Phenolic compounds and antioxidant capacity of canned pequi pulp (Caryocar spp)

Compostos fenólicos e capacidade antioxidante da polpa do pequi (Caryocar spp) em conserva

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Abstract
The aim of the study was to evaluate the extracts, canning liquid (CL) and phenolic acids fractions of canned pequi pulp concerning the phenolic contents and antioxidant capacity (AC). The alcoholic extracts (ALCE) and aqueous (AQE) were obtained by sequential extraction and CL was only filtered. The fractions rich in free phenolic acids (FPA), soluble esters (SPA) and insoluble (IPA) were isolated with solvents, hydrolysis and acidification. The total phenolic content (TPC) and its composition by liquid chromatography were determined. AC was measured by DPPH· methods, IC50 values of 533.62, 1376.28 and 1542.74 µg/mL for ALCE, AQE and CL, respectively, and oxidation inhibition percentage (% OI) inferior than the butylated hydroxytoluene (BHT) synthetic antioxidant by β-carotene/linoleic acid system. It was found that the extracts and CL of canned pequi have small amount of TPC (18.06 mg GAE/ 100 g), however fractions have significant amount (1134.75 mg GAE/ 100 g), with special focus on FPA. The p-coumaric acid was the major phenolic constituent of the extracts, and especially of fractions. It was also detected in CL, showing the migration of this phenolic into surrounding liquid. The extracts showed by DPPH· method, IC50 values of 533.62, 1376.28 and 1542.74 µg/mL for ALCE, AQE and CL, respectively, and oxidation inhibition percentage (% OI) inferior to the butylated hydroxytoluene (BHT) synthetic antioxidant by β-carotene/linoleic acid system. The fractions showed high AC by DPPH· method and OI% higher than BHT in all tested concentrations. The study shows that extracts and mainly the canned pequi fractions have phenolic with good AC, which are important for the promotion of health.

Additional keywords: antioxidants; bioactive compounds; canning liquid; migration; processing.

Resumo
O objetivo do estudo foi avaliar os extratos, líquido da conserva (LC) e frações de ácidos fenólicos da polpa de pequi em conserva quanto aos teores de fenólicos e capacidade antioxidante (CA). Os extratos alcoólico (EALC) e aquoso (EAO) foram obtidos por extração sequencial, e o LC foi apenas filtrado, já as frações, ricas em ácidos fenólicos livres (AFL), ésteres solúveis (AFS) e insolúveis (AFI), foram isoladas com solventes, hidrólise e acidificação. Foram determinados o conteúdo de fenólicos totais (TPC) e sua composição por cromatografia líquida. A CA foi medida pelas metodologias DPPH·, IC50 valores de 533,62, 1376,28 e 1542,74 µg/mL para ALCE, AQE e CL, respectivamente, e a inibição de oxidação (% IO) inferior ao antioxidante sintético butilhidroxitolueno (BHT) pelo sistema β-caroteno/ácido linoleico. As frações apresentaram elevada CA pelo método DPPH· e % IO superior ao BHT em todas as concentrações testadas. O estudo demonstra que os extratos e, principalmente, as frações do pequi em conserva possuem fenólicos com boa CA, os quais são importantes para a promoção da saúde.

Palavras-chave adicionais: antioxidantes; compostos bioativos; líquido da conserva; migração; processamento.
Introduction

Pequi is a typical species of the Brazilian Cerrado region, from the Caryocar genus and Caryocaraceae family (Vera et al., 2007) (Figure 1). The edible and more appreciated part of pequi is named inner mesocarp and corresponds to a layer of pulp generally thick, which involves almond and woody endocarp, varying in color from intense orange, yellow or even white, and it is rich in oil (Souza & Salviano, 2002), containing from 20.0 to 33.40% of lipids, of which 61.35% of its constituent fatty acids are unsaturated fatty acids (mainly oleic acid). The pulp is highly caloric, providing about 360 kcal per 100 g (Azevedo-Meleiro & Rodriguez-Amaya, 2004, Lima et al., 2007).

