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Physicochemical Characteristics and Antioxidant Potential of a Fruit Beer Produced with Juçara (Euterpe edulis Martius) Fruit Pulp

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HIGHLIGHTS

- Juçara pulp (10, 20, and 40%) was added to the fruit beer production.
- Most physicochemical properties of beers were not affected by juçara pulp addition.
- Beer with 20% of juçara pulp added at maturation showed the best bioactive potential.
- Juçara pulp has enormous potential for application in beer production.

Abstract: Juçara fruit (*Euterpe edulis* Martius) is rich in nutrients and antioxidant compounds, a potential ingredient to meet consumer demands for beers with bioactive potential. In this study, different concentrations (10, 20, and 40%) of juçara fruit pulp were added during the brewing steps (mash, fermentation, and maturation). Physicochemical parameters (carbohydrate profile, real extract, pH, alcohol content, glycerol, and color), total phenolic content, and scavenging effect on 2,2-diphenyl-1-picrilhidrazil (DPPH) radical were evaluated during the brewing process and in the beers produced. The sugars, real extract, pH, ethanol, and glycerol did not vary significantly between beers produced with different juçara concentrations. Juçara beers had an average alcohol content of 3.6% (w/v) and a pH of 4.43. The color varied between 3.2 and 16 Standard Reference Method depending on the fruit pulp concentration and the step in which it was added. The addition of juçara fruit pulp increased the total phenolic content (49.57–80.17 mg of gallic acid equivalent 100 mL^{-1}) and the antioxidant effect on DPPH (1785–3971 mmol ascorbic acid equivalent 100 mL^{-1}) in the beers, especially when added in the final production stages. In conclusion, this study demonstrated that the beer produced with 20% of juçara fruit pulp added at maturation presented the best results and suggested that

this fruit has enormous potential for use in the brewing process, promoting an increase in the bioactive compounds.

Keywords: superfruit; phenolic compounds; bioactive potential; physicochemical characteristics; açaí.

INTRODUCTION

Beer is one of the most consumed alcoholic beverages around the world. It is a fermented cereal-based beverage produced from barley, hops, water, and yeast, supplemented or not with other cereals or other sources of carbohydrates[1,2].

Moderate beer drinking has been associated with improved human health, possibly due to its non-alcoholic components, mainly phenolic compounds from malt and hops [3]. The chemical structure of the phenolic compounds is related to their antioxidant potential, preventing many oxidative stress-related diseases [4,5]. Fruits are a good source of phenolic compounds; thus, they can increase the antioxidant activity of the beer [5].

Among the various beer adjuncts, the addition of fruits has been known for centuries and is a trend nowadays. Fruit beer is one of the categories described in the Beer Judge Certification Program. This special-type beer is elaborated with any fruit or combination of fruits to obtain a product with a specific sensory profile, with a fruity character, balanced with the beer base style [6]. Studies carried out with beers elaborated with the addition of star fruit [7], goji berry [8], Cornelian cherry [4], persimmon [9], and grape must [10] showed improvement in the organoleptic, nutritional, and antioxidant characteristics.

Juçara (*Euterpe edulis* Martius) is a mono-stemmed palm native of the Atlantic Rainforest. For a long period (peak in the 1970s), the juçara palm was exploited for the extraction of palm hearts, and considering that it does not produce tillers and regrowth, the species almost extinguished [11,12]. In this way, the use of juçara fruit for human consumption has been encouraged as a sustainable form of exploitation since it does not result in the plant death [13]. Juçara fruits are small and rounded (weigh about 1 g and 1 to 1.5 cm in diameter) with a violet-black color when ripe [12]. . Juçara fruit has been identified as a “superfruit” due to its nutritional (fatty acids, dietary fiber, protein, and minerals) and bioactive (mainly phenolic compounds) composition. The total lipid content in juçara fruits is between 9.75 and 27.36%, with a predominance of monounsaturated fatty acids (45.5%–56.8% of the total lipid content), mainly oleic acid [14,15]. Protein varies between 3.07 and 5.34%, while dietary fiber ranges from 4.39–5.40% [15,16]. Regarding minerals, the majority are potassium (173–564 mg 100 g^{-1}), calcium (30.7–260 mg 100 g^{-1}), and magnesium (15.4–73.3 mg 100 g^{-1}) [16,17]. Juçara fruit is also rich in phenolic compounds, especially anthocyanins. Total phenolic content, in general, is higher than 5,500 mg gallic acid equivalents 100 g^{-1} in dry weight (DW), while total monomeric anthocyanins vary between 245 and 531 mg cyanidin 3-glucoside equivalents 100 g^{-1} DW[18]. These compounds are related to high antioxidant capacity described for this fruit. Schulz and coauthors [19] demonstrated that juçara fruits in optimal maturity had values of inhibition of DPPH radical and ferric reducing ability higher than 100 and 400 mg ascorbic acid equivalents 100 g^{-1} DW, respectively. In addition, the high antioxidant capacity, other potential health benefits have been described, such as anti-inflammatory, neuroprotective, cardioprotective, and chemopreventive activity [18,20,21].

