-1}$). En la evaluación de la estabilidad oxidativa de los aceites enriquecidos con este extracto, se encontró que para la concentración del 1% es posible obtener valores de período de inducción (IP) cercanos a las muestras adicionadas con BHT.

PALABRAS CLAVE: *Extracto hidroalcohólico; Oxidación de lípidos; Período de inducción (IP); Pluchea quitoc*

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1. INTRODUCTION

Lipid oxidation is a spontaneous and inevitable phenomenon, with direct implication in the commercial value of fatty compounds and all products that are formulated from them (e.g. foods, cosmetics, medicines) (Carocho *et al.*, 2018; Shahidi, 2005). The prevention or delay of lipid oxidation is considered one of the main factors in vegetable oil deterioration, which can be affected by the addition of antioxidants which preserve the quality and extend the shelf-life of the products (Klein *et al.,* 2020; Mohamed *et al.,* 2018; Ramalho and Jorge, 2006).

Nowadays, synthetic antioxidants are the most commonly used by industries, and include butylhydroxyanisol (BHA), butylhydroxytoluene (BHT), tert-butylhydroquinone (TBHQ) and propyl gallate (GP). However, the continued use of these antioxidants has been the subject of debate because of the possibility of causing toxic effects in the body, which makes the use of these compounds questionable, as shown by recent data in the literature (Mikołajczak *et al*., 2020; Akoh and Min, 2008; Gunstone, 2011). Therefore, butylated hydroxyanisole (BHA) was removed from the list of compounds generally recognized as safe (GRAS) (Li *et al.,* 2021). Currently, due to the great demand for natural ingredients in various applications, consumers have demanded the gradual substitution of synthetic compounds by natural ones (Taghvaei *et al*., 2014; Rostagno and Prado, 2013). Consequently, the search for and evaluation of natural materials with antioxidant properties has become a trend, with plants and extracts receiving special focus.

Thus, in an attempt to substitute synthetic antioxidants, many scientific researchers have been looking at options of natural and sustainable raw materials. mainly those that possess antioxidant and antimicrobial activities in their extracts and can produce a protective action in food (Shahidi and Ambigaipalan, 2015). Extracts obtained from plants are constantly used as functional foods, ingredients, additives (dyes, antioxidants, etc.) or as final products (nutraceuticals and supplements), and many of them have high antioxidant power (Javadian *et al.,* 2017; Shahidi and Ambigaipalan, 2015). Some procedures have been used to enrich or incorporate antioxidants in vegetable oils and have provided the development of functional edible oils with potential health-promoting potential, as they are improved with extracts rich in phenolic compounds (Fregapane *et al*., 2020).

Due to questions and certain insecurity related to the consumption of synthetic antioxidants, research has focused on obtaining natural products with antioxidant activity which serve to replace synthetics (Sousa *et al.,* 2014; Sousa *et al.,* 2019). Interest in natural phenolic compounds as antioxidants has grown rapidly in recent years due to evidence of nutritional properties for human health. Phenolic compounds, in addition to having high antioxidant activity, reduce the risk of certain types of diseases through consumption in foods (Bravo, 1998; Wu *et al.,* 2019)

Many plants are recognized for their antioxidant properties, and it has been observed that the extracts of some species have potential equivalent to synthetic or isolated natural antioxidants. A strategy currently employed is to carry out tests with combinations of natural antioxidants (extracts or isolates) with synthetic antioxidants in order to minimize the use or decrease the concentration of synthetics in formulations (Li *et al*., 2021). Some results have shown to be very promising, such the combination of extracts with traditional antioxidants, highlighting the synergistic effect (Hraš *et al.,* 2000; Marinova *et al.,* 2008; Thoo *et al.,* 2013; Yanishlieva *et al.,* 2006). In most cases, the antioxidant capacity of the extracts is attributed to phenolic compounds, which are important in the development of plants which are efficient in preventing auto-oxidation (Angelo and Jorge, 2007).

As well as to the protective action against oil oxidation, the preparation of oils enriched with extracts is a good strategy to ensure the intake of bioactive polyphenols through the diet (Fregapane *et al*., 2020). The development of these functional oils can help prevent chronic diseases (such as cardiovascular disease, immune weakness, aging disorders and degenerative diseases) and improve the quality of life for many consumers by reducing health costs (Reboredo-Rodríguez *et al*., 2017). Many efforts are being made to contribute to the developmental processes of functional oils, including computational modeling of the enrichment process with phenolic compounds/extracts, kinetic and thermodynamic studies and the application of artificial neural networks, which enable evaluation and improve the quality of vegetable oils treated with phytochemicals (Gülmez and Sahin, 2019; Sahin *et al.,* 2017; Sahin *et al.,* 2019; Sahin *et al.,* 2020; Samli *et al.,* 2020).

