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Shelf life and retention of bioactive compounds in storage of pasteurized *Passiflora setacea* pulp, an exotic fruit from Brazilian savannah

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TPTZ: 2,4,6-Tris(2-pyridyl)-*s*-triazine (PubChem CID: 77258)
Ascorbic acid (PubChem CID: 54670067)
Putrescine (PubChem CID: 1045)
Orientin (PubChem CID: 5281675)
Isoorientin (PubChem CID: 114776)
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Epicatechin (Pubchem CID: 72276)

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ABSTRACT

We estimated the shelf life of pasteurized *Passiflora setacea* pulp, by accelerated tests based on microbiological, nutritional, functional, and sensory variables. The pulp was pasteurized using the binomial of 82 °C/20 s and stored at 25, 35, and 45 °C. The quality of the pulp was evaluated at the beginning of storage and every two days, up to 12 days. Vitamin C, flavonoids, ORAC and FRAP antioxidant activity, sensory and microbiological evaluations were carried out. On the last day of the accelerated tests, pasteurized pulp had counts of <1.0 CFU/g, 6.70 CFU/g, and 6.30 CFU/g of molds and yeasts, for temperatures of 25 °C, 35 °C, and 45 °C, respectively. The estimated shelf-life values at 5 and -15 °C ranged from 13.4 to 18.3 days for vitexin, to 60.5 and 184 days, for acceptance.

1. Introduction

The "Cerrado" is a Brazilian savannah biome with a high diversity of plants, characterized by its closed forests and trees with crooked trunks, in addition to the scarcity of rain, high frequency of fire in the dry season, low humidity and high temperatures (Filardi et al., 2018). The edaphoclimatic conditions of this biome result in plants resistant to extreme stress conditions, with a very active secondary metabolism and, consequently, a high amount of antioxidant compounds (Siqueira, Rosa, Fustinoni, Sant'Ana, & Arruda, 2013). Among these plants, we highlight the *Passiflora* genus, which has around 130 species cataloged, although not all native (Braga, Junqueira, & Faleiro, 2010). *Passiflora edulis* is the most well-known and grown as a crop species, consumed in many countries. An important wild species in Brazil is *Passiflora setacea*, regionally known for its sedative properties, which gives it the popular name of sleeping passion fruit. The BRS Pérola do Cerrado variety of *P. setacea* comes from a genetic improvement by polycross breeding of various wild fruits and was launched by the Brazilian Agricultural Research Corporation in 2013 (Embrapa, 2013).

A previous study by our research group demonstrated the acute effect of the consumption of *P. setacea* juice on the prevention of inflammatory interleukin-17A increase, on the reduction of insulin resistance biomarkers and on the modulation of genes involved in inflammation, cell adhesion and cytokine-cytokine receptor, which may help in the prevention of cardiometabolic diseases (Duarte et al., 2020). This effect may be related to the content of phytochemicals. According to Arabbi, Genovese, and Lajolo (2004), the average daily ingestion of flavonoids by the Brazilian population is about 60–106 mg/day. Sanchez et al. (2020) found about 848 mg/100g of epicatechin, 19.9 mg/100g of orientin, 212 mg/100g of isoorientin, 5.45 mg/100g of vitexin, 57.8 mg/100g of isovitexin and 14.6 mg/100g of hesperitin mg/100g of *P. setacea* pulp.

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De Carvalho, Oliveira, Melo and Costa (2018) have shown the effect of climate conditions and training system on the content of *P. setacea* flavonoids, with no influence on the sensory acceptance of the pulp. Processing is another factor that influences the content of bioactive compounds. Our study about the effect of pasteurization conditions on the retention of bioactive compounds allowed us to choose a binomial with little loss of flavonoids, vitamin C and bioactive amines, besides few sensory alterations (Sanchez et al., 2020), but the effect of storage on these compounds remains unclear. The shelf life, defined as the time in which the food is safe for human consumption, under chemical, physical, microbiological and sensory conditions (IFST, 1993) can be estimated by accelerated test. This uses critical conditions, such as extreme temperatures, so that changes in food occur more quickly, which makes it possible to extrapolate the moment when unacceptable limits of quality are reached through mathematical models (Grizotto, Berbari, Moura, & Claus, 2006; Kilcast & Subramaniam, 2000). In this case, the food is stored for a pre-established time at high temperatures to accelerate its deterioration and to have an estimate of the time in which the food complies with the established parameters of microbiological, nutritional, functional and sensory quality for human consumption.

To the best of our knowledge, few studies have evaluated the quality of fresh and pasteurized fruit derivatives during storage, with emphasis on the bioactive content and sensory properties. Dos Reis, Facco, Flôres, and Rios (2018) evaluated bioactive compounds and antioxidant activity in fresh and pasteurized *Passiflora caerulea* juice and subsequent storage at 8 °C. These authors observed the significant retention of bioactive compounds during storage for 4 days. Studies in strawberry jam stored for 120 days at 20 °C showed that the total phenolic content decreased 12% after 30 days of storage and the anthocyanin content at the end of storage was 50% lower. At the end of the storage conditions, there was a degradation of catechin and epicatechin contents of 42.8% and 31.2%, respectively (Pineli, Moretti, Chiarello, & Melo, 2015).

The evaluation of degradation/retention of antioxidants and other bioactive compounds during storage is of extreme relevance in a context of functional food, especially considering exotic and seasonal fruit, whose availability to consumers throughout the year depends on processing and storage. Therefore, the objective was to determine the shelf life of *P. setacea* pulp submitted to rapid pasteurization processing and to evaluate the effects of storage on functional, nutritional and sensory properties, using accelerated tests.

