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Abstract: The aim of this work was to produce and evaluate the quality of craft beers of the Witbier type, with different levels of acerola pulp (Malpighia emarginata) added. The production process involved milling, mashing, boiling, and adding hops, coriander, and acerola pulp, followed by yeast inoculation, fermentation, carbonation, bottling, and maturation. The physical-chemical analyses carried out included soluble solids content, pH, total acidity, dry matter, mineral matter, foam stability, color, determination of phenolic and total flavonoid contents, antioxidant activity, and acceptability testing. The experimental design was completely randomized, with four treatments and four repetitions, namely T1, T2, T3, and T4, with 0%, 5%, 10%, and 20% (v/v) of pulp inclusion, respectively. The addition of acerola resulted in beers with higher acidity and lower pH, a higher percentage of mineral matter, and more stable foam. There were no significant differences in the dry matter and flavonoid content between the beers. The antioxidant activity and phenolic compound content increased linearly with the increase in acerola concentration.

Keywords: Physical-chemical analysis; Antioxidant activity; Fruit beer; Phenol contents; FRAP; ABTS

1. INTRODUCTION

Beer, being one of the oldest and most popular fermented beverages, is highly valued for its quality, which includes sensory, physical-chemical, and biochemical properties.[1] Its components and its production process originate a drink rich in micronutrients and with antioxidant and anti-inflammatory properties.[2] According to Matsubara et al., (2016), beer has a diuretic effect that helps in kidney functioning and the control of hypertension, while its moderate consumption helps in the prevention of coronary diseases.[3]

In Brazil there are about 1500 breweries registered with the Ministry of Agriculture, Livestock, and Supply (MAPA), guaranteeing the country the third place among the largest beer producers in the world.[4-5] According to Decree No. 9.902/19, beer is a beverage obtained by the alcoholic fermentation of malt wort or its extract, previously cooked with the addition of hops or its extract, and part of the malt or malt extract can be partially replaced by brewing adjuncts.[6] For fermentation, yeast strains of the genus *Saccharomyces* are commonly used, especially *Saccharomyces cerevisiae* (Ale yeasts) and *Saccharomyces pastorianus* (Lager yeasts).[7]

According to Brazilian legislation, the mandatory ingredients in the production of beer include water, malt or its extract, and hops (except "fruit beer"), with the optional addition of brewing adjuncts, which can replace up to 45% of the weight of the beer. Primitive extract, vegetable, and animal raw materials suitable for human consumption, and yeasts and other fermenting microorganisms can be used (Instrução Normativa do MAPA nº 65/2019).

According to Rosa and Afonso (2015), the water used in brewing must have a controlled pH, free of turbidity, and be clean. The use of pH adjustment for each style of beer and the calibration of water properties through the manipulation of mineral salt concentrations are possible due to new technologies and have resulted in improved water quality for each type of beer.[7]

Malt is the grain resulting from the barley malting process. During this process enzymatic activation occurs, especially the enzymes that degrade starch to simple sugars, the α, and β-amylases. Malt has the role of providing aroma and flavor, color, foam, and body of the beer.[9]

Hops contain essential oils, polyphenols, and resin in their constitution, contributing to antioxidant activity, aroma, flavor, bitterness, and retention of beer foam.[2] The bitterness of hops is related to their concentration of α-acids, which during the boiling process are isomerized to iso-α-acids, on the other hand, their essential oils give the aroma to beer.[10]

Yeasts are responsible for the fermentation process for the conversion of raw material sugars into ethyl alcohol. Depending on the type of yeast used, beers can be classified into two types: Ale (high fermentation) and Lager (low fermentation). Ale-type beers are characterized by high fermentation, the yeast species used is *S. cerevisiae*, which accumulates in the upper part during the fermentation process.[9] According to Melo and Soares (2019), in top-fermentation beers, substances with fruity aromas and spices are released, which contribute to the type of beer.[11]

As per the Beer Judge Certification Program (BJCP), Witbier beers belong to the Ale family and are Belgian wheat beers. Their ingredients include unmalted wheat, light barley malt, and spices like coriander seeds and sweet orange peel that enhance their sweet aroma. These beers range in color from pale yellow to golden yellow, have a consistent foam that retains well and possess a hazy appearance with a refreshing citrus taste. They also have a low to medium-low hop bitterness.[12]

As the understanding of chemical processes and hygiene improved, beer production processes have expanded, resulting in improvements in beer quality, ingredient variety, alcohol content, and other characteristics over time.[13] Craft beer production is steadily increasing, thanks to the vast range of raw materials and the potential for creating new formulations during brewing. This results in craft beers offering diverse characteristics such as flavor, aroma, color, and bitterness, among others, that differ from those of industrial beers.[14-15] Known as premium or special beers, craft beers have increased their consumer audience, looking for a quality product, with differentiated and unique flavors. In this way, investment in research and innovation in this area is essential, in addition, this sector proves to be an interesting business possibility for entrepreneurs.[16-17]

The use of fruits as an adjunct in the production of beers results in the diversity of products with distinct characteristics that arouse the interest of the consumer. Brazil has a great diversity of fruits and is also one of the largest producers in the world.[18] Being rich in minerals, fibers, and vitamins, in addition to essential nutrients, they collaborate to improve the quality of the product, its nutritional value, and its biochemical properties due to the presence of phenolic compounds and bioactive compounds that contribute to the increase of antioxidant activity.[19] The fruits used in the production of beers guarantee the diversity of aromas, specific flavors, colors, bitterness, and foam quality, which generally present satisfactory acceptance by consumers.[20]

