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# Physicochemical characterization, bioactive compounds, in vitro antioxidant activity, sensory profile and consumer acceptability of fermented alcoholic beverage obtained from Caatinga passion fruit (*Passiflora cincinnata* Mast.)

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## ABSTRACT

The aim of this research was to evaluate the quality of fermented alcoholic beverage from passion fruit produced using passion fruit species obtained from the Brazilian Caatinga biome (*Passiflora cincinnata* Mast.). Four treatments were elaborated using fruit pulp in two different ripening stages (unripe and ripe), with and without dilution of the pulp. The following analyses were conducted to characterize the passion fruit wines: pH, density, soluble solids, titratable and volatile acidity, sulfur dioxide, dry extract, alcohol content, total sugar, color parameters (L\*, a\*, b\*, C\* and h) and bioactive compounds (ascorbic acid and phenolic compounds). The antioxidant potential as determined in vitro by DPPH, FRAP and ORAC assays. The overall acceptance and purchase intention of the beverages were evaluated using a consumer test. The physicochemical compositions of the samples were different, except for volatile acidity and ascorbic acid. The total phenolic compounds content was considered relevant, >700 mg GAE L<sup>-1</sup>. Twenty-one phenolic compounds, among phenolic acids, flavonols, flavanols and stilbenes were identified and quantified by HPLC-DAD-FD. The sensory score obtained was considered satisfactory. This study showed the feasibility of using the Caatinga passion fruit to produce fermented alcoholic beverage, which could be inserted on the market as a new product.

## 1. Introduction

The passion fruit, genus *Passiflora*, belongs to the family *Passifloraceae*, and includes approximately 530 tropical and subtropical species, of which 150 to 200 native species are found in Brazil and 70 of these produce edible fruits (Carvalho et al., 2017).

Brazil is the main world producer and consumer of passion fruit, and stands out amongst the major producers of the concentrated juice, the yellow passion fruit, cultivated throughout almost all the national territory, being responsible for more than 90% of the national production (CEPLAC, 2019). Nevertheless, other species present promising sensory and nutritional characteristics, and could be more explored for consumption, amongst which the wild Brazilian species Caatinga stands out,

as *Passiflora cincinnata* Mast. (Braga et al., 2006).

The Caatinga passion fruit occurs frequently and spontaneously in the Caatinga (states of Pernambuco and Bahia) and Cerrado (states of Minas Gerais and Goiás) Brazilian biomes, but is more frequent in the Caatinga. The Caatinga passion fruit can also be found in other South American countries such as Argentina, Bolivia, Paraguay, Colombia and Venezuela. It represents a wild species of passion fruit, producing a sour fruit and showing resistance to drought and to a series of diseases and/or pests that strike the common passion fruit (Araújo et al., 2016, 2018; Oliveira Junior et al., 2010).

When ripe the Caatinga passion fruit has a greenish-yellow skin, whitish-yellow to whitish pulp, soluble solids contents in the range from 8 to 13°Brix, about 88% moisture content, pH value of approximately

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3.0 and acidity (from 2.0 to 3.0%). The fruit is extremely aromatic, showing exotic aroma and flavor and good nutritional value, being a source of potassium, iron, phosphorus and calcium and of the vitamins A, C (approximately 10 mg 100 g<sup>-1</sup>) and the B complex (Aidar et al., 2016; Araújo et al., 2019a).

Unfortunately, official data on the production and marketing of Caatinga passion fruit is still scarce in Brazil. Currently, the production of Caatinga passion fruit is being carried out by cooperatives of family farmers and traditional communities, who work in different segments of the productive chain: use of the plant for ornamental purposes; seed and seedling production; commercialization of the *in natura* fruit; and agro-industrial processing for gastronomical use in the food and cosmetic industries. There are approximately 24 family agro-industries just in the State of Bahia which process Caatinga passion fruit producing pulps, jams, sweetmeats, ice creams and liqueurs. The mean productivity of the Caatinga passion fruit is 17 tons per hectare/crop, and can reach over 30 tons per hectare. On the other hand, the mean yield in pulp from the fruit is 50% when extracted using a rotary pulping machine, allowing for the production of at least 7.5 thousand liters of fermented alcoholic beverage per hectare/crop, but this type of beverage can be further diluted with water, providing an even higher product yield (Araújo et al., 2018; Araújo et al., 2016; Jesus & Faleiro, 2016). However, its use to produce other alcoholic beverages of greater value such as fermented of passion fruit is still commercially unknown.

Thus the aim of the present study was to evaluate the feasibility of elaborating fermented alcoholic beverage from Caatinga passion fruit, determining its physicochemical quality, composition with respect to bioactive compounds and antioxidant activity and its consumer acceptance as a beverage.

## 2. Material and methods

### 2.1. Passion fruit sampling

Caatinga passion fruits (180 kg) in an intermediate ripeness stage were acquired on the local farmer in the Sub-middle San Francisco Valley (Petrolina, Pernambuco State, Brazil), being selected considering a greenish, firm skin, no squashed areas or skin cuts and the apparent absence of microbial contamination. Part of the fruits were sanitized and pulped in the intermediate ripeness stage and the rest were maintained at 26±2 °C until completely ripe (greenish-yellow skin color). The fruits were prepared by first washing under running water and then immersing in a 200 mg L<sup>-1</sup> sodium hypochlorite solution for 15 min, before rinsing under running water. The pulp and seeds were then removed and the fruits pulped in an electric pulping machine with a size 10 mesh sieve (Macanuda, model DMJI-05, Brazil). The yield in pulp from the Caatinga passion fruit was about 40% (to fruits in the intermediate ripeness stage) and 56% (to fruits in the mature ripeness stage).

### 2.2. Physicochemical characterization of the fruit pulp

The fruit pulp was characterized in triplicate analysis, as: density - direct reading of the samples using an electronic hydrostatic balance (Gibertini, model Super Alcomat); pH - direct reading of the samples using a pH meter (Hanna Instruments, model HI 2221); soluble solids - direct reading using a portable refractometer (Atago, Pocket Refractometer model PAL-3); titratable acidity - titration with 0.1M NaOH in an automatic titrator (Metrohm, model Tritino Plus 848, Switzerland); reducing sugars - by titration with Fehling reagents A and B (AOAC, 2012).

### 2.3. Elaboration of the passion fruit fermented alcoholic beverage

Four treatments were considered as from *in natura* pulp and *in natura* pulp diluted with 20% water based on the weight of the pulp and two ripeness stages (unripe and ripe): T1 = *in natura* pulp, unripe fruit; T2 =

*in natura* pulp, ripe fruit; T3 = diluted fruit pulp, unripe fruit; T4 = diluted fruit pulp, ripe fruit. The elaboration of the four treatments was carried out in three repetitions.

The must (Caatinga passion fruit pulp with or without dilution – 8 kg) was prepared in microvinification glass carboy (10L) with airlock S-shaped valve. Potassium metabisulfite (0.1 g L<sup>-1</sup>) (AMAZON, Brazil) and a pectinolytic enzyme (2 mL 100 L<sup>-1</sup>) (AEB, Spain) were added and the jars maintained at 16±2 °C for 24h. Bentonite (0.5 g L<sup>-1</sup>) (AMAZON, Brazil) was then added to promote clarification while incubated at 5±2 °C for 24h. At the end of the *debouillage* (clarification) step, the musts were racked before starting the alcoholic fermentation. Chaptalization, aiming at a final alcohol content of 8% v/v, was effected by adding sucrose in the form of crystal sugar (104.50 g L<sup>-1</sup> was added in T1, 99.70 g L<sup>-1</sup> in T2, 104.70 g L<sup>-1</sup> in T3 and 100 g L<sup>-1</sup> in T4). Alcoholic fermentation occurred at 24±2 °C after adding the yeast *Saccharomyces cerevisiae* var. bayanus Maurivin PDM (400 mg L<sup>-1</sup>) (MAURIVIN, Australia) and ammonium phosphate (200 mg L<sup>-1</sup>) (Gesferm®, AMAZON, Brazil) as activator.

The fermentative process took six days (144 h) for the treatments elaborated with fruit pulp processed in the intermediary state of ripeness (T1 and T3) and four days (96 h) for the treatments elaborated with fruit pulp processed in the fully ripe state (T2 and T4). The S-shaped airlock valve was used throughout the fermentative period, and open reassembling carried out twice a day (removing the valve and replacing 40% of the volume of the jar 5 times). Throughout the whole fermentative period the density was measured daily using a hydrostatic electronic scale (Gibertini, Super Alcomat model), as also the soluble solids content by direct reading using a portable refractometer (Atago, Pocket refractometer model PAL-3), and fermentation was considered to have finished when the readings of these parameters remained constant for three consecutive days. Having verified the end of fermentation, the alcohol content was determined by densitometry and the direct reading of the samples on a hydrostatic electronic scale (Gibertini, Super Alcomat model) after distillation of the sample using an oenological distiller (Gibertini, Super DEE model).