The *P. quitoc* species is a shrub belonging to the Asteraceae family, popularly known as "quitoco". This species is common in the tropical region, located in different countries of Latin America, including the south of Brazil. It is a perennial, erect and aromatic plant with a characteristic mild odor. In the literature it is possible to find records of the popular use of this plant as an agent to combat respiratory and stomach disorders. It is carminative, with indications for the home treatment of digestive problems and colds and also acts as a stimulant. Other studies have shown that *P. quitoc* extracts have antimicrobial, anti-inflammatory, antioxidant and anticancer effects (Simionatto *et al.,* 2007b; Guilhon and Muller, 1996).

In order to contribute to studies on the possibilities of natural antioxidants, in the present study the effectiveness of the *P. quitoc* hydroalcoholic extract was compared to the synthetic antioxidant butylhydroxyltoluene (BHT) and flavonoid rutin, for delaying the oxidation of the vegetable oils soybean and corn.

2. MATERIALS AND METHODS

2.1. Chemicals

Ethanol, methanol, glacial acetic acid, sodium acetate, acetonitrile (99.9%), caffeic acid (98.0%), ferulic acid (98.0%), gallic acid (97.5%), rosmarinic acid (98.0%), *p*-coumaric acid (98.0%), rutin (94%), quercetin (95.0%), campesterol (65%), β-sitosterol (95%), lupeol (94%), lupeol acetate (95.0%), 2,2-diphenyl-1-picrylhydrazyl (DPPH), (±) -6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), ethyl benzothiazoline-6-sulfonic acid diammonium 2,2'-azino-bis (ABTS), sodium chloride, aluminum chloride, sodium acetate, sodium carbonate, potassium persulfate and butylhydroxyltoluene (BHT), were all purchased from Sigma-Aldrich, São Paulo - SP, Brazil. Additive-free vegetable oils were supplied by the company Cocamar, Maringá-PR, Brazil.

2.2. Plant material

The aerial parts of *P. quitoc* were collected in Ibarama-RS, Brazil, in January 2018. The collected material was dried in a place with low luminosity and humidity, at 35 ºC for 5 days, and maintained at room temperature for further processing. The voucher specimen was deposited in the Herbarium of the Faculty of Biological Sciences of the Federal University of Grande Dourados - UFGD (voucher no. 8507).

2.3. Preparation of hydroalcoholic extract

The *P. quitoc* aerial part (1.0 kg) was crushed and subjected to exhaustive extraction with ethanol/water (70/30, 2L) at room temperature and with occasional stirring. The extraction process was carried out over 10 days, and every 2 days in maceration, the hydroalcoholic extract was filtered and stored in amber bottles. The hydroalcoholic extract was obtained by removing the solvents in a rotary evaporator (50 °C) under vacuum. The drying was carried out at room temperature, 10 mL of the extract were deposited onto plates and kept for 48 hours. This process resulted in a solid, which was removed and conditioned.

2.4. Determination of the total phenolic content

The total phenolic content was determined according to the Folin–Ciocalteu's reagent method (Djeridane *et al.,* 2006). In a short period of time, 100 µL of the aqueous extract solution (1 $mg \cdot mL^{-1}$) were added to 1000 µL of ultrapure water and 500 µL of the Folin–Ciocalteu's reagent (1/10) in water. After 1 min 1500 µL of Na_2CO_3 (20% w/v) were added. The final mixture was shaken and incubated for 2 hours in the dark. The absorbance was read by a spectrophotometer (FENTO 700 PLUS) (λ=760 nm). Gallic acid (Sigma-Aldrich, USA) was used as standard at concentrations varying from 5 to 1000 $\mu g \cdot mL^{-1}$, $r^2 = 0.9992$. The results were expressed in mg of gallic acid per g of dry weight of aqueous extract. All tests were performed in triplicate.

2.5. Evaluation of free radical scavenging activity

2.5.1. DPPH assay

The free radical scavenging activity of extracts was evaluated by the modified 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and according to the Trolox equivalent antioxidant capacity (TEAC) (Simionatto *et al*., 2007a; Klein *et al*., 2020). A stock solution of DPPH in 0.004% methanol was prepared and 50 µL of the concentrations 1.25 $mg \cdot mL^{-1}$, 0.625 $mg \cdot mL^{-1}$ and 0.312 $mg \cdot mL^{-1}$ of *P. quitoc* extracts were added to 5 mL of the DPPH solution. After 30 minutes of incubation in a dark environment and at room tem-

perature, the absorbance was analyzed at a wavelength of 517 nm. Rutin was used as standard. The calculation to verify the inhibition of free radical DPPH (I%) is described in the equation:

$$I\% = [(Aa - Ab) / Aa] \cdot 100$$

With Aa being the control Absorbance and Ab the absorbance of the reaction. The extract concentration ($\mu g \cdot mL^{-1}$) giving 50% inhibition of the DPPH radicals (IC50) was obtained by linear regression analysis by interpolation.

