Optimization of total carotenoids extraction from pequi pulp (*Caryocar brasiliense* Camb.) using a mixture planning method

Otimização da extração de carotenoides totais da polpa de pequi (*Caryocar brasiliense* Camb.) usando método de planejamento de misturas

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ABSTRACT
The pequi is a native fruit of the Brazilian Savanna and the consumption of its carotenoid-rich pulp has been associated with several health benefits. However, there are considerable differences in carotenoid content reported for this fruit, mainly due to the diversity of extraction methods used in the recovery of these compounds from pequi pulp. The objective of this study was to evaluate the efficiency of different organic solvents and mixtures in the extraction of total carotenoids from pequi pulp. For the carotenoids extraction, pure solvents and the mixtures of acetone, ethyl alcohol and petroleum ether were used. The mixture planning was used to determine the proportions of the solvents in each treatment. To our knowledge, this is the first study to identify that the most efficient treatment for total carotenoid extraction from pequi pulp is pure petroleum ether solvent (234.40 µg/g). In contrast, ethyl alcohol was the solvent that extracted the lowest concentration of total carotenoids (124.03 µg/g). Pure acetone, one of the most used solvents in the extraction of carotenoids from pequi, showed intermediate efficiency (166.07 µg/g). Thus, pure petroleum ether is the solvent recommended for total carotenoid extraction, mainly in the samples rich in carotenoid and lipids, such as pequi pulp.

Keywords: Bioactive compounds; Carotenes; Cerrado; Phytochemical analysis; Mixture design; Native fruit.

RESUMO
O pequi é um fruto nativo do Cerrado e o consumo de sua polpa rica em carotenoides tem sido associado a diversos efeitos benéficos à saúde. Entretanto, existem diferenças consideráveis nas concentrações de carotenoides relatadas para este fruto, decorrentes, sobretudo, da diversidade de métodos de extração utilizados na recuperação desses compostos da polpa do pequi. O objetivo deste trabalho foi avaliar a eficiência de diferentes solventes orgânicos e misturas na extração de carotenoides totais da polpa de pequi. Para a extração dos carotenoides foram utilizados solventes puros e misturas de acetona, álcool etílico e éter de petróleo. O delineamento de misturas foi utilizado para determinar as proporções dos solventes em cada tratamento. De forma inédita, este estudo identificou que o solvente mais eficiente para a extração de carotenoides totais da polpa de pequi é o éter de petróleo puro (234,40 µg/g). Em contraste, o álcool etílico foi o solvente que extraiu menores concentrações de carotenoides totais (124,03 µg/g). A acetona pura, um dos solventes mais utilizado na extração de carotenoides do pequi, apresentou eficiência intermediária (166,07 µg/g). Logo, recomenda-se o uso de éter de petróleo puro para extração de carotenoides totais, principalmente, em amostras ricas em carotenoides e lipídios, como a polpa de pequi.

Palavras-chave: Compostos bioativos; Carotenos; Cerrado; Análise de fitoquímico; Planejamento de misturas; Fruto nativo.

1 INTRODUCTION

The pequi (*Caryocar brasiliense* Camb.) is a native fruit of the Brazilian Savanna (Cerrado). The pequi pulp (internal mesocarp) is appreciated and used in traditional Brazilian cuisine, especially because of its intense, complex, and peculiar flavour. It also has a high concentration of carotenoids, good-quality lipids (55-60% monounsaturated fatty acids), dietary fiber, zinc and magnesium, and it is a source of minerals and polyphenols (Batista & Sousa, 2019; Nascimento-Silva & Naves, 2019). Studies using animals supplemented with oil extracted from pequi pulp found some biological activities, such as hepatoprotective (Palmeira et al., 2016; Vale et al., 2019), cardioprotective (Oliveira et al., 2017), hypocholesterolemic (Silva et al., 2020), anticarcinogenic (Almeida et al., 2012; Miranda-Vilela et al., 2014), and analgesic and anti-inflammatory effects (Jorge Júnior et al., 2020). Those effects might be related to antioxidant and anti-inflammatory activity, inhibition of lipid oxidation, and DNA damage (Nascimento-Silva & Naves, 2019). Studies that tested pequi whole pulp (Almeida et al., 2012) or pulp extract (Colombo et al., 2015) showed antioxidant and antigenotoxic effects. Such beneficial health effects have been attributed to the bioactive compounds present in the pulp, especially carotenoids (Miranda-Vilela et al., 2014; Palmeira et al., 2016).