When fermentation stopped the liquid was transferred to another glass carboy (first racking) and the free SO<sub>2</sub> content corrected to 50 mg L<sup>-1</sup> using potassium metabisulfite (AMAZON, Brazil) prior to clarification and protein stabilization at 2±1 °C for 25 days. Fining agents were added as follows: first day – bentonite (1400 mg L<sup>-1</sup>) (AMAZON, Brazil); twenty-first day – silica (500 mg L<sup>-1</sup>) (EVER, Brazil); and the 20-s day – gelatin (100 mg L<sup>-1</sup>) (EVER, Brazil). After this stage, the second racking was carried out, followed by correction of the free SO<sub>2</sub> content to 50 mg L<sup>-1</sup>, the addition of sucrose to a final content of 90 g L<sup>-1</sup> in the fermented beverage, and the addition of the preservative potassium sorbate (200 mg L<sup>-1</sup>) (ALPHATEC, Brazil). The fermented alcoholic beverage was then transferred to 750 mL olive green glass bottles, a nitrogen gas flush used to fill the headspace, the bottles closed with cork stoppers and stored in a horizontal position in an air-conditioned wine cellar (16±2 °C).

### 2.4. Physicochemical characterization of the passion fruit fermented alcoholic beverage

The physicochemical characterization of the alcoholic beverages was carried out in triplicate according to the methodologies proposed by AOAC (2012) and OIV (2019), determining the following parameters: pH, from the direct reading of the samples using a pH meter (Hanna Instruments, model HI 2221); density, from the direct reading of the samples using a hydrostatic electronic scale (Gibertini, model Super Alcomat, Italy); titratable acidity, by titration with 0.1M NaOH using an automatic titrator (Metrohm, model Tritino Plus 848, Switzerland); volatile acidity, by titration with 0.1M NaOH and phenolphthalein as the indicator after distillation of the sample using an oenological distiller (Gibertini, model Super DEE, Italy); free and total sulfur dioxide (SO<sub>2</sub>) using the Ripper titrimetric method with 0.02 N iodine solution and a

starch indicator; and dry extract and alcohol content, by densimetry and the direct reading of the samples on a hydrostatic electronic scale (Gibertine, model Super Alcomat) after distillation of the sample using an oenological distiller (Gibertini, model Super DEE, Italy).

## 2.5. Colorimetric evaluation

The colorimetric parameters ( $L^*$ : luminosity [white (0) to black (100)],  $a^*$ : red/green coordinate [red (+) to green (-)],  $b^*$ : yellow/blue coordinate [yellow (+) to blue (-)],  $C^*$ : chromaticity or saturation, and the  $h$  angle: hue) were determined using the CIELAB and CIEL $^*C^*h$  systems, as measured by the Delta Vista portable spectrophotometer (Delta Color, model Delta Vista 450G).

## 2.6. Determination of the ascorbic acid content (vitamin C)

The ascorbic acid content was determined by titrating a sample extract (in an oxalic acid solution) using Tilman's solution (2,6-dichlorophenol-indophenol) (Strohecker & Henning, 1967).

## 2.7. Determination of the total phenolic compounds content (TPC)

The TPC was determined using a colorimetric analysis with a saturated solution of sodium carbonate and the Folin-Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO, USA), as described by Singleton & Rossi (1965), with modifications. The absorbance (760 nm) was measured using a spectrophotometer (Multiskan GO, Thermo Scientific, Waltham, MA, USA), and the reading compared with a calibration curve ( $R^2 = 0.994$ ) of gallic acid – GAE (purity > 97%, Sigma-Aldrich, St. Louis, MO, USA).

## 2.8. Determination of phenolic compounds by HPLC-DAD-FD

The methodologies proposed by Natividade et al. (2013) and Costa et al. (2020) validated under the same analytical conditions, were used as follows: twenty-two phenolic compounds were quantified one by one by high performance liquid chromatography – HPLC – using a Waters model Alliance e2695 chromatograph equipped with a Gemini-NX C18 column (150 mm  $\times$  4.60 mm  $\times$  3  $\mu$ m) with a Gemini-NX C18 pre-column (4.0 mm  $\times$  3.0 mm), both from Phenomenex® (USA), and simultaneously coupled to diode array – DAD (280, 320 and 360 nm) and fluorescence - FD (excitation at 280 nm and emission at 360 nm) detectors. Using gradient elution, the mobile phase was constituted of 0.85% ortho-phosphoric acid (Sigma-Aldrich, St. Louis, MO, USA) as phase A and acetonitrile (HPLC grade, J. T. Backer, Madrid, Spain) as phase B. The gradient process was started at time 0 min with 100% of solvent A and adjusted for 93% of solvent A and 7% of solvent B in 10 min; 90% of solvent A and 10% of solvent B in 20 min; 88% of solvent A and 12% of solvent B in 30 min; 77% of solvent A and 33% of solvent B in 40 min; 65% of solvent A and 35% of solvent B in 45 min; and 100% of solvent B in 55 min with a total run time of 60 min. The oven temperature was maintained at 40 °C and the flow at 0.5 mL min<sup>-1</sup>. Twenty microliters of each sample were automatically injected by the equipment. The samples were injected after filtration through a 13 mm diameter nylon membrane with a pore size of 0.45  $\mu$ m (Analítica, SP, Brazil), without prior preparation. The results of validation parameters are shown in Table S1.

The gallic and caffeic acid standards were acquired from Chem Service (West Chester, USA), *trans*-caftaric, chlorogenic and *p*-coumaric acids and *cis*-resveratrol from Sigma-Aldrich (St. Louis, MO, USA), and the remaining phenolic compounds (ferulic acid, kaempferol-3-O-glucoside, quercetin-3- $\beta$ -D-glucoside, isorhamnetin-3-O-glucoside, myricetin, rutin, (+)-catechin, (-)-epicatechin, (-)-epicatechin gallate, (-)-Jepigallocatechin gallate, procyanidin A2, B1 and B2, *trans*-resveratrol, piceatannol and viniferin) from Extrasynthese (Geney, France). Ultrapure water was employed, purified in a PURELAB Option Analítica

water ultra-purifier (São Paulo, SP).

## 2.9. Antioxidant activity

Different methods were used to evaluate the antioxidant activity of the samples.

The FRAP (Ferric Reducing Antioxidant Power) assay was carried out using the method described by Rufino et al. (2006), with modifications described by Oliveira et al. (2020). The FRAP reagent was prepared from 0.3M acetate buffer (pH 3.6) in 10 mM TPTZ [2,4,6-tris (2-pyridyl)-s-triazine] (Sigma-Aldrich, Milan, Italy) diluted in 40 mM HCl (ALPHATEC, Brazil) and a 20 mM ferric chloride solution (Anidrol, Brazil). A 20  $\mu$ L aliquot of the sample was added to the flask followed by 180  $\mu$ L of the FRAP reagent, the mixture stirred, incubated at  $37 \pm 2$  °C for 30 min, and the absorbance (620 nm) obtained using a microplate reader (Fluor star Omega model, BMG LABTEC, Germany).

The DPPH (2,2'-diphenyl-1-picrylhydrazyl) radical capture assay was carried out using the method described by Brand-Williams et al. (1995), with the modifications described by Rufino et al. (2009). A 180  $\mu$ L aliquot of an 80  $\mu$ M DPPH (Sigma-Aldrich, Hamburg, Germany) solution was added to 20  $\mu$ L of sample, and the absorbance read at 515 nm after 30 min using a microplate reader (Fluor star Omega model, BMG LABTEC, Germany).

The antioxidant ORAC (Oxygen Radical Absorbance Capacity) assay was carried out according to the method described by Davalos et al. (2004), with modifications described by Oliveira et al. (2020). An 20  $\mu$ L aliquot of the sample was added to 120  $\mu$ L fluorescein solution (Synth, Brazil), to which 60  $\mu$ L of 178 nM AAPH [dicloreto de 2,2'-azobis (2-amidinopropano)] (Sigma-Aldrich, St. Louis, MO, USA) solution was added to start the reaction. The fluorescence intensity was measured kinetically every 1 min (excitation: 285 nm, emission: 520 nm) using a microplate reader (Fluor star Omega model, BMG LABTEC, Germany). By the three assays the calibration curves was prepared with Trolox-TE (hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (Sigma-Aldrich, Hamburg, Germany) and the results expressed as  $\mu$ mol TE L<sup>-1</sup>.