For consumption, juçara fruits are usually macerated and mixed with water in a de-pulping machine to remove their seeds and obtain a thick pulp, similar to the açaí (*Euterpe oleraceae* Mart. and *Euterpe precatoria* Mart.) from the Amazonian region [12]. This pulp can be consumed directly, frozen, freeze-dried, or in formulations of beverages, ice creams, dairy products, jelly, desserts, and supplements [21].

The potential of juçara fruit as a food ingredient and its high nutritional and bioactive value can be associated with the current trend of producing fruit beers to meet consumer demands for novel health-promoting products. These products include decreased-calorie, low-alcohol, alcohol-free, fruity-flavored, gluten-free, and functional beers [1,22].

In fact, the juçara fruit has potential for application in the production of special-type beers, as it has functional potential, is a viable product in terms of industrial raw material, and its use is important for the environmental restoration of the species. In this context, this study aimed: (i) to develop a fruit beer with juçara fruit pulp (*Euterpe edulis* Martius); (ii) to evaluate physicochemical parameters during the brewing process and in beers produced; (iii) to evaluate antioxidant capacity and total phenolic content during the brewing process and in beers produced; and (iv) to verify the best concentration and the brewing step for adding the juçara fruit pulp.

MATERIAL AND METHODS

Biological and raw material

The pasteurized, defatted, acidified, and frozen (-18 °C) juçara fruit pulp was produced by a specialized company from ripe and healthy fruits harvested in Garuva, Santa Catarina, Brazil (26° 01' 37" S 48° 51' 18" O). Before the addition in the elaboration of the beers, the juçara fruit pulp was thawed at 4 ± 2 °C and homogenized. An aliquot was collected to analyze its physicochemical parameters, total phenolic content, and antioxidant capacity.

Pilsen malt (2 to 3 SRM - Standard Reference Method) and Northern Brewer Hops (8.4% acid-acid) were purchased from Cooperativa Agrária Agroindustrial (Guarapuava, Brazil - 25° 23' 42" S 51° 27' 28" O) and WE Consultoria (Porto Alegre, Brazil), respectively. Filtrated water from the distribution systems of the city of Florianópolis (27°25′25″S 48°32′48″W, Brazil) was used for the brewing process. The dry yeast Safale US-05 (*Saccharomyces cerevisiae*) from Fermentis (Lesaffre, France) was selected for the fermentation process.

Brewing

The development of the beer was based on the Beer Judge Certification Program (BJCP) 2015 Style Guidelines[23]. The fruit beer using juçara fruit pulp was developed with a Blond Ale recipe as background. The brewing process was performed according to Daniels [24]. The experiments were performed in duplicate.

The calculations of the amount of water, malt, and hops were performed according to Daniels [24]. According to the manufacturer's recommendations, the yeast (0.5 g/kg dry yeast/wort) was rehydrated in the wort for 30 minutes at 25 °C and inoculated in the fermenters flasks. According to Brazilian food law [25], the percentage of the juçara fruit pulp was based on the malt mass (i.e.: 10, 20, and 40 g of juçara pulp/100 g malt). The water calculation is based on the final volume of beer and water losses during the manufacturing process. The water loss ratio is approximately 1.67 L/Kg (water/malt), and there is still a reduction in the process, up to about 42.2%, i.e., 8.1% by evaporation; 9.9% in the TRUB (cluster of proteins coagulated with hops residues); 4.2% of volume reduction and 20.0% on equipment. Thus, the amount of water needed in the brewing process was calculated as follows (Equation 1).

$$Water\ (L) = Vb\ +\ (1.67\ \times MM) + (0.42\ \times Vb) \quad (1)$$

In which, Vb is the desired volume (L) of beer and MM is the malt mass (Kg).

The malt calculation was performed according to Equation 2. The Pilsen malt was chosen for the fruit beer style according to the BJCP (2015), which density average is 1.050; therefore, 50 was assumed to be the *TG* (Target Gravity).

$$Malt\ (Kg) = \left(\frac{Vb\ (L)\ \times TG\ \times 0.26}{PE\ \times E}\right) \times 0.45 \quad (2)$$

In which, the *PE* is the extract potential, expressed in GU (Gravity units), for Pilsen malt it is 1035 (35 GU) and *E* is the efficiency of the process; in this case, with a value of 65%, an average of craft beer productions.

The hops calculation is based on the α-acid concentration, α-acid utilization, and boiling time (Equation 3).

$$Hops\ (g) = \left(\frac{IBU\ \times Vb}{1000\ \times \alpha a \times U}\right) \quad (3)$$

In which, *IBU* (International Bitter Units). 10 IBU was set for this fruit beer, ***αa*** is the α-acids percentage of the hop chosen (0.084), and *U* is the α-acids utilization [24].