Figure 1 - Pequi fruit (Caryocar brasiliense Camb.) and its parts. Adapted from Cardoso et al. (2013).

Pequi contains phytochemicals compounds with antioxidant properties, such as carotenoids and phenolic which is one of secondary metabolites groups of plants more numerous and ubiquitous (Soobratteé et al., 2005, Lima et al., 2007, Lima, 2008, Oliveira, 2009). Lima et al. (2007) found in fresh pequi pulp 209 mg/100 g of total phenolic, values higher than those found in most fruit pulp consumed in Brazil, which characterizes pequi pulp as a food with good antioxidant capacity (AC), that is also demonstrated by the good in vitro AC of fruit using different methods.

The pequi pulp has served as a raw material for small agribusiness, such as canning. Canning are highly appreciated by the population, and besides being one of the main forms of fruit conservation (Ferreira, 2007). Data on compounds with AC in canned pequi pulp are scarce in the literature. Thus, the objective of this study was to evaluate the contents of phenolic compounds and antioxidant capacity of canned pequi pulp.

Material and methods

Canned pequi pulp sample was acquired randomly in April 2008 in the market of the municipality of São Paulo, Brazil. Canning was in water-based liquid, salt and citric acid, within validity period, stored at room temperature and away from light following the manufacturer recommendations.

The pulp was separated from its respective conserving liquid, being filtered and stored in a glass bottle at -22 °C. The pulp was frozen in ultrafreezer at -80 °C and then, lyophilised (MP TFS system, Dura-Top™, New York, United States) (temperature –83 °C, vacuum -24 mT), grounded in mill (IKA A11 basic, Wilmington, United States), standardized at sieve of 60 mesh, homogenized and stored in plastics pots at -22 °C.

Preparation of pequi extracts

The ether (ETE), alcoholic (ALCE) and aqueous (AQE) extracts of canned pequi pulp were prepared according to Jardini & Mancini-Filho (2003) with modifications. Initially, 10 g of lyophilized pulp was weighed and 100 mL of solvent was added following a 1:10 ratio (sample: solvent). Solvents respect the following order: ethyl ether, ethyl alcohol and distilled water. Samples with solvents were placed in erlenmayers capped and protected from light and kept under constant agitation for 1 hour at room temperature. After this time, the first obtained extract (ether) was filtered on Buchner funnel using vacuum pump and the yield of the filtered material (supernatant) was measured and solvent was added to complete the volume following the initial ratio (1:10). The
residue from the extraction was collected, dried, weighed and subjected to extraction with ethyl alcohol (95%) following the same proportion and previous procedure. Then, the residue of the alcohol extraction was subjected to the same extraction with distilled water. The AOE needed additional centrifugation (DuPont Instruments Sorvall RC5C; United States) at 5000 rpm for 20 minutes at room temperature. The canning liquid (CL), after filtered, it was directly analyzed without extraction. All extracts were stored in amber pot at -22 °C.

Preparation of pequi phenolic acid fraction

Isolation of pequi phenolic acid fraction was carried out, rich in free phenolic acids (FPA), soluble esters of phenolic acids (SPA) and insoluble esters of phenolic acids (IPA) using solvents with different polarities according to the method described by Krygier et al. (1982) with modifications. For the isolation of FPA, 1 g of dried and defatted sample were carried out six extractions (20 mL/min) with tetrahydrofuran (THF) in turrax (IKA DI 25 Basic, Staufen, Germany). The supernatants obtained from each extraction were filtered, dried over anhydrous sodium sulfate and collected in flask protected from light. The solvent of extraction was completely removed using rotary evaporator (Micronal, b525, São Paulo, Brazil) at 40 °C. The material obtained (FPA) was resuspended in 5 mL of methanol and packed in amber pot and maintained at -22 °C.