## 2.10. Sensory analysis

The research was submitted to the Ethics in Research Committee of the Federal University of Vale do São Francisco - Brazil (CAAE 4417021.5.0000.5189). Prior to the test, the consumers signed a Free and Clarified Term of Consent. Sixty volunteers (33 women and 27 men, with ages ranging from 21 to 62 years old), not trained, were recruited, and the acceptance test carried out in individual booths under white light, with the temperature controlled at  $23 \pm 1$  °C in the Sensory Analysis Laboratory of Embrapa (Petrolina, PE, Brazil). The samples of passion fruit fermented alcoholic beverage were presented in a monadic form in a single session at a temperature of  $10 \pm 2$  °C, in ISO wine glasses containing 30 mL of sample, coded with three digits. In order to avoid tiredness and sensory fatigue, water and unsalted water biscuits were served to the consumers among the samples evaluations. The samples were served to the participants in balanced order of presentation following the complete balanced block design suggested by Macfie et al. (1989) to four products. All the consumers evaluated the overall acceptability of the passion fruit fermented alcoholic beverage using the hybrid hedonic scale (Villanueva & Da Silva, 2009), anchored with the terms "1 = disliked extremely" and "9 = liked extremely" at the left and right endpoints, respectively, and also stated whether they would or would not buy the product if it were put on sale using the Purchase Intent Test, with a five-point categorical scale (1 = certainly would not buy, 2 = possibly would not buy, 3 = have doubts if I would buy, 4 = possibly would buy and 5 = certainly would buy), as proposed by Meilgaard et al. (2006).

In the same evaluation session, the CATA - Check-all-that-apply method (Ares et al., 2014) was used to describe the sensory profile of the product by the consumers, from a questionnaire containing 16

previously selected terms by a focus group. The consumers were instructed to indicate by marking with an 'x' the terms of the questionnaire that they judge that characterized each sample. The CATA terms included characteristics related to the appearance, aroma, flavor and buccal textural sensations, such as: floral aroma, green aroma, passion fruit aroma, sweet aroma, citric aroma, refreshing aroma, not very aromatic, intense aroma, attractive color, golden yellow color, astringent, acid, sweetish, equilibrated, passion fruit flavor and well bodied.

### 2.11. Statistical analysis

Three bottles of each treatment were used to carry out the physicochemical analyses, quantification of the phenolic compounds and to evaluate the antioxidant activity in triplicate, one bottle for each replicate of the elaboration. The number of sources of variation used in the One-way ANOVA obtained from the physicochemical analyses, quantification of the phenolic compounds and evaluation of the antioxidant activity, were two: treatments (degrees of freedom = 3) and batches elaborated (degrees of freedom = 2). However, for the sensory analysis the sources of variation were the treatments (degrees of freedom = 3) and consumers (degrees of freedom = 59). The data was submitted to ANOVA and Tukey's means test ( $p \leq 0.05$ ) to using the statistical software Statistical Analytical Systems - SAS (SAS Institute, Cary, NC, USA, University Edition, 2017). For the consumer test, the elaboration triplicates for each treatment were mixed in equal proportions. The data was submitted to ANOVA and Fisher's LSD means test ( $p \leq 0.05$ ) using the same statistical software and as sources of variation the treatments (degrees of freedom = 3) and consumers (degrees of freedom = 59). The CATA results were analyzed with the XLStat software (Addinsoft Inc., Anglesey, UK, 2015). As the responses to each term is binary (1 = term marked by the consumer; 0 = term not marked by the consumer), was used nonparametric Cochran's Q Test ( $p \leq 0.05$ ) and comparisons based on the Marascuilo approach. Correspondence Analysis (CA) was performed to identify differences and similarities among the treatments used to elaborate the fermented alcoholic beverage from Caatinga passion fruit. Additionally, the graphics were obtained with the Sigma Plot version 14.0 (Systat Software, Inc., San Jose, CA, USA). Correlation between the phenolic compounds and antioxidant activity was performed using Spearman correlation and XLStat.

## 3. Results and discussion

### 3.1. Physicochemical characterization of the fruit pulp

It can be seen that the parameters of the physicochemical composition evaluated for the *in natura* passion fruit pulp showed significant differences between the ripeness stages (Table 1). The increases in pH and in the soluble solids - SS and reducing sugars - RS contents with the increase in ripeness of the fruits were attributed to starch and pectin

**Table 1**

Physicochemical composition of the frozen passion fruit pulp from fruits in two ripeness stages (intermediate and ripe).

Variables <sup>a,b</sup>	Frozen passion fruit pulps	
	Intermediate	Ripe
Density (g mL <sup>-1</sup> )	1.04 ± 0.00b	1.05 ± 0.00a
pH	3.06 ± 0.01b	3.27 ± 0.01a
SS (°Brix)	10.77 ± 0.06b	11.60 ± 0.00a
TA (% citric acid)	4.32 ± 0.23a	3.43 ± 0.01b
SS/TA ratio	2.50 ± 0.12b	3.38 ± 0.01a
RS (g L <sup>-1</sup> )	39.17 ± 0.84b	44.26 ± 0.12a

<sup>a</sup> Means ± standard deviation ( $n = 3$ ) followed by different letters, in the same line, represent statistically significant differences between the pulps in the physicochemical composition according to the Tukey's means test ( $p \leq 0.05$ ).

<sup>b</sup> SS = soluble solids. TA = titratable acidity. RS = reducing sugars.

hydrolyses, the synthesis of secondary compounds (as phenolic compounds) and to the reduction of organic acids (Chitarra & Chitarra, 2005; Vilas-Boas, 1999). Concomitantly, for the SS/TA ratio, the values of Table 1 confirms that the fruits in the ripe stage of maturation showed greater sweetness, a characteristic justified by the decrease of acidity and increase in the soluble solids contents when the fruits ripened.

### 3.2. Physicochemical composition of the passion fruit fermented alcoholic beverage

Table 2 shows the results obtained for the physicochemical parameters evaluated.

The pH value of the samples varied from 2.99 to 3.21 (Table 2), thus conforming to the value recommended for white wines. Satisfactory pH levels for wines are between 3.1 and 3.6 (Lins & Sartori, 2014). Thus the pH values of the fermented Caatinga passion fruit beverages are similar to those of commercial red wines produced from world famous grape varieties such as Cabernet Sauvignon and Merlot (Lins & Sartori, 2014).

The density is related to the alcohol and reducing sugar contents of alcoholic beverages, showing a proportionally inverse response to the

**Table 2**

Color and Physicochemical composition of the fermented alcoholic passion fruit beverage.

Physicochemical variables <sup>a,b</sup>	Wines from Caatinga passion fruit <sup>c</sup>			
	T1	T2	T3	T4
pH	3.05 ± 0.01b	3.20 ± 0.03a	2.99 ± 0.01c	3.21 ± 0.01a
Density (g mL <sup>-1</sup> )	1.0458 ± 0.0001a	1.0443 ± 0.0001b	1.0412 ± 0.0000c	1.0409 ± 0.0004d
TA (meq L <sup>-1</sup> )	527.50 ± 3.34a	498.35 ± 3.79b	498.35 ± 3.79b	429.86 ± 1.26c
VA (meq L <sup>-1</sup> )	6.46 ± 0.63a	6.67 ± 0.62a	6.09 ± 0.13a	5.53 ± 0.01a
Free sulfur dioxide (mg L <sup>-1</sup> )	26.11 ± 0.00c	30.55 ± 0.30a	27.99 ± 0.30b	30.72 ± 0.00a
Total sulfur dioxide (mg L <sup>-1</sup> )	28.16 ± 0.00d	92.93 ± 0.68b	74.24 ± 0.0c	94.72 ± 0.00a
Dry extract (g L <sup>-1</sup> )	145.00 ± 0.26a	142.47 ± 0.32b	136.10 ± 0.10c	131.80 ± 0.10d
Alcohol content (% v/v)	7.15 ± 0.05c	7.61 ± 0.09b	8.12 ± 0.03a	6.99 ± 0.02d
Color L*	51.06 ± 0.04b	51.10 ± 0.27b	51.35 ± 0.08b	52.23 ± 0.20a
Color a*	-2.50 ± 0.02a	-2.53 ± 0.02a	-2.67 ± 0.02b	-2.77 ± 0.05c
Color b*	19.27 ± 0.03b	19.96 ± 0.10a	17.93 ± 0.06c	14.97 ± 0.10d
Color C*	19.43 ± 0.04b	20.12 ± 0.10a	18.13 ± 0.06c	15.23 ± 0.11d
Color h	97.40 ± 0.05c	97.24 ± 0.03d	98.50 ± 0.06b	100.48 ± 0.12a
Ascorbic acid (mg 100g <sup>-1</sup> )	4.55 ± 0.45a	4.40 ± 0.26a	4.77 ± 0.27a	4.27 ± 0.27a
Total phenolic compounds (mg GAE L <sup>-1</sup> )	735.94 ± 17.29a	805.84 ± 102.52a	817.02 ± 78.06a	819.51 ± 91.67a

<sup>a</sup> Means ± standard deviation ( $n = 3$ ) in the same line followed by the same letters are not statistically significantly different according to the Tukey's means test ( $p \leq 0.05$ ).