2. Material and methods

2.1. Processing and storage of Passiflora setacea pulp

Fruit of *P. setacea* BRS Pérola do Cerrado were harvested in 2018 in the experimental field of Embrapa Cerrados, Planaltina, Brasília, Federal District, with an approximate altitude of 1050 m during the dry season. They were washed, sanitized with 100 mg/L solution of active chlorine for 10 min and selected by discarding injured fruits or those outside the appropriate maturation point. The pulp was extracted in the depulper (Mundinox, Lambari, MG, Brazil) and packed in plastic bags of 1000 g each, sealed with thermal sealer. The *P. setacea* pulp was stored at −18 °C, until the next day for High Temperature Short Time type pasteurization, adopting the binomial 82 °C/20 s, in a bench pasteurizer developed at EMBRAPA Cerrados (Celestino & Sanchez, 2018). Pasteurized pulp was packed in 50 g polyethylene bags and stored at −80 °C. Frozen samples were then submitted to storage under accelerated conditions in a temperature-controlled chamber at temperatures of 25 °C, 35 °C and 45 °C, for 0, 2, 4, 6, 8, 10 and 12 days, in order to estimate the shelf life under refrigeration and freezing, at 5 °C and −15 °C, respectively, by using kinetic modeling. Therefore, every 2 days, microbiological analyses, antioxidant activity and sensory analysis were performed.

2.2. Quality analysis of Passiflora setacea pulp during storage in accelerated tests

2.2.1. Microbiological analysis

Filamentous fungi and yeasts, aerobic mesophiles, total coliforms, thermotolerant coliforms and *Salmonella* spp. were analyzed in the pasteurized pulp, in accordance with Brazilian legislation on microbial standards (Brasil, 2018, 2019a, 2019b).

2.2.2. Flavonoid profile

Flavonoids were quantified according to the method described by De Carvalho, De Oliveira, and Costa (2018) and adapted by Sanchez et al. (2020). Briefly, the extracts were obtained by ethanol extraction, 5 g of pulp and 10 mL of ethanol: water solution (1: 1). The extract was mixed with 0.2 mL of 50% methanol, 1.2 mL of HCl (1.2 mol/L) and 0.25 mL of *tert-butylhydroquinone* (TBHQ, 0.4 g/L), finally, the extracts were sonicated and filtered through a millex filter,0.45 mm HV. Extracts were analyzed in HPLC using the Shimadzu® system, equipped with a high-pressure pump, model LC-10AT VP, an automatic sampler, model SIL-10AF and a detector of UV visible diode matrix, model SPD-M10A (Shimadzu®, Kyoto, Japan), using wavelengths of 270, 340 and 380 nm. The mobile phases were (A) Water: Tetrahydrofuran: Trifluoroacetic acid (99.79: 0.2: 0.01) and (B) acetonitrile for epicatechin, vitexin, isovitexine, orientin and isoorientin. A run was performed with 80% A 20% B, with a flow rate of 0.5 mL/min for 25 min. The standard curves were made with concentrations of 0.10 mg/mL, 0.08 mg/mL, 0.06 mg/mL, 0.04 mg/mL, 0.02 mg/mL, 0.01 mg/mL, and 1 mg of the standard was dissolved in 50% methanol, which was finally filtered through a 0.45 mm Millex filter.

2.2.3. Vitamin C

Vitamin C was quantified as described by Sanchez et al. (2020). The quantification of DHA (dehydroascorbic acid) was performed by the difference in the total AA (ascorbic acid) content; for conversion of DHA to AA, 1.0 mL of 0.5 mol/L Trizma buffer solution (pH 9.0) containing DTT was added 40 mmol/L. The mobile phase was composed of monobasic sodium phosphate 1.0 mmol/L (NaH_2PO_4) and EDTA 1.0 mmol/L, pH adjusted to 3.0 with phosphoric acid (H3PO4), in isocratic elution at a flow rate of 1.0 mL/min. Detection was performed at 245 nm and ascorbic acid was used as a standard, with a curve ranging from 0 to 100 μg/mL (De Carvalho, De Oliveira, & Costa, 2018).

2.2.4. Antioxidant activity

The antioxidant activity was determined by FRAP and ORAC methods. Initially, extracts were obtained according to Laurrarri, Rupérez, & Saura-Calixto (1997), modified by Sanchez et al. (2020). The analysis of antioxidant activity by the FRAP method was determined according to Benzie and Strain (1996) and with modifications proposed by Pulido, Bravo, and Saura-Calixto (2000), adopting an absorbance reading at 595 nm. Results were expressed in μmol equi. Trolox/100g.

The analysis of antioxidant activity by the ORAC method was carried out according to (Wang, Cao, & Prior, 1997), with some modifications proposed by Prior, Wu, and Schaich. (2005), in which 150.0 μL of fluorescein 63.0 mmol/L were pipetted, followed by 25 μL of sample, after which the plate was incubated in the equipment at 37 °C for 5 min. Then 125.0 μL of 178.0 mmol/L AAPH solution was added and incubated for another 10 min in the equipment. The measurements were made every minute with λ_{ex} 485 nm and λ_{em} 520 nm for 50 min. Trolox was used as standard and 96-well black plates were read in a SpectraMax M2 spectrofluorometer, Molecular Devices (San Jose, USA).

2.2.5. Sensory evaluation

Thirty evaluators were recruited, who signed the free and informed consent form, completed the questionnaire of demographic data (age, sex, educational level) and consumption patterns of fresh or industrialized passion fruit nectars. Only consumers with a minimum

consumption of passion fruit pulp, juice or nectar of about once a month were included in the study. The analyses were submitted to the ethics committee and approved with rapporteur number 2.500.174 and CAAE 74299317.9.0000.0030.