For extraction of SPA, the residue from FPA extraction was subjected to six new extractions (20 mL/5 min) with a solution of methanol: acetone: water (7: 7: 6) in turrax. The supernatant obtained from each extract was filtered through anhydrous sodium sulfate and collected in a flask protected from light. The solvent of extraction was completely removed using rotary evaporator at 40 °C until the aqueous phase. Subsequently, the hydrolysis was carried out to release the soluble esters bound to proteins and/or polypeptides, measuring the volume of the sample and adding an equal amount of NaOH 4 N, remained under stirring in nitrogen atmosphere and protected from light for 1 hour away from light for further reading at 720 nm in a spectrophotometer (Thermo Spectronic® 20, Genesys® Rochester, United States). The results were expressed as milligrams of total phenolic acid equivalent per 100 g of dried sample (mg GAE/100 g) for extracts or per 100 mL (mg GAE/100 mL) for CL.

Determination of total phenolic content (TPC)

The TPC was spectrophotometrically determined using Folin-Ciocalteau reagent, according the method described by Swain & Hills (1959) using standard curve of gallic acid at concentrations of 2.5, 5, 10, 20, 40, 80 and 100 µg. Different sample aliquots (100, 125, 500 and 20 µL of ALCE, AOE, LC and fractions) diluted in water (final volume of 500 µL) were added to 8 mL of distilled water and 0.5 mL of Folin-Ciocalteau reagent. The solution was mixed by vortex and after 3 minutes, it was added 1 mL of saturated solution of sodium carbonate. The mixture was stirred and left to rest for 1 hour away from light to further reading at 720 nm in a spectrophotometer (Thermo Spectronic® 20, Genesys® Rochester, United States). The results were expressed as milligrams of total phenolic acid equivalent per 100 g of dried sample (mg GAE/100 g) for extracts or per 100 mL (mg GAE/100 mL) for CL.

Determination of dry extract content (DE)

The DE was determined gravimetrically by arranging a 0.5 mL aliquot of the extracts and fractions in pre-weighed watch glass, followed by drying at 105 °C for 2 hours to determine the amount of dry matter used in AC test.

Identification of phenolic compounds in the extracts and fractions

The extracts and fractions of pequi were analyzed using high performance liquid chromatography (HPLC) for the determination of phenolic according to Pereira et al. (2004) and Tiberti et al. (2007). The HPLC analyses were performed using chromatograph (Shimadzu, LC20AT, Quioto, Japão), with SIL – 20AC automatic injector, CBM-20a controller, CTO-20a column oven and SPD-M20A diode array detector. The column was a Shim-pack VP ODS-2 (25cm x 0.6 cm, 5 µm particle, Shimadzu) and C18 stationary phase. It was used as mobile phase 0.1% trifluoroacetic acid (v/v) in deionized water as A eluant and acetonitrile as B eluant. The gradient program was: 5% of A (10 min), 5-100% of B (40 min), 100% of B (5 min), 5% of B (5 min). The oven temperature was maintained at 35 °C and the eluent flow was maintained at 1 mL/min during analysis. Detection was performed between 190 and 400 nm. The recovery of phenolic compounds was analyzed in triplicate by adding the concentrations of similar patterns in the analyzed samples. Patterns of phenolic compounds were used: quer cetin, catechin, chlorogenic acid, apigenin, epicatechin, caffeic acid, gallic acid, vanillic acid, trans cinnamic acid, protocatechuc acid, hydroxybenzoic acid, ferulic acid, benzoic acid, salicylic acid, o-coumaric acid to
coumaric, synaptic acid, gentisic acid, quinic acid and ellagic acid (Sigma-Aldrich).