Acerola (*Malpighia emarginata* DC) is a tropical fruit, produced mainly in the northeast region of Brazil, has a high content of vitamin C, and is a source of anthocyanins and carotenoids, which have antioxidant action.[21] In fruits, the most common antioxidants are phenolic compounds. Phenolic compounds play important roles in the formation of flavor, aroma, astringency, and body and can contribute to ensuring the antioxidant potential of the diet, in addition to improving foam stability and ensuring longer shelf life than in beers with lower antioxidant activity.[22]

The present work aimed to produce and evaluate craft beers of the Witbier type with levels of inclusion of acerola pulp on the physicochemical properties, the content of phenolic and flavonoid compounds, and antioxidant activity. The introduction of Witbier craft beer infused with acerola pulp opens the door to sustainability, allowing brewers to source ingredients locally and promote ecological responsibility. The addition of acerola to beer, in addition to adding nutritional value, makes it possible to offer consumers a beer with a different flavor and aroma. Through comprehensive characterization and analysis, this pioneering effort not only enriches the realm of craft brewing, but also advances scientific understanding, thereby expanding the narrative of innovation in the beverage industry.

2. MATERIAL AND METHODS

2.1. Material

The following ingredients were used to produce a Witbier craft beer: Chateau Pilsen 2-Row malt from Castle Malting (Beloeil, Belgium), flaked wheat, East Kent Golding hops (Kent, UK) for bittering and aroma (α-acid 4.6%), coriander, and Saccharomyces cerevisiae commercial brewer's yeast (SafAle T58 – Fermentis, Marcq-en-Baroeul, France). Mineral water and previously pasteurized acerola pulp purchased from local shops were used in the beer manufacturing process.

2.2. Beer production

2.5 kg of Chateau Pilsen 2-Row malt from Castle Malting, 2.5 kg of flaked wheat, 25 g of East Kent Golding hops for bittering and aroma (α-acid 4.6%), 10 g of coriander, and 8 g of S. cerevisiae commercial brewer's yeast were used to produce a Witbier craft beer. Fruit sanitization followed Bezerra's (2009) methodology,[24] starting with pre-washing and sanitizing with sodium hypochlorite (10 mL L^{-1} for 20 min), then they were manually pulped and packed in polyethylene bags. Pasteurization of the pulp was adapted according to the methodology of Igual et al. (2014),[23] with heating of 2 L of acerola pulp in a thermostatic bath until reaching 30 ºC followed by a conditioning in a microwave oven at maximum power (1000 W) for 5 min. In this condition, the sample reached 83 ºC.

After the pasteurization treatment, the pulp was packed in polyethylene plastic bags. The pasteurized and bottled fruit pulps were stored in the refrigerator at 4 °C until the next day when the beer was produced.

Water mineral used in mash was adjusted related to its mineral content to Ca^{2+} 55 mg L^{-1}, Mg^{2+} 3 mg L^{-1}, Na^{+} 7 mg L^{-1}, Cl^{-} 59 mg L^{-1}, SO_4^{2-} 51 mg L^{-1}, and alkalinity equivalent to 35 mg L^{-1} HCO_3^{-}. Adjustments was made using brewfather plataform and salts $CaCl_2$, NaCl, $MgSO_4$, $MgCl_2$ and $Ca(OH)_2$ (Synth, Diadema, Brazil).

The malt and wheat grains were ground using a cereal grain mill (B03 – Botini grain crusher). To prepare the wort, 20 L of water was added to a single vessel (Brewhome 20 L – Brewbeer, São Paulo, Brazil) that was heated to 50 ºC. Soon after, the ground grains were added to it and kept for 20 min (protein break and ferulic rest) at this temperature. Then, the temperature was raised to 65 °C for 60 min for the saccharification process. Subsequently, the must temperature was raised to 76 ºC for 10 min to inactivate the enzymes and to reduce wort viscosity. During all the mentioned steps, the continuous recirculation of the wort was maintained. Then, the malt was washed with 8.8

L of water previously heated to 76 ºC. Soon after, the boiling step began, which lasted 60 min. At the beginning of the boiling process, hops were added expecting as result 15 IBU of bitterness, and after 55 min of boiling, coriander was added.

To cool down the wort, a plate-type heat exchanger was used with water as the coolant. Next, 16 fermenters were filled with 0.768 L of wort and 0.5 grams of yeast each. In order to normalize plato degree among the different conditions evaluated, complementary diluted grain wort was created by diluting grain wort from mashing with water to achieve the same plato degree as the acerola pulp (6 ºP), and then this solution and/or acerola pulp was added to each fermenter to complete the final volume (960 mL, Table 1). In this way, all condition started fermentation at the same plato degree (13,4 ºP). Four fermenters were designated for each pulp concentration percentage (0%, 5%, 10%, and 20% V/V), resulting in a total of 16 fermenters. The fermentation process was conducted at 20°C for 9 days (Figure 1).

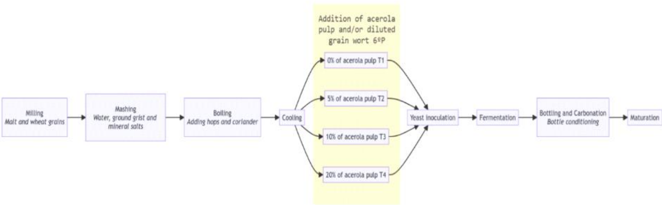

Figure 1. Basic flow of beer production.