<sup>b</sup> TA = titratable acidity. VA = volatile acidity. L\* = luminosity, white (0) to black (100). a\* = red component, red (+) to green (-). b\* = yellow component, yellow (+) to blue (-). C\* = (Chroma) color chromaticity. h = (hue) color tone angle. GAE = expressed in gallic acid equivalents.

<sup>c</sup> T1 = passion fruit fermented alcoholic beverage elaborated from the frozen pulp of fruits in the intermediate ripeness stage without dilution. T2 = passion fruit fermented alcoholic beverage elaborated from the frozen pulp of ripe fruits without dilution. T3 = passion fruit fermented alcoholic beverage elaborated from the frozen pulp of fruits in the intermediate ripeness stage with dilution. T4 = passion fruit fermented alcoholic beverage elaborated from the frozen pulp of ripe fruits with dilution.



alcohol content and direct response to the soluble solids content present (Manfroi et al., 2010), varied from 1.0458 to 1.0409 g cm<sup>3</sup> (Table 2) and the soluble solids contents were significantly lower in the treatments obtained using diluted pulp but did not differ for the treatments obtained using whole pulp (Table 2). The alcohol content (% v/v) ranged from 6.99 to 8.12 (Table 2) and varied between the treatments. However, although the amount of sucrose added during chaptalization aimed at a minimum value of 8% of alcoholic content, only T3 reached this value. The passion fruit fermented alcoholic were classified as sweet, since the total sugar contents were adjusted to 90 g L<sup>-1</sup> and the legislation establishes a minimum content of 3 g L<sup>-1</sup> for this classification (BRASIL, 2012, p. 12). Similar alcohol contents to those obtained in the present study were reported by Reddy and Reddy (2005) on producing alcoholic beverages from different mango varieties, with values of 7–8% (v/v); and by Araújo et al. (2011) who reported an alcohol content of about 6% (v/v) for a fermented cashew beverage.

The titratable acidity, in the range from 429 to 527 meq L<sup>-1</sup>, was above the maximum limit (130 meq L<sup>-1</sup>) established by the Brazilian legislation to fruit wines (BRASIL, 2012, p. 12). The volatile acidity (VA) of the samples varied from 5.53 to 6.67 meq L<sup>-1</sup>, with no significant differences between the treatments (Table 2) and below the maximum limit (20 meq L<sup>-1</sup>) defined by the same legislation. In other papers published about alcoholic beverages obtained from tropical fruits and about red and white wines, the authors reported lower values for the titratable acidity than those observed for the fermented Caatinga passion fruit beverage (Bessa et al., 2018; Boeira et al., 2020; Lins & Sartori, 2014; Paula et al., 2012; Silva et al., 2015). However, the volatile acid contents were similar to those presented by Silva et al. (2015), who found values between 5.89 and 6.17 meq L<sup>-1</sup>, indicating the healthiness of the fruit at the moment of harvesting and processing (Silva et al., 2008).

The free sulfur dioxide concentrations differed significantly amongst the samples, varying between 26 and 30 mg L<sup>-1</sup> (Table 3), these values being close to those considered ideal for wine conservation, which should be about 30 mg L<sup>-1</sup> (Silva et al., 2008).

Total sulfur dioxide is widely used in wineries to protect wines from oxidative effects and microbial deterioration (Boeira et al., 2020), the maximum permitted concentration being 350 mg L<sup>-1</sup> of total SO<sub>2</sub> (BRASIL, 2012, p. 12). Thus all the formulations presented values below the permitted maximum (Table 2).

The dry extract contents were similar for all the treatments (Table 2), varying from 131 to 145 g L<sup>-1</sup>. The dry extract content in the beverages represent the concentration of the total solids, including non-volatile organic substances (such as sugars and fixed acids) and minerals and it is directly proportional to the perception of “body” in the wine (Bia-soto et al., 2014). A wine with a dry extract above 30 g L<sup>-1</sup> is classified as a well-bodied beverage (Castilhos & Del Biachi, 2011). In addition, the amounts of sugar added to the musts during alcoholic fermentation for chaptalization and before bottling, increased the dry extract content of the product (Boeira et al., 2020; Oliveira, Souza & Mamede, 2011). Thus the results obtained here indicated that the passion fruit fermented alcoholic beverage were very well bodied.

### 3.3. Colorimetric evaluation

The color parameters of the samples varied between the treatments (Table 2) as follows: from 51.06 to 52.23 for luminosity (L\*), -2.50 to -2.77 for the red/yellow component (a\*), 14.97 to 19.96 for the green/blue component (b\*), 15.23 to 20.12 for chromaticity (C\*) and 97.24 to 100.48 for the hue angle (h). Luminosity was significantly higher for T4, the treatment elaborated with diluted ripe pulp, but the other treatments showed no difference between them. The treatments elaborated with *in natura* pulp (T1 and T2) were less intense for yellow and for the hue angle and more intense for green and chromaticity. In general, the variations between the treatments could be justified by the addition of water to the pulps (T3 and T4), diluting the medium, and to the degree

**Table 3**

HPLC-DAD-FD phenolic compound profile of the fermented alcoholic passion fruit beverage.

Phenolic compounds (mg L <sup>-1</sup> ) <sup>a,b</sup>	RT <sup>c</sup> (min)	λ <sup>d</sup> (nm)	Wines from Caatinga passion fruit <sup>e</sup>			
			T1	T2	T3	T4
Caffeic acid	29.91 ± 0.18	320	1.92 ± 0.04b	2.88 ± 0.01a	1.73 ± 0.03c	1.94 ± 0.00b
Trans-caftaric acid	19.45 ± 0.20	320	0.95 ± 0.02a	0.76 ± 0.01c	0.84 ± 0.07b	0.67 ± 0.03d
Chlorogenic acid	25.34 ± 0.17	320	0.50 ± 0.01b	0.50 ± 0.00b	0.47 ± 0.01c	0.54 ± 0.01a
ρ-Coumaric acid	33.80 ± 0.17	320	1.17 ± 0.09a	1.01 ± 0.14a	1.03 ± 0.05a	0.63 ± 0.02b
Ferulic acid	39.18 ± 0.05	320	0.52 ± 0.00a	0.45 ± 0.01b	0.47 ± 0.04b	0.37 ± 0.01c
Gallic acid	9.66 ± 0.28	280	7.07 ± 0.15a	4.02 ± 0.30c	5.26 ± 0.22b	3.08 ± 0.17d
Total phenolic acids	–	–	12.12 ± 0.08a	9.61 ± 0.17b	9.80 ± 0.14b	7.22 ± 0.17c
Kaempferol-3-O-glucoside	45.87 ± 0.11	360	0.26 ± 0.01b	0.31 ± 0.00a	0.27 ± 0.01b	0.31 ± 0.01a
Quercetin-3-β-D-glucoside	43.62 ± 0.03	360	7.62 ± 0.16a	7.66 ± 0.14a	7.57 ± 0.08a	7.13 ± 0.00b
Isorhamnetin-3-O-glucoside	46.37 ± 0.02	360	0.58 ± 0.06a	0.50 ± 0.00b	0.58 ± 0.00a	0.28 ± 0.00c
Myricetin	46.97 ± 0.02	360	0.49 ± 0.02a	0.51 ± 0.00a	0.51 ± 0.03a	0.48 ± 0.00a
Rutin	42.98 ± 0.04	360	3.84 ± 0.03c	4.37 ± 0.06a	3.44 ± 0.05d	4.08 ± 0.05b
Total Flavonols	–	–	12.79 ± 0.22	13.35 ± 0.09a	12.37 ± 0.14b	12.27 ± 0.05b
(+)-Catechin	22.27 ± 0.20	320F	0.66 ± 0.09a	0.62 ± 0.00a	0.70 ± 0.12a	0.67 ± 0.11a
(-)-Epicatechin	28.43 ± 0.15	320F	1.48 ± 0.00a	1.21 ± 0.03b	1.34 ± 0.01	1.40 ± 0.21 ab
(-)-Epicatechin gallate	41.50 ± 0.04	280	2.34 ± 0.16c	6.60 ± 0.16a	2.31 ± 0.22c	3.31 ± 0.14b
(-)-Epigallocatechin gallate	32.44 ± 0.19	280	1.82 ± 0.03	2.17 ± 0.32a	1.46 ± 0.15bc	1.31 ± 0.20c
Procyanidin A2	44.76 ± 0.03	320F	2.20 ± 0.56a	1.38 ± 0.03b	2.52 ± 0.02a	1.15 ± 0.23b
Procyanidin B1	21.70 ± 0.17	320F	2.03 ± 0.28bc	2.58 ± 0.05a	1.78 ± 0.22c	2.49 ± 0.46 ab
Procyanidin B2	27.28 ± 0.15	320F	2.42 ± 0.48a	2.72 ± 0.08a	2.47 ± 0.02a	2.28 ± 0.04a
Total Flavonols	–	–	12.94 ± 0.43b	17.28 ± 0.18a	12.58 ± 0.41b	12.60 ± 0.43b
Trans-resveratrol	48.32 ± 0.02	320	0.28 ± 0.00a	0.27 ± 0.00b	0.27 ± 0.00a	0.26 ± 0.00c
Cis-resveratrol	48.35 ± 0.03	280	0.35 ± 0.01a	0.34 ± 0.01a	0.32 ± 0.01a	0.27 ± 0.00a
Piceatannol	–	320	–	–	–	–