Brewing: experimental process

In the first stage of the production process, 9.8 kg of barley malt were milled in a mill wet dry grinder (model 643888, Welljoin, China), preserving the seed husk (filter element) [26]. Then the mashing was carried out in a brew kettle (60 L), with the addition of the grounded malt in 47 L in drinking water at 62 °C for 30 min, after the temperature was elevated (1 °C/min) to 72 °C for 20 minutes. Finally, the mixture was conducted for 10 minutes at 78 °C. The wort was manually recirculated for 10 minutes, then transferred to a brew kettle and boiled for 60 minutes with the addition of 15.28 g of hops at the first minute of the process. After this process, the whirlpool was performed for 5 minutes. The wort was then cooled at 25 °C and fractioned for the different experiments. Another batch was processed with identical parameters, only changing the addition

of 1.9 kg of the juçara fruit pulp, replacing the same mass of water, at the beginning of mashing. Figure 1 and Table 1 provide details of the experimental process.

The iodine test was performed at the end of the brewing to confirm the starch saccharification [27]. After brewing and fruit addition, all worts were diluted at 12 °Brix with water at 25 °C, previously sterilized. The fermentation (8 days) and maturation (14 days) were carried out in 5 L flasks, with 20% headspace, at 18 °C and 5 °C, respectively. Matured beers were not subjected to carbonation methods, as degassing is one of the steps for sample preparation.

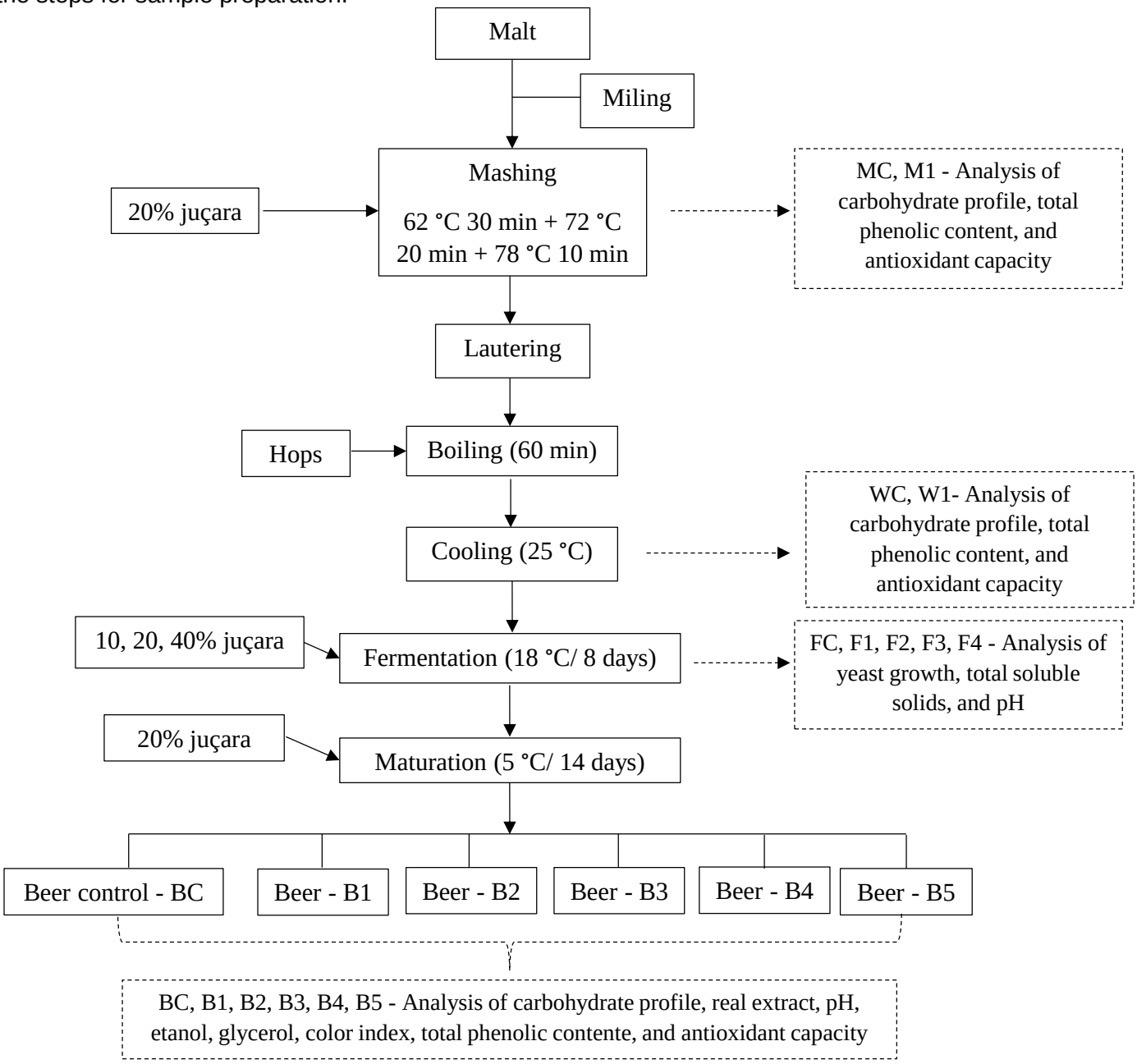

Figure 1. Diagram of the manufacturing process of fruit beer with juçara fruit pulp.