Acceptance tests were carried out in the Dietetic Technique Laboratory of the Faculty of Health Sciences of the University of Brasília. Participants received approximately 20 mL of each of the twelve samples (pasteurized *P. setacea* pulp subjected to accelerated tests at temperatures 25, 35 and 45° for 0, 2, 4 and 6 days), at 10 ± 1 °C. The samples were served in plastic cups marked with random three-digit codes. The limit of six (6) days of storage of the pulps was adopted, considering the results obtained regarding the microbiological analyses. The samples were presented to 51 consumers simultaneously, in a randomized order. The consumers were selected among students and staff of the University of Brasilia, who stated a liking for passion fruit juices and tended to consume it at least once a month; most of them consumed passion fruit nectar twice a week, being classified as high users. Samples were stored at −80 °C each day for simultaneous presentation. Water and unsalted crackers were served along with the samples to cleanse the palate. The acceptance test for the *P. setacea* pulp was performed using the 9-point structured hedonic scale.

2.3. Estimated shelf life of pasteurized P. setacea pulp from accelerated tests

The kinetics of food quality changes generally follows zero-, first-, or second-order reactions (Ling, Tang, Kong, Mitcham, & Wang, 2014), as these allow the extrapolation of storage conditions (Escobar & Meeker, 2006). The shelf-life was estimated from the evaluation of pasteurized *P. setacea* pulp in accelerated tests at temperatures of 25, 35, and 45 °C, by analyzing the quality of the product at the beginning of the storage and every two days, until 12 days, for flavonoid profile, vitamin C content and antioxidant activity (FRAP and ORAC). For sensory shelf life, samples stored for up to six days were used, because of the microbial damage after this period.

From the data obtained for each analyzed variable, the first order and second order kinetic models were tested at temperatures of 25, 35 and 45 °C. Table 1 shows the different kinetic reaction models and their integrated and linearized equations (Wright, 2004). The determination coefficient (r^2) was used to define the appropriate kinetic model to be considered for the set of variables.

By knowing the k value (reaction rate constant) of the kinetic reaction model that best fitted the set of variables, the reaction activation energy (E_a) and the temperature acceleration factor (Q_{10}) were determined.

The Arrhenius model was used to evaluate the effect of temperature changes on the reaction rate (Equation (1)). Thus, it was possible to obtain the activation energy of the reaction for each variable analyzed.

$$lnk = ln\, k_0 - \frac{E_a}{R.T} \qquad \text{Equation 1}$$

in which:

E_a = activation energy (cal mol^{-1});

R = universal gas constant – 1.987 cal mol^{-1} K^{-1};

T = absolute temperature (K).

From the activation energy values, it was possible to define the variables to be considered for determining the shelf life of *P. setacea* pulp. In this stage, three different groups were considered: I) flavonoids and vitamin C; II) antioxidant activity (FRAP and ORAC); and III) sensory analysis. For groups I and II, the variables with the highest activation energy were used to determine the shelf life of the *P. setacea* pulp. For this, initially the values of Q_{10}, the temperature acceleration factor or the ratio between the constant of the temperature reaction rate with a 10 °C interval were calculated, using Equation 2.

$$Q_{10} = exp\left(\frac{E_a}{R} x \frac{10}{T.(T+10)}\right) \qquad \text{Equation 2}$$

in which:

E_a = activation energy (cal mol^{-1});

R = universal gas constant – 1.987 cal mol^{-1} K^{-1};

T = absolute temperature (K).

After obtaining the Q_{10} values, it was possible to estimate the shelf life of the *P. setacea* pulp for the three groups previously established. For groups I and II, the limit was defined as a reduction to 50% of the concentration value of the reference variable at the beginning of storage. For group III (sensory analysis), grade 5.0 was adopted as the cut-off point or minimum acceptable grade. We used the temperatures of 5 °C and −15 °C to extrapolate and estimate shelf-life under refrigerated and freezing conditions. According to Fu and Labuza (1997), frozen foods deteriorate during storage by different modes or mechanisms, but microorganisms are usually not a problem since they cannot grow at freezing temperatures. In the literature, there is no psychrophile which grows at pH conditions found in *P. setacea* pulp (pH = 3) and in freezing storage. Therefore, estimations were calculated only for variables of groups I to III, as described above.

Table 1
Models of kinetics reaction and respective integrated and linearized equations.

Order	Differential Equations	Integrated and Linearized Equations
0	$\frac{dC}{dt} = -k$	$C = C_0 - kt$
1	$\frac{dC}{dt} = -kC$	$lnC = lnC_0 - kt$
2	$\frac{dC}{dt} = -kC^2$	$\frac{1}{C} = \frac{1}{C_0} + kt$

Fonte: Wright, 2004.
C = concentration of different compounds; t = time (day); k = reaction rate constant (1/day).

2.4. Experimental design and statistical analyses

The experiment was carried out in a completely randomized design, in a 3 × 7 factorial scheme, with three temperatures (25, 35 and 45 °C) and seven storage periods (0, 2, 4, 6, 8, 10 and 12 days). The results obtained regarding the flavonoid profile, vitamin C content, antioxidant activity and sensory acceptance were subjected to analysis of variance (ANOVA) and, when significant, Tukey's test ($p < 0.05$) was adopted afterwards. All the statistical analyses were run in the XLTAT program (Addinsoft, France).

3. Results and discussion

The shelf life of *P. setacea* pulp, as of other functional foods, must be estimated regarding not only microbiological and sensory aspects, but also considering the retention of the key bioactive compounds.

