GRASAS Y ACEITES 71 (4)
October–December 2020, e385
ISSN-L: 0017-3495
https://doi.org/10.3989/gya.0803192

Production of lipids and natural antioxidants from passion fruit seeds

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Submitted: 13 August 2019; Accepted: 29 November 2019; Published online: 27 October 2020

SUMMARY: The wild passion fruit species *Passiflora setacea*, *Passiflora alata*, and *Passiflora tenuifila* are native to the Brazilian biomass. The seed waste generated from the extraction of passion fruit juice contains functional polyunsaturated fatty acids and phenolic compounds. The aims of this study were to obtain lipids and natural antioxidants from passion fruit seeds. Passion seed oils were extracted using a lab-scale continuous press and their oxidative stability was evaluated using the Rancimat® method. Higher antioxidant extract capacity was observed when using an ethanol-water solution (70:30) at 45 ºC. In these cases, the total phenolic contents expressed as gallic acid equivalents from *P. setacea*, *P. alata*, and *P. tenuifila* cakes were approximately 1800, 600 and 900 mg·100g^{-1} of extract. Induction periods increased up to two-fold when adding these extracts to their respective seed oil. Therefore, passion fruit seed extract can contribute to increasing the oxidative stability of polyunsaturated oils.

KEYWORDS: *Natural antioxidants;* Passiflora alata*;* Passiflora setacea*;* Passiflora tenuifila*; Phenolic compounds; Sustainable technology*

RESUMEN: ***Producción de lípidos funcionales y antioxidantes naturales a partir de semillas de maracuyá.*** Las especies de maracuyá silvestre *Passiflora setacea, Passiflora alata y Passiflora tenuifila* son nativas de la biomasa brasileña. El desecho de semillas generado después de la extracción del jugo de maracuyá contiene ácidos grasos poliinsaturados funcionales y compuestos fenólicos. Los objetivos de este estudio fueron obtener lípidos y antioxidantes naturales de las semillas de maracuyá. Los aceites de semillas de la pasión se extrajeron usando una prensa continua a escala de laboratorio y su estabilidad oxidativa se evaluó usando el método Rancimat®. Se observó una mayor capacidad antioxidante del extracto cuando se usó una solución de etanol-agua (70:30) a 45 ºC. En estos casos, el contenido fenólico total expresado como equivalentes de ácido gálico de las tortas de *P. setacea, P. alata y P. tenuifila* fue de aproximadamente 1800, 600 y 900 mg·100g^{-1} de extracto. Los períodos de inducción aumentaron hasta dos veces al agregar estos extractos a sus respectivos aceites de semillas. Por lo tanto, el extracto de semillas de maracuyá puede contribuir a aumentar la estabilidad oxidativa de los aceites poliinsaturados.

PALABRAS CLAVE: *Antioxidantes naturales; Compuestos fenólicos;* Passiflora alata*;* Passiflora setacea*;* Passiflora tenuifila*; Tecnología sostenible*

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Citation/Cómo citar este artículo: Reis CC, Mamede AMGN, Soares A, Freitas SP. 2020. Production of lipids and natural antioxidants from passion fruit seeds. *Grasas Aceites* **71** (4), e385. https://doi.org/10.3989/gya.0803192

1. INTRODUCTION

Passion fruits are native to America and grow in tropical and subtropical regions. About 150 native species are found in the Middle East and in northeastern Brazil, 60 of which are known to be edible (Dhawan *et al*., 2004; Bernacci *et al*., 2003; Cunha *et al*., 2002). Unfortunately, only two are currently commercially important, *Passiflora edulis* Sims f. flavicarpa Deg. (yellow passion fruit) and *P. edulis* f. edulis Sims (purple passion fruit) (Narain *et al*., 2012). However, other species display promising sensorial and functional characteristics and can be commercially exploited, such as *P. setacea*, *P. alata* and *P. tenuifila*, native Brazilian biome fruits, particularly in the Atlantic Rainforest, Cerrado and Caatinga biomasses.

Brazil is the largest passion fruit juice producer and consumer worldwide. In 2016, about 700 thousand tons of yellow passion fruit were produced (IBGE, 2016). At least 40% of the production is destined to the juice or pulp industries. Therefore, the passion fruit juice industry discards huge amounts of bagasse, composed mainly of husks and seeds that may still contain high amounts of bioactive compounds, particularly lipids and phenolics. Currently, these natural compounds are used as functional ingredients in food, contributing to the sustainable development of food and biomass production and processing.

