

## Effect of acerola pulp on the quality of witbier beer: Characterization and analysis

## Efeito da polpa de acerola na qualidade da cerveja witbier: Caracterização e análise

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### ARTICLE

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### ABSTRACT

This research focused on the analysis of Witbier-style craft beers with the addition of different concentrations of acerola pulp: 0% (T1), 5% (T2), 10% (T3), and 20% (T4) (v/v). The brewing process included milling, mashing, boiling, fermentation with hops, coriander, and acerola pulp, followed by carbonation, bottling, and maturation. The physicochemical analyses conducted included soluble solids, pH, acidity, dry matter, ash content, foam stability, color, total phenolic content, flavonoids, and antioxidant activity. The addition of acerola pulp significantly influenced apparent attenuation, alcohol content, and soluble solids. The T3 formulation exhibited the highest attenuation (87%) and alcohol content (6.17% alcohol by volume (ABV)) exceeding the expected range for Witbier (4.5-5.5% ABV). Soluble solids decreased with increased pulp addition, while total acidity increased and pH decreased, indicating higher acidity. Visually, all samples maintained the characteristic light tone of Witbier. In terms of foam stability, the T4 sample showed the best results. Biochemical analysis revealed an increase in total phenolic content and antioxidant activity with acerola addition, with T4 being the richest in these compounds. Flavonoid content remained stable across all samples, indicating less influence of the pulp in this aspect. It is concluded that the addition of acerola pulp enriches Witbier, enhancing its functionality with higher acidity, foam stability, and antioxidant properties, making it a unique option in the craft beer market.

### RESUMO

#### Palavras-chave:

*Malpighia emarginata*

Análise físico-química

Atividade antioxidante

Cerveja artesanal

Esta pesquisa se concentrou na análise de cervejas artesanais do tipo Witbier com adição de diferentes concentrações de polpa de acerola: 0% (T1), 5% (T2), 10% (T3), e 20% (T4) (v/v). O processo de fabricação incluiu moagem, mosturação, fervura, fermentação com lúpulo, coentro e polpa de acerola, seguido de carbonatação, engarrafamento e maturação. As análises físico-químicas realizadas incluíram sólidos solúveis, pH, acidez, matéria seca, teor de cinzas, estabilidade da espuma, cor, teor fenólico total, flavonoides e atividade antioxidante. A adição de polpa de acerola influenciou significativamente a atenuação aparente, o teor alcoólico e os sólidos solúveis. A formulação T3 apresentou a maior atenuação (87%) e teor alcoólico (6,17% álcool por volume (ABV)), acima do intervalo esperado para Witbier (4,5-5,5% ABV). Os sólidos solúveis diminuíram com o aumento da polpa, enquanto a acidez total aumentou e o pH diminuiu, indicando maior acidez. Visualmente, todas as amostras mantiveram o tom claro característico da Witbier. Em termos de estabilidade da espuma, a amostra T4 apresentou os melhores resultados. As análises bioquímicas revelaram aumento do conteúdo fenólico total e da atividade antioxidante com a adição de acerola, sendo a T4 a mais rica nesses compostos. O conteúdo de flavonoides permaneceu estável em todas as amostras, indicando menor influência da polpa nesse aspecto. Conclui-se que a adição de polpa de acerola enriquece a Witbier, melhorando sua funcionalidade com maior acidez, estabilidade de espuma e propriedades antioxidantes, tornando-a uma opção diferenciada no mercado de cervejas artesanais.

## INTRODUCTION

Beer is one of the oldest alcoholic beverages, valued for its quality, encompassing sensory, physical-chemical, and biochemical characteristics. (LARGE et al., 2020). The ingredients and brewing process create a beverage that is rich in micronutrients and possesses antioxidant and anti-inflammatory properties (CHIVA-BLANCH et al., 2015; GAETANO et al., 2016). Matsubara et al. (2016) note that beer has a diuretic effect that supports kidney function and helps manage hypertension, while moderate consumption can aid in the prevention of coronary diseases.