Table 1. Symbols and descriptions of the samples evaluated in the study.

Production stage	Symbol	Description
Raw material	J	Juçara fruit pulp
Mashing	MC	Final mash control
	M1	Final mash with 20% of juçara fruit pulp
Boiling	WC	Final wort control
	W1	Final wort with 20% of juçara fruit pulp
Fermentation	FC	Fermentation control
	F1	Fermentation with 20% of juçara fruit pulp added at mash
	F2	Fermentation with 10% of juçara fruit pulp
	F3	Fermentation with 20% of juçara fruit pulp
	F4	Fermentation with 40% of juçara fruit pulp
Final product	BC	Control beer
	B1	Beer with 20% of juçara fruit pulp added at mash
	B2	Beer with 10% of juçara fruit pulp added at fermentation
	B3	Beer with 20% of juçara fruit pulp added at fermentation
	B4	Beer with 40% of juçara fruit pulp added at fermentation
	B5	Beer with 20% of juçara fruit pulp added at maturation

Analytical determinations

The total soluble solids, pH, and cell viability were analyzed during fermentation (samples collected every 12 hours for the first 96 hours and every 24 hours up to 192 hours). The fermentation kinetics was determined by counting the yeast cell in a Neubauer chamber with an optical microscope (Trinocular CX31, Olympus, Japan). The cell viability was analyzed with the addition of 0.01 % methylene blue [28].

The juçara fruit pulp, mash, and worts samples were analyzed for carbohydrate profile, total phenolic content, and antioxidant capacity. The beer samples were analyzed for carbohydrate profile, real extract, pH, ethanol, glycerol, color index, total phenolic content, and antioxidant capacity.

The pH was determined according to the Beer 9 method [29], the total soluble solids (°Brix) by direct reading on a portable refractometer (model RSG-100ATC, Grandindex, China), and the real extract was determined according to the method reported in Instituto Adolfo Lutz [30].

The color was determined by reading on a UV-visible spectrophotometer (model 8425A, Hewlett-Packard, Germany) of each final beer sample, at 430 nm and 700 nm, against the distilled water sample (white) [31]. The color was expressed in SRM units (Standard Reference Method).

The carbohydrate profile (maltose, glucose, fructose, and maltotriose), glycerol, and ethanol were determined by High Performance Liquid Chromatography (HPLC) (Prominence SPD-20A/20AV UV-Vis, Shimadzu, USA) [32]. Samples of beers, worts, and juçara fruit pulp were diluted in distilled water 1:1 (v/v). An Aminex HPX-87H column (300 × 4.6 mm) from Phenomenex (Torrance, USA) was used. The following parameters were adopted: injection volume of 20 µL, elution temperature of 60 °C, flow rate of 0.5 mL/min, mobile phase of 5 mM H_2SO_4, and thermostatic detector at 35 °C. The concentration of the analyzed compounds was determined on the calibration curve integrated with Chromax 10.0 software (Pol-Lab, Poland).

Total phenolic content was determined by the Folin-Ciocalteu method [33] and the results were expressed as mg gallic acid equivalents (GAE) per 100 mL of sample. The antioxidant capacity was evaluated by the free radical-scavenging capacity on the DPPH radical according to Brand-Williams and coauthors [34] with modifications by Kim and coauthors [35]. A 2.9 mL aliquot of DPPH radical was homogenized with 0.1 mL of the sample or standard and kept in the dark place, at room temperature, for 30 minutes. The absorbance was measured at 515 nm using a UV–Vis spectrophotometer (SB 1810–60 S, Spectro Vision, China). The results were expressed as mmol ascorbic acid equivalent (AAE) per 100 mL.

Statistical analysis

The experimental results were submitted to analysis of variance (ANOVA), and the means were compared using Tukey's test at 95% confidence level, using the software Statistica 10.0 (StatSoft, Tulsa, USA).

RESULTS AND DISCUSSION

Yeast growth, total soluble solids, and pH

The yeast growth and total soluble solids contents during the brewing process are presented in Figures 2a and 2b. The inoculation rate obtained for all experiments varied from 6.86 to 6.96 log CFU/mL, the optimal value for the beginning of the fermentation process [29]. The highest numbers of viable yeast cells were quantified between 36 and 60 h (6.95 to 7.44 log CFU/mL). From 96 h of fermentation, the values were lower than 6.60 log CFU/mL, showing no statistically significant difference (p>0.05) between them until the end of the evaluated period. Yeast growth data showed no influence of the juçara fruit pulp in the beer fermentation. The addition of fruit at this stage could have caused metabolic stress in the yeast [36,37] that was not identified in our study.