**Determination of in vitro antioxidant capacity**

The DPPH• scanning analysis (2,2-diphenyl-1-picrylhydrazyl) was performed according to Blois (1958) and Brand-Williams et al. (1995). Different amounts of sample (25, 50, 100 and 200 µg of extract and 6.25, 12.5, 25 and 50 µg of fraction) diluted in methanol (final volume of 0.5 mL) were added to 1.5 mL of methanolic solution of DPPH• 6 × 10⁻⁵ mol/L. The DPPH• reduction was measured at 517 nm in a spectrophotometer after 30 min of resting in the dark. The decrease in optical density values of samples was correlated with the control (no sample), and thus, it was established antioxidant protection percentage given the DPPH• according to the equation: % of protection = 100 - (Abs sample x 100)/Abs control. The inhibition capacity values of 50% of the radical (IC₅₀) were calculated using the line equation obtained from a curve prepared with different concentrations of the sample.

The AC by β-carotene/linoleic acid system was determined as described by Miller (1971) with modifications. For the analysis, initially emulsion was prepared in the following proportions: 120 μL of β-carotene/chloroform solution (20 mg/mL), 70 μL of linoleic acid, 22 drops of Tween 40 and 1 mL of chloroform. From this emulsion, a total of 5 mL were taken, which were placed in tubes with amounts of butylated hydroxytoluene (BHT) synthetic antioxidant and samples (50, 100, and 200 ppm of the extracts and 10, 20 and 40 ppm of fractions) alone or associated with BHT to evaluate the synergistic effect on 1:1 ratio (sample: BHT). The quantities were established from previous studies using concentrations between 10 and 200 ppm. Subsequently, absorbance readings were performed using spectrophotometer at 470 nm. The tubes were placed in a water bath at 50 °C and further readings were performed at the end of two hours. The discoloration was observed by absorbance measure and results were expressed as percentage of oxidation inhibition (% OI) calculated compared to absorbance decay of control (considered as 100% of oxidation).

**Statistical analysis**

The test results were analyzed by univariate ANOVA followed by Honest Significant Difference (HSD) Tukey test at 5 % of significance which was applied to compare the means. Linear correlation of Pearson (r) between the parameters was performed in order to analyze the strength of connection between two data sets. All analyzes were performed in triplicate, and the results expressed as the mean ± standard deviation (SD). Statistica 7.1 program was used (Statsoft, Tulsa, Oklahoma, USA) for all statistical calculations and Software Prism 5.0 (GraphPad) for plots.

**Results and discussions**

Contents of TPC and DE of canned pequi pulp are presented in Figure 2.

![Figure 2](image-url)  
*Figure 2 - Total phenolic content (TPC) and dry extract (DE) from extracts and canning liquid (CL) (A) and free (FPA), soluble (SPA) and insoluble (IPA) phenolic acids fractions (B) from canned pequi pulp. Results expressed as mean and standard deviation, n = 3. Different letters represent statistically different results (p <0.05) according to HSD Tukey test. CL*: TPC (mg GAE/100 mL) and DE (g/100 mL). GAE: Gallic acid equivalent.*
The current study demonstrated phenolic presence in canned pequi pulp with a significant difference (p<0.05) between alcoholic (6.89 mg GAE/100 g), aqueous (8.96 mg GAE/100 g) extracts and CL (2.21 mg GAE/100 g). The TPC sum of the extracts was 18.06 mg GAE/100 g, less than that found by Lima (2008) in methanol/water extract of fresh pequi pulp (209 mg GAE/100g). This result may be related to extraction method or processing which canned pequi pulp was submitted resulting in TPC loss. In addition, other factors must be further investigated, including variation of species, cultivar or other environmental factors.

TPC of analyzed pequi were similar to those found in the AQE of bacuri (10.35 mg GAE/100g) and in hydroalcoholic extract of bacuri (Platonia insignis) and caja (Spondias mombin) (7.73 and 6.62 mg GAE/100g, respectively), however below the contents found in the extracts of avela (Malpighia glabra), cashew (Anacardium occidentale), guava (Psidium guajava) and tamarind (Tamarindus indica) (449.63, 165.07, 20.21 and 23.35 mg GAE/100g, respectively) (Vieira et al., 2011).