At the end of fermentation, the beer was submitted to the carbonation and maturation process for 40 days, in glass bottles with a capacity of 250 mL, in which 5 mL of a solution of 6 g L^{-1} of sugar with 5 mL of lemon was added.

Table 1. Craft beer formulations added with different concentrations in percentage (v/v) of acerola pulp.

Treatments	Beer wort volume (L)	Acerola pulp volume (L)	Diluted grain wort 6 ºP volume (L)	Final Volume (L)
0% of acerola pulp (T1)	0.768	0.000	0.192	0.960
5% of acerola pulp (T2)	0.768	0.048	0.096	0.960

10% of acerola pulp (T3)	0.768	0.096	0.048	0.960
20% of acerola pulp (T4)	0.768	0.192	0.000	0.960

2.3. Physicochemical analysis

2.3.1. Degree Plato (ºP)

The soluble solids content was measured in an analog refractometer (Vodex VX25-40, Southampton, US). For the content of soluble solids during fermentation kinetics, readings were performed during the fermentation period of 9 days.

Original gravity and final gravity were calculated according to equations 1 and 2 below:[25]

$$OG = 1,000 + (P_i x 4) \quad (1)$$

$$FG = 1.001843 - 0.002318474 x P_i - 0.000007775 x P_i^2 - 0.000000034 x P_i^3 + 0.00574 x P_f + 0.00003344 x P_f^2 + 0.0000000 x P_f{}^3 \quad (2)$$

2.3.2. Apparent attenuation (%)

Apparent attenuation (AA) was calculated from the original and final gravity values of the beers (expressed as ºP) according to equation 1 below:[25]

$$AA = \frac{OG - FG}{OG} x 100 \quad (3)$$

where, OG= Original Gravity; FG= Final Gravity.

2.3.3. Estimation of alcohol content (% ABV)

The estimated alcoholic strength (% ABV) was calculated from the original and final gravity values of the beers using the simplified equation 2. This equation is commonly used to a fast and roughly ABV estimation.

$$ABV = (OG - FG) x 131.25 \quad (4)$$

where, OG= Original Gravity; FG= Final Gravity. Both expressed as specific gravity.

2.3.4. Total acidity

According to the methodology of Celso et al. (2013), the beer samples were placed in a beaker and then placed in the ultrasound device for 90 s to decarbonate the beer.[26]

In a beaker, 20 mL of the beer sample, 80 mL of distilled water, and 3 drops of phenolphthalein were added. Then, the sample was titrated with a 0.1 mol L^{-1} NaOH solution, with the aid of a magnetic stirrer, until a stable pink color was obtained. The sodium hydroxide solution was standardized with potassium bi-phthalate P.A, obtaining a normality value for the NaOH solution of 0.0846 mol L^{-1}. The acidity was calculated using equation 5:

$$TA\left(meEqL^{-1}\right)=\frac{1000xVnxN}{V} \quad (5)$$

where Vn = volume of NaOH solution used (mL); N = normality of the NaOH solution; V = sample volume (mL).

According to AOAC 950.07.25, the acidity expressed in lactic acid considers that 1 mL 0.1 mol L^{-1} NaOH is equivalent to 0.0090 g of lactic acid, thus considering the adjustment in the concentration of the NaOH solution obtained in the standardization (1 mL 0.0846 mol L^{-1} NaOH is equivalent to 0.007614 g of lactic acid), to express the acidity in lactic acid, use the following equation 6:

$$(6) \quad Acidity \in lacticacid=\left(\frac{Vnx\,0.007614}{V}\right)x\,1000g\ lactic\ acid$$

where Vn = volume of NaOH solution used (mL); V = sample volume (mL).

2.3.5. Determination of dry matter and mineral matter

The analysis of dry matter and mineral matter (ash) of beer was adapted according to the methodology of Cunha et al., (2023).[27] For the determination of the dry matter, the crucibles were previously identified and weighed on an analytical balance to obtain the tare value, 40 mL of the samples were placed in the crucibles, subsequently taken to an oven at 105 °C for 17 h, then transferred into a desiccator for 30 min for final weighing. For the determination of mineral matter (ash) the crucibles were placed in the muffle at 600 ºC for 4 h and weighed to determine the mineral matter.

2.3.6. pH analysis

The pH analysis was carried out using a HANNA EDGE® LCD 5.5 pH meter (Tamboré Barueri, Brazil), previously calibrated with pH 7.0 and 4.0 standard solutions.

2.3.7. Color analysis

Color analysis was performed according to Clements et al., (2001),[28] using the Tristimulus system using the L*a*b* color space proposed by the Commission Internationale de l'Eclairage (CIE), also known as CIELab.

Beer samples were previously filtered using the membrane filtration method [28], with a thickness of 0.35 mm and porosity of 0.6 µm. To carry out the analysis, a UV spectrophotometer (UV-1100 – Kasuaki, Jiangsu, China) was used. Transmittance spectra were obtained in an acquisition range (380-780 nm) with a gap of 5 nm. From the transmittance spectra, the color values L* a* b*, associated with luminosity, red to green and yellow to blue, respectively, were calculated. The equations below were used to calculate the tristimulus X, Y, Z values and, from these, the L* a* b* values.