In agreement with the results of yeast growth, a significant reduction in the total soluble solids content can be observed between 12 and 60 hours, evidencing the exponential phase of the microorganism's growth (Figure 2b). After 72 hours, total soluble solids were less than 7 °Brix, and no significant variation was observed until the end of the evaluated period. The decrease in total soluble solids is expected throughout the process since it is related to the consumption of wort sugars by yeast [38]. Similar results were obtained for other beers produced with fruits, such as persimmon [39] and soursop [38], which, after fermentation, showed total soluble solids of 6.8 and 6.5 °Brix, respectively.

A decrease in the pH (Figure 2c) was observed for all experiments during fermentation, with final pH between 4.30 to 4.78. This decrease is probably due to synthesis of organic acids by yeasts and carbon dioxide production [39]. For the beers, the pH was 4.43 ± 0.10, with no statistical difference between the samples (p>0.05) (Table 3). These results are similar to other fruit beers (cherry, raspberry, peach, apricot, grape, plum, and apple) and conventional beers, which showed a pH of 3.56 to 4.42 [40]. The pH below 5.0 has an important function in beer to inhibit contamination by other microorganisms, besides promoting the stability of the foam and anthocyanins [41,42]. It is important to highlight that pH of the juçara fruit pulp was 4.070 ± 0.007 and that the water used in the brewing process had a pH of 6.15 ± 0.05. Thus, the pH adjustment of the wort was not necessary, as pH was 5.70 ± 0.06 for the final wort control and 5.61 ± 0.16 for the final wort with 20% of juçara fruit pulp, and literature report values between 5.7 and 5.9 [43]. These data suggest that adding juçara fruit pulp had no great influence on the pH of the beers and that the decrease of this parameter in the final product is mainly related to alcoholic fermentation.

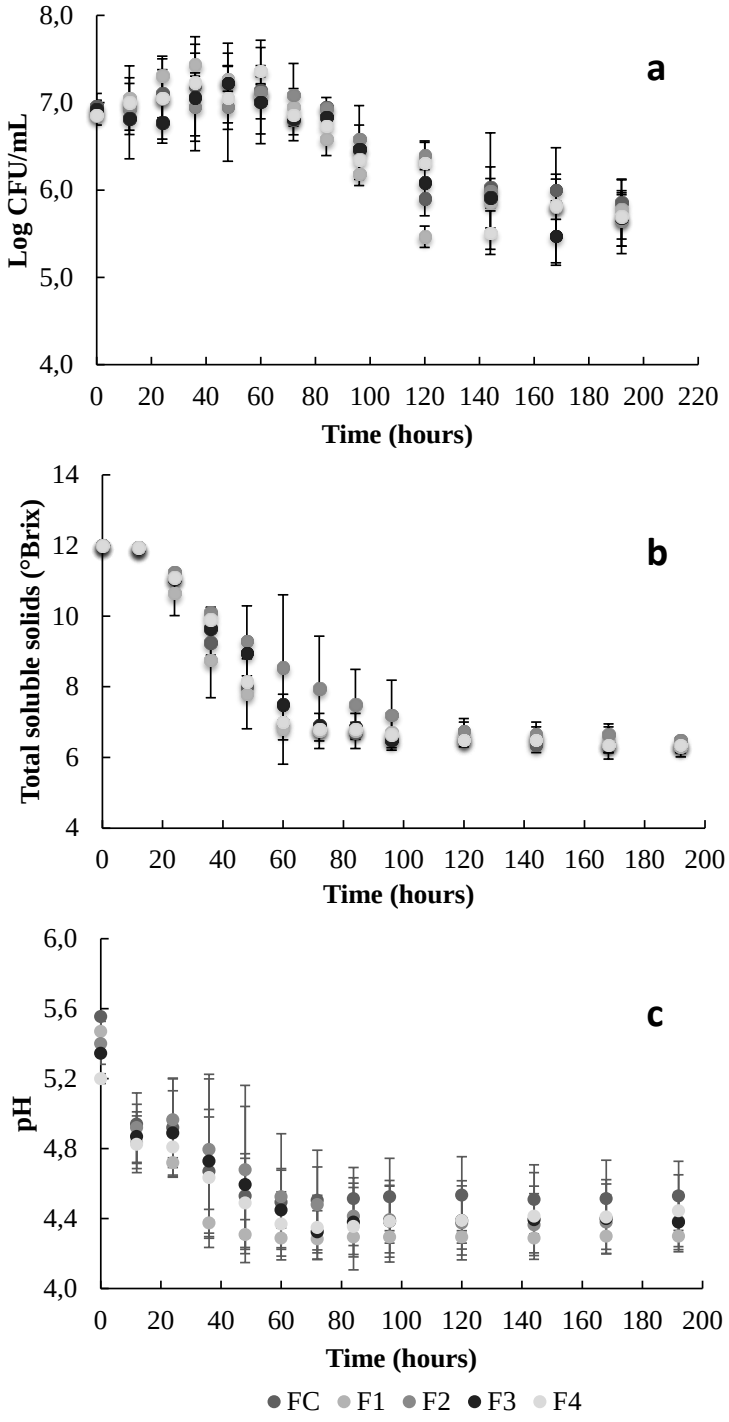

Figure 2. Yeast growth (a), total soluble solids (b), and pH (c) during the fermentation of beers prepared with juçara fruit pulp.