According to the Brazilian Ministry of Agriculture, Livestock, and Supply (MAPA), Brazil is the third-largest beer producer in the world (BRASIL, 2023; KORDIALIK-BOGACKA, 2022; TRONINA et al., 2020). Brazilian legislation mandates water, malt or its extract, and hops (except for “fruit beer”) as essential ingredients for beer production. Optional brewing adjuncts can replace up to 45% of the beer's weight (BRASIL, 2019). Thus, the inclusion of ingredients such as tropical fruits fosters the diversification of styles and enhances the value of regional products.

Rosa and Afonso (2015) emphasize that brewing water must have a controlled pH, be clean, and free of turbidity. Technologies such as reverse osmosis and pH adjustment systems allow for precise manipulation of mineral salt composition, improving water quality tailored to various beer styles (MUSICÒ et al., 2019). Malt, from barley's malting process, activates enzymes like  $\alpha$ - and  $\beta$ -amylases to convert starches into simple sugars, essential for beer's aroma, flavor, color, foam, and body (BAMFORTH, 2023). In addition to playing a crucial role in the sensory characteristics of beer, malt usage also provides flexibility in the formulation of specific recipes. One example is the substitution of unmalted wheat for wheat malt in Witbier, which affects flavor, color, and production costs (BELCAR et al., 2022).

Hops, which contain essential oils, polyphenols, and resins, contribute to beer's bitterness through the conversion of  $\alpha$ -acids into iso- $\alpha$ -acids during boiling, while their essential oils shape the aromatic profile (KAPPLER et al., 2010). These compounds collectively enhance the beer's antioxidant properties, aroma, flavor, bitterness, and foam stability, characteristics that can be impacted by the addition of regional ingredients such as tropical fruits (DURELLO et al., 2019; SANTOS et al., 2024). Yeasts are essential for fermentation, converting sugars into ethyl alcohol. Beers are classified into Ale (top fermentation) and Lager (bottom fermentation) types. Ale beers, fermented at higher temperatures with *Saccharomyces cerevisiae* yeast, develop fruity and spicy aromas (VIDGREN et al., 2010; BOKULICH; BAMFORTH, 2013; BASSO; ALCARDE, 2016; BOURBON-MELO et al., 2021). According to the Beer Judge Certification Program (BJCP), Witbier is a Belgian wheat ale with unmalted wheat, barley malt, coriander seeds, and sweet orange peel, giving it a sweet aroma,

pale to golden color, persistent foam, hazy appearance, refreshing citrus flavor, and low to medium-low hop bitterness (STRONG; ENGLAND, 2021).

Advancements in chemical processes and hygiene have improved beer production, enhancing quality, ingredient diversity, and alcohol content (CARLOS et al., 2020). The rise in craft beer is driven by diverse raw materials and innovative brewing methods, resulting in unique flavors, aromas, colors, and bitterness levels, distinguishing them from industrial beers (ASSIS et al., 2020; RAMOS; PANDOLFI, 2019). Known as premium or specialty beers, craft beers attract consumers seeking high-quality, distinctive flavors. Thus, research and innovation in this sector present significant opportunities for entrepreneurs (RAMOS; PANDOLFI, 2019; SILVA et al., 2019).

Incorporating fruits as adjuncts in beer production creates unique products that captivate consumers. Brazil, recognized as one of the world's leading fruit producers, stands out for its diverse array of options (CUNHA et al., 2023). Fruits are rich in minerals, fibers, vitamins, and essential nutrients, enhancing product quality, nutritional value, and biochemical properties. They also contain phenolic and bioactive compounds that boost antioxidant activity (PAZ CARMONA et al., 2022). The use of fruits in beer enriches the beverage with various aromas, flavors, colors, bitterness, and foam quality, generally leading to high consumer satisfaction (PINTO, 2015).

Acerola (*Malpighia emarginata* DC) is a tropical fruit predominantly cultivated in the northeastern region of Brazil. It is renowned for its high vitamin C content and is also a rich source of anthocyanins and carotenoids, which possess antioxidant properties (MACIEL et al., 2010). The primary antioxidants found in fruits are phenolic compounds. These compounds play crucial roles in developing flavor, aroma, astringency, and body, and they also contribute to the antioxidant potential of the diet. Additionally, they enhance foam stability and extend the shelf life of beers compared to those with lower antioxidant activity (MITIĆ et al., 2014).