(7)

(8)

(9)

(10)

(11)

(12)

where T(λ)= transmittance value at each wavelength λ, S(λ)= the spectral power distributions at each λ for the illuminant pattern C (Table 4). x10(λ), y10(λ), z10(λ)= color matching functions, standard observer C at each wavelength λ (Table 4). X, Y, Z = tristimulus values representing red, blue, and green.

According to the American Society for Testing and Materials (ASTM, 2001),[29] the white point values are:

$$X_n = 97.285,\ Y_n = 100.00,\ Z_n = 116.145$$

(13)

where k corresponds to the normalization factor and can be calculated directly from Table 4.

2.3.8. Foam analysis

Foam analysis was performed according to Nyarko et al., (2021).[30] To pour the beer in a standardized way, tweezers with an oval claw were used, fixed at a height of 30 cm to universal burette support. The beer was poured into a tall beaker with a volume of 1 L. To measure the foam, a ruler was fixed in a vertical position next to the beaker. The beers stored in 250 mL bottles were poured so that they flowed to the bottom of the beaker continuously, with records of the foam height and liquid beer height at elapsed times of 0.5, 15, 30, 45, 60, 90, 120, 150, and 180 s. To help record the decay of the foam, a Canon EOS Rebel SL2 camera was used.[30]

Four samples were used per treatment and the average data were used to calculate the foam values according to the following equations:

For foam height (F) in centimeters for each measuring time (t):

$$F=T - L \tag{14}$$

Where F= foam height; L= beer liquid; T= total height.

The mean foam height data were used to obtain the intercept (a) and slope (b) values, correlating the least squares of the natural logarithm of the foam height and the measurement time according to the following equation:

$$\ln(F_t)=a+bt \tag{15}$$

Other foam attributes were calculated using the coefficients determined by equation 13.

$$\text{Head (after the first minute of pouring), cm}=e(a+b) \tag{16}$$

$$\text{Half-life (HL), min} = \ln(0.5)/b \tag{17}$$

$$\text{Life, min} = -a/b \tag{18}$$

$$\text{Density (g.mL-1)}= (L2.5-L1)/Head1 – Head2.5 \tag{19}$$

$$\text{Quality (Q)} = 10 \text{ x HL x Density} \tag{20}$$

$$\text{NHL-1, min} = (10 \text{ x HL})/\text{Head} \tag{21}$$

$$\text{NHL-0, min} = (10xHL)/F0=(10xHL)/(ea) \tag{22}$$

2.4. Biochemical analyzes

2.4.1. Content of total phenolic compounds (TPC)

For the quantification of total phenolics compounds, the Folin-Ciocalteau method was used according to the protocol reported by Julião et al. (2020).[31] The analysis was performed in triplicate, with the reaction conducted in a basic medium composed of 250 µL of Folin-Ciocalteau reagent (Sigma-Aldrich, St. Louis, US), 50 µL of diluted beer sample (1:10, v/v) and 1 mL of distilled water. After 5 min, 750 µL of 20% sodium carbonate (w/v) and 2.95 mL of distilled water were added. The quantification was performed by measuring the absorbance in a spectrophotometer at 798 nm. The result was obtained through linear regression through the standard curve of gallic acid (Sigma-Aldrich, St. Louis, US) and expressed in milligram equivalent of gallic acid per liter of the sample (mgEq GA L^{-1}).

2.4.2. Total flavonoid content (TFC)

The determination of the total content of flavonoids was carried out according to the procedure reported by Salgueiro et al. (2014).[32] The test was performed in triplicate, submitted to the medium containing 2 mL of diluted beer sample (1:10, v/v) and 2 mL of 2% (m/v) methanolic aluminum chloride solution, for each sample a so-called blank sample was prepared using 2 mL of non-alcoholic beer. diluted and 2 mL of distilled water. After 30 min of incubation, absorbance readings were taken at 415 nm using distilled water as a blank. Total flavonoid content, expressed in milligrams of quercetin per liter (mgEq Q L^{-1}), was calculated using a seven-point calibration curve.

2.4.3. Ferric Reducing Antioxidant Power (FRAP)

The antioxidant power of reduction of ferric ion Fe^{3+} (FRAP) was determined according to the methodology of Rufino et al., (2007).[33] The test was performed in triplicate, submitted to reactions in the proportion of 200 µL of diluted beer sample (1:10, v/v), except for the control beers, in which 50 µL of the undiluted sample, 150 µL of distilled water, and 4 mL of FRAP reagent were used. The quantification was carried out by measuring the absorbance at 593 nm. The result was obtained using a seven-point standard curve of FeSO4 and expressed in mmol Fe^{2+} per L of the sample (mmol Fe^{2+} L^{-1}).

2.4.4. DPPH assay

The scavenging capacity of the stable radical 2,2-diphenyl-picrylhydrazyl (DPPH) (Sigma-Aldrich, St. Louis, US) was performed according to Al-Sayyed et al., (2022).[34] The test was performed in triplicate, submitted to reactions in the proportion of 200 µL of beer sample and 2.8 mL of DPPH 8x10-5mol L-1ethanolic solution reagent, absorbance reading was performed at a wavelength of 517 nm. Results were calculated from a five-point analytical curve of a-tocopherol (Sigma-Aldrich, St. Louis, US) and expressed in milligrams of a-tocopherol per L of the sample (mgEq T L^{-1}).