Wort and beer characterization

Carbohydrate profile and real extract

Table 2 shows the maltose, glucose, fructose, and maltotriose contents for juçara fruit pulp, wort, beers, and for all procedures studied (MC, M1, FC, F1, F2, F3, and F4). For final beers (BC, B1, B2, B3, B4, and B5), fructose was the only sugar found. In fact, the yeasts metabolize almost all fermentable sugars in the wort, mainly into ethyl alcohol and carbon dioxide [24]. In agreement with the literature [44,45], maltose was the predominant sugar in the wort in all processes. This sugar is released in the malt mashing by saccharification of starch and is the major sugar in brewing beer [8].

An increase in the sugar concentrations of MC to WC and M1 to W1 was observed, probably due to the boiling process conducted for 1 h. Whereas the juçara fruit pulp has a low sugar content, the different

formulations used had no significant effect on the sugar profile. A low contribution of the pulp with carbohydrates also influenced the results of real extracts since were no significant (p>0.05) differences between beers (Table 2).

Table 2. Carbohydrate profile of the juçara fruit pulp, worts, and beers.

Sample	Maltose (g L^{-1})	Glucose (g L^{-1})	Fructose (g L^{-1})	Maltotriose (g L^{-1})
J	Nd	0.05 ± 0.071 ^{d}	0.92 ± 0.113 ^{c}	Nd
MC	71.29 ± 1.532 ^{ab}	13.12 ± 0.522 ^{b}	4.15 ± 0.130 ^{b}	8.96 ± 0.518 ^{abc}
M1	70.99 ± 1.090 ^{ab}	12.64 ± 0.625 ^{b}	4.15 ± 0.402 ^{b}	8.63 ± 0.438 ^{abc}
WC	92.31 ± 29.161 ^{ab}	17.19 ± 0.874 ^{a}	7.04 ± 0.242 ^{a}	17.75 ± 9.392 ^{a}
W1	94.49 ± 5.231 ^{a}	18.08 ± 1.225 ^{a}	7.43 ± 0.887 ^{a}	11.56 ± 1.503 ^{ab}
FC	58.40 ± 5.233 ^{ab}	9.65 ± 0.747 ^{bc}	5.26 ± 0.809 ^{ab}	7.88 ± 0.727 ^{bc}
F1	55.04 ± 3.251 ^{b}	9.12 ± 0.651 ^{c}	5.02 ± 0.382 ^{ab}	6.73 ± 0.856 ^{bc}
F2	57.57 ± 18.590 ^{ab}	10.87 ± 0.559 ^{bc}	6.39 ± 0.000 ^{ab}	7.95 ± 0.433 ^{bc}
F3	62.40 ± 5.930 ^{ab}	10.58 ± 0.178 ^{c}	6.51 ± 0.246 ^{ab}	7.83 ± 0.225 ^{bc}
F4	56.38 ± 10.530 ^{b}	9.55 ± 1.838 ^{c}	6.25 ± 1.983 ^{ab}	6.97 ± 0.909 ^{bc}
BC	Nd	Nd	0.58 ± 0.165 ^{c}	Nd
B1	Nd	Nd	0.75 ± 0.072 ^{c}	Nd
B2	Nd	Nd	0.57 ± 0.184 ^{c}	Nd
B3	Nd	Nd	0.58 ± 0.103 ^{c}	Nd
B4	Nd	Nd	0.62 ± 0.119 ^{c}	Nd
B5	Nd	Nd	0.91 ± 0.451 ^{c}	Nd

$^{a-d}$ Different letters in the same column represent significant differences between samples by the Tukey test (p < 0.05). Nd: not detected.

Alcohol and glycerol

The concentration of alcohol in the beers prepared in this study, with juçara fruit pulp, varied between 3.4 and 3.8% (w/v), with no statistically significant difference (p>0.05) between the fruit concentration (Table 3). Unlike other researches that produced beers with fruits with higher sugar contents, such as banana [46] and grape [10], the juçara fruit pulp addition did not influence the alcohol concentration of the beers, probably because of the low sugars (potential alcohol).

The average alcohol content of the beers was lower than those found for conventional beers (5.0 and 8.0%, w/v), as well as for other fruit beers (banana, grape, goji berry, cherry, raspberry, peach, plum, and orange), which had values >5.0% (w/v) [5,8,10,46]. Therefore, the produced juçara beers can contribute to the current trend to produce functional beers low in alcohol. Its consumption can promote the health of consumers since moderate alcohol consumption can provide benefits, especially cardioprotective effects [47].

Table 3 also presents glycerol contents for the beer samples, which did not differ statistically between treatments, on average 1.96 g L^{-1}. This value is within the normal range for beers (1 - 3 g L^{-1}) and close to the average found for Cornelian cherry beer (1.65 g L^{-1})[4]. Glycerol is an important indicator of the quality of fermented beverages, imparting beer viscosity, flavor intensity and influencing the resistance of yeasts to osmotic stress [48].