The AQE showed higher contents of TPC when compared to ALCE, which can be explained by its higher polarity compared to ethanol (water> ethanol> acetone). Roginsky & Lissi (2005) state that TPC and AC reduced when solvent polarity decreases.

TPC in the ether extract, as well as its AC could not be determined due to its immiscibility to reactants, thus leading to solution turbidity.

In CL, as observed in Figure 2, it was also detected TPC presence, although in smaller quantities, indicating that there was dissolution of these polar compounds in the liquid. According to Sikora et al. (2008), the dissolution of the polyphenols in canning water depends on processing type and plant size (fractionation). Due to the presence of these, and possibly other polar compounds in CL, it is assumed that they have AC.

Regarding DE content, values of 1.56 g/100 g were found in ALCE, 2.46 g/100 g in AQE and 2.95 g/100 mL in CL. Greater presence of dry matter in AQE was observed, indicating higher yield even in relation to ALCE and may have relation to higher TPC on AQE, although no statistically significant correlation has been found. The DE content of CL confirms the dissolution of the pulp components to liquid, which may or may not have interesting AC.

DE content of fractions differ statistically with increased yield to FPA (2.40 g/100 g), followed by IPA (0.75g/100g) and SPA (0.67g/100g). No correlation was found between the DE and TPC variables.

Despite the extracts exhibit low amounts of TPC, the fractions of phenolic acids of pequi presented statistically different and significant amounts. The FPA fraction had the highest content: 465.16 mg GAE/100 g, followed by SPA (400.83 mg GAE/100 g) and IPA (268.76 mg GAE/100 g). Together the fractions showed TPC of 1134.75 mg GAE/100 g, confirming TPC presence in high amounts in canned pequi pulp, in addition demonstrating the high yield and efficient purification of phenolic acids of fractions compared to extracts.

There was no data in the literature on TPC of pequi fractions, however, lower value was reported in FPA fraction of apple pulp cv. Gala (230.39 mg/100 g) (Soares et al., 2008). The results presented in this study are in agreement with the study of Sun et al. (2002) who investigated fruits (cranberry, apple, red grape, strawberry, lemon, peach, orange, banana, pear and pineapple) and reported that soluble and free phenolic fractions had higher amounts of phenolic than the soluble and connected fraction. In the same study, only FPA fraction of cranberry (507 mg/100 g) showed TPC higher than canned pequi. These results lead us to consider canned pequi as potential source of bioactive phenolic compounds.

Identification by HPLC of the phenolic pequi pulp can be seen in Table 1.

<table>
<thead>
<tr>
<th>Phenolic (µg/g of dry sample)</th>
<th>Extracts</th>
<th>Fractions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aqueous Extract</td>
<td>Canning liquid</td>
</tr>
<tr>
<td>Catechin</td>
<td>0.88 ± 0.05</td>
<td>n.d.</td>
</tr>
<tr>
<td>p-coumaric acid</td>
<td>2.97 ± 0.1</td>
<td>6.34 ± 0.2</td>
</tr>
<tr>
<td>Sympathetic acid</td>
<td>3.20 ± 0.1</td>
<td>n.d.</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>3.61 ± 0.2</td>
<td>n.d.</td>
</tr>
<tr>
<td>∑ Phenolic</td>
<td>10.66 ± 0.2</td>
<td>6.34 ± 0.2</td>
</tr>
</tbody>
</table>

Results expressed as mean and standard deviation, n = 3. N.d.: not detected. Different letters in the same line (fractions) represent results statistically different (p <0.05) according to HSD Tukey test. ∑ Phenolic: Sum of phenolic.