3.1. Microbiological quality of pasteurized pulp of P. setacea submitted to accelerated tests

With regard to mold and yeast counts in pasteurized pulp, the mean values remained below the value of 3.30 log CFU/g for 12 days of storage at 25 °C and for 8 and 10 days of storage, at temperatures of 35 and 45 °C, respectively (Table 2). It should be noted that the reference value of 3.30 log CFU/g (2.0×10^3 CFU/g) is recommended by Normative Instruction nº 49/2018, of the Ministry of Agriculture, Livestock and Supply (Brasil, 2018), being valid for fruit pulp that has undergone heat treatment.

The aerobic mesophilic counts increased after 10 days of storage, to 25 and 35 °C, and after 12 days, to 45 °C (Table 2). The count of this type of microorganism is important to determine the quality of the food, given that a high count indicates that the processing was carried out

Table 2
Microbiological analyzes of pasteurized *Passiflora setacea* pulp submitted to accelerated conditions at temperatures of 25, 35 and 45 °C, for 12 days.

Temperature (°C)	Storage time (days)						
	0	2	4	6	8	10	12
	Molds and yeasts (log CFU/g)						
25	<1.0[a]	<1.0[a]	<1.0[a]	<1.0[a]	<1.0[a]	<1.0[a]	<1.0[a]
35	<1.0[a]	<1.0[a]	<1.0[a]	<1.0[a]	2.48	6.30[a]	6.70[a]
45	<1.0[a]	<1.0[a]	<1.0[a]	<1.0[a]	<1.0[a]	<1.0[a]	6.30[a]
Aerobic mesophiles (log CFU/g)							
25	<1.0[a]	<1.0[a]	1.48	1.70	1.95	5.30[a]	3.48
35	<1.0[a]	<1.0[a]	<1.0[a]	1.30	2.08	5.70[a]	5.85[a]
45	<1.0[a]	<1.0[a]	<1.0[a]	<1.0[a]	1.30	1.30[a]	6.70[a]
Salmonella spp. (absence or presence)							
25	absence	absence	absence	absence	absence	absence	absence
35	absence	absence	absence	absence	absence	absence	absence
45	absence	absence	absence	absence	absence	absence	absence
Total coliforms (MPN/g)							
25	<3,0[a]	<3,0[a]	<3,0[a]	<3,0[a]	<3,0[a]	<3,0[a]	<3,0[a]
35	<3,0[a]	<3,0[a]	<3,0[a]	<3,0[a]	<3,0[a]	<3,0[a]	<3,0[a]
45	<3,0[a]	<3,0[a]	<3,0[a]	<3,0[a]	<3,0[a]	<3,0[a]	<3,0[a]
Thermotolerant coliforms (MPN/g)							
25	<3,0[a]	<3,0[a]	<3,0[a]	<3,0[a]	<3,0[a]	<3,0[a]	<3,0[a]
35	<3,0[a]	<3,0[a]	<3,0[a]	<3,0[a]	<3,0[a]	<3,0[a]	<3,0[a]
45	<3,0[a]	<3,0[a]	<3,0[a]	<3,0[a]	<3,0[a]	<3,0[a]	<3,0[a]

[a] - Estimated data.

inappropriately or that the product was manufactured with contaminated raw material. Aerobic mesophilic counts greater than 1.0×10^6 CFU/g (6.0 log CFU/g) are indicative that the food has undergone some sanitary alteration (De Carvalho, 2010). This limit was achieved only at 12 days of storage at 45 °C.

Regarding the detection of *Salmonella* spp., it was found that the

Table 3
Average values for flavonoid profile, vitamin C content and antioxidant activity in *Passiflora setacea* pasteurized pulp subjected to accelerated tests at temperatures of 25, 35 and 45 °C, for 12 days.