2.4.5. ABTS assay

Determination of antioxidant activity was performed using the method described by Rufino et al., (2007).[33] The test was performed in triplicate, submitted to reactions in the proportion of 100 µL of the diluted sample (1:10), 200 µL of distilled water, and 2.7 mL of ABTS solution (Sigma-Aldrich, St. Louis, US). Absorbance readings were taken at 734 nm using distilled water as a blank. Data were calculated from a five-point calibration curve of α-tocopherol (Sigma-Aldrich, St. Louis, US) and expressed in mg α-tocopherol per L of the sample (mgEq T L^{-1}).

2.5. Statistical analysis

Data from physical-chemical analyses, except for the analysis of color, phenolic compounds, and antioxidant activity were submitted to analysis of variance and, when significant ($P<0.05$), regression analysis was performed. Data analysis was performed using the SISVAR 5.6 statistical software.

3. RESULTS AND DISCUSSION

3.1. Physicochemical analysis

3.1.1. Fermentative kinetics of soluble solids content

Figure 2 shows the attenuation profile of the wort during the fermentation of the monitored beers produced.

Figure 2. Degree Plato (ºP) during the fermentation kinetics of the brewer's wort of craft beers with different concentrations of acerola pulp.

The degree plato of the samples were reduced according to the consumption of fermentable sugars present in the samples by the yeasts. In 14 h of fermentation, the soluble solids dropped from 13.4 (ºP) to values close to 10 (ºP) for all samples. During the whole period, it is possible to observe the gradual reduction of the soluble solids content, in the first 60 h of the fermentation the maximum deceleration of the consumption of substrate by the yeasts occurred.

Among the beers with the addition of acerola pulp, the kinetics of the T3 sample showed a lower degree plato (ºP) at the end of fermentation compared to the T2 and T4 samples, the control sample had the highest soluble solids content. Fernandes (2017) obtained similar results when evaluating the degree plato (ºP) during the fermentation kinetics of craft beers added with acerola Blonde Ale, the samples with acerola obtained equal and lower values of degree plato (ºP) at the end of fermentation, in comparison with beer without adding pulp.[35] In view of this, it is possible to observe that the difference in the residual sugars of the beers with acerola pulp in relation to the control beer, may be related to the differences in wort and its concentration of fermentable and non-fermentable sugars.

It is possible to observe in the graph that the fermentation of the T4 beer attenuated faster, with about 85 h than the T3 and T2 beers, which presented attenuation at 108 h and 157 h, respectively. The T1 beer attenuated with about 44 hours of fermentation. The values of apparent attenuation and alcohol content at the end of fermentation showed significant differences ($P<0.05$) (Table 2).

Table 2. Physical-chemical analyzes of craft beers with the addition of different levels of acerola pulp.

Variables	Average±SD

	0% of acerola pulp (T1)	5% of acerola pulp (T2)	10% of acerola pulp (T3)	20% of acerola pulp (T4)
Apparent attenuation (%)	79.25 ± 0.00^{b}	84.02 ± 2.01^{a}	87.04 ± 0.00^{a}	85.09 ± 3.90^{a}
Alcoholic graduation (% ABV)	5.64 ± 0.00^{b}	$5.91 \pm 0.20^{a,b}$	6.17 ± 0.00^{a}	$6.04 \pm 0.34^{a,b}$
Soluble solids (ºP)*	7.00 ± 0.00^{a}	$6.65 \pm 0.10^{a,b}$	6.40 ± 0.00^{b}	6.55 ± 0.30^{b}
Total acidity (mEq L$^{-‘}$)	37.86 ± 0.24^{d}	42.51 ± 2.09^{c}	49.17 ± 1.97^{b}	61.02 ± 2.96^{a}
Total acidity (g of lactic acid L^{-1})	3.41 ± 0.02^{d}	3.83 ± 0.19^{c}	4.43 ± 0.18^{b}	5.49 ± 0.27^{a}
Dry matter (%)	4.43 ± 0.07^{a}	4.40 ± 0.06^{a}	4.46 ± 0.02^{a}	4.44 ± 0.05^{a}
Mineral matter (%)	3.69 ± 0.06^{c}	$3.97 \pm 0.06^{b,c}$	$4.20 \pm 0.28^{a,b}$	4.57 ± 0.34^{a}
pH	4.50 ± 0.03^{a}	4.20 ± 0.00^{b}	4.04 ± 0.01^{c}	3.89 ± 0.01^{d}

*Values not ethanol corrected. Means followed by the same letters in the lines, do not differ significantly by Tukey's test, at 5% probability ($P<0.05$). SD (standard deviation).

Beer T3 showed higher apparent attenuation and alcohol content at the end of fermentation of 87% and 6.17%, respectively. The range of alcoholic strength for Witbier beers, according to the Beer Judge Certification Program (BJCP) (de Borba et al., 2020),[12] is 4.5 to 5.5%, therefore, the values obtained in this work did not fall within the standard range for this style. These results are similar to those found by Costa et al., 2020,[36] when producing Witbier beers with added ginger, they obtained alcoholic strength values between 6.5% and 6.7%, in addition to that, the control sample also did not present values within the standard for this style.