The Trolox equivalent antioxidant capacity (TEAC) was performed using a standard curve constructed with concentrations of 2000 μM, 1500 μM, 1000 μM, 500 μM and 100 μM of Trolox. A DPPH 0.004% solution was used as the reagent and the concentrations of the hydroalcoholic extract of *P. quitoc* were 5 $mg \cdot mL^{-1}$, 2.5 $mg \cdot mL^{-1}$, 1.25 $mg \cdot mL^{-1}$, 0.625 $mg \cdot mL^{-1}$ and 0.312 $mg \cdot mL^{-1}$. The values obtained were expressed in μMtrolox/g of crude extract.

2.5.2. ABTS assay

The activity of elimination of free radical extracts was also determined by ABTS assay. The percentage of inhibition was evaluated according to the methodology described by Re *et al*. (1999) and the method described by Rufino *et al.* (2007). The ABTS radical was prepared from the reaction of 140 mM potassium persulfate with 7 mM ABTS, stored in the dark at room temperature for 16 hours, and then diluted in 95% ethyl alcohol until the absorbance value of 0.700 ± 0.020 at the wavelength 734 nm.

To measure the percentage of antioxidant inhibition, sample aliquots of 30 μL at concentrations of 1.25 $mg \cdot mL^{-1}$, 0.625 $mg \cdot mL^{-1}$ and 0.312 $mg \cdot mL^{-1}$ were transferred to test tubes and added with ABTS (3 mL). After 6 minutes of reaction in a dark place at room temperature, the absorbances were analyzed at 734 nm using ethanol as blank and rutin as standard. The calculation to verify the inhibition of free radical ABTS (I%) is described in the equation:

$$I\% = [(Aa - Ab) / Aa] \cdot 100$$

The extract concentration ($\mu g \cdot mL^{-1}$) giving 50% inhibition of the DPPH radicals (IC50) was obtained by linear regression analysis by interpolation.

The Trolox equivalent antioxidant capacity (TEAC), was performed using a standard curve constructed at 2000 μM, 1500 μM, 1000 μM, 500 μM and 100 μM Trolox concentrations, using the ABTS radical as reagent, and the extracts at concentrations of 5 $mg \cdot mL^{-1}$, 2.5 $mg \cdot mL^{-1}$, 1.25 $mg \cdot mL^{-1}$, 0.625 $mg \cdot mL^{-1}$ and 0.312 $mg \cdot mL^{-1}$. The values obtained are expressed in μMtrolox/g crude extract.

2.6. HPLC analysis of *P. quitoc* extract

The sample was solubilized in water: methanol (7:3 v:v) filtered through 0.45 μm ultrafilter. and evaluated in a LC analytical column (LC-6AD Shimadzu, Kyoto, Japan) with the assistance of a photodiode array detector (DAD) system which was monitored between wavelengths λ = 200-800 nm. In an LC analytical apparatus; the column was ODS HYPERSIL (C-18, 150 mm long x 4.6 mm diameter, Thermo Electron Corporation, United States). The flow rate and the injection volume were 1 $mL \cdot min^{-1}$ and 20 μL, respectively. All chromatographic analyses were performed at 25 ºC. Eluent A was composed of a binary mobile phase of water with 6% acetic acid and 2 mM sodium acetate, and eluent B was composed of acetonitrile and the following gradient was applied: 0 min 5% B; 20 min 15% B; 30 min 60% B; and 40 min 100% B. Standard samples of gallic acid, ferulic acid, caffeic acid, ferric acid, rosmarinic acid, *p*-coumaric acid, rutin, quercetin, luteolin and apigenin were used (Sigma), prepared in methanol-water (7:3 v:v) in 1000 $\mu g.mL^{-1}$ concentration. The elution method developed for LC aimed at the identification of phenolic compounds with the assistance of the DAD scanning detector in the spectral range of 200-800 nm did not reveal interferences in the retention times. The absorption spectra and the retention time of the standards were the parameters for the identification and quantification of the compounds. Co-injection experiments, in which extracts and standard aliquots were mixed and diluted to a known volume, were also carried out to unequivocally identify the compounds. The calibration curves were determined by linear regression using LC. The linearity for standards was assessed for 10 concentration ranges. The average standard errors for the peak areas of replicated injections (n = 5) were less than 2%, thus showing good repeatability of the calibration curve. The respective coefficients of determination (r^2) were 0.9994 for caffeic

acid, ferulic acid and gallic acid and $r^2 = 0.9996$ for rutin and quercetin.

2.7. Chromatographic analysis by GC-MS

Sample preparation for GC-MS analysis was as follows; 100 mg of the *P. quitoc* extract were added to 1 mL of water and 1 mL of hexane and followed by separation of the hexane fraction. 2 mL of hexane were added to the aqueous fraction and the process was repeated again. These two hexane fractions were dried and suspended in 1.0 mL of hexane. For GC-MS analysis the solution was first filtered through a 0.45 μm ultrafilter.