The carotenoid content of pequi pulp (*Caryocar* spp.) seems to depend especially on the fruit species (Nascimento-Silva & Naves, 2019). However, studies with pequi pulp of the same species (*C. brasiliense* Camb.) showed discrepant results for the total carotenoid content. In the literature, values ranging from 37.08 μg/g (Ribeiro et al., 2014) to 270.3 μg/g (Cordeiro et al., 2013) have been reported, as well as intermediate concentrations of 72.5 μg/g (Lima et al., 2007), 97.83 μg/g (Boas et al., 2012), and 187.0 μg/g (Ribeiro et al., 2014). The great range among the values found may be explained, in part, by the different carotenoid extraction methods used. Among the issues involved in the carotenoid extraction technique, the solvent used is one of the most important to ensure greater analytical recovery, which is associated with the polarity of the compounds present in the food matrix (Amorim-Carrilho et al., 2014). Considering the importance of the fruit as a source of carotenoids, as well as its potential health benefits, the aim of this study was to investigate the efficiency of different organic solvents and their mixtures in the extraction of total carotenoids from pequi pulp using a mixture planning method.

2 MATERIAL AND METHODS

2.1 MATERIAL AND SAMPLE PREPARATION

The pequi fruits were collected at Hidrolândia municipality, Goiás state, Brazil (16° 57' 48" S and 49° 11' 2" W), in the harvest period, during December. Ripe and intact fruits collected after natural fall from the tree were selected.

The fruits were washed in running water, and submerged in sodium hypochlorite (2.5%) for 15 min. Then, the fruit was pulped (internal mesocarp or yellow pulp) with the aid of a stainless-steel knife, and the pulp was frozen in liquid nitrogen, freeze dried, and powdered in an analytic food mill (A11-IKA, Staufen, Germany). The pulps were homogenized and stored in polyethylene bags, protect from light in a dark package and stored at -20 °C for no more than one month.

2.2 EXTRACTION OF CAROTENOIDS AND THE EXPERIMENTAL DESIGN

The extraction was carried out from 0.5 g of sample and followed the method described by Rodriguez (2001), with modifications in the solvents used and with the exclusion of evaporation and saponification steps. In order to optimize the solvent extraction, a three-component mixture experiment was employed using pure acetone (AC), pure petroleum ether

(PE), and pure ethyl alcohol (EA) following the mixture planning showed in Table 1. This augmented simplex centroid planning can be visualized in Figure 1 and the simplex mixture space can be modelled using the special cubic model (Equation 1):

$$y = \sum_{i=1}^{p} \beta_i x_i + \sum \sum_{i<j}^{p} \beta_{ij} x_i x_j + \sum \sum \sum_{i<j<k} \beta_{ijk} x_i x_j x_k \quad (1)$$