Temperature (°C)	Storage time (days)						
	0	2	4	6	8	10	12
Flavonoids profile							
Isoorientin (mg/100g)							
25	61.78 ± 0.55 [a]	53.66 ± 0.42 [Ab]	52.07 ± 2.61 [Ab]	47.13 ± 1.64 [Bc]	36.79 ± 0.45 [Ad]	33.26 ± 0.60 [Ae]	25.56 ± 1.56 [Af]
35	61.78 ± 0.55 [a]	52.35 ± 2.66 [Ab]	51.20 ± 2.36 [Ab]	51.17 ± 0.72 [Ab]	29.31 ± 0.93 [Bc]	28.49 ± 0.36 [Bcd]	27.09 ± 0.39 [Ad]
45	61.78 ± 0.55 [a]	45.56 ± 1.87 [Bb]	43.27 ± 0.33 [Bc]	30.95 ± 0.61 [Bd]	19.70 ± 0.48 [Ce]	17.47 ± 0.88 [Cf]	14.13 ± 1.29 [Bg]
Orientin (mg/100g)							
25	12.79 ± 0.12[a]	12.28 ± 0.31[Aa]	11.58 ± 1.29[Ab]	7.27 ± 0.93[Bc]	7.22 ± 0.83[Ac]	0.33 ± 0.03[Ad]	0.25 ± 0.25[Ae]
35	12.79 ± 0.12[a]	11.20 ± 0.05[Bb]	9.02 ± 1.33[Bc]	7.80 ± 0.66[Bd]	0.52 ± 0.00[Ce]	0.05 ± 0.00[Bf]	0.00 ± 0.00[Bg]
45	12.79 ± 0.12[a]	9.75 ± 1.28[Cb]	9.62 ± 0.47[Bb]	8.41 ± 0.98[Ac]	1.54 ± 0.00[Bd]	0.33 ± 0.07[Ae]	0.00 ± 0.00[Bf]
Vitexin (mg/100g)							
25	5.74 ± 0.20[a]	3.60 ± 0.09[Bb]	3.10 ± 0.26[Bc]	2.85 ± 0.27[Bd]	2.68 ± 0.10[Ad]	1.87 ± 0.04[Ce]	1.64 ± 0.13[Ae]
35	5.74 ± 0.20[a]	3.62 ± 0.12[Bb]	3.57 ± 0.10[Bb]	2.62 ± 0.05[Bc]	2.50 ± 0.05[Ac]	2.13 ± 0.15[Bd]	1.33 ± 0.01[ABe]
45	5.74 ± 0.20[a]	5.54 ± 0.03[Aab]	5.34 ± 0.03[Ab]	4.05 ± 0.37[Ac]	2.84 ± 0.14[Ad]	2.71 ± 0.12[Ad]	1.27 ± 0.01[Be]
Isovitexin (mg/100g)							
	0	2	4	6	8	10	12
25	14.36 ± 0.11[a]	12.93 ± 0.16[Bb]	11.96 ± 0.31[Ac]	11.94 ± 1.08[Ac]	11.10 ± 0.64[Ac]	4.42 ± 0.44[Bd]	4.38 ± 0.70[Ae]
35	14.36 ± 0.11[a]	14.33 ± 0.46[Ab]	9.51 ± 0.70[Bc]	8.53 ± 0.47[Cd]	6.53 ± 0.42[Ce]	3.83 ± 0.14[Cf]	3.63 ± 0.06[Bf]
45	14.36 ± 0.11[a]	12.92 ± 0.44[Bb]	11.62 ± 0.42[Ac]	10.86 ± 0.94[Bd]	10.86 ± 0.57[Bd]	9.09 ± 0.36[Ae]	4.26 ± 0.19[Af]
Epicatechin (mg/100g)							
25	98.44 ± 3.75[a]	92.25 ± 0.97[Ab]	86.61 ± 4.49[Ac]	83.83 ± 3.43[Ad]	82.46 ± 4.91[Ae]	71.18 ± 0.31[Af]	67.63 ± 0.35[Ag]
35	98.44 ± 3.75[a]	74.78 ± 0.29[Bb]	72.00 ± 1.26[Bc]	70.80 ± 0.51[Bd]	70.40 ± 0.83[Bd]	67.00 ± 0.79[Be]	64.74 ± 1.97[Bf]
45	98.44 ± 3.75[a]	73.31 ± 1.03[Cb]	68.70 ± 2.71[Cc]	66.97 ± 0.25[Cd]	64.32 ± 0.25[Ce]	60.12 ± 0.26[Cf]	56.07 ± 2.17[Cg]
Vitamin C (mg/100g)							
25	21.97 ± 0.37[a]	21.13 ± 0.09[Aa]	20.47 ± 0.07[Ab]	19.80 ± 0.07[Ac]	18.69 ± 0.11[Ad]	18.45 ± 0.05[Ad]	18.25 ± 0.02[Ad]
35	21.97 ± 0.37[a]	20.71 ± 0.04[Bb]	20.49 ± 0.05[Ab]	19.34 ± 0.22[Ac]	18.60 ± 0.05[Ad]	18.48 ± 0.25[Ad]	18.15 ± 0.02[Ad]
45	21.97 ± 0.37[a]	20.61 ± 0.08[Bb]	20.29 ± 0.04[Ab]	19.02 ± 0.17[Ac]	18.50 ± 0.03[Ad]	18.32 ± 0.03[Ad]	17.86 ± 0.25[Be]
FRAP (μmol equi. Trolox/100g)							
25	202.63 ± 16.33[a]	180.97 ± 2.51[Ab]	155.97 ± 3.49[Ac]	140.97 ± 1.87[Ad]	129.58 ± 1.10[Ae]	119.86 ± 1.87[Af]	107.77 ± 1.57[Ag]
35	202.63 ± 16.33[a]	171.66 ± 3.75[Bb]	149.72 ± 2.51[Bc]	136.94 ± 0.63[Bd]	127.08 ± 0.41[Be]	116.94 ± 0.48[Bf]	105.00 ± 0.41[Bg]
45	202.63 ± 16.33[a]	164.44 ± 1.20[Cb]	145.69 ± 1.73[Cc]	132.50 ± 1.81[Cd]	123.75 ± 0.83[Ce]	112.22 ± 2.83[Cf]	99.44 ± 3.75[Cg]
ORAC (μmol equi. Trolox/100g)							
25	1165.12 ± 47.89[a]	1075.08 ± 64.68[Ab]	1057.65 ± 43.90[Ab]	1053.34 ± 31.12[Ab]	986.17 ± 19.61[Ac]	812.13 ± 16.39[Ad]	626.88 ± 39.89[Ae]
35	1165.12 ± 47.89[a]	837.52 ± 15.68[Cb]	806.70 ± 66.60[Bbc]	797.02 ± 18.86[Bc]	780.17 ± 35.23[Bc]	599.80 ± 7.16[Bd]	504.10 ± 41.88[Be]
45	1165.12 ± 47.89[a]	961.22 ± 74.35[Bb]	775.25 ± 46.91[Cc]	733.34 ± 47.39[Cc]	685.19 ± 64.68[Cd]	609.94 ± 36.96[Be]	432.18 ± 10.43[Cf]

** Different capital letters in the vertical indicate a statistical difference ($p < 0.001$) between the fresh and pasteurized treatments, at temperatures 25 °C, 35 °C and 45 °C. Different lower-case letters in each horizontal row indicate a statistical difference ($p < 0.001$) in the vitexin content of the same treatment in relation to the shelf-life days. P-value according to Tukey's test ($p < 0.001$)..

pasteurized pulp met the standard established by Brazilian legislation, when stored at temperatures of 25, 35 and 45 °C, for up to 12 days (Brasil, 2019b) as well as the estimated counts of total coliforms and thermotolerant coliforms, which were below 3 MPN/g of pulp (estimated data) (Table 2).