This study produced and evaluated Witbier-style craft beers with varying acerola pulp amounts, focusing on physicochemical properties, phenolic and flavonoid content, and antioxidant activity. Adding acerola pulp enhances sustainability by using locally sourced ingredients and promoting ecological responsibility, while also increasing nutritional value. This approach offers unique flavor and aroma profiles. Through characterization and analysis, this innovative effort enriches the craft brewing field and advances scientific knowledge, contributing to the ongoing narrative of innovation within the beverage industry. The main objective was to assess the impact of adding acerola pulp on the physicochemical quality, sensory characteristics and antioxidant activity of Witbier-style beers.

## MATERIAL AND METHODS

The Witbier craft beer was made using the following ingredients: Chateau Pilsen 2-Row malt from Castle Malting in Beloeil, Belgium; flaked wheat; East Kent

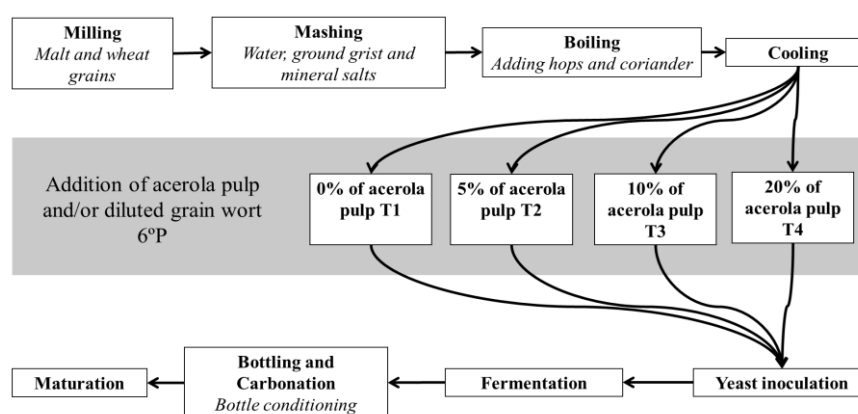
Golding hops from Kent, UK, for bitterness and aroma ( $\alpha$ -acid 4.6%); coriander; and *Saccharomyces cerevisiae* commercial brewer's yeast (SafAle T58) from Fermentis in Marcq-en-Baroeul, France. The beer production also involved mineral water and acerola sourced from local retailers.

### Beer production

The Witbier craft beer was brewed using 2.5 kg of Chateau Pilsen 2-Row malt, 2.5 kg of flaked wheat, 25 g of East Kent Golding hops ( $\alpha$ -acid 4.6%), 10 g of coriander, and 8 g of *S. cerevisiae* yeast. Fruit sanitization followed Bezerra (2009) method, which involved pre-washing and sanitizing with sodium hypochlorite (10 mL L<sup>-1</sup> for 20 min), followed by manual pulping and packaging in polyethylene bags. The pulp was pasteurized using an adapted method from Igual et al. (2014), which involved heating 2 L of acerola pulp in a thermostatic bath to 30 °C, then microwaving at 1000 W for 5 min, reaching 83 °C.

After pasteurization, the pulp was sealed in polyethylene plastic bags and stored in a refrigerator at 4 °C until the following day, when the beer production commenced. The mineral water used for the mash was adjusted to the following mineral content: Ca<sup>2+</sup> 55 mg L<sup>-1</sup>, Mg<sup>2+</sup> 3 mg L<sup>-1</sup>, Na<sup>+</sup> 7 mg L<sup>-1</sup>, Cl<sup>-</sup> 59 mg L<sup>-1</sup>, SO<sub>4</sub><sup>2-</sup> 51 mg L<sup>-1</sup>, and an alkalinity equivalent to 35 mg L<sup>-1</sup> HCO<sub>3</sub><sup>-</sup>. Adjustments were made using the Brewfather platform with salts CaCl<sub>2</sub>, NaCl, MgSO<sub>4</sub>, MgCl<sub>2</sub>, and Ca(OH)<sub>2</sub> (Synth, Diadema, Brazil).

The malt and wheat grains were milled using a Botini B03 grain crusher. To prepare the wort, 20 L of water were heated to 50 °C in a Brewhome 20 L vessel. The ground grains were added and held at this temperature for 20 minutes (protein break and ferulic rest). The temperature was then increased to 65 °C for 60 minutes to allow saccharification. The temperature was further raised to 76 °C for 10 minutes to inactivate enzymes and reduce wort viscosity, with continuous recirculation maintained throughout. The malt was then rinsed with 8.8 L of water heated to 76 °C. The wort was boiled for 60 minutes, with hops added at the start to achieve 15 IBU bitterness and coriander added 55 minutes into the boil.