Agro-industry pomace, such as peels and seeds, is of industrial interest due to its high contents in lipids, protein, fiber, sugar, vitamins, minerals and bioactive compounds, including phenolic compounds, phytosterols and carotenoids. In this case, these agroindustry residues become raw materials of interest to the chemical industry, particularly to the pharmaceutical, food and cosmetic sectors (Silva and Jorge, 2014). In addition, waste disposal is restricted by legal regulations. Thus, the processing of these materials has been shown to be technically and economically viable in several production chains (Lowe and Buckmaster, 1995).

Data reported in the literature indicate that passion fruit pomace contains phenolic compounds of significant industrial interest (Mirabella *et al*., 2014).

Passion fruit processing in the food industry discards up to 40% of the fruit, with seeds corresponding to 6–15% (Manica, 2005). Passion fruit oils are rich in monounsaturated and polyunsaturated fatty acids, mainly linoleic acid (C18: 2 n-6). According to Paula (2015), *P. setacea* BRS Pérola do Cerrado and *P. alata* BRS Sweet Honey lipids contain between 57 and 59% of this fatty acid. The fatty acid composition depends, among other factors, on the fruit species and climatic characteristics of the producing region.

Passion fruit seeds contain high amounts of piceatannol (3,4,3',5'-tetrahydroxytans-stilbene) in their phenolic composition. Piceatannol is a resveratrol hydroxylate which presents strong antioxidant activity (Uchida-Maruki *et al*., 2015; Matsui *et al*., 2010). According to the reported data, the high piceatannol content in passion fruit seeds exerts positive effects on cultured dermal cells with respect to melanogenesis inhibition and collagen synthesis.

Due to the phytotherapeutical properties of native passion fruit species' pulp and leaves, Embrapa has been developing new passion fruit genotypes for classical genetic improvement, focusing on increasing fruit consumption, improving pleasant sensory characteristics, increasing field resistance and improving functional properties. Howerver, reported data on lipids from P. *setacea*, P. *alata* and P. *tenuifila* are rare. The main aims of this study were to obtain lipids and natural antioxidants from passion fruit seeds by applying sustainable technology.

2. MATERIALS AND METHODS

2.1. Materials

The passion fruit species (*Passiflora alata* BRS Doce Mel, *Passiflora setacea BRS* Peróla do Cerrado and *Passiflora tenuifila*) were cultivated in the experimental Embrapa Cerrados fields, located at Planaltina-DF/Brazil. The fruit was harvested when the skin color of the fruit reached about 20% yellow (between 40 and 60 days after inflorescence). The assays were carried out using homogenized seed lots between January and May, 2016. After fruit pulping, all the *Passiflora* seeds were stored at −20 °C until use. After thawing, the seeds were washed under tap water to remove the pulp and mucilage residues adhered to the seeds, followed by autoclaving at 120 °C for 20 minutes in order to reduce microbial loads.

2.2. Seed drying kinetics

Passion fruit seeds were dried in a convective dryer (Hauber – DMS-P) at 50 °C and 2-g samples were weighed at 30 minute intervals until constant weight over three successive weights. The drying temperature was optimized in previous experiments for *P. alata* and *P. setacea* seed drying (Paula, 2015). Physical air properties were monitored by the dew point of a Cole-Parmer Traceable® hygrometric thermometer. The room temperature of the air was 22.7 ± 1 °C and humidity was approximately 50% and the temperature and humidity inside the dryer were 45 ± 1 °C and 15%, respectively. Drying was carried out in triplicate.

2.3. Mass diffusion coefficient

In order to estimate the effective water diffusivity (D_{eff}) during seed drying, the dimensionless moisture (MR) and time (t) data were adjusted by applying the second Fick diffusion law model (Eq. 1) in

spherical coordinates, considering up to three term models of the infinite series solution, using the Statistica software (v13.0).

$$Mr = \frac{M - Ms}{Mo - Ms} = \frac{9}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \exp\left(-\frac{(2n+1)^2 \pi^2 D_{eff}}{r^2} t\right) \quad \text{[Eq. 1]}$$