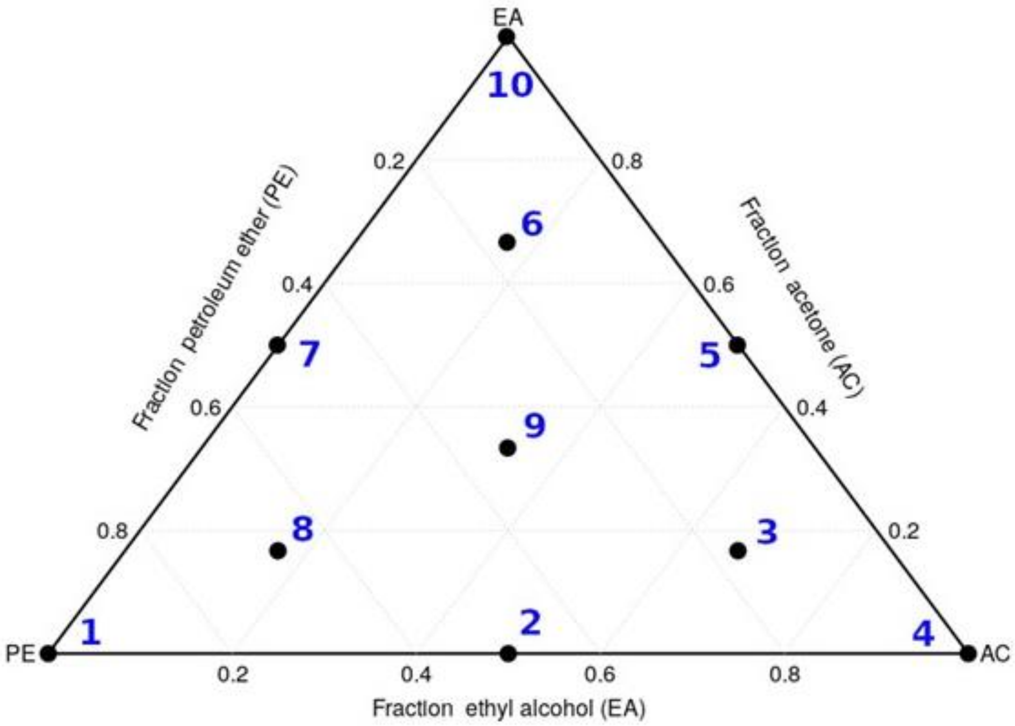
Where p is the number of components (solvents); x_p, the solvent composition; β_i, the linear coefficient; and the coefficients β_{ij} representing the quadratic blending effects in the model (Lawson, 2015).

Table 1. Mixture planning for total carotenoids extracted from pequi pulp.

Run	Mixture composition (AC:PE:EA)#
1	0:1:0
2	1:1:0
3	4:1:1
4	1:0:0
5	1:0:1
6	1:1:4
7	0:1:1
8	1:4:1
9	1:1:1
10	0:0:1

#AC – acetone; PE – petroleum ether; EA – ethyl alcohol.

Figure 1. Mixture planning for carotenoid extraction using acetone (AC), petroleum ether (PE), and ethyl alcohol (EA). Runs are numbered (in blue) according to Table 1.



Chemical solvents were chosen in order to extract the main carotenoids in pequi pulp (Rodriguez-Amaya, 2001). Carotenoid quantification was based on the readings for the sample extracts in a UV/Vis spectrophotometer with visible wavelength between 350 and 550 nm. The wavelength range was the same for all experiments and was selected based on the main types of carotenoids present in the pequi and its ultraviolet absorption data reported by Rodriguez-Amaya (2001). The wavelength at which highest absorbance was observed was employed to determine the total carotenoid content of the extracts. Total carotenoid concentration, in µg/g, of the sample was estimated by (Equation 2):

$$\text{Total carotenoids content } (\mu g/g) = \frac{A \times V \times 10^4}{A1\% \times P} \quad (2)$$

Where A = Extract absorbance; V = Total extract volume (100 mL); $A^{1\%}$ = 2592 (β-carotene extinction coefficient); P = sample weight (g).

2.3 STATISTICAL ANALYSIS

All the replicates were cautiously randomized and performed under similar environmental conditions. Data were expressed as mean ± standard deviation. The analysis of variance test (one-way ANOVA) was carried out to assess the model fit. For comparison between experiments, the Tukey test ($p < 0.05$) was used. The calculations were performed using the SPSS software, version 17.0. The simplex planning data were treated in the R software environment (R Core Team, 2018) using the package mixexp (Lawson & Willden, 2016).