(continued on next page)

Table 3 (continued)

Phenolic compounds (mg L <sup>-1</sup> ) <sup>a,b</sup>	RT <sup>c</sup> (min)	λ <sup>d</sup> (nm)	Wines from Caatinga passion fruit <sup>e</sup>			
			T1	T2	T3	T4
Viniferin	43.79		0.89 ± 0.04a	0.61 ± 0.03b	0.68 ± 0.05b	0.61 ± 0.03b
	0.03			0.03b		0.03b
	51.01	320	ND	ND	ND	ND
	± 0.00					
Total stilbenes	–	–	1.52 ± 0.05a	1.21 ± 0.02b	1.28 ± 0.04b	1.13 ± 0.03b

<sup>a</sup> Means ± standard deviation ( $n = 3$ ) in the same line followed by different letters represent statistically significant differences between samples in the phenolic compound content according to the Tukey's means test ( $p \leq 0.05$ ).

<sup>b</sup> ND = not detected.

<sup>c</sup> RT = retention time.

<sup>d</sup> λ = wavelength.

<sup>e</sup> T1 = passion fruit fermented alcoholic beverage elaborated from the frozen pulp of fruits in the intermediate ripeness stage without dilution. T2 = passion fruit fermented alcoholic beverage elaborated from the frozen pulp of ripe fruits without dilution. T3 = passion fruit fermented alcoholic beverage elaborated from the frozen pulp of fruits in the intermediate ripeness stage with dilution. T4 = passion fruit fermented alcoholic beverage elaborated from the frozen pulp of ripe fruits with dilution.

of ripeness of the fruits (Intermediate and ripe). In addition, the phenolic compound content and antioxidant activity, also related to the degree of ripeness, show a strong correlation with the color of the fruit pulp (Zielinski et al., 2014).

### 3.4. Ascorbic acid (vitamin C) content

The ascorbic acid content of the samples was about 4 mg 100 g<sup>-1</sup> (Table 2), with no difference between the treatments. Silva et al. (2020) characterized the Caatinga passion fruit pulp (cv. BRS Sertão Forte) and found 11 and 17 mg 100 g<sup>-1</sup> of vitamin C in the intermediate and ripe stages, respectively. Larger amounts were found in the following passion fruit varieties: purple *Passiflora edulis* (32 mg 100 g<sup>-1</sup> fresh weight), yellow *Passiflora edulis* (24 mg 100 g<sup>-1</sup> fresh weight), *Passiflora maliformis* (15 mg 100 g<sup>-1</sup> fresh weight) and *Passiflora glandulosa* Cav. (57.76 mg 100 g<sup>-1</sup> fresh weight) (Ramaiya et al., 2012; Lima-Neto et al., 2017). Alcoholic fermentation and other phases of the fruit processing to obtain of the fermented alcoholic beverage influenced the vitamin C content, since this vitamin is easily degraded. Explaining the drastic reduction in ascorbic acid presented in Table 2. Even so, the alcoholic passion fruit beverage can be considered as a source of vitamin C.

### 3.5. Total phenolic compound content (TPC)

Similar amounts of total phenolic compound contents were found in all the treatments (Table 2) (>700 mg GAE L<sup>-1</sup>), values higher than those reported by Lima-Neto et al. (2017) and Zielinski et al. (2014) for the varieties yellow *Passiflora edulis* (276 mg GAE Kg<sup>-1</sup>) and *Passiflora glandulosa* Cav. (205.5 mg GAE kg<sup>-1</sup>) when the authors analyzed *in nature* fruits. Silva et al. (2020) characterized the Caatinga passion fruit (cv. BRS Sertão Forte) and also found values lower than those found in the present study, of 530 and 410 mg GAE kg<sup>-1</sup> for the intermediate and ripe stages, respectively. While Santos et al. (2021) found 365 and 476.1 mg kg<sup>-1</sup> of total phenolic compounds for *P. cincinnata* and *P. edulis*, respectively.

The increase observed in the phenolic compound content of the alcoholic fermented beverage obtained from Caatinga passion fruit (Table 2) could be explained by the possible influence on the phenolic fraction unleashed by the action of the commercial yeast, pectinolytic enzyme and other enological consumables added, and by various reactions occurring during the pre and post-fermentative processes. In

addition, various factors affect these reactions, such as the duration of the clarification, fermentation and stabilization steps, the pH value, temperature, mineral concentrations, quantity of dissolved oxygen and the extent of homogenization of the must during fermentation (Morero-Arribas & Polo, 2009).

Although the total polyphenol compound contents present in white wines are about 10 times lower than those present in red wines (Vaccari et al., 2009), the alcoholic Caatinga passion fruit beverages showed contents approximately 1.5–3 times lower than commercial red wines of the varieties Cabernet Sauvignon, Merlot and Syrah (Lins & Sartori, 2014). On the other hand, the total phenolic compound contents of the alcoholic Caatinga passion fruit beverages were about twice the values found in commercial white wines of the varieties 'Verdejo' and 'Malvasia' (Paixão et al., 2007) and about four times higher than those of the commercial white wine made from the grape variety 'Sauvignon Blanc' (Baiano et al., 2012). This confers merit on this passion fruit species, indicating the potential of the matrix as a source of bioactive compounds.

### 3.6. Phenolic components

A large number of phenolic compounds were found in the passion fruit fermented alcoholic beverage (Table 3), twenty-one compounds being identified and quantified by HPLC-DAD-FD, belonging to four main groups: phenolic acids, flavanols, flavonols and stilbenes. Of the compounds tested, only viniferin was not identified in the samples. The phenolic profile varied between the treatments, larger amounts of total phenolic acids and total stilbenes being found in the treatment elaborated with *in natura* pulp in the intermediate stage of ripeness (T1), followed by the treatment elaborated with ripe *in natura* pulp (T2) and with diluted pulp in the intermediate ripeness stage (T3), which did not differ from each other. Larger amounts of total flavanols (flavanols and flavonols) were found in T2, but the total flavonols in T1 and T2 not differed significantly. In summary, the state of ripeness of passion fruit was determinant for the phenolic compounds profile of the beverage. Dilution appears to cause a significant loss in the total phenolic compound content.

The phenolic compounds are the secondary metabolites of plants responsible for the antioxidant capacity of fruits, and hence their regular consumption provides prevention against chronic degenerative diseases and reduces the risk of some types of cancer (Huang et al., 2012; Paikrao et al., 2010). Some studies have shown a strong correlation between the amounts of bioactive compounds in fruits, including the phenolic compounds, and arid and semi-arid climatic conditions, where the plants are submitted to excessive exposure to heat and sunlight (de Carvalho et al., 2018; Siqueira et al., 2013).

Previous studies concerning the biological activity of *Passiflora* species showed a high antioxidant capacity *in vitro* assays, reporting that the antioxidant capacity of the fruit was attributed to the phenolic compounds present in the fruit pulp and their respective chemical structures, mainly the flavonoids (Rotta et al., 2019; Santos et al., 2021; Silva et al., 2020; Zeraik et al., 2010; Zou et al., 2016).

In this context, the results obtained in the present study express the elevated antioxidant potential of Caatinga passion fruits, corroborating with reports in the literature about BRS Sertão Forte Caatinga passion fruits, where Silva et al. (2020) identified significant phenolic acid and flavonoid contents, independent of the state of ripeness of the fruit. The phenolic acid contents of the fermented Caatinga passion fruit beverages were higher than those found in the fruits of different *Passiflora* species (*P. edulis*, *P. alata* and *P. ligularis*) (Rotta et al., 2019). No previous study evaluated the phenolic profile of alcoholic beverages elaborated with Caatinga passion fruit.