2.4. Grinding and seed oil extraction

After drying, *P. alata* and *P. setacea* seeds were ground using a knife mill (Macron MA-048). This step was not necessary for *P. tenuifila* seeds, as they displayed a reduced mean diameter (dp < 3 mm). The seeds' moisture was adjusted between 12 and 15% in sealed dissectors containing distilled water, as recommended by Evangelista and Cermak, 2007. Crushed passion fruit seeds were processed in a continuous press (Oekotec - Germany, model CA59G), at room temperature of 22 ± 1 °C and compression ratio of 4.4 to obtain the first-extraction crude oil, which was then decanted for 24 h in a dark cabinet. A micropipette (Labmate Soft 1–5 μL) was used to separate the clarified oil. The clarified fraction was stored at −18 °C until analysis. To improve the extraction efficiency, the pressed cake was recycled and reprocessed at 30 ± 2 °C. At the end of extraction, the residual oil in pressed cake was recovered by Soxhlet using petroleun ether as solvent and the efficiency was then calculated. The defatted pressed cake was reserved for subsequently extraction of phenolic compounds.

2.5. Phenolic compound extraction from pressed cake

For phenolic compound extraction, the pressed cake was ground using a knife mill (Macron MA-048) coupled to a 1 mm diameter circular mesh sieve. The crushed sample, with particle diameter of less than 2 mm, was then homogenized with ethanol at a 1:5 ratio (pressed cake:ethanolic solution).

The experimental design was carried out with three different aqueous-ethanol solutions selected from the preliminary test at the best selected extraction parameters of 30:70, 50:50 and 70:30 ethanol:water. The suspension was maintained in a heated bath at 45 °C for 30 minutes at a stirring rate of 600 rpm. After extraction, the solids and soluble compounds were separated by vacuum filtration. The liquid phase containing the bioactive compounds was stored at –18.0 ± 0.5 °C until analysis.

2.6. Analytical methods

Oxidative Stability: The oxidative stability of passion seed oils was determined using Rancimat® 743 (Metrohm, model 743) equipment, according to EN 14112, under a 10 $L \cdot h^{-1}$ flow at 110 °C, using 3 g of each oil sample, in duplicate. These test data were expressed as an induction period that represented the time interval until the sample reached a high oxidation level.

Antioxidant Capacity: The antioxidant capacity of passion seeds oils was determined according to a modified methodology developed by the Embrapa Tropical Agroindustry for fruit analysis (Rufino, 2007), which uses the DPPH (2,2-diphenyl-1-picryl-hidrazil) radical method, adapted for vegetable oils. The DPPH reagent was added at five different dilutions of the original oil in isopropyl alcohol, which then remained for one hour in the dark prior to a spectrophotometric analysis at 515 nm ((BelFotonix SP 1105). The Inhibitory Concentration (IC50 - mass necessary to reduce the initial concentration of the DPPH radical by 50%) was determined from the absorbance readings.

The antioxidant capacity of the pressed cake was evaluated by the ABTS method, expressed as Trolox equivalents (TEAC) according to Re *et al.*, (1999) and Rufino *et al.*, (2007). The ABTS radical was chemically obtained by ABTS oxidation by potassium persulfate. Aliquots of 5 mL of the aqueous 7 mM ABTS solutions were mixed with 88 μL of 140 mM potassium persulfate in amber bottles. The flasks were kept at room temperature for 14 h for complete reaction and radical stabilization, and a concentrated radical solution was obtained. The concentrated ABTS + solution was diluted in 95% ethanol to an absorbance of 0.700 ± 0.020 at 734 nm (BelFotonix SP 1105) to form the test reagent.

Trolox® (300–2000 μM in 95% ethanol) and the extracts were transferred to test tubes where they were left to react for 6 minutes with the diluted ABTS + solution at a 1:10 ratio at room temperature, followed by immediate spectrophotometric absorbance determinations at 734 nm (BelFotonix SP 1105). The discoloration of the mixed solution indicated that the antioxidant compounds in the extract inhibited the radical ABTS cations. A quantitative relationship between absorbance reduction at 734 nm and antioxidant concentrations present in the sample was noted. The results were expressed as μmol Trolox·g^{-1} of extract *Total Phenolic (TP) Content*: The total phenolic (TC) content of the pressed cake extract was determined by the spectrophotometric method proposed by Singleton and Rossi (1965), using the Folin-Ciocalteu reagent. This method is based on the oxi-reduction reaction, which forms a blue compound with the phenol. Briefly, 0.5 mL of each sample was mixed with 2.5 mL of the 10% Folin-Ciocalteu reagent and vortexed for 2 min. After homogenization, 2 mL of a saturated sodium carbonate solution (7.5%) were added. The solution was then incubated for 15 min at 50 °C in a water bath, and immediately cooled in an ice bath

for 30 seconds. The absorbance of the mixture was determined at 760 nm using a spectrophotometer (BelFotonix SP 1105). Phenolic compound quantification was performed using a gallic acid calibration curve. The results were expressed as mg gallic acid equivalent per 100 g of sample (GAE.100.g^{-1}).