To identify the compounds present in the sample, it was also evaluated by mass spectrometry (GC-MS). The GC-MS analysis was carried out on a GC-2010 Plus (Shimadzu, Kyoto, Japan), equipped with a mass spectrometry detector (GC-MS Ultra 2010), using LM-5 (5% phenyldimethylpolysiloxane), fitted with a capillary column of fused silica (15 m length x 0.2 mm id, and 0.2 μm-thick film). The analysis was performed under the following conditions: helium make up gas (99.999% and flow rate 1 $mL \cdot min^{-1}$), 1 μL of injection volume, split ratio (1:20), initial oven temperature adjusted to 150 ºC and heating from 150 ºC to 280 ºC at 15 $ºC \cdot min^{-1}$ and held at 280 ºC for 15 min. The injector temperature was 280 °C and the quadrupole detector temperature was 300 °C. An electron impact ionization voltage of 70 eV, a mass range of 45-600 nm *m/z* and a scanning interval of 0.3 s were the MS scanning parameters.

Compounds identification was accomplished by comparing the mass spectra obtained in the NIST21 and WILEY229 libraries. In some cases, reference compounds were co-chromatographed and the identification of the components was made by comparison of their retention times with standards. Standards for stigmasterol, campesterol, β-sitosterol, lupeol and lupeol acetate (Sigma) were prepared in hexane in the concentration of 1000 $\mu g \cdot mL^{-1}$. The compound concentrations were determined by external calibration. The linearity of standards was assessed for 5 concentration ranges. The average standard errors for the peak areas of replicated injections (n = 5) was less than 2%, thus showing good repeatability of the calibration curve. The respective coefficients of determination (r^2) were 0.9996 for stigmasterol, campesterol, β-sitosterol and lupeol and $r^2 = 0.9994$ for lupeol acetate.

2.8. Preparation of *P. quitoc* extract - enriched soybean and corn oils

Samples of soybean and corn oils were enriched with the *P. quitoc* hydroalcoholic extract to reach concentrations of 0.62, 1.25 and 2.5% (extract in oil). The extracts were added directly to the oils, followed by slow stirring for homogenization. Samples of soy and corn oils were also enriched with the synthetic antioxidant BHT at 0.02% (w/v) concentration (Brazil-Anvisa, 2005). BHT was added directly to the oils, followed by slow stirring until dissolved.

2.9. Rancimat test

The Rancimat test was used to assess the oxidative stability of the additive oils (with BHT and *P. quitoc* extract) and of the additive-free oils. The analyses were performed using a Rancimat apparatus (Metrohm, model 893, Herisau, Switzerland). The analyses were performed in a fixed amount of oil sample (3 g) at 110 °C and 10-$L \cdot h^{-1}$ air flow (Tinello and Lante, 2020). The oxidative stability was expressed as the induction period (IP) corresponding to the time (h). The induction period was determined using the software provided by the equipment manufacturer (StabNet).

2.10. Schaal oven test and evaluation of acid value

The Schaal oven test, which aims to assess the effect of accelerated storage conditions on the oxidative stability of oils, was performed as described by Yang *et al.* (2016) in soybean and corn oils without any addition (C), with *P. quitoc* hydroalcoholic extract (1%, 2.5% and 5.0%), and with butylated hydroxytoluene (BHT) as synthetic antioxidant at 0.02% (w/w) concentration, which corresponds to the maximum level established by Codex Alimentarius (2019). In detail, the oil samples were accurately weighed (40 g ± 0.01 g) in beakers and stored in an oven at a constant temperature of 62 °C for 28 days. Every 7 days the samples were collected and submitted to analysis.

2.10.1. Acid value

For the measurement of the acid value, the official method of AOCS (1993) was followed. 15 g of each oil were weighed into a container, dissolved in 50 mL of ethanol/diethyl ether (1:1 v/v) and then titrated with potassium hydroxide in the presence of phenolphthalein until persistence of pink coloring.

2.11. Statistical analysis

Results are presented and expressed in terms of mean and standard deviations. Significant differences were calculated using ANOVA in combination with the Duncan's test with significance level of α=0.05, supplemented, when necessary, by the Tukey test with Statistic Software (version 7.0).

3. RESULTS AND DISCUSSION

3.1. Characterization of the *P. quitoc* hydroethanolic extract

The hydroalcoholic extract of the *P. quitoc* aerial part was obtained st a yield of 10.2%. This yield can be considered good and comparable to the extracts obtained from from *Schinus molle* (fruits and leaves) (12 and 16%), *Psidium firmum* (11.5%) and lower than *Rosmarinus officinalis* (16-19%) (Klein *et al.,* 2020; Peres *et al.,* 2013, Wang *et al.,* 2018). The extract was initially characterized following the Folin-Ciocalteau method for the presence of phenolic compounds attributed to the concentration of 314 mg GAE (gallic acid equivalent)/g of *P. quitoc* extract. This result serves to classify this plant as rich in phenolic compounds at levels close to the extracts widely known and already highlighted as having high contents of these compounds. Several works highlight the phenol content identified in peanut skin extracts, ginger (*Zingiber officinale*), turmeric (*Curcuma longa*) and rosemary, which have phenol contents close to those found in the *P. quitoc* hydroalcoholic extract (Franco *et al.*, 2018; Tinello and Lante, 2020; Wang *et al.*, 2018).