3 RESULTS AND DISCUSSION

To our knowledge, the present study is the first to apply a mixture planning model to identify the most efficient solvent for extracting carotenoids in the pequi pulp. In the pequi fruit, this method was used only in one study to evaluate the yield of the oil extracted from the pequi pulp using different solvents, without carotenoids extraction (Nepomucena et al., 2013). Among the experimental runs (Table 2), pure petroleum ether was the most efficient solvent for carotenoids extraction from the pequi pulp, followed by the binary (equimolar) mixture of petroleum ether and acetone. The lowest extraction efficiency was obtained using pure ethyl alcohol.

Table 2. Concentration of total carotenoids extracted from pequi pulp with different solvents and their mixtures.

Run	Carotenoid content (µg/g of fresh weight)[#]
1	229.04 ± 7.6 [a]
2	195.54 ± 6.1 [b]
3	170.05 ± 8.4 [c]
4	166.07 ± 0.6 [c]
5	157.82 ± 4.2 [cd]
6	150.91 ± 7.5 [de]
7	148.24 ± 3.8 [de]
8	146.97 ± 3.1 [de]
9	141.42 ± 5.0 [e]
10	124.98 ± 1.5 [f]

[#]Values are expressed as mean ± standard deviation. Different letters indicate statistically different values (Tukey test, $p < 0.05$).

The special cubic model was fitted to the mixture planning data (Table 1 and Figure 1) resulting in (Equation 3):

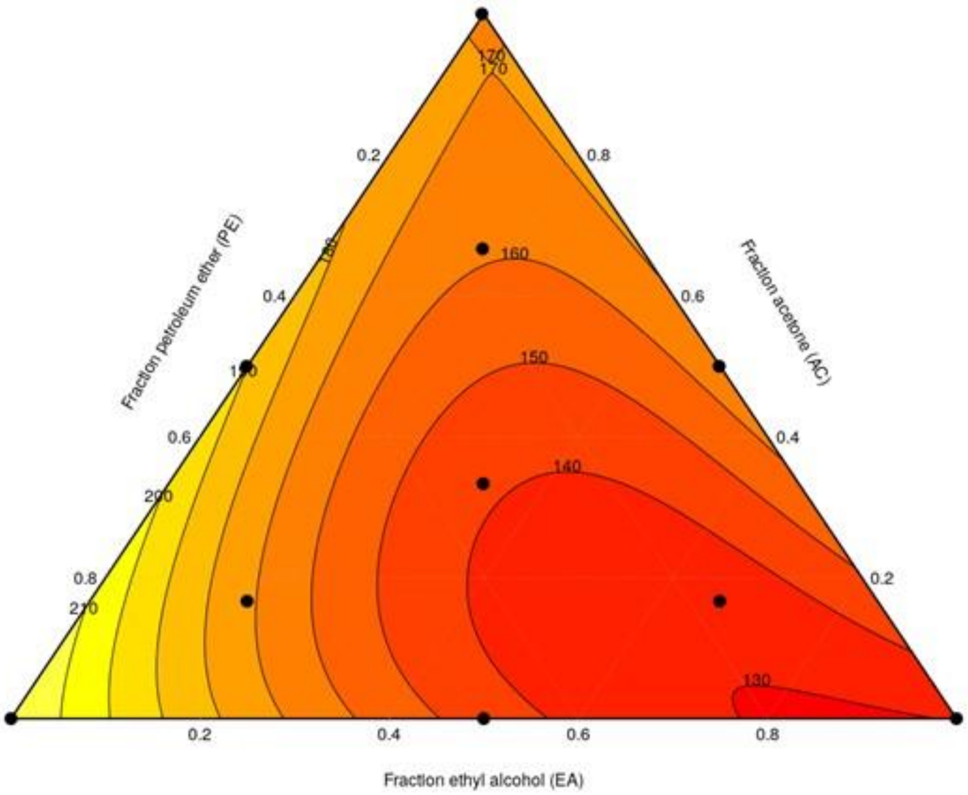
$$y = 4.43\, x_{ac} + 3.52\, x_{pe} + 1.24\, x_{ea} + 6.90\, x_{ac}x_{pe} + 8.95\, x_{ac}x_{ea} + 5.31\, x_{pe}x_{ea} + 25.55\, x_{ac}x_{pe}x_{ea} \quad (3)$$