Table 3. Physicochemical properties of beers prepared with juçara fruit pulp (real extract, pH, color index, ethanol, and glycerol content).

Sample	Real extract (% m/v)	pH	Ethanol (g 100 mL^{-1})	Glycerol (g L^{-1})	Color index (SRM)
BC	4.58 ± 0.040 ^{a}	4.74 ± 0.064 ^{a}	3.68 ± 0.448 ^{a}	1.98 ± 0.263 ^{a}	3.2 ± 0.020 ^{a}
B1	4.76 ± 0.170 ^{a}	4.31 ± 0.035 ^{a}	3.67 ± 0.088 ^{a}	2.07 ± 0.120 ^{a}	4.9 ± 0.031 ^{b}
B2	4.64 ± 0.335 ^{a}	4.43 ± 0.170 ^{a}	3.78 ± 0.009 ^{a}	1.99 ± 0.286 ^{a}	5.9 ± 0.064 ^{b}
B3	4.63 ± 0.377 ^{a}	4.48 ± 0.141 ^{a}	3.46 ± 0.249 ^{a}	1.20 ± 1.335 ^{a}	8.5 ± 0.011 ^{c}
B4	4.48 ± 0.521 ^{a}	4.47 ± 0.057 ^{a}	3.83 ± 0.069 ^{a}	2.22 ± 0.296 ^{a}	16.0 ± 0.028 ^{e}
B5	4.42 ± 0.052 ^{a}	4.44 ± 0.120 ^{a}	3.53 ± 0.006 ^{a}	2.28 ± 0.065 ^{a}	11.1 ± 0.071 ^{d}

$^{a-e}$ Different letters in the same column represent significant differences between samples by the Tukey test (p < 0.05).

Color

Color is an important characteristic of the beer since it brings style information and is a primary sensory element impacting the purchase decision [49].

The results of the color analysis of the beer samples are shown in Table 3. Considering that juçara fruit contains high contents of anthocyanins, which are pigments responsible for their dark purple color when ripe [18], the beers showed a significant increase in the color index. The sample B4 presented a higher color index (16.0 SRM) due to the higher pulp concentration (40%). Studies carried out with beers produced with other fruits rich in anthocyanins, such as aronia berry [50] and raspberry [51], also showed an increase in color compared to the control. This increase is mainly related to the solubility of anthocyanins and increased extraction of these pigments by heating. However, extraction and thermal degradation of the anthocyanins from the fruits are competing [42, 53]. Jahn and coauthors [50] observed a decrease in the color after 45 min of boil performed during aronia beer brewing process. These data corroborate the results of the present study since the samples B1, B3, and B5, with the same pulp concentration (20%), showed statistically different results, probably due to heat treatment [41,52]. Compared to B5 (beer made with added pulp in the last stage of the production process - maturation), B1 (beer produced with pulp submitted to heat treatment at 100 °C for 1 hour) showed a 55.8% reduction in the color index.

In addition to the temperature, during the process conducted with juçara fruit pulp added to the mash, part of the pigment was adhered to the malt bagasse and was discarded after mashing, causing a further reduction of the color of B1. For sample B3 (produced with 20% of juçara fruit pulp added at fermentation), when compared to B5, a significant reduction in the color index by 23.4% was observed. This attenuation probably was caused by the adsorption of anthocyanin to the yeast cell walls, which were removed from the beer before the maturation stage [53].

TPC and antioxidant capacity

The results obtained for TPC and antioxidant capacity are shown in Table 4. The addition of juçara fruit pulp, regardless of the method used and/or added concentration, promoted a significant increase in the TPC of the beers. These results are consistent with earlier findings, which showed that the TPC in fruit beers, such as aronia, raspberry, apricot, and apple, was considerably higher than the standard beers [5,50]. However, the values of TPC obtained in this study for juçara beers (49.57 to 80.17 mg GAE 100 mL^{-1}) were higher than those found for the mentioned fruit beers (up to 46.5 mg GAE 100 mL^{-1}. Juçara fruit is a recognized source of phenolic compounds, being called "super fruit" due to this characteristic [12], which justifies its higher value of phenolic compounds in relation to other fruit beers. This can be an important advantage for beer produced with juçara fruit pulp since beers with high phenolic antioxidants content exhibit longer shelf life, better flavor and foam stability [51]. The beers B1, B3, and B5 (20% of juçara fruit pulp added at mash, fermentation, and maturation, respectively) presented statistically (p<0.05) different values of TPC (Table 4). B1 beer presented the lowest value. The heat treatment (boiling) probably caused a decrease in these compounds. B1 showed no significant difference from beer made with only 10% juçara fruit pulp (B2), demonstrating that adding the pulp in the mashing process reduced 50% of TPC. Therefore, adding the juçara fruit pulp at mash is not recommended for TPC preservation. Still, the B3 sample did not present a significant reduction in TPC when compared to B5. Relevant factor since anthocyanin adsorption occurs in the yeast cell wall (removed from the process), which could have decreased the TPC [53]. Similar data were reported in the evaluation of beers with the addition of Cornelian cherry. The best results were obtained for samples with the fruit added during fermentation and maturation [4].