The extract was analyzed by HPLC, and initially detected the presence of seven peaks of greater intensity. With the use of standards and spectral analysis, it was possible to identify five compounds. Figure 1 shows the chromatographic profile of the extract and the identification of gallic acid (peak 1), caffeic acid (peak 2), ferulic acid (peak 3), rutin (peak 4) and quercetin (peak 5). It is worth noting that these phenolic compounds found in the *P. quitoc* extract have already been reported as good natural antioxidants (D'Andrea, 2015; Shahidi and Ambigaipalan, 2015). Considerable research has been done with the objective of obtaining extracts enriched with classes of phenolic compounds such as flavonoids, and organic phenolic acids, among others (Taghvaei *et al*., 2014).

The hydroalcoholic extract of *P. quitoc* was also subjected to extraction with hexane and after filtration, analyzed by gas chromatography (Figure 2). Through this analysis it was possible to identify and quantify five compounds: campesterol (22.0 $mg \cdot g^{-1}$), stigmasterol (26.5 $mg \cdot g^{-1}$), β-sitosterol (57.1 $mg \cdot g^{-1}$), lupeol (47.4 $mg \cdot g^{-1}$) and lupeol acetate (48.9 $mg \cdot g^{-1}$).

Table 1. Compounds identified by liquid chromatography (HPLC) the hydroalcoholic extract of the aerial parts of *P. quitoc.*

RT (min)	Peak	Compounds	Hydroalcoholic extract ($mg.g^{-1}$ ± SD)
2.31	1	Gallic acid	159.6 ± 1.1^{c}
6.31	2	Caffeic acid	82.6 ± 1.2^{b}
8.83	3	Ferrulic acid	217.5 ± 1.0^{e}
21.75	4	Rutin	69.8 ± 0.4^{a}
24.42	5	Quercetin	162.7 ± 0.2^{d}

Data are shown as mean ± standard deviation (SD). Different letters in the same lines represent significant differences ($p < 0.05$); equal letters do not differ significantly. Duncan test ($p < 0.05$) was used for the comparison of means. All experiments were carried out in triplicate. RT: retention time.

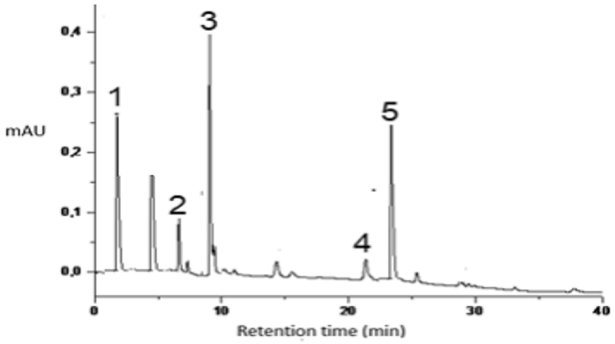

Figure 1. Chromatographic profile (280 nm) of the hydroalcoholic extract of *P. quitoc*. Identification of compounds: gallic acid (1), caffeic acid (2), ferrulic acid (3), rutin (4), quercetin (5).

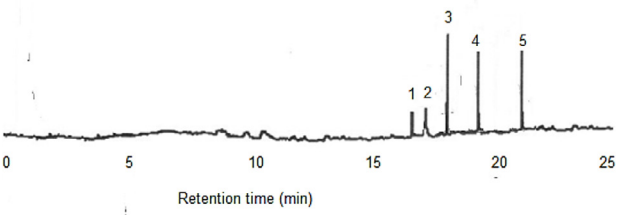

Figure 2. Chromatogram (GC-MS) of the hexane fraction obtained from the hydroethanolic extract of *P. quitoc*. Identification of compounds: campesterol (1), stigmasterol (2), β-sitosterol (3), lupeol (4) and lupeol acetate (5).