Having an adjusted multiple R^2 of 0.9981, a measure of goodness-of-fit for the linear regression between experimental and predicted values.

The carotenoid content as a function of different solvent proportions is shown in the contour surface plot (Figure 2). According to the curves' standard colour (*heatmap*), yellow areas represent a high carotenoid content in the extract, and red areas correspond to low carotenoid content. The highest average concentration obtained with pure petroleum ether was 229.04 ± 7.60 µg/g, with the maximum extraction point of 234.4 µg/g of fresh pulp. In contrast, the extract obtained with pure ethyl alcohol was the least efficient, with an average concentration obtained of 124.98 ± 1.50 µg/g, and minimum extraction point of 124.03 µg/g (Figure 2). Pure ethyl alcohol reduced the efficiency of the extraction. This might be due to the fact that the carotenoids present in pequi pulp are more soluble in organic solvents with a more non-polar nature, such as petroleum ether and hexane (Amorim-Carrilho et al., 2014).

Pure acetone is the most widely used solvent in the literature for total carotenoid extraction from the pequi pulp (Cordeiro et al., 2013; Lima et al., 2007; Ribeiro et al., 2014). However, in the present study, pure acetone was the fourth most efficient treatment for carotenoid extraction. This behaviour may be explained by the fact that the fruit has a rich lipid matrix (Nascimento-Silva & Naves, 2019) which can represent up to one-third of the fruit pulp. Considering that carotenoids are more lipophilic compounds, there is a positive correlation between the lipid content and carotenoid concentration in pequi pulp (Ribeiro et al., 2014). Therefore, the results of this study highlight the influence of food matrix composition on the extraction efficiency of the carotenoids.

Figure 2. Mixture planning response surface for the total carotenoid extraction. Contour lines represent model predicted carotenoid contents, which varied from 130 (red) to 210 (yellow) (μg/g).



The highest total carotenoid content found in this study was higher than those reported by Lima et al. (2007) (72.5 μg/g) and Ribeiro et al. (2014) (from 37.08 to 187.00 μg/g) for the pulp of *C. brasiliense* native to different regions of the Cerrado. Considering that Lima et al. (2007) and Ribeiro et al. (2014) used acetone as extraction solvent, the use of a more non-polar solvent, such as petroleum ether, is likely to be more efficient in recovering the carotenoids from pequi pulp.

In the present study, an experimental design using mixture planning was employed, which allowed optimization of the number of assays and the carotenoid extraction. Thus, we suggest the use of this experimental design for carotenoid extraction in complex food matrices, such as the pequi pulp. In addition, further investigation is needed regarding the factors that may influence the carotenoid extraction in lipid-rich food matrices. An inefficient carotenoid recovery may be a confounding factor for the definition of extract doses to be used in *in vitro* and *in vivo* experimental studies, and for interpreting their biological effects.

4 CONCLUSION

The mixture planning method assisted in optimizing the number of experiments and made it possible to affirm that, among the solvents tested, the pure petroleum ether was the most effective solvent for extracting carotenoids from the pequi pulp. In contrast, the solvent that recovered the lowest carotenoid content was pure ethyl alcohol. Therefore, the use of pure petroleum ether in carotenoid-rich food matrices with a high lipid content, such as pequi pulp, is recommended when aiming for greater extraction efficiency of these bioactive compounds.

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