Regarding microbiological quality, despite the absence of *Salmonella* spp. and insignificant counts of total and thermotolerant coliforms, the maximum storage period for pasteurized pulp of *P. setacea*, suitable for all temperatures adopted in the present study, is eight days. Time greater than eight days implies a higher mold and yeast count than that allowed by Brazilian legislation, which is 3.30 log CFU/g for heat-treated fruit pulp (Brasil, 2018).

3.2. Flavonoid profile, vitamin C content and antioxidant activity of pasteurized P. setacea pulp submitted to accelerated tests

There was a significant reduction in the flavonoid profile ($p < 0.05$) of the different compounds during storage, at different temperatures (Table 3). As expected, as the temperature increased, the reduction in the levels of isoorientin, orientin and epicatechin became more intense. However, the temperature of 45 °C presented a higher retention of vitexin for up to 10 days, whereas a more marked reduction in the content of isovitexin was observed at a temperature of 35 °C, during 12 days of storage. Flavonoids are based on a fifteen-carbon compound consisting of two benzene rings linked through a heterocyclic pyran ring (Kumar & Pandey, 2013). Flavonoid classes differ in the oxidation level and C-ring substitution pattern. Flavonoid content can be determined by the conversion or release of glycosidic or phenolic compounds when subjected to temperature changes, which produces the increase or decrease in the content of flavonoids (Sharma et al., 2015). Depending on the replacement in the carbon ring, there will be an isomer of the main structure, making a flavonoid more sensitive or resistant to the effect of temperature. It is important to note that the presence of orientin was not detected in the pasteurized pulp after 12 days of storage, at temperatures of 35 and 45 °C. Thus, orientin stood out as the most sensitive compound during storage at temperatures equal to or greater than 35 °C.

There was a significant reduction in the vitamin C content ($p < 0.05$) as the storage time increased, at different temperatures (Table 3). A significant difference in vitamin C content with the increase of temperature was found only on the second and twelfth days. Reductions in vitamin C levels after 12 days of storage remained between 16.93% (25 °C) and 18.87% (45 °C). It is noteworthy that the reductions in the levels of vitamin C were lower than those observed in the flavonoid profile.

Hoffmann et al. (2017) found similar results regarding the stability of bioactive compounds in the pulp and nectar of *Butia odorata* submitted to pasteurization (100 °C/15 min) and subsequently stored at −18 °C for 3 months. There were reductions in the levels of phenolic compounds and flavonoids, but there was no significant reduction in the vitamin C content during storage. On the other hand, Tembo, Holmes, and Marshall (2017) evaluated the vitamin C content in pasteurized *Adansonia digitata* pulp stored at 30 °C and observed a 93.3% reduction after 60 days of storage.

Regarding antioxidant activity by FRAP and ORAC methods, in general, a significant reduction ($p < 0.05$) was observed during storage, with this trend being more intense at 45 °C (Table 3). In the case of antioxidant activity by ORAC, reduction percentages were obtained varying between 46.20% (25 °C) and 62.91% (45 °C), after 12 days of storage. For the antioxidant activity by FRAP, there was not such a significant difference when comparing the results obtained at different temperatures, after 12 days of storage. The percentages of reduction in antioxidant activity by FRAP remained between 46.81% (25 °C) and 50.93% (45 °C).

Similar results to the present study were observed by Touati, Barba, Louaileche, Frigola, and Esteve (2016), who found a significant reduction in antioxidant activity by ORAC in pear and grape nectars, thermally processed, after three days of storage, at 25 and 37 °C. According to these authors, the reductions in antioxidant activity were 11% and 23% at temperatures of 25 °C and 37 °C, respectively, for pear nectar. In the case of grape nectar, the reductions in antioxidant activity were 29% and 35% at temperatures of 25 °C and 37 °C, respectively. Tembo et al. (2017) found reductions in antioxidant activity by FRAP in pasteurized pulps (72 °C/15 s) of baoba (*Adansonia digitata*) and stored for 42 days, at 15 °C (34%) and 30 °C (19%). On the other hand, Londoño, Ramos, Alzate, Rojano, and Celis (2019) did not observe a significant reduction in antioxidant activity by ORAC in pasteurized mango juice (85 °C/10 min) stored at 4 °C, for up to 64 days.

Table 4 shows the global acceptance data for pasteurized *P. setacea* pulp, stored at temperatures of 25, 35 and 45 °C, for 6 days. It is noteworthy that the maximum storage time considered for sensory analysis was defined taking into account the results obtained in the microbiological analyses (Table 2), in such a way that the tasters were provided with a safe product. In addition, a change in the color of the product was visually observed for storage times longer than 6 days, especially at a temperature of 45 °C. Thus, samples referring to storage times greater than 6 days were considered unsuitable for use in sensory analysis. In this context, although a slight downward trend was observed in the scores attributed by the tasters for global acceptance, as the temperature and storage time increased, the results of the analysis of variance did not indicate a significant difference. On the other hand, Buvéa et al. (2018) observed a significant difference when they submitted to sensory analysis pasteurized strawberry juices (95 °C/2 min) stored at 20 °C, for up to 32 weeks. For sensory analysis, these authors considered the aroma and obtained significant variation when comparing samples that were not stored with those stored for a week or longer.