B4 sample (beer with 40% of juçara fruit pulp added at fermentation) showed a higher TPC (80.17 mg GAE 100 mL^{-1}); however, there was no significant difference with B5 beer (20% of juçara fruit pulp added at maturation) (74.05 mg GAE 100 mL^{-1}) (Table 4). The addition of the pulp at a concentration of 20% showed the best yield and indicated the saturation point of the phenolic enrichment. It is important to highlight that the TPC values of B4 and B5 were higher than those reported for other fruit beers produced with goji berry, cherry, plum, grape, peach, and apple (33.5 – 63.1 mg GAE 100 mL^{-1}) [5,8].

Whereas the phenolic compounds are important antioxidants in beer, acting as receptors for free radicals [5,54], adding juçara fruit pulp increased the antioxidant capacity of the studied worts and beers. As shown in Table 3, the wort with 20% of juçara fruit pulp (W1) increased by 54.1% compared to wort control (WC). However, the B1 beer, generated by the W1 fermentation, showed the lowest antioxidant capacity due to the higher TPC losses by boiling. In agreement, Jahn and coauthors [50]also observed that beer produced with the aronia berry added after boiling is more effective in preventing losses in antioxidant capacity and consequently can increase the product stability.

B4 and B5 beers showed the highest values. However, unlike the TPC, B4 and B5 samples were statistically different. B4 presented an increase in antioxidant capacity of 331% in relation to the control, while for B5, this increase was 255%. Although B3 and B5, both with 20% pulp, did not show a statistical difference to TPC, B3 beer showed a significantly lower value for antioxidant capacity. It is important to consider that phenolic compounds are metabolized and modified during fermentation, which may decrease antioxidant capacity [55]. Still, during fermentation, the levels of anthocyanins can be reduced by 50%, affecting the antioxidant capacity of the beers [56]. Therefore, it is not recommended to add the pulp to this stage.

Thus, despite the higher antioxidant capacity observed for B4 beer, the best result was for beer B5, considering the higher yield concerning the concentration of pulp added.

Table 4. Total phenolic content and antioxidant capacity of the worts and beers prepared with juçara fruit pulp.

Sample	Total phenolic content (mg GAE 100 mL^{-1})	DPPH radical scavenging (mmol AAE 100 mL^{-1})
J	336.2 ± 56.99 ^{a}	7.901 ± 1.08 ^{a}
MC	52.33 ± 5.812 ^{d}	0.761 ± 0.004 ^{g}
M1	64.82 ± 14.906 ^{c}	1.949 ± 0.229 ^{e}
WC	69.78 ± 7.545 ^{c}	1.797 ± 0.081 ^{c}
W1	89.23 ± 11.052 ^{b}	3.321 ± 0.240 ^{b}
BC	43.08 ± 1.648 ^{e}	1.198 ± 0.151 ^{f}
B1	49.57 ± 0.021 ^{d}	1.785 ± 0.139 ^{e}
B2	52.68 ± 3.338 ^{d}	1.958 ± 0.223 ^{e}
B3	69.26 ± 3.132 ^{c}	2.452 ± 0.104 ^{d}
B4	80.17 ± 5.961 ^{b}	3.971 ± 0.023 ^{b}
B5	74.05 ± 0.389 ^{bc}	3.059 ± 0.253 ^{c}

$^{a-g}$ Different letters in the same column represent significant differences between samples using Tukey test ($p < 0.05$). AAE: ascorbic acid equivalent. GAE: gallic acid equivalent.

CONCLUSION

Juçara fruit is a berry with great nutritional and bioactive value due to its rich content of essential nutrients (mainly fatty acids, protein, dietary fiber, and minerals) and a great variety of phenolic compounds. Therefore, this study investigated the influence of the addition of juçara fruit pulp on the quality characteristics, phenolic content, and antioxidant potential of beer. Considering the low sugar content, most physicochemical properties of the wort and beer were not affected by the addition of juçara fruit pulp. On the other hand, its addition significantly increased the color, the phenolic compounds, and the antioxidant capacity of the beers, but with variations according to the brewing step in which it was added (mash, fermentation, or maturation). In fact, significant losses of these compounds occur during the heat treatment applied in the mashing and during the fermentation. Therefore, the incorporation of 20% of juçara fruit pulp during the maturation is the best option, as it ensures a greater preservation of the bioactive compounds and greater antioxidant capacity.

This was the first work that evaluated the addition of juçara fruit pulp in beer and demonstrated its enormous potential for application in the brewery sector. The addition of this product can enrich the beneficial effects already reported for the consumption of traditional beer by incorporating additional bioactive compounds with potential health benefits. In addition, the data from this study may contribute to the consumer market and expand the production of this fruit, a promising and sustainable raw material, with benefits for the Atlantic Forest preservation and regeneration of the species' population.

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