2.7. Addition of phenolic extract to passion fruit seed oils

The extracts obtained from the passion fruit seeds *P. alata*, *P. setacea* and *P. tenuifila* in 70:30 ethanol-water solutions were selected to increase the oxidative stability of their respective oils. To maintain bioactive properties, the solvents were removed from the extracts by forced convection at an air temperature of 21 °C.

The dried extracts were mixed with passion fruit seed oils at 10% mass/mass ratio, according to preliminary tests. To evaluate the oxidative stability of the mixtures, 3 g of each sample were then transferred to a Rancimat reaction tube, and the analyses were performed in duplicate using an air flow at 10 L.h^{-1} and 110 °C.

2.8. Statistical analyses

All analytical determinations were carried out at least in triplicate, except for oxidative stability trials. An analysis of variance (ANOVA) followed by Fisher's LSD test was performed using the Statistica® v.13.0 software.

3. RESULTS AND DISCUSSION

3.1. Seed drying kinetics

The estimated drying rate parameters and corresponding R-squared results are presented in Table 1. In addition, kinetic curves at 50 °C which fitted the experimental data for passion fruit seed drying by Fick's second law are displayed in Figure 1.

The following equations were used to determine P. *alata* (Eq. 1.a), *P. setacea* (Eq1.b) and *P. tenuifila* (Eq. 1.c):

$$Mr = \frac{9}{\pi^2}\left(\begin{array}{l}\left(\exp\left(-\pi^2 * 3.06 * 10^{-9} * t\right)\right)+\frac{1}{9}\\ *\exp\left(-9\pi^2 * 3.06 * 10^{-9} * t\right)+\frac{1}{25}\\ *\exp\left(-25\pi^2 * 3.06 * 10^{-9} * t\right)\end{array}\right) \quad [1.a]$$

$$Mr = \frac{9}{\pi^2}\left(\begin{array}{l}\left(\exp\left(-\pi^2 * 4.62 * 10^{-9} * t\right)\right)+\frac{1}{9}\\ *\exp\left(-9\pi^2 * 4.62 * 10^{-9} * t\right)+\frac{1}{25}\\ *\exp\left(-25\pi^2 * 4.62 * 10^{-9} * t\right)\end{array}\right) \quad [1.b]$$

$$Mr = \frac{9}{\pi^2}\left(\begin{array}{l}\left(\exp\left(-\pi^2 * 4.66 * 10^{-9} * t\right)\right)+\frac{1}{9}\\ *\exp\left(-9\pi^2 * 4.66 * 10^{-9} * t\right)+\frac{1}{25}\\ *\exp\left(-25\pi^2 * 4.66 * 10^{-9} * t\right)\end{array}\right) \quad [1.c]$$

TABLE 1. Effective diffusion coefficient (D_{eff}) during mass transport in the drying process of passion fruit seeds

Sample	Average radius (mm)	D_{eff} ($m^2.s^{-1}$) × 10^{10}	R^{2*}
Passiflora alata	1.9±0.5	1.14±0.82	0.98
Passiflora setacea	1.3±0.5	0.82±0.87	0.98
Passiflora tenuifila	1.3±0.4	0.83±0.68	0.98

*R^2 regression coefficient. Each point represents mean value of three replicates ± SD value

The diffusion coefficient for *P. alata* seeds was higher than the other two assessed species. It was possible to estimate the drying time of the passion fruit seeds from the fitted equation, in order to achieve the best moisture for pressing passion fruits seeds between 10 and 15%.

3.2. Oil extraction

The oil contents of dried passion fruit seeds were 19, 16 and 23% for *P. alata*, *P. setacea* and *P. tenuifila*, respectively (Table 2). Paula (2015) reported a lipid content of 22.5 and 32.2% for *P. alata* and *P. setacea*, respectively. This difference may be due to seasonality effects, particle size and moisture. The efficiencies of the continuous oil extraction process, calculated by the ratio of the oil mass obtained for the total oil in the seeds, were 57% to 67%, as displayed in Table 2.