3.2. Evaluation of the antioxidant activity of hydroethanolic extract

Table 1 shows the results of the antioxidant activity against the DPPH and ABTS radicals, where it can be seen that the extract has high activity. The IC50 values for the extract were 13.2 $\mu g \cdot mL^{-1}$ and 5.6 $\mu g \cdot mL^{-1}$, in the tests with DPPH and ABTS radicals, respectively. According to the results, there is a variation in the IC50 values obtained by both methods. This variation has also been observed in other studies on the evaluation of the antioxidant properties of extracts using the radicals DPPH, ABTS and other methods (Franco *et al.,* 2018; Pedro *et al.,* 2018; Tinello and Lante, 2020). The high activity of the extract is verified against the two radicals in comparison to the standard of synthetic antioxidant BHT and to the natural flavonoid rutin, whose extracts presented IC 50 values close to the values for these standard compounds. Another parameter analyzed for the antioxidant potential of *P. quitoc* extract was the determination of the Trolox Equivalent Antioxidant Capacity (TEAC). The TEAC results also showed that the extract had significant activity, obtaining the values of 1024.8 and 1943.43 mM Trolox/g of hydroalcoholic extract, for the tests with the radicals DPPH and ABTS, respectively. The extract showed higher TEAC compared to the flavonoid rutin using the ABTS method. This behavior can be related to a probable synergistic effect of the phenolic compounds present in the extract of *P. quitoc*, acting in the inhibition of the radical ABTS. The TEAC values obtained for the *P. quitoc* extract and standards can be compared to those obtained from green tea ethanolic extract (3028 mM Trolox·g^{-1}), green tea aqueous extract (1723 mM trolox·g^{-1}) and extracts obtained from *Mangifera indica* (2750 mM trolox·g^{-1}) (Leite *et al.,* 2012; Sánchez-Camargo *et al.,* 2020). The results obtained in the present study are relevant to the data on antioxidant activity, demonstrating that *P. quitoc* extract has great capacity to eliminate radicals, suggesting that the extract could act as a preventive or blocker of the formation of reactive oxygen species chains, which could delay damage to certain systems and materials.

Some plant extracts are already mentioned in the literature as promising natural antioxidants, verified by several methods. Plant extracts that can be prepared from herbs, leaves, vegetable residues and fruit residues stand out as important sources of phenolic compounds (Sharma *et al.*, 2019). It is also intensively mentioned in the literature that the content of simple phenols and phenolic acid compounds is linked to the antioxidant effects of plant extracts (Bodoira *et al.*, 2017).

This antioxidant action potential may be associated with the phenolic compounds present in the *P. quitoc* extract. The chromatographic analysis showed that the extract consisted of three groups of compounds, two of which were phenolic, flavonoids and phenolic acids. Many authors have already reported the ability of phenolic extracts, flavonoids and phenolic acids to eliminate ABTS and DPPH radicals (Zahran and Najafi, 2020; Wang *et al.,* 2018; Klein *et al.,* 2020). Among the phenolic compounds, flavonoid quercetin and ferulic acid were the most representative compounds of the *P. quitoc* extract with concentrations of 162.7 $mg \cdot g^{-1}$ and 217.5 $mg \cdot g^{-1}$, respectively. These compounds (quercetin and ferulic acid) are reported in the literature because both have important antioxidant effects, and present high activity (D'Andrea, 2015; Shahidi and Ambigaipalan, 2015). All other phenolic components (gallic acid, caffeic acid and rutin) of the *P. quitoc* hydroalcoholic extract are also highlighted for their important antioxidant effects (Shahidi and Ambigaipalan, 2015), which justify the high activity of this extract. The an-

Table 2. Concentration which inhibits 50% of radicals (IC50 values in $\mu g.mL^{-1}$) and Trolox Equivalent Antioxidant Capacity (TEAC) values (in $\mu mol.g^{-1}$) according to the ABTS and DPPH methods for the hydroalcoholic extract of *P. quitoc.*

Samples	Method	
	DPPH	ABTS
IC50 - extract *P. quitoc*	13.2±0.6[a]	5.6±0.2[b]
IC50-rutin	9.2±0.5[b]	16.0±0.3[a]
IC50-BHT	9.6±0.6[b]	4.2±0.3[c]
TEAC – extract *P. quitoc*	1024.8±24.8[c]	1943.43±27.4[b]
TEAC-rutin	2588.61±36.7[b]	966.4±16.2[c]
TEAC-BHT	2981.09±41.2[a]	2531.62±36.1[a]

Data are shown as mean ± standard deviation (SD). Different letters in the same column represent significant differences ($p < 0.05$); the same letters do not differ significantly. The Duncan test ($p < 0.05$) was used for the comparison of means. All experiments were carried out in triplicate. IC50: concentration which inhibits 50% of DPPH/ABTS radicals. TEAC: Trolox equivalent antioxidant capacity.

tioxidant potential of phenolic compounds depends on the number and arrangement of hydroxyl groups in the molecules. Phenolic substances, such as those found in the extract of *P. quitoc*, can act as antioxidants by donating hydrogen atoms to lipid radicals and produce lipid derivatives and phenoxyl radicals, which are more stable and have less potential for self-oxidation (Kiokias *et al.,* 2008). The other compounds characterized are steroids (campesterol, stigmasterol, β-sitosterol) and triterpenoids (lupeol and lupeol acetate).

3.3. Soybean and corn oils enriched with hydroalcoholic extract: Analysis of oxidative stability by Rancimat and acid value

After the characterization of the *P. quitoc* hydroalcoholic extract and verification of potential antioxidant activity (against DPPH and ABTS radicals), the extract was evaluated for its activity in the inhibiting of lipid oxidation, once added to vegetable oils. The Rancimat analysis was performed at 110 °C and the induction period was evaluated until the end point of the samples' stability. Different concentrations of the extract were added to soybean and corn oils and the effects of these oils enriched with the extracts were compared to the synthetic standard BHT. Table 4 presents the results of the induction period of soybean and corn oils added with different concentrations of *P. quitoc* hydroethanolic extract, with BHT, and without the addition of any antioxidants.