**Figure 1.** Basic flow of the production process for Witbier beer with acerola pulp.

To cool the wort, a plate-type heat exchanger was used with water as the cooling medium. Subsequently, 16 fermenters were each filled with 0.768 L of wort and 0.5 grams of yeast. To standardize the degrees Plato across different conditions, a diluted grain wort was prepared by mixing wort from mashing with water to match the degree Plato of the acerola pulp (6 °P). This diluted solution or acerola pulp was then added to each fermenter to achieve a final volume of 960 mL (Table 1), ensuring all conditions started fermentation at the same degree Plato (13.4 °P). Four fermenters were allocated for each pulp concentration (0%, 5%, 10%, and 20% V/V), totaling 16 fermenters. The fermentation was carried out at 20 °C for 9 days (Figure 1).

At the end of fermentation, the beer underwent carbonation and maturation for 40 days in 250 mL glass bottles. Each bottle received 5 mL of a solution containing 6 g L<sup>-1</sup> of sugar and 5 mL of lemon juice.

### Physicochemical analysis

#### Degree Plato (°P)

A analog refractometer (Vodex VX25-40, Southampton, US) was used to measure the soluble solids content. Throughout the 9-day fermentation period, readings were taken to track the soluble solids during the fermentation kinetics. The original gravity and final gravity were determined using equations 1 and 2 as outlined by Briggs (2004).

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

**Table 1.** Craft beer formulations with varying concentrations of acerola pulp (v/v).

Treatments	Beer wort volume (L)	Acerola pulp volume (L)	Diluted grain wort 6 °P volume (L)	Final volume (L)
0% (v/v) of acerola pulp (T1)	0.768	0.000	0.192	0.960
5% (v/v) of acerola pulp (T2)	0.768	0.048	0.096	0.960
10% (v/v) of acerola pulp (T3)	0.768	0.096	0.048	0.960
20% (v/v) of acerola pulp (T4)	0.768	0.192	0.000	0.960

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

where, OG= Original Gravity; P<sub>i</sub>: Initial Plato Degree; FG= Final Gravity.

### Apparent attenuation (%)

Apparent attenuation (AA), which reflects the conversion of sugars into alcohol and carbon dioxide, was used as a fermentation parameter. AA values were calculated based on the original and final gravity values of the beers (measured in °P), following the calculation outlined in equation 3 below (BRIGGS, 2004). Actual attenuation was not calculated in this study.

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

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

### Estimation of alcohol content (% ABV)

The estimated alcohol content (% ABV, or alcohol by volume) was derived from the original and final gravity values of the beers using the simplified equation 4. This formula provides a quick and approximate estimation of the ABV (MARTINEZ et al., 2019).

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

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

### Total acidity

Total acidity (TA) was determined by titration using the potentiometric method, as outlined in the official AOAC Method 950.07 (AOAC, 2010). Beer samples were de-carbonated with an ultrasound device for 90 seconds. In a beaker, a mixture of 20 mL of beer, 80 mL of distilled water, and 3 drops of phenolphthalein was prepared. This mixture was then titrated with a 0.1 mol L<sup>-1</sup> NaOH solution using a magnetic stirrer until a stable pink color was achieved. The NaOH solution was standardized with potassium bi-phthalate to reach a normality of 0.0846 mol L<sup>-1</sup>. Acidity was determined using equation 5 provided.

$$TA \text{ (meEqL}^{-1}\text{)} = \frac{1000 \times V_n \times N}{V} \quad (5)$$

where, V<sub>n</sub> = volume of NaOH solution used (mL); N = normality of the NaOH solution; V = sample volume (mL).

In accordance with AOAC 950.07.25, acidity expressed as lactic acid is calculated based on the fact that 1 mL of 0.1 mol L<sup>-1</sup> NaOH is equivalent to 0.0090 g of lactic acid. For the NaOH solution adjusted during standardization (1 mL of 0.0846 mol L<sup>-1</sup> NaOH is equivalent to 0.007614 g of lactic acid), the acidity in terms of lactic acid can be determined using the following equation 6:

$$\text{Acidity } \in \text{ lactic acid} = \left( \frac{V_n \times 0.007614}{V} \right) \times 1000 \text{ g lactic acid} \quad (6)$$

where, V<sub>n</sub> = volume of NaOH solution used (mL); V = sample volume (mL).