The compounds found in canned pequi pulp were: catechin, p-coumaric, sympathetic and ellagic acids. In AQE, ellagic acid has been found in greater amounts (3.61 mg/g), followed by sympathetic and p-coumaric acids and catechin (3.20, 2.97 and 0.88 g/g, respectively). In ALCE was not possible to identify phenolic compounds. Lima (2008) also found ellagic acid as the main constituent of AQE in freshpequi pulp, however, with higher values (32.2 µg/g) than those found in this study. Besides ellagic, p-coumaric (27.4 µg/g) and gallic (9.56 µg/g) acids were also detected by the author.

| Table 1 - Phenolic compounds present on extracts and fractions of free (FPA), soluble (SPA) and insoluble (IPA) phenolic acids from canned pequi pulp. | }
When phenolic compounds of canned pequi were added, the value of 17.0 µg/g was obtained, less than that found in fresh pulp (Lima, 2008), which can be attributed to the extraction method, to the loss by processing or even to migration of these compounds for CL. In the present study, the ρ-coumaric was detected in CL (6.34 µg/g), showing this phenolic acid migration to the liquid. Other polar compounds that were not evaluated in this study may have migrated to the dissolution liquid, thus influencing antioxidant properties of samples.

In all fractions ρ-coumaric and synaptic acids were found. ρ-coumaric was detected in greatest amount (24.08, 28.23 and 30.87 µg/g in FPA, SPA and IPA, respectively), followed by synaptic (11.25, 13.10 and 17.43 µg/g, in FPA, SPA and IPA, respectively) (Table 1). The IPA fraction showed the highest amount of phenolics compared to others, and the ρ-coumaric acid is the main constituent. The greatest presence of phenolic in this fraction can be attributed to the disconnection of the structures to which they were adhered. According to Lima (2008), many phenolic acids are strongly linked to sugars or to other cellular structures of plants, requiring hydrolysis and acidification to its extraction. The same author has detected in IPA of fresh pequi, higher amount of phenolic (75.35 µg/g, composed of ellagic, ρ-coumaric, gallic and 4 hydroxybenzoic acids).

The sum of phenolic compounds of canned pequi fractions resulted in a total of 124.96 µg/g, with ρ-coumaric contributing with 67% (83.18 µg/g) and synaptic with 33% (41.78 µg/g). The total phenolics found in this study was higher than that found in the sum of fresh pequi fractions (88.37 µg/g) (Lima, 2008), which may be related to differences in the purification methodology of these compounds, leading us to consider canned pequi as a food with interesting antioxidant potential, rich in phenolics. Phenolic compounds are known for its high AC also in other fruits and vegetables. The ρ-coumaric acid, for example, has excellent AC, higher to activity of vitamins C and E against reactive oxygen species. It has wide range of biological activities, such as protection against coronary heart disease, anti-inflammatory activity, antitumor, antimutagenic and antimicrobial (Karthikeyan et al., 2015).

In Table 2 is described AC of canned pequi by DPPH* reduction method.

Table 2 - Antioxidant capacity from extracts and fractions of free (FPA), soluble (SPA) and insoluble (IPA) phenolic acids from canned pequi pulp using reduction of DPPH* method.

<table>
<thead>
<tr>
<th>Samples</th>
<th>DPPH* – IC50 (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Extracts</strong></td>
<td></td>
</tr>
<tr>
<td>Alcoholic</td>
<td>533.62 ± 7.48</td>
</tr>
<tr>
<td>Aqueous</td>
<td>1376.28 ± 165.32</td>
</tr>
<tr>
<td>Canning liquid</td>
<td>1542.74 ± 3.59</td>
</tr>
<tr>
<td><strong>Fractions</strong></td>
<td></td>
</tr>
<tr>
<td>FPA</td>
<td>112.58 ± 3.12</td>
</tr>
<tr>
<td>SPA</td>
<td>n.d.</td>
</tr>
<tr>
<td>IPA</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

Results expressed as mean and standard deviation, n = 3. Different letters in the same column (extracts) represent statistically different results (p <0.05) according to HSD Tukey test. DPPH*: 2,2-diphenyl-1-picrylhydrazyl. IC50: inhibitor capacity of 50% of the radical. n.d.: not detected.