According to the results, considering antioxidant-free oils, it is noted that corn oil had greater oxidative stability in comparison to soybean oil, the difference being statistically evidenced, according to the Tukey test ($p < 0.05$). It can be attributed to the differences in the oils' compositions; corn oil has a higher content of saturated compounds and less content of unsaturated compounds in comparison to soybean oil, parameters that contribute to the greater stability of corn oil (Dweck and Sampaio, 2004). In oils enriched with extracts, it was observed that there was an increase in the induction period (IP), demonstrating the effectiveness of the extract in protecting the oil in relation to oxidative degradation. Analyzing corn oil specifically, the control sample showed a significant difference compared to the oil samples incorporated into the extract, with an increase in the induction period (IP) from 1.2 h, 1.4 h and 2.0 h to 1.0 2.5 and 5.0%, concentrations, respectively. As for soybean oil, the increases in induction periods in relation to the control sample were 0.7 h, 1.4 h and 1.7 h at concentrations of 1.0, 2.5 and 5.0%, respectively. Once the action of *P. quitoc* extract was compared to the synthetic antioxidant BHT, promising effects were also observed, since in both oils evaluated, the inhibition of oxidation was more effective with the use of the extract. The increase in the induction period (IP) of the oils added with BHT in relation to the control was 0.7 h and 0.4 h for corn and soybean oils, respectively; whereas with a concentration of 1% of extract, the oils presented increases in the induction period of 1.2 h and 0.7 h in relation to the control for corn and soybean oils, respectively. Therefore, oils already enriched with extract at a concentration of 1% performed better than the antioxidant BHT.

The acid value was selected as a parameter, with the objective of evaluating the stability of soybean and corn oil samples with or without antioxidants during storage at 62 °C for 28 days (Schall oven test). Acceptable acid values for vegetable oils must be below 0.6 mg KOH per g of oil, as established by the ANVISA (National Health Surveillance Agency) standard for vegetable oils (Brazil-Anvisa, 2005). Table 4 shows the results obtained from the determination of acid values. By comparing the acid values of oils without additives to the oils enriched with extracts, it was noted that the additive oils showed a beneficial effect in reducing acid values. According to the values, it appeared that all samples had an acid value below 0.6 mg $KOH \cdot g^{-1}$ at the time of preparation (day zero), demonstrating that the addition of

Table 3. Oxidative stability (Induction Period) of soybean and corn oils at different concentrations of *P. quitoc* extract.

Samples	Induction period (h)	
	Corn oil	Soybean oil
pure oil (control)	$9.5 \pm 0,54^{Ab}$	$7.3 \pm 0,11^{aA}$
Oil + BHT	$10.2 \pm 0,02^{baB}$	$7.7 \pm 0,42^{Ba}$
Oil + 1% of extract	$10.7 \pm 0,51^{cbB}$	$8.0 \pm 0,23^{cA}$
Oil + 2.5% of extract	$10.9 \pm 0,11^{dcB}$	$8.7 \pm 0,15^{Da}$
Oil + 5% of extract	$11.5 \pm 0,13^{edB}$	$8.9 \pm 0,11^{Da}$

Data are shown as mean ± standard deviation (SD). Values with same lowercase letters in same column and values with the same uppercase letters within the same row were not statistically different ($p < 0.05$). Duncan test ($p < 0.05$) was used for the comparison of means. All experiments were carried out in triplicate. BHT: butylhydroxyltoluene. Analysis conditions: 3 g of sample, at 110 °C and 10-$L \cdot h^{-1}$ air flow.

TABLE 4. Effect on the acid value of antioxidant-free corn and soybean oils, added with BHT and enriched with the hydroalcoholic extract of *P. quitoc* during 28 days storage at 62 °C.