The values of soluble solids differed statistically ($P<0.05$) with a linear reduction in the ºBx contents of the beers ($P<0.05$) with the addition of acerola pulp, estimating a reduction of 0.0197% for each unit of acerola pulp added to beer. The samples with the inclusion of acerola pulp had lower ºBx values than the control beer. According to Mutz, et al., (2021), beers added with fruit pulp have most of the soluble solids composed of fermentable sugars.[37]

Regarding the total acidity expressed in (mEq L^{-1}) and (g L^{-1} of lactic acid), a linear increase ($P<0.05$) of 1.1758 and 0.1058, respectively, was observed for each 1% addition of acerola pulp added to beer. The T1 beer had the lowest acidity and the T4 beer had the highest acidity. These results are in line with those obtained by Pinto (2015),[38] who found an increase in beer acidity with the addition of acerola pulp. The total acidity in beers may be related to the type of production and raw materials used, in addition, the organic acids produced during alcoholic fermentation are also responsible for the increase in acidity and drop in pH.

There was a linear decrease in the pH values of the beers with the addition of acerola pulp, estimated at 0.0290 for each 1% of added pulp. The T4 beer obtained a more acidic pH of 3.89, a result equivalent to the total acidity. The pH results for all samples followed similar behavior to those verified for total acidity, so that the higher the level of inclusion of acerola pulp, the lower the pH. Values within this range were obtained by Pinto et al., 2015,[38] ranging from 4.10 to 4.24 for Pale Ale beers, with the addition of acerola and pineapple pulp. According to Fernandes (2017),[35] the fruits added to beer production tend to decrease the pH, reducing the probability of contamination and preserving the characteristics of the beer.

Regarding the mineral matter (dry extract) of the analyzed samples, there were no significant differences ($P<0.05$), with an average of 4.42%. According to Sousa and Fogaça (2018),[39] dry matter is related to the resulting amount of solids present in the beer composition, which undergo a process and water evaporation, a good quality beer has values above 3.0% of dry matter. Thus, all samples produced have the indication of good quality in relation to this aspect. The results are similar to the values found by Brito Júnior (202),[9] who obtained average dry extract values of 4.5 for six Fruit Beers produced with juçara fruit pulp. Souza et al., (2020) obtained a real extract value lower than that found in this study of 3.58% in Pilsen beer with acerola pulp.[40]

In the analysis of the mineral matter, a linear increase of 0.0433 was observed for each 1% addition of pulp. The values obtained in the present study were higher than that obtained by Ngafwan et al., (2021),[41] of 2.38% for craft beer in the Blonde Ale style with the addition of araçá-boi. Fruits are important sources of essential minerals for the human diet, acerola has a composition of minerals such as calcium, magnesium, and potassium, among others.[42]

3.1.2. Color analysis

Regarding the results of the color analysis, Table 3 presents the average values of the color parameters, where L* indicates variation in luminosity, ranging from white (L=100) to black (=0), a* which varies from red (+a*) to green (-a*) and b* which indicates the color variation from yellow (+b*) to blue (-b*). The average values of L* differed ($P<0.05$) between the samples, being between 92.90 and 86.98 close to white (L=100), characterizing the beers with a light hue. There was a significant difference between a* values ($P<0.05$), samples T1, T2, and T4 obtained negative values, and sample T3 obtained a positive a* value, the values of all samples were close to zero. The b* parameter also differed significantly ($P<0.05$), the samples showed positive values ranging from 35.28 to 18.18, indicating a yellow color.

Table 3. Mean and standard deviation of CIE Lab-System color attributes (L*, a*, b*) for control and acerola-added craft beer samples.

Treatment	Average±SD		
	L*	a*	b*
0% of acerola pulp (T1)	92.90 ± 0.46^{a}	-0.60 ± 0.32^{b}	18.18 ± 0.34^{d}
5% of acerola pulp (T2)	88.48 ± 0.41^{b}	-0.24 ± 0.12^{ab}	27.30 ± 0.44^{c}
10% of acerola pulp (T3)	86.98 ± 1.32^{b}	0.10 ± 0.32^{a}	30.51 ± 0.62^{b}
20% of acerola pulp (T4)	88.30 ± 0.93^{b}	-0.42 ± 0.13^{ab}	35.28 ± 1.01^{a}

Means followed by the same letters in the columns, do not differ significantly by Tukey's test, at 5% probability ($P<0.05$). SD (standard deviation), a*(green-red chroma), b*(blue-yellow chroma), L*(brightness).

However, despite the significant statistical differences observed between the samples for the color parameters, the differences were small, resulting in little visual difference in the appearance of the produced beers. Thus, analyzing the values of a* in relation to those of b*, all samples showed a more pronounced yellow color. These color characteristics, according to the BJCP (de Borba et al., 2020),[12] are within the standard of Witbier-type beers, which have a straw-yellow to very light golden color.

3.1.3. Foam analysis

Foam is one of the most important attractive features of beer for consumers. Its stability is related to the external quality of the beers and is determined by the adequate addition of hops, the presence of metallic ions in the wort, and the existence of hydrophobic surface-active proteins (polypeptides) of the albumin class.[43-44] Table 4 shows the average results of the evaluated foam attributes presented: foam height, half-life, life, density, and normalized half-life (NHL-1 and NHL-0).

Table 4. Mean and standard deviation for the attributes of the foam analysis performed with the control craft beer samples and with the addition of acerola pulp.