It was observed that the canned pequi pulp extracts showed the ability to reduce DPPH* radical. The ALCE reduction ability (IC50 533.62 µg/mL) was higher than AQE (IC50 1376.28 µg/mL) (Table 2). This result is in disagreement with some authors that, analyzing different food matrices, found higher AC in AQE compared to ALCE due to the higher water polarity and, consequently, greater ability to drag antioxidant compounds (Vidal et al., 2006, Roesler et al., 2007, Lima, 2008, Vieira et al., 2011). In this study, the observed result occurred probably due to the effects of processing over phenolic, causing loss or even enhancing the migration of antioxidant compounds to the surrounding liquid, as noted in phenolic values previously described. CL, in turn, showed radical reduction capability comparable to AQE (1542.74 and 1376.28 µg/mL, respectively) (Table 2). This result confirms the AC of phenolic compounds and/or other compounds present in CL.

Even with the possible AC losses due to processing, canned pequi activity was superior to that described for AQE (IC50 188500 µg/mL) (Morais et al., 2013) and for ALCE (IC50 820.7 µg/mL) (Lima, 2008) of fresh pequi which may be related to addition of citric acid to canned pequi during processing or to the presence of other polar compounds that were not evaluated in this study. These compounds may have contributed to the increased antioxidant activity of processed samples. The results also demonstrate that canned pequi has good ability to reduce DPPH* and hence, good AC. The AQE, e.g., has better AC when compared to the AQE of bacuri and tamarind.
(IC\textsubscript{50} 4700.24 and 2193.79 µg/mL, respectively), in addition to have AC similar to caja (IC\textsubscript{50} 535.53 µg/mL), second only to guava, acerola and cashew (IC\textsubscript{50} 24.42, 154.95 and 433.36 µg/mL, respectively) (Vieira et al., 2011).

In Table 2 is also described the reduction ability of FPA from canned pequi pulp, which was IC\textsubscript{50} of 112.58 µg/mL, superior value than to those found in FPA of fresh pequi (14.30 µg/mL) (Lima, 2008) and also to that described for babassu (Attalea speciosa) mesocarp (48.77 µg/mL) (Vieira, 2011) indicating AC inferior. In the other fractions (SPA and IPA), it could not possible to detect activity. The results of AC assessments by β-carotene/linoleic acid system of canned pequi pulp are described in Table 3.

**Table 3 - Antioxidant capacity from extracts and fractions from free (FPA), soluble (SPA) and insoluble (IPA) phenolic acids from canned pequi pulp by β-carotene/linoleic acid system.**

<table>
<thead>
<tr>
<th>Samples</th>
<th>% Oxidation inhibition Concentration (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
</tr>
<tr>
<td><strong>Extracts</strong></td>
<td></td>
</tr>
<tr>
<td>Alcoholic</td>
<td>n.a.</td>
</tr>
<tr>
<td>Alcoholic + BHT</td>
<td>n.a.</td>
</tr>
<tr>
<td>Aqueous</td>
<td>n.a.</td>
</tr>
<tr>
<td>Aqueous + BHT</td>
<td>n.a.</td>
</tr>
<tr>
<td>CL</td>
<td>n.a.</td>
</tr>
<tr>
<td>CL + BHT</td>
<td>n.a.</td>
</tr>
<tr>
<td><strong>Fractions</strong></td>
<td></td>
</tr>
<tr>
<td>FPA + BHT</td>
<td>81.95 ± 1.75\textsuperscript{cd}</td>
</tr>
<tr>
<td>SPA + BHT</td>
<td>57.15 ± 5.55\textsuperscript{b}</td>
</tr>
<tr>
<td>SPA + BHT</td>
<td>86.77 ± 0.27\textsuperscript{de}</td>
</tr>
<tr>
<td>SPA + BHT</td>
<td>79.37 ± 1.19\textsuperscript{e}</td>
</tr>
<tr>
<td>IPA + BHT</td>
<td>87.58 ± 0.29\textsuperscript{e}</td>
</tr>
<tr>
<td>IPA + BHT</td>
<td>80.43 ± 0.86\textsuperscript{de}</td>
</tr>
<tr>
<td>BHT</td>
<td>5.46 ± 1.18\textsuperscript{a}</td>
</tr>
</tbody>
</table>