Samples	Acid value (mg KOH·g^{-1})				
	Day 0	Day 7	Day 14	Day 21	Day 28
Corn oil					
Pure oil (control)	0.47 ± 0.4^{cA}	2.04 ± 0.01^{cB}	2.72 ± 0,02^{cC}	2.72 ± 0,01^{cC}	9.50 ± 0,01^{dD}
Oil + BHT	0.24 ± 0.1^{aA}	0.68 ± 0.02^{aB}	0.68 ± 0,02^{aB}	1.36 ± 0,01^{bC}	4.75 ±0,03^{bD}
Oil+1% extract	0.40 ± 0.01^{bA}	0.68 ± 0.01^{bC}	1,36 ±0,02^{bC}	1.36± 0,04^{aB}	8.15 ± 0,01^{cD}
Oil + 2.5% extract	0.40 ± 0.03^{bA}	1.36 ± 0.01^{bB}	1.36 ± 0,01^{bB}	1.36 ± 0,02^{bB}	2.72 ± 0,02^{aC}
Oil + 5% extract	0.40 ± 0.01^{bA}	1.36 ± 0.01^{bB}	1.36 ± 0,01^{bB}	1.36 ± 0,01^{bB}	2.72 ± 0,01^{aC}
Soybean oil					
Pure oil (control)	0.54 ± 0.01^{dA}	4.07 ± 0.02^{dB}	5.43 ± 0,06^{cC}	8.82 ± 0,02^{dD}	14.26 ± 0,02^{eE}
Oil + BHT	0.34 ±0.01^{aA}	0.68 ± 0.02^{aB}	0.68 ± 0,05^{aB}	3.39 ± 0,03^{cC}	5.43 ± 0,01^{aD}
Oil + 1% extract	0.44 ± 0.01^{cA}	1.36 ± 0.01^{cC}	2.04 ± 0,01^{bB}	2.04 ± 0,01^{aC}	9.50 ± 0,03^{dD}
Oil + 2.5% extract	0.40 ± 0.02^{bA}	1.36 ± 0.04^{bB}	1.36 ±0,01^{bB}	3.39 ± 0,01^{cC}	8.15 ± 0,02^{cD}
Oil + 5% extract	0.40 ± 0.01^{bA}	1.36 ± 0.01^{bB}	1.36 ± 0,03^{bB}	2.72 ± 0,02^{bC}	6.11 ± 0,02^{bD}

Data are shown as mean ± standard deviation (SD). Values with the same lowercase letter in the same column and values with the same uppercase letters within the same row were not statistically different ($p < 0.05$). All experiments were carried out in triplicate.

the extract to the oil samples did not affect this parameter. The tendency to increase the acid value of soybean and corn oils samples was slow in the first 14 days, while a sharp increase was observed until the end of thermal storage.

The antioxidant-free oils showed the highest acid values, and presented increases in all analyses of the period for soybean and corn oils, reaching high values on day 28, at 9.50 and 14.26 mg KOH·g^{-1}, respectively. With the extract addition, the samples showed a less accelerated behavior in the variation in acid values, which also presented lower indexes in comparison to oils without additives, suggesting that the extracts contributed to the control of this parameter.

The highest extract concentrations added to the oils (5%) provided samples with less acidity at the end of the analysis period (day 28), showing better behavior than the BHT standard for corn oil, with an acid value of 2.72 mg KOH·g^{-1}; whereas for the oil with BHT standard the acid value was 4.75 mg KOH·g^{-1}. For soybean oil, samples with BHT and 5% extracts also showed similar acid values, with 5.43 mg KOH·g^{-1} and 6.11 mg KOH·g^{-1}, respectively. According to the results obtained, soybean oil showed the highest acidity values at the end of the analysis period (day 28). The lipid composition of each oil can influence these acidity values. There are differences in the proportions of saturated/unsaturated compounds in soybean and corn oils, which are approximately 15%/84% and 18%/81%, respectively (Dweck and Sampaio, 2004). This higher proportion of unsaturated compounds in soybean oil may contribute to less oil stability, which may result in higher acidity values. In addition, the oils differ in terms of their contents of polyunsaturated compounds. Soy oil has approximately 58%, while corn oil has 45% (Dweck and Sampaio, 2004). Oils that contain a greater abundance of polyunsaturated fatty acids are vulnerable to oxidative degradation (Sharma *et al.,* 2019).

Generally, there was an increase in the acid value with the time of exposure of the sample to heat. This behavior is in agreement with other studies found in the literature (Yldirim, 2009; Souza *et al.,* 2014; Souza *el al.,* 2019). For the oils analyzed, the greatest variation in acid values occurred in the period of 21-28 days, both for oils with extracts or with BHT. In this period of analysis, it was observed that the oils added with the *P. quitoc* extract had significantly lower acid values than the oil which was free of antioxidants, demonstrating the positive effect of the extract in the control of acidity.

4. CONCLUSIONS

The hydroalcoholic extract of the aerial part of *P. quitoc* has a composition with high content of

phenolic compounds. Tests related to the evaluation of antioxidant activity demonstrated high activity for this extract. It was possible, for the first time, to determine the behavior of this extract in the lipid oxidation inhibition in vegetable oils (soybean and corn). It can be concluded that the *P. quitoc* hydroalcoholic extract can be used as stabilizer against the oxidation reactions of these oils. Under conditions of accelerated oxidation (Rancimat), all tested concentrations (1%, 2.5% and 5%) showed better effects as inhibitors of oil oxidation, providing an increase in induction periods in comparison to the samples exempt of antioxidants and samples containing the antioxidant BHT. In the highest concentration tested (5%), the *P. quitoc* hydroalcoholic extract also promoted control of the acid value of the oil samples, and these samples presented lower indexes than the control and standard samples. The protective effect of *P. quitoc* hydroalcoholic extract against the damage of primary oxidative reactions in soybean and corn oils may be highly correlated to its content of phenolic compounds and may be an important source of phytonutrients.

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