Attributes	Average±SD			
	0% of acerola pulp (T1)	5% of acerola pulp (T2)	10% of acerola pulp (T3)	20% of acerola pulp (T4)
Head* (cm)	4.01±0.18^{a}	2.78±0.64^{b}	3.54±0.32^{a,b}	3.72±0.86^{a,b}
Half-life (min)	1.09±0.90^{b}	1.20±0.22^{b}	1.49±0.07^{a,b}	1.92±0.38^{a}
Life (min)	3.17±0.14^{b}	2.80±0.71^{a,b}	3.70±0.23^{a,b}	4.66±1.30^{a}
Density (g mL$^{-1)}$	0.19±0.04^{a,b}	0.16±0.01^{b}	0.17±0.02^{b}	0.23±0.0 ^{a}
Quality	2.08±0.45^{b}	1.92±0.46^{b}	2.47±0.30^{b}	4.36±0.97^{a}
NHL-1 (min)	2.72±0.34^{c}	4.38±0.37^{a,b}	4.23±0.47^{b}	5.24±0.60^{a}
NHL-0 (min)	1.44±0.26^{c}	2.42±0.19^{b}	2.65±0.33^{b}	3.60±0.38^{a}

*Estimated height of foam after one minute from pouring. SD (standard deviation). NHL-1: Normalized half-life 1. NHL-0: Normalized half-life 0. Means followed by the same letters in the lines, do not differ significantly by Tukey's test, at 5% probability ($P<0.05$).

According to the results of foam height during the first minute after serving the beer, T1 had the highest mean height compared to the other beers with acerola. Regarding the half-life and life of the beers, sample T4 showed the longest times for both. The half-life is related to the stability of the foam, which together with its quantity influence the lifetime. Thus, the

T4 beer presented a more stable foam than the others, in addition, it is possible to observe that by increasing the inclusion of acerola pulp, there is an increase in the half-life of the foam.

The calculated density values for 1 min ranged from 0.34 g mL^{-1} for T2 beer to 0.22 g mL^{-1} for T4 beer. The density of the foam is caused by important components such as hops and proteins, the rapid dispersion of the foam can indicate the small amount of these components. In this work, the calculated density is dimensionless, but the unit g mL^{-1} is assigned because it is assumed that the beer density is ~1 g mL^{-1}.

The quality assessment carried out in this work was proposed by Nyarko et al., (2021),[30] to provide a general assessment of foam performance, considering density and half-life values. Thus, beer T2 had the highest average quality value of 4.44, and beer T1 had the lowest average of 3.53.

Normalized half-life values represent a corrected expected value for samples with a height (NHL-1) or initial foam (NHL-0) of 10 cm, or 10 times a single-point slope of the half-life for the height of the half-life. foam. In the analysis of normalized half-life 1 (NHL-1) and normalized half-life 0 (NHL-0), the T4 sample had the highest mean compared to the other samples. It is observed that sample T1 produced more foam, but the foam produced by sample T4 showed greater stability.

3.2. Biochemical analyzes

The results of the biochemical analyzes of total phenolic compounds, and antioxidant activity by FRAP, DPPH, and ABTS methods, and the flavonoid content are presented in Table 5.

Table 5. Average of the acceptability test performed with the control craft beer samples and those with acerola added.

Variables	Average±SD			
	0% of acerola pulp (T1)	5% of acerola pulp (T2)	10% of acerola pulp (T3)	20% of acerola pulp (T4)
Total phenolic compounds (mgEg GA L^{-1})	399.6±24.64[d]	908.1±97.49[c]	1,549.2±184.72[b]	2,486.4±266.06[a]
FRAP	1.01±0.05[b]	0.84±0.19[b]	2.23±0.16[b]	5.34±1.32[a]

(mmol Fe^{2+} L^{-1})				
Total flavonoids (mgEq Q L^{-1})	463.57±5.88^{a}	466.68±14.17^{a}	407.04±96.08^{a}	477.25±33.48^{a}
DPPH (mgEq GA L^{-1})	129.10±22.25^{c}	168.53±22.05^{bc}	210.13±13.95^{b}	368.38±12.91^{a}
ABTS (mgEq T L^{-1})	90.76±42.57^{b}	212.89±6.54^{b}	777.71±9.99^{a}	765.98±163.43^{a}

Means followed by the same letters in the lines, do not differ significantly by Tukey's test, at 5% probability P<0.05). SD (standard deviation).

There was a linear increase (P<0.05) of 105.1582 for each 1% addition of acerola pulp added to beer. The beers with the addition of acerola had higher concentrations of total phenolics when compared to the control beer, the T4 beer had the highest average. These data show the direct effect of the addition of acerola pulp on the phenolic profile of the beers produced. The results found in this study are higher than those found by Zhao et al. (2010) in their analysis of 34 samples of commercial beers, which showed a variation in the content of phenolic compounds between 152.01 mg GAE L^{-1} and 339.12 mg GAE L^{-1}.[51] The high concentration of phenolic compounds, in addition to coming from the malt and hops present in the beer, reflects the inclusion of acerola pulp, showing its influence on the number of phenolic compounds in the beer. In this sense, acerola appears to be a brewing adjunct capable of increasing the concentration of total phenolic compounds in beers.