#### 3.6.1. Phenolic acids

The phenolic acid group is constituted of the hydroxybenzoic (HBA) and hydroxycinnamic (HCA) acids, found mainly in the free form. HPLC-

DAD-FD allowed for the identification and quantification of the following phenolic acids: caffeic, caftaric, chlorogenic,  $p$ -coumaric, ferulic and gallic (Table 3). Of these, gallic acid was present in greater amounts. The largest amount of total phenolic acids was found in the intermediate ripeness stage of the *in natura* pulp (T1) and the smallest amount in the diluted ripe pulp (T4), distinguishing it from the other treatments. The values for T2 and T3 were similar showing that the composition of the ripe *in natura* pulp was similar to that of the diluted intermediate ripeness stage pulp.

The literature also cites gallic acid as one of the HBAs found in greater concentration in wine, originating in the grapes and also being formed by hydrolysis of the tannins (Moreno-Arribas & Polo, 2009). To the contrary of Table 3, which highlights T1 followed by T3 as the treatments giving rise to the alcoholic Caatinga beverage showing the largest amounts of gallic acid (7.07 and 5.26 mg L<sup>-1</sup>, respectively), the results presented by Silva et al. (2020) show lower concentrations of this acid in Caatinga passion fruit, especially *in natura* in the intermediate stage (1.67 mg L<sup>-1</sup>). Considering this, one can assume that during the processing of the passion fruit, the gallic acid concentration increased due to hydrolysis of the tannins. In addition, making a parallel with the phenolic acid profile of Caatinga passion fruit determined by Silva et al. (2021) and Santos et al. (2021), it was shown that during processing of the fruit to produce an alcoholic fermented beverage, there was a possible loss of chlorogenic, *trans*-caftaric, *p*-coumaric and ferulic acids, and increase in the caffeic acid content.

### 3.6.2. Flavonoids (flavanols and flavanols)

In this study, the compounds flavanols identified were: kaempferol-3-O-glucoside, quercetin-3- $\beta$ -D-glucoside, isorhamnetin-3-O-glucoside, myricetin and rutin (Table 3). The phenolic compound present in largest amounts (>7.00 mg L<sup>-1</sup>) in all the samples was quercetin-3- $\beta$ -D-glucoside, with significant variation between the *in natura* and diluted pulp samples, but not between the ripeness stages. On the other hand, myricetin showed no variation between the ripeness stages or between the *in natura* and diluted pulps.

As compared to the flavonoid profile identified by Silva et al. (2020) in Caatinga passion fruit, there was a reduction in the quercetin-3- $\beta$ -D-glucoside and rutin contents, these compounds possibly having been degraded during the production of the alcoholic beverage. According to Moreno-Arribas and Polo (2009), since the flavonoids are easily oxidized, the exposure to oxygen before alcoholic fermentation during the preparation of white wines, is sufficient to reduce these compounds in the must. In addition, the pectinolytic enzymes added to the must to improve the quality and yield of the beverage, can also degrade the flavonoids.

Seven flavanols were identified (Table 3), as follows: (+)-catechin, (-)-epicatechin, (-)-epicatechin gallate, (-)-epigallocatechin gallate, procyanidin A2, procyanidin B1 and procyanidin B2, the flavanols being the most prevalent group. The compound (-)-epigallocatechin gallate stood out with an amount of 6.60 mg L<sup>-1</sup> in the treatment using ripe *in natura* pulp (T2), nearly three times the amount detected in the intermediate stage pulp (2.34 mg L<sup>-1</sup>). As consequence, there was no difference in the amounts of this component quantified in the intermediate stage pulps (2.3 mg L<sup>-1</sup> in both T1 and T3). (+)-Catechin and procyanidin B2 showed no variation between the different ripeness stages or between the *in natura* and diluted pulps. Procyanidin A2 remained constant even after dilution of the pulp, more being detected in the intermediate stage. Small amounts of procyanidin B1 (0.12 mg kg<sup>-1</sup>), (-)-epicatechin (0.02 mg kg<sup>-1</sup>), (-)-epicatechin gallate (0.15 mg kg<sup>-1</sup>) and procyanidin B2 (1.40 mg kg<sup>-1</sup>) were also found in the *in natura* Caatinga passion fruit by Santos et al. (2021), suggesting an increase in these flavanols during preparation of the alcoholic beverage. On the other hand, the epigallocatechin gallate (3.06 mg kg<sup>-1</sup>) and (+)-catechin (1.64 mg kg<sup>-1</sup>) concentrations were higher in the fruit than in the alcoholic beverage.

### 3.6.3. Stilbenes

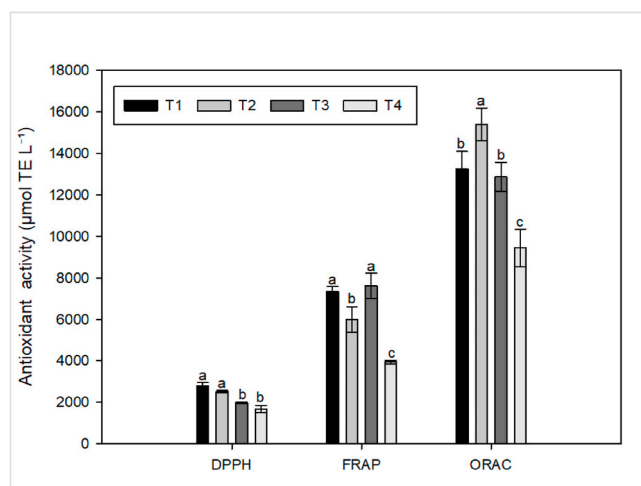
The stilbenes are of great interest due to their relevant antioxidant, anti-carcinogenic and anti-mutagenic potentials (Buiarelli et al., 2007; Moreno-Arribas & Polo, 2009). The stilbenes were the least prevalent of the phenolic compounds detected, the following three compounds being identified: *trans*-resveratrol, *cis*-resveratrol and piceatannol. Piceatannol was present in the largest amounts in all the samples (Table 3). *Trans*-resveratrol was the compound quantified with the smallest amounts (<0.3 mg L<sup>-1</sup>), showing no variation between the samples elaborated with pulp in the intermediate stage (T1 and T3), which showed larger amounts than the treatments prepared with ripe pulp.

The *trans* and *cis* resveratrol contents found in this study, although low, were in agreement with the concentration range (0.1–0.8 mg L<sup>-1</sup>) commonly reported for white wines. Considering that no viniferine was found in the beverages obtained, this infers that in addition to the matrix (fruit) not containing a significant amount of this stilbene, there was no polymerization of the monomer resveratrol by way of the activity of peroxidase (Moreno-Arribas & Polo, 2009), this reaction being responsible for the synthesis of this compound. On the other hand, there were possible losses of the compound piceatannol as a function of processing, since Silva et al. (2020) found larger amounts in *in natura* Caatinga passion fruit (>1.37 mg L<sup>-1</sup>).

### 3.7. Antioxidant activity

Fig. 1 shows the results obtained for antioxidant activity as verified using the different trials (DPPH, FRAP and ORAC).

The DPPH method showed that the ripeness stage did not interfere ( $p \leq 0.05$ ) with the antioxidant activity of the fermented samples (Fig. 1), but dilution of the pulp significantly reduced the activity (T1 = T2 < T3 = T4); from 2783 to 1981  $\mu\text{mol TE L}^{-1}$  (T1 and T3, respectively) and from 2527 to 1679  $\mu\text{mol TE L}^{-1}$  (T2 and T4, respectively). The FRAP and ORAC methods showed that the ripeness stage of the fruit presented non-similar results for antioxidant activity (T1  $\neq$  T2 and T3  $\neq$  T4) for both methods, and dilution of the pulp in the intermediate stage (T3) did not result in a reduction in antioxidant activity when compared with the *in natura* pulp, giving results of 7612  $\mu\text{mol TE L}^{-1}$  for T1 and 7348  $\mu\text{mol TE L}^{-1}$



**Fig. 1.** *In vitro* antioxidant activity of the fermented alcoholic passion fruit beverage as determined by the DPPH, FRAP and ORAC assays. Different letters indicate significant differences according to the Tukey's means test ( $p \leq 0.05$ ). **Captions:** T1 = passion fruit fermented alcoholic beverage elaborated from the frozen pulp of fruits in the intermediate ripeness stage without dilution. T2 = passion fruit fermented alcoholic beverage elaborated from the frozen pulp of ripe fruits without dilution. T3 = passion fruit fermented alcoholic beverage elaborated from the frozen pulp of fruits in the intermediate ripeness stage with dilution. T4 = passion fruit fermented alcoholic beverage elaborated from the frozen pulp of ripe fruits with dilution.