These results are typical of continuous oil extraction from lignin-rich seeds (up to 40% in dry matter and lipid content of less than 30%). In addition, the extraction yield for *P. setacea* was lower, in contrast to the data reported by Paula (2015), possibly due to the harvest season. The overall yield after heated cake pressing was increased by about 10%. According to reported data, cold-pressed oils require no further refinement and can be consumed after simple decantation, presenting superior sensory characteristics and nutritional qualities when compared to hot-pressed oils (Emir, Gunes and Yılmaza, 2014).

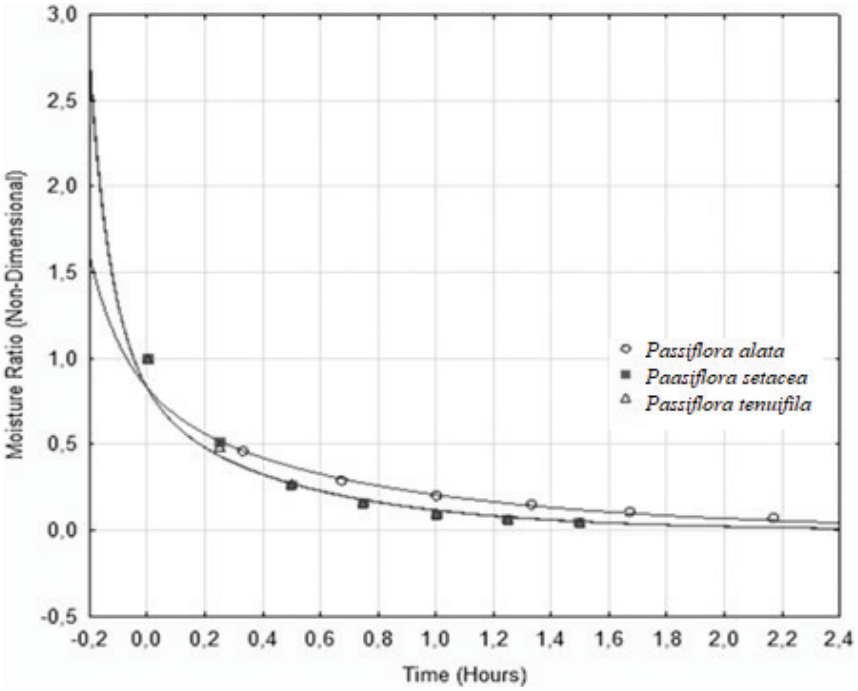

FIGURE 1. Drying curves of the three species of passion fruit seeds. The experimental data are points and the fit model is a continuous line. The *P. setacea* and *P. tenuifila* curves intersect.

TABLE 2. Moisture, oil content and oil extraction yield from cold-pressed passion fruit seeds

Sample	Moisture after drying (%)	Oil content (%)	Oil Extraction yield[1] (%)
Passiflora alata	15.0±0.6[a]	19±1[b]	67±1[a]
Passiflora setacea	11.0±0.1[b]	16±1[c]	57±1[c]
Passiflora tenuifila	10.0±0.2[c]	23±1[a]	63±1[b]

[1]The yield of the oil extraction is the ratio of the oil extracted in cold pressing and the total oil in the sample.
[a,b,c,]Different letters in the same column indicate significant differences ($p < 0.05$) according to ANOVA one way Fisher LSD test.
Each point represents the mean value of two replicates ± SD value.

TABLE 3. Oxidative stability and antioxidant capacity by DPPH inhibition of *Passiflora* seed oils

Samples	Induction period(h)	DPPH inhibition (%)	IC50 mg oil, mg DPPH^{-1}
Passiflora alata	3.52±0.14[c]	43±1[b]	1.09±0.24[b]
Passiflora setacea	7.32±0.04[a]	53±3[a]	1.09±0.03[b]
Passiflora tenuifila	6.87±0.10[b]	24±3[c]	2.60±0.05[a]

[a,b,c,]Different letters in the same column indicate significant differences ($p < 0.05$) according to ANOVA one way Fisher LSD test.
Each point represents mean value of three replicates ± SD value.