Results expressed as mean and standard deviation, n = 3. Different letters in the same column represent statistically different results (p <0.05) according to HSD Tukey test. n.a.: not assessed. ppm: parts per million. CL: Canning liquid. BHT: butylated hydroxytoluene.

In the co-oxidation test of β-carotene/linoleic acid, all canned pequi extracts had lower percentage of protection to BHT (63.55, 76.44 and 84.03% at concentrations 50, 100 and 200 ppm, respectively) at three concentrations used in the study (Table 3). It was noted that the AEQ obtained AC similar to ALCE, while CL showed lower AC, which is consistent with previous analyzes. Furthermore, it can be observed that the activity increased with increasing extract concentration.

When the extracts were associated with BHT, there was an increase in its AC, but they remained with lower activity to pure BHT, indicating the absence of synergism, especially in CL had lower results compared to others.

Lima (2008) found values of % OI in fresh pequi of 0.75, 5.7 and 18.3 % in ALCE and 72.9, 84.9 and 90.8 % in AEQ for concentrations 50, 100 and 200 ppm, respectively. The results of this study demonstrate that ALCE of canned pequi preserves its AC, with even better results than fresh pequi, however, AEQ loses activity, possibly due to processing.

The purified phenolic acid fractions had higher OI% than BHT in all tested concentrations. In combination with BHT, fractions showed OI% lower than isolated fractions, thus not observing, synergism. Similar results were also reported by Lima (2008).

The FPA fraction had high AC by β-carotene/linoleic acid method, which can be attributed to its high content of TPC. The IPA fraction, although lower content of TPC, showed superior performance than FPA regarding the % OI in the same tested quantities, which may be related to the high content of p-coumaric and synaptic acids. This result indicates pequi as a source of these phenolic acids and justifies the high AC found in this study. The highest % OI of IPA was also observed for fresh pequi (Lima, 2008).

It is important to note the existence of differences in action methodologies of each method, with different behaviors and responses. Correlations were performed between TPC, DPPH and β-carotene/linoleic acid methods and significantly positive and strong associations were found between the TPC and β-carotene/linoleic acid variables (r ≥ 0.90 and p <0.05) for extracts, demonstrating that TPC can act in preventing oxidation of linoleic acid.

The present study demonstrates the presence of phenolic compounds with antioxidant capacity...
in canned pequi, although more studies with larger samples are needed, in addition to signal the need for knowledge of content of phytochemical compounds present in processed foods, as well as the factors that can change them in order to maintain or increase the concentrations of these compounds in food.

Conclusions

The canned pequi pulp contains phenolic compounds and has antioxidant potential suggesting possible biological effects. The aqueous extract and the fraction of free phenolic acids of pequi pulp had the highest amounts of total phenolics, similar to that found in sources such as bacuri, caja and other fruits. From phenolic compounds, catechin and p-coumaric, synaptic and ellagic acids were identified and, among these, p-coumaric acid was present in higher concentration in fractions, in addition to being present in canning liquid, showing the migration of this compound.

The alcoholic and aqueous extracts and fractions studied showed antioxidant capacity, regardless of the applied test, however, fractions are highlighted when assessed by β-carotene/linoleic acid system, where activity was superior to butylated hydroxytoluen synthetic antioxidant at the tested concentrations. The alcoholic extract showed greater potential for reducing the DPPH* in relation to aqueous extract and canning liquid demonstrated ability both to reduce and to prevent the oxidation of linoleic acid, confirming the dissolution of phenolic compounds with antioxidant capacity in the liquid.

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