L<sup>-1</sup> for T3 according to FRAP, and of 13258 µmol TE L<sup>-1</sup> for T1 and 12871 µmol TE L<sup>-1</sup> for T3 according to ORAC. In the ripe stage, dilution of the pulp significantly reduced the antioxidant activity from 5995 µmol TE L<sup>-1</sup> for T2 to 3942 µmol TE L<sup>-1</sup> for T4 according to FRAP, and from 15412 µmol TE L<sup>-1</sup> for T2 to 9443 µmol TE L<sup>-1</sup> for T4 according to ORAC.

The minimum values found in this study agree with the values reported in the literature by Ramaiya et al. (2012), Zielinski et al. (2014) and Silva et al. (2020), who reported maximum antioxidant activity of 1685 µmol TE L<sup>-1</sup> in passion fruit pulp (*Passiflora maliformis*) according to the DPPH assay, 3915 µmol TE L<sup>-1</sup> (*Passiflora edulis*) according to the FRAP assay and 6810 µmol TE L<sup>-1</sup> (*Passiflora cincinnata* Mast. BRS Sertão Forte) according to the ORAC assay. Thus the maximum values found for the passion fruit wines according to the DPPH, FRAP and ORAC assays provided results of 2783 µmol TE L<sup>-1</sup>, 7612 µmol TE L<sup>-1</sup> (treatment T1) and 15412 µmol TE L<sup>-1</sup> (treatment T2), respectively, for the three methods (Fig. 1), values relatively higher than those reported in the literature. The greater antioxidant activity of the alcoholic fermented passion fruit beverage in relation to that of the *in natura* fruit could be explained by the presence of a higher total phenolic content in the beverage (Table 2).

In general, fruits present large variations in their antioxidant activity when evaluated by different methods, and hence the variations observed in this study for the antioxidant activity, by *in vitro* antioxidant methods, are related to the chemical structure and action mechanisms (with respect to the free radicals) of the phenolic compounds involved in the evaluation method applied (Lima-Neto et al., 2017). Consequently, (Lins & Sartori, 2014) emphasized the fact that wine is a complex mixture of different compounds, and that the degree of combination between the phenolic compounds and the molecular arrangements present in the matrix can influence the response to free radical scavenging to a large extent.

The results presented in this study demonstrate that the Caatinga passion fruit shows expressive antioxidant activity and could be used as a source of natural antioxidants, as expected considering the fact that previous studies ((Ramaiya et al., 2013); Zielinski et al., 2014; de Carvalho et al., 2018; Silva et al., 2020) already indicated that the fruits of various passion fruit (*Passiflora*) cultivars were rich in compounds that

act in the elimination of free radicals.

In addition, although no previous studies with fermented passion fruit beverages were found in the literature, the antioxidant activities of the beverages produced in the present study showed higher antioxidant activities than the commercial white wines elaborated with different grape varieties (Baiano et al., 2012; Silva et al., 2015).

It is known that the variation in the results for the variety used in this study (*Passiflora cincinnata* Mast.), as compared to other varieties of fruit, could be strongly influenced by the genetics of the fruit, the climate, the developmental stages (fruit ripening), as well as the cultivation system and soil conditions (Chitarra & Chitarra, 2005; Ramaiya et al., 2012).

The correlation between antioxidant capacity and phenolic compounds and ascorbic acid contents was performed using Spearman correlation (Table 4). The positive correlations were found in this study according to the arbitrary scale defined by Granato et al. (2014). Considering the DPPH assay, the total phenolic compounds and *cis*-resveratrol showed a perfect correlation ( $r = 1.00$ ) with antioxidant capacity, and *trans*-resveratrol showed strong correlation ( $r = 0.90$ ). While to the FRAP assay, was observed a perfect correlation ( $r = 1.00$ ) among the ascorbic acid and procyanidin A2 contents and antioxidant activity, and a strong correlation ( $r = 0.90$ ) to chlorogenic acid and isorhamnetin-3-*O*-glucoside contents. Finally to ORAC assay, quercetin-3- $\beta$ -D-glucoside and (-)-epigallocatechin gallate were the compounds that presented a perfect correlation ( $r = 1.00$ ) with antioxidant activity and the total phenolic compounds obtained a moderated correlation ( $r = 0.64$ ). In addition, phenolic acids was the class of phenolic that presented the highest amount of compounds with moderate correlation ( $0.50 \leq r < 0.80$ ) with antioxidant activity by DPPH and FRAP methods. Highlighting the fact that the alcoholic passion fruit beverage obtained using T1 and fruits with an intermediate stage of ripeness was that showing the highest phenolic acid content (Table 3) and the highest values for antioxidant activity by the DPPH and FRAP methods (Fig. 1). Consequently, the trend in this case means that a higher concentration of these compounds in the beverage promotes an increment in their antioxidant activity. The results shown in Table 4 corroborate that indicated in the literature, which indicates that the antioxidant capacity of citric fruits is correlated with the ascorbic acid and phenolic compound

**Table 4**

Spearman correlation between the phenolic compounds and ascorbic acid with the *in vitro* antioxidant activity of the fermented alcoholic passion fruit beverage as measured by the DPPH, FRAP and ORAC assays.

Compounds	DPPH		FRAP		ORAC	
	Spearman r <sup>a</sup>	p-values	Spearman r	p-values	Spearman r	p-values
Total phenolic compounds	<b>1.00</b>	<b>0.08</b>	0.16	0.75	0.64	0.33
Ascorbic acid	0.16	0.75	<b>1.00</b>	<b>0.00</b>	0.04	0.92
Caffeic acid	0.00	1.00	0.64	0.33	0.16	0.75
<i>Trans</i> -caftaric acid	0.64	0.33	0.64	0.33	0.16	0.75
Chlorogenic acid	0.10	0.75	<b>0.90</b>	<b>0.08</b>	0.10	0.75
$\rho$ -Coumaric acid	0.64	0.33	0.64	0.33	0.16	0.75
Ferulic acid	0.64	0.33	0.64	0.33	0.16	0.75
Galic acid	0.64	0.33	0.64	0.33	0.16	0.75
Kaempferol-3- <i>O</i> -glucoside	0.40	0.33	0.54	0.33	0.01	0.92
Quercetin-3- $\beta$ -D-glucoside	0.64	0.33	0.04	0.92	<b>1.00</b>	<b>0.00</b>
Isorhamnetin-3- <i>O</i> -glucoside	0.40	0.42	<b>0.90</b>	<b>0.08</b>	0.10	0.75
Myricetin	0.10	0.75	0.40	0.42	0.40	0.42
Rutin	0.00	1.00	0.64	0.33	0.16	0.75
(+)-Catechin	0.36	0.42	0.16	0.75	0.64	0.33
(-)-Epicatechin	0.04	0.92	0.00	1.00	0.16	0.75
(-)-Epicatechin gallate	0.00	1.00	0.64	0.33	0.16	0.75
(-)-Epigallocatechin gallate	0.64	0.33	0.04	0.92	<b>1.00</b>	<b>0.00</b>
Procyanidin A2	0.16	0.75	<b>1.00</b>	<b>0.00</b>	0.04	0.92
Procyanidin B1	0.00	1.00	0.64	0.33	0.16	0.75
Procyanidin B2	0.16	0.75	0.16	0.75	0.64	0.33
<i>Trans</i> -resveratrol	<b>0.90</b>	<b>0.08</b>	0.40	0.42	0.40	0.42
<i>Cis</i> -resveratrol	<b>1.00</b>	<b>0.00</b>	0.16	0.75	0.64	0.33
Piceatannol	0.40	0.42	0.54	0.33	0.01	0.92

<sup>a</sup> Spearman correlation (r): very weak (almost none):  $0.10 \leq r$ ; moderate correlation:  $0.50 \leq r < 0.80$ ; strong correlation:  $0.80 \leq r < 1.00$ ; perfect correlation:  $r = 1.00$  (Granato et al., 2014). The perfect and strong correlations are presented in bold type.



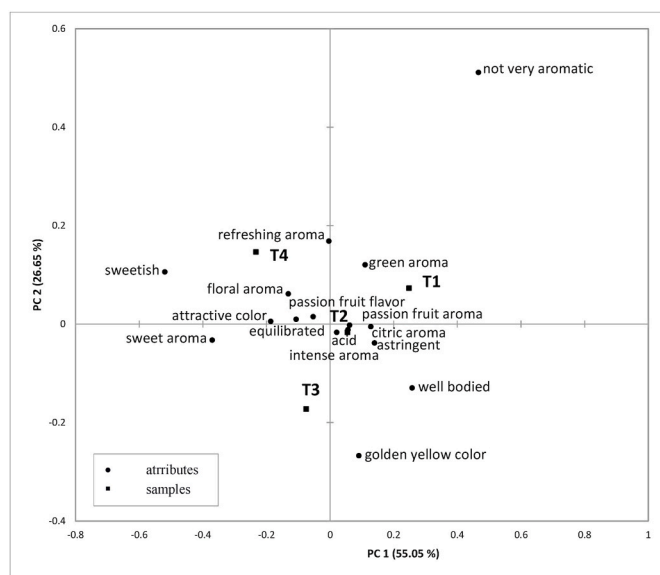
contents (Del Caro et al., 2004; Rekha et al., 2012; Silva et al., 2020).