3.3. Estimated shelf life of P. setacea pasteurized pulp using accelerated tests

The adjusted equations and the respective coefficients of determination of the zero, first and second order kinetic models, referring to the different qualitative variables of the pasteurized pulp, are presented in Tables 1S–9S of the Supplementary Material. In general, the first order kinetic model presented an adequate adjustment, presenting equations with the highest or the second highest determination coefficients. According to Zhi et al. (2017), generally the zero order or first order kinetic models have been used to evaluate qualitative changes in food during processing and storage, with the first order kinetic model being the most widely used. Thus, we decided to use the k values (constant of the reaction rate) of the first order kinetic model to obtain the activation energy (E_a) of the qualitative variables of the pasteurized pulp.

Table 5 shows the values of E_a (activation energy) obtained for the three groups of qualitative variables in the pasteurized pulp submitted to accelerated tests. Group I was formed by flavonoids and vitamin C contents. However, for group I, E_a values were obtained only for isoorientin, vitexin and epicatechin. Orientin and isovitexin compounds

Table 4
Global acceptance of pasteurized *Passiflora setacea* pulp subjected to accelerated tests at temperatures of 25, 35 and 45 °C, for 12 days.

Storage time (days)	Temperature (°C)			p-value
	25	35	45	
0	6.4 ± 1.75	6.30 ± 1.84	6.40 ± 1.50	0.133[b]
2	6.47 ± 1.99	5.05 ± 2.24	6.23 ± 2.09	
4	6.03 ± 1.86	5.87 ± 2.35	6.00 ± 1.98	
6	6.10 ± 2.00	5.60 ± 2.34	5.43 ± 1.85	
p-value	0.398[a]			0.968[c]

[a] Temperature source of variation.
[b] Time source of variation.
[c] Time * temperature source of variation in ANOVA.

Table 5
Estimated activation energy (E_a, cal/mol) of groups of variables (I, II and III) for the pasteurized *Passiflora setacea* pulp submitted to accelerated tests.

Group	Variables	E_a (cal/mol)
I	Isoorientin (mg/100g)	5508
	Vitexin (mg/100g)	2199
	Epicatechin (mg/100g)	2245
II	ORAC (μmol equi. Trolox/100g)	4484
III	Acceptance	8643

Group I - referring to flavonoids and vitamin C; Group II - referring to antioxidant activity; and Group III - referring to sensory analysis (acceptance).

did not have E_a values calculated, since there was not a continuous increase in the k module (constant of the reaction rate) as the temperature increased from 25 to 45 °C, considering the first-order kinetic model. For these two compounds, the highest k value (in the module) was obtained at 35 °C. For vitamin C, there was no significant difference in k values with increasing temperature, with k values (in the module) for the first order kinetic model ranging between 0.0170 and 0.0172. For group I, the highest E_a value was 5508.36 mol mol^{-1}, obtained when isoorientin was analyzed. On the other hand, the lowest E_a value was calculated for vitexin, being equivalent to 2198.81 mol mol^{-1}. It is noteworthy that E_a is defined as the amount of energy required for a given reaction, and the lower its value, the faster the reaction (Calixto, 2013; Jafari, Ganje, Dehnad, Ghanbari, & Hajitabar, 2017).

As for the variables in group II (antioxidant activity), E_a was calculated only for ORAC, with a value equivalent to 4483.86 mol^{-1} cal (Table 5). The k values of FRAP antioxidant activity were relatively close at the temperatures of 25 and 35 °C, which prevented the correct adjustment of these data to the Arrhenius model. For this variable, the k-module values for the first order kinetic model were equal to 0.0518 and 0.0519, for 25 and 35 °C, respectively. The E_a value of group III concerning sensory evaluation or acceptance was equal to 8642.85 cal mol^{-1}, the highest achieved among all the quality parameters.

Table 6
Estimated shelf life of pasteurized *Passiflora setacea* pulp at different temperatures.

Group	Variable	Adjusted equations[a]	R^2	Q_{10}	Temperature (°C)	Shelf life (days)
I	Isoorientin	$\hat{y} = -0.1273x + 4.1399$	0,97	–	45	5.6
				1.33	35	7.4
				1.35	25	10.0
				1.45	5	19.51[b]
				1.49	−15	42.2[b]
	Vitexin	$\hat{y} = -0.1176x + 1.9648$	0,86	–	45	8.2
				1.12	35	9.1
				1.13	25	10.3
				1.16	5	13.4[b]
				1.17	−15	18.3[b]
	Epicatechin	$\hat{y} = -0.0384x + 4.4598$	0,84	–	45	14.7
				1.12	35	16.5
				1.13	25	18.6
				1.16	5	24.5[b]
				1.18	−15	33.5[b]
II	ORAC	$\hat{y} = -0.0716x + 7.0283$	0,94	–	45	9.2
				1.33	35	12.3
				1.35	25	16.6
				1.45	5	32.3[b]
				1.49	−15	70.0[b]
III	Acceptance	$\hat{y} = -0.0265 + 1.8720x$	0,91	–	45	9.9
				1.50	35	14.9
				1.55	25	23.0
				1,71	5	60.50[b]
				1.78	−15	184.5[b]

[a] For the first order kinetic model, considering ln (C), with C being the value obtained for the analyzed variable.
[b] Values estimated by extrapolation.