3.3. Oxidative stability and antioxidant capacity

As displayed in Table 3, the induction periods (IP) for *P. setacea* and *P. tenuifila* seed oils at 110 ºC were 7.32 h and 6.87 h, respectively, which were typical IP for passion fruit seed oils (Paula *et al*., 2015). However, *P. alata* presented a lower IP 50% (3.53 h), which could be explained by the lower oleic acid and higher linoleic acid contents in *P. alata* seed oil (Santana *et al.*, 2015). Due to the two double bonds present in linoleic acid, this acid is 40 times more unstable than oleic acid, which contains only one double bond (Damodaran and Parkin, 2017).

The IC_{50}, defined as the amount of antioxidant required to reduce DPPH concentrations by 50%, is considered a good antioxidant capacity measure (inversely proportional). Regarding DPPH results, the oil from *P. setacea* seeds presented the highest antioxidant capacity, followed by *P. alata* and *P. tenuifila*. These values were consistent with the literature data reported by Santana *et al*., (2015), and could be explained due to the higher vitamin E content in *P. setacea* (215.3 mg·100 g^{-1}) and *P. alata* (114.8 mg·100 g^{-1}) seed oil compared to *P. tenuifila* seed oil (80 mg·100 g^{-1}).

3.4. Pressed cake total phenolic (TP) content and antioxidant capacity (TEAC)

The most efficient solid-liquid extraction of bioactive compounds from the pressed cake was achieved using 70:30 ethanol-water solutions at

TABLE 4. Total phenolic (TP) contents and antioxidant capacity (TEAC) of the ethanolic extract from pressed cake

Sample	ethanol: water ratio	Total phenolic	TEAC(μmol Trolox·g^{-1})
Passiflora alata	30:70	293.5±41.2^{f}	242.96±18.0^{g}
	50:50	477.2±35.7^{e}	568.24±107.8^{ef}
	70:30	596.9±10.9^{d}	670.19±95.5^{de}
Passiflora setacea	30:70	220.5±6.0^{g}	200.03±3.06^{g}
	50:50	1159.9±29.9^{b}	1032.64±121.4^{b}
	70:30	1814.4±50.3^{a}	1569.14±190.3^{a}
Passiflora tenuifila	30:70	445.9±28.2^{e}	451.27±23.4^{f}
	50:50	886.7±87.2^{c}	739.88±86.3^{d}
	70:30	909.0±25.1^{c}	878.04±51.5^{c}

^{a,b,c,d,e,f,g}Different letters in the same column indicate significant differences ($p < 0.05$) according to the ANOVA one way Fisher LSD test.
Each point represents the mean value of three replicates ± SD value.

45 °C. This was probably due to the greater solubility of the polar compounds in the passion fruit seed cake in this aqueous and ethanolic solution. The highest values for total TP contents and TEAC for the pressed cake extracts are displayed in Table 4. For the selected experimental conditions, the ethanolic *P. setacea* extract presented higher TP contents and TEAC when compared to the other species.

Total phenols from *P. setacea*, *P. alata*, and *P. tenuifila* cakes were approximately 1800, 600 and 900 mg GAE·100 g^{-1} of extract, respectively. The TP of the extract obtained at a 70:30 ratio from the *P. setacea* cake was 3- and 2-fold higher than the *P. alata* and *P. tenuifila* extracts, respectively (Table 4). A strong positive Pearson correlation (R=0.97) with antioxidant activity was observed, since the results for the antioxidant capacity in the same experimental conditions for the *P. setacea* cake were 2.3- and 1.8-fold higher than for *P. alata* and *P. tenuifila*, respectively.

According to Vasco *et al*., (2008) the ethanolic extracts obtained in the present study from pressed *P. alata* and *P. tenuifila* cakes can be treated as medium phenolic contents, while the extracts obtained at 50:50 and 70:30 ethanol:water ratios from pressed *P. setacea* cake are treated as high phenolic contents.

4. CONCLUSIONS

P. setacea, *P. alata*, and *P. tenuifila* passion seed oils obtained by pressing presented high quality indexes, probably due to the presence of phenolic compounds in the cake. Furthermore, it was possible to enhance the oxidative stability of all samples by adding 10% of the ethanolic extract obtained from defatted cake, which are rich in natural antioxidants. Future studies should be conducted to evaluate the best ethanolic extract to oil ratio.

ACKNOWLEDGMENTS

The authors would like to thank the Federal University of Rio de Janeiro (UFRJ/EQ), Embrapa Cerrados, Embrapa Food Technology and Brazilian National Research Council (CNPq) for supporting this study.

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