An analysis of the correlation between the phenolic compound profile and the antioxidant activity is of relevant importance for the beverage characterization, contributing to the indication of the compounds that possibly influence the functional potential of the product (Lima et al., 2014). However, a determined compound does not always present correlation with the antioxidant activity by all the methods employed (Di Majo et al., 2008), as can be seen in this study and in the literature (Padilha et al., 2017; Rufino et al., 2010). So it is not so simple to choose the most appropriate method to determine the in vitro antioxidant capacity, suggesting that this analysis should occur as from a combination of different assays, the DPPH, FRAP and ORAC methods frequently being used in combination (Shahidi & Zhong, 2015; Zou et al., 2016).

### 3.8. Sensory analysis

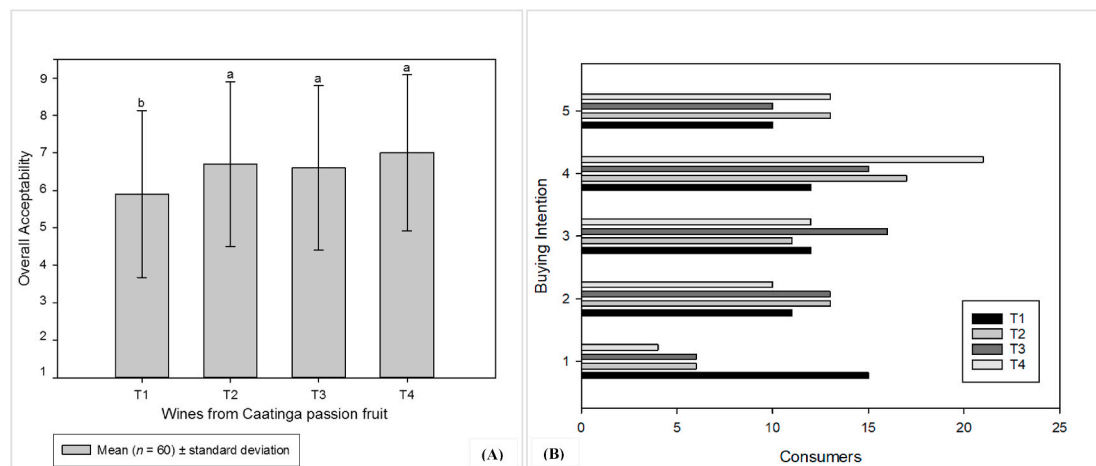
The overall acceptance of the samples (Fig. 2A and Table S2) showed that sample T4 had a more expressive mean for acceptance, being placed in the category “liked slightly = 6.0”, and not differing from samples T2 and T3, which received means of 5.7 and 5.6, respectively. The sample T1 received the worse average of overall acceptability, a mean of 4.9, thus being classified in the category of “neither liked nor disliked = 5.0” on the hibrid hedonic scale and 45% of the consumers rejected this treatment. Samples T2 and T4 received the highest scores in the Purchase Intention Test (Fig. 2B). However, on analyzing samples T1 and T3, at least 40% of the consumers affirmed they would possibly buy the samples. With respect to the category “have doubts if I would buy”, sample T3 showed the greatest predominance of uncertainty of the tasters as to his purchase intention.

Using the CATA technique, the consumers were asked to describe the sensory profiles of the alcoholic fermented passion fruit beverages (Fig. 3 and Table S3). Table S3 indicated that some terms were equally perceived amongst the beverages obtained, such as astringent, well-bodied, passion fruit flavor and equilibrated, and the aromas floral, green, passion fruit, citric, refreshing and intense, while other terms (attractive color, sweet aroma, not very aromatic, sweetish and golden yellow color) showed significant differences ( $p \leq 0.05$ ) between the samples. In this context, sample T1, which was awarded the lowest mean values for acceptance (Fig. 2A and Table S2) was indicated as the



**Fig. 3.** Correspondence Analysis obtained from the sum of the CATA terms cited for the sensory descriptive evaluation of the fermented alcoholic beverages from Caatinga passion fruit. **Captions:** T1 = passion fruit fermented alcoholic beverage elaborated from the frozen pulp of fruits in the intermediate ripeness stage without dilution. T2 = passion fruit fermented alcoholic beverage elaborated from the frozen pulp of ripe fruits without dilution. T3 = passion fruit fermented alcoholic beverage elaborated from the frozen pulp of fruits in the intermediate ripeness stage with dilution. T4 = passion fruit fermented alcoholic beverage elaborated from the frozen pulp of ripe fruits with dilution.

sourest and least sweet beverage, showing the least attractive color, also being indicated as the sample of less intense aroma. It can also be inferred that some of the sensory characteristics cited may be considered undesirable by the consumers when present in the beverages. This treatment emphasized the total acidity according to Table 2 ( $527.50 \text{ meq L}^{-1}$ ), and this excessive acidity possibly reduced the perception of the sweetness resulting from the sugar added to the alcoholic beverage just before bottling. For its part, the treatment that received the highest mean for overall acceptability, T4, was the sample with less citations for



**Fig. 2.** Results of the consumer test ( $n = 60$ ): (A) Means for the overall acceptability of the fermented alcoholic passion fruit beverage using the hybrid hedonic scale anchored with the terms “1 = disliked extremely” and “9 = liked extremely”. Different letters indicate significant difference according to Fisher’s LSD means test ( $p \leq 0.05$ ). (B) Purchase intention of the passion fruit wines. **Captions:** T1 = passion fruit fermented alcoholic beverage elaborated from the frozen pulp of fruits in the intermediate ripeness stage without dilution. T2 = passion fruit fermented alcoholic beverage elaborated from the frozen pulp of ripe fruits without dilution. T3 = passion fruit fermented alcoholic beverage elaborated from the frozen pulp of fruits in the intermediate ripeness stage with dilution. T4 = passion fruit fermented alcoholic beverage elaborated from the frozen pulp of ripe fruits with dilution. Purchase Intention: 1 = Certainly would not buy, 2 = Possibly would not buy, 3 = Have doubts if I would buy, 4 = Possibly would buy and 5 = Certainly would buy.

the presence of a golden yellow color, presenting a higher value for L\* and a hue angle (h) further from 90° (yellow color) according to Table 2.

In sequence, according to the Correspondence Analysis (CA) obtained using the sum of the CATA terms (Fig. 3), it can be seen that sample T4 showed the most distinct sensory profile, notably as compared to sample T1. Sample T4 was mainly described using the terms refreshing, sweet and floral aromas, attractive color, equilibrated and sweetish. Thus it can be inferred that the appearance, aroma and flavor influenced the acceptance of the alcoholic passion fruit beverage, and to obtain a better product from the sensory point of view, one should use ripe fruits and dilute the pulp with water.

#### 4. Conclusions

The production of alcoholic beverages is a feasible alternative to add value to native Caatinga fruits, to date little explored economically. With the exception of total acidity, the results obtained for the basic physicochemical parameters are in agreement with the limits established by Brazilian legislation. The identification and quantification of the phenolic compounds present in the samples produced revealed a great number of compounds and promising antioxidant activity, although significant losses were observed amongst the treatments with the increase in ripeness of the fruits in parallel with dilution of the pulp during the processing. Further studies are required in order to adequate the deviation in total acidity from the values cited in the legislation. The composition of the Caatinga passion fruit pulps indicated a potential for the development of new and exotic food products.

#### Credit author statement

The authors Renata Torres dos Santos e Santos, Aline Camarão Telles Biasoto, Ana Cecília Poloni Rybka, Clivia Danubia Pinho da Costa Castro, Saulo de Tarso Aidar, Graciele Campelo Broges e Flávio Luis Honorato da Silva declare to be responsible for the preparation of the manuscript entitled “Physicochemical characterization, bioactive compounds, in vitro antioxidant activity and consumer acceptability of fermented alcoholic beverage obtained from Caatinga passion fruit (*Passiflora cincinnata* Mast.)”. The first two authors were responsible for the idea of the project and experimental planning, data collection and for writing the article, the sixth author assisted in the analysis of antioxidant activity in vitro, the seventh author performed the data analysis, and the other authors assisted in the acquisition of the fruits, processing and writing of the article. February 10th, Petrolina.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.lwt.2021.111714>.

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