A similar result was found by Dalla Santa et al., (2020) where the addition of red pulp pitaya in Fruit Bier beers with different levels of inclusion increased the content of total phenolic compounds in the beers.[45] According to Silva (2018), in beers, phenolic compounds are important sources of antioxidants, capable of preventing the degradation and oxidation of the beverage, in addition to helping with colloidal stability, flavor, and sensory properties.[46]

According to Callemien and Collin (2009), the phenolic compounds in beer contribute to human health, acting as cardioprotectors, with an antioxidant effect, inhibiting platelet activity and vasodilation, having anticancer activity, anti-inflammatory activity, and estrogenic activity, in addition to being able to help to prevent neurodegenerative diseases such as Alzheimer's and Parkinson's.[47]

Silva et al,. (2022) analyzed the total phenolic content and antioxidant capacity of craft beers with added fruit produced in Portugal. These craft beers had high levels of total phenolic compounds, between 343.8 and 2,172.5 mg GAE L^{-1}. These results are very similar to those found in this work, where the values varied between 399.2 and 2,486.4 mg GAE L^{-1}, with the highest values obtained in the drink with the highest addition of acerola pulp.[48]

The antioxidant activity by the iron reduction method (FRAP) and by 2,2-diphenyl-1-picrylhydrazyl radical capture (DPPH) showed significant differences ($P<0.05$) between the samples. Analyzing the results of the samples for these methods, it is possible to observe that the addition of acerola pulp provided a linear increase ($P<0.05$) in the antioxidant activity of 449.8984 and 12.0928, respectively, for each 1% of pulp added, there was a positive match between the results of total phenolic compounds and the antioxidant activity measured by these methods.

Sorbo and Broetto (2019), producing Pilsner-type craft beers with passion fruit pulp added,[49] and Hübner (2019), producing Catharina Sour-style craft beers with pitaya and ginger, obtained results similar to those presented in this study, according to the increase in the level of inclusion of passion fruit pulp. fruit obtained an increase in the content of total phenolic compounds and antioxidant activity.[50]

Using the ABTS 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical capture method, the antioxidant activity of beers expressed in α-tocopherol L^{-1} ranged from 90.76 to 777.71. In this method, the T3 beer had the highest average of antioxidant activity, and the control beer the lowest, different results from those found by the FRAP and DPPH methods, but these methods have correlation coefficients of $r^2 = 99.4$ and $r^2 = 96.9$, respectively, being greater than the ABTS method ($r^2 = 74.6$), thus the FRAP and DPPH methods are better related to the results of phenolic compounds. According to Zhao et al. (2010),[51] the antioxidant activities present in beer depend on its composition and the conditions of the test systems performed, thus, different methods of determining antioxidant activity, based on different reaction mechanisms, can present different results.

Antioxidants present in the diet, such as phenolic compounds, can help prevent diseases linked to oxidative stress, in addition, the antioxidant activity and low alcohol content of beers are important for their nutritional properties (Zhao et a., 2010).[51] The results of the analysis of flavonoids did not show significant differences ($P<0.05$), with an average of 453.64 mg Eq Q L^{-1}, indicating that the flavonoid content is mostly linked to the base raw material of the beers and not the addition of acerola pulp. Flavonoids contribute to the astringency and bitterness of the beer. The values of flavonoids found in this study were higher than that identified by Souza et al., (2020) in a craft beer of the Pilsen type with the addition of acerola pulp, in which it obtained an average of 68.9 (mgEq Q L^{-1}).[40] It is likely that the concentration of other groups of phenolic compounds present in acerola, such as carotenoids, anthocyanins, and phenylpropanoids, among others, is higher than the concentration of flavonoids, which resulted in the high concentration of phenolic compounds obtained in samples with pulp.

In this work, there was practically no variation in the reducing activity of beer without the addition of fruit (control) and beer with 5% pulp, however, there was a significant increase in the addition of 10 to 20% pulp. The reducing activity ranged from 0.84 (5% pulp) to 5.34 (20% pulp). This increase in reducing activity is associated with an increase in acerola pulp content, since this fruit is rich in vitamin A and C, thiamin, riboflavin, niacin, rutin, quercetin, cyanidin, catechin, kaempferol, anthocynins and flavonoids.[52-54]

Ditrych et al. (2015) evaluated the antioxidant properties of sixteen beers produced in Poland, where they found greater reducing potentials in dark beers (5.0 to 8.8 mmol Fe^{2+} L^{-1}). The reducing powers of artisanal and large-scale lager beers ranged between 2.9 and 3.8 and between 2.0 and 2.4 mmol Fe^{2+} L^{-1}, respectively. In addition, it is worth noting that dark beers (Bock and Porter) require a greater load of malt (source of antioxidants) when produced.[55]

Conclusion

The beers with pulp inclusion showed higher acidity, lower pH, lower soluble solids, and higher % ash when compared to the control. Fermentative kinetics showed that the presence of acerola pulp in the wort resulted in lower soluble solids at the end of the fermentation process for beers with the fruit, compared to the control beer. The addition of acerola pulp promoted an increase in the concentration of phenolic compounds and the antioxidant capacity of the beers, especially the T4 beer. Thus, moderate consumption of beers with these properties may be more beneficial to human health. As for the color, there was little visual difference, presenting a straw yellow color in all the beers produced. The acerola pulp added to the beers contributed to the stability and quality of the foam produced when compared to the control beer. Acerola pulp, used as an adjunct in the production of Witbier-type beers, adds value to the final product and has important properties to be explored in the brewing industry.

Author Contributions

Cecila L. Sousa: Methodology, investigation, writing the original draft. Lilian S. M. da Costa: Conceptualization, project administration, validation, writing review, and editing. Lorena P. Ferreira: Validation, data curation. Karita J. S. Silva: Supervision, resources. Marcia M. R. Azevedo: formal analysis, data curation. Cléo R. Bressan: Validation, formal analysis. Kashif Gul data curation, investigation. Sumeet Malik: Software, data curation. Paulo S. Taube: Reviewing and data analysis

Conflicts of interest

There are no conflicts to declare.

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