Table 6 shows the times for the shelf life of the *P. setacea* pulp, considering the different groups of variables, at temperatures of 25, 35 and 45 °C, in addition to the temperatures of 5 °C and −15 °C (obtained by extrapolation). The temperature of −15 °C was included as a previous insight, since it is a market condition applied in Brazil for fruit pulps and widely used. For isoorientine, shelf-life values were estimated, for the interval between 5.6 days (45 °C) and 10 days (25 °C) whereas epicatechin showed longer shelf life, with variation between 14.7 days (45 °C) and 18.6 days (25 °C). For 5 °C, shelf-life values of 24.5 and 13.4 days were estimated for epicatechin and vitexin, respectively.

When the shelf life was analyzed at −15 °C, the lowest value of 18.3 days was obtained for vitexin and the longest for isoorientine, of 42.2 days. This is much lower than the current shelf-life practiced for fruit pulps in Brazil, from 4 months (actual life of the product in retail, from distribution to consumer) to one year (usual label shelf life, personal information from retail sector).

Considering the antioxidant activity by ORAC, shelf-life varied from 9.2 days (45 °C) to 16.6 (25 °C), and values at 5 and -15 °C of storage were estimated as 32.3 and 70 days of storage, respectively. The highest shelf-life was obtained for acceptance variable ranging from 9.9 days (45 °C) to 23 days (25 °C) and estimations of 60.5 and 184.5 days at 5 °C and −15 °C, or approximately two months in refrigeration and six months in frozen storage.

Still referring to the shelf lives shown in Table 6, it is important to compare the values obtained with those referring to microbiological analysis. Considering the mold and yeast count (Table 2), the pasteurized *P. setacea* pulp met the standard established by Brazilian legislation (Brasil, 2018) for at least 12 days at 25 °C, and for 8 and 10 days, at temperatures 35 and 45 °C respectively. At 25 °C, the estimated shelf life, considering the mold and yeast count, was higher than those obtained when isoorientine (10.0 days) and vitexin (10.3 days) were considered. At 35 °C, the shelf lives for epicatechin (16.5 days), antioxidant activity by ORAC (12.3 days) and acceptance (14.9 days) were higher than that obtained from the mold and yeast count (8 days). Leizerson and Shimon (2005) also used different variables to estimate the shelf life of pasteurized orange juice (90 °C/50 s) stored at 4 °C. These authors used microbiological criteria, concentration of vitamin C and sensory analysis. Differently from the results obtained in the present study, Leizerson and Shimon (2005) observed that the sensory analysis implied shorter shelf life (50 days), while for the microbiological quality and the concentration of vitamin C, shelf-lives equal to 105 and 79 days were estimated, respectively.

The main focus of our study is the evaluation of the retention of bioactive compounds during the storage of *P. setacea* pulp that could support an eventual functional and/or health claim associated with the content of the phenolic compounds evaluated. Evidently, the complexity of establishing shelf-life makes microbiological and sensory analysis essential as quality criteria. As raised by Wibowo, Buvé, Hendrickx, Loey, and Grauwet (2018), the large number of variables analyzed with different natures could demand the application of statistical tools for multivariate analysis, with Principal Component Analysis or PLS Regression. However, in our study it was observed that the samples from a sensory point of view did not show significant changes. A PLS regression using sensory data as dependent variables and the other variables as independent would contribute little to the main focus of the study, which would be to assess, under satisfactory sensory conditions, which pulp shelf life could ensure a claim to a functional property on the label of the product associated with the retention of the bioactive compounds studied here. A limitation of our study is the extrapolation of temperature to freezing conditions. Future studies could start from this initial insight by adjusting the temperature ranges to estimate freezing. Like any kinetic model, the estimated condition must also be actually tested for a more accurate shelf-life. However, these drawbacks do not limit the relevance of the results presented, since this is an unprecedented study on a fruit from Brazilian biodiversity with great functional

potential, already used in popular medicine and whose consumption can be boosted by a genetic improvement program and the launch of the cultivar BRS Pérola do Cerrado, leading to the need for processing and storage, as it is a seasonal fruit.

4. Conclusions

According to the results obtained, it is possible to conclude that: i) the first order kinetic model adequately described the different qualitative variables of the pasteurized pulp of *P. setacea* submitted to accelerated tests during storage; ii) the lowest activation energy values were obtained for vitexin and epicatechin, variables belonging to the groups of flavonoids, indicating that these compounds are more affected by temperature than the others; iii) the shortest estimated shelf life values for pasteurized pulp of P. *setacea* stored at 5 and -15 °C were 13.4 and 18 days, respectively, when the variable vitexin was considered, while the longest shelf life values were 60.5 and 184 days, for sensory analysis. For a functional and/or health claim, more studies are necessary to increase the retention of the bioactive compounds and extend the shelf-life as a functional food under refrigerated and freezing conditions.

Author declaration

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

We further confirm that any aspect of the work covered in this manuscript that has involved either experimental animals or human patients has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

We understand that the Corresponding Author is the sole contact for the Editorial process (including Editorial Manager and direct communications with the office). She is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs. We confirm that we have provided a current, correct email address which is accessible by the Corresponding Author.

CRediT authorship contribution statement

Lívia de Lacerda de Oliveira: Supervision, Conceptualization, Investigation, Methodology, Formal analysis, Validation, Writing – original draft, Writing – review & editing. **Beatriz Alejandra Ortega Sanchez:** Conceptualization, Investigation, Methodology, Formal analysis, Writing. **Isadora Costa Celestino:** Formal analysis, Investigation. **Sônia Maria Costa Celestino:** Conceptualization, Methodology, Formal analysis. **Ernandes Rodrigues de Alencar:** Formal analysis, Validation, Writing – original draft, Writing – review & editing. **Ana Maria Costa:** Supervision, Conceptualization, Methodology, Investigation.

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Appendix A. Supplementary data

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