The acidity in beer is expressed in lactic acid because this compound is widely used to adjust the acidity of the

beverage. This parameter is essential for the characterization, standardization, and identification of possible frauds, as well as for controlling undesirable changes caused by microorganisms (ALVES, 2014). Furthermore, the standardization of total acidity in lactic acid, as described by AOAC Method 950.07 (AOAC, 2010), ensures consistent and comparable results, facilitating the analysis and quality control in beer production. This is particularly relevant in studies involving the addition of ingredients, such as fruits, which can alter the sensory and chemical profile of the beer.

### Determination of dry matter and mineral matter

The analysis of dry matter and mineral matter (ash) in beer followed an adapted method from Cunha et al. (2023). Crucibles were first weighed to determine the tare. Then, 40 mL of beer samples were added and heated at 105°C for 17 hours. After cooling, the final weight was recorded to measure dry matter. For ash content, the crucibles were heated in a muffle furnace at 600°C for 4 hours, then weighed to determine the mineral matter.

### pH analysis

The pH analysis was performed using a HANNA EDGE® LCD 5.5 pH meter (Tamboré Barueri, Brazil), which was calibrated beforehand with standard solutions of pH 7.0 and 4.0.

### Color analysis

Color analysis was conducted using the Tristimulus system in the CIELab color space (CLEMENTS, 2001). Beer samples were first filtered through a membrane with 0.35 mm thickness and 0.6 μm porosity. A UV spectrophotometer (UV-1100 – Kasuaki, Jiangsu, China) measured the transmittance spectra between 380 and 780 nm at 5 nm intervals. The L\*, a\*, and b\* color values, representing luminosity ranging from white (L=100) to black (=0), the color variation from red (+a\*) to green (-a\*), and the color variation from yellow (+b\*) to blue (-b\*), respectively, were derived from these spectra using the provided equations 7-12.

$$X = k \sum_{\lambda=380}^{780} T_{(\lambda)} S_{(\lambda)} x_{10(\lambda)} \quad (7)$$

$$Y = k \sum_{\lambda=380}^{780} T_{(\lambda)} S_{(\lambda)} y_{10(\lambda)} \quad (8)$$

$$Z = k \sum_{\lambda=380}^{780} T_{(\lambda)} S_{(\lambda)} z_{10(\lambda)} \quad (9)$$

$$L^* = 116 \left( \frac{Y}{Y_n} \right)^{1/3} - 16 \quad (10)$$

$$a^* = 500 \left[ \left( \frac{X}{X_n} \right)^{1/3} - \left( \frac{Y}{Y_n} \right)^{1/3} \right] \quad (11)$$

$$b^* = 200 \left[ \left( \frac{Y}{Y_n} \right)^{1/3} - \left( \frac{Z}{Z_n} \right)^{1/3} \right] \quad (12)$$

where T(λ)= transmittance value at each wavelength λ, S(λ)= the spectral power distributions at each λ for the illuminant pattern C (Table 1S) (supplementary materials). x<sub>10</sub>(λ), y<sub>10</sub>(λ), z<sub>10</sub>(λ)= color matching functions, standard observer C at each wavelength λ (Table 1S). X, Y, Z = tristimulus values representing red, blue, and green.

According to the American Society for Testing and Materials (ASTM, 2001), the white point values are  $X_n = 97.285$ ,  $Y_n = 100.00$  and  $Z_n = 116.145$ .

The constant  $k$  (normalizing factor) is calculated as described in equation 13.

$$k = 100 / \sum_{\lambda=380}^{780} S(\lambda) Y_{10(\lambda)} \quad (13)$$

where  $k$ : corresponds to the normalization factor and can be calculated directly from Table 1S.

### Foam analysis

Foam analysis followed Nyarko et al. (2021). Beer was poured from a standardized height of 30 cm into a 1 L beaker using tweezers with an oval claw. Foam height and liquid level were measured at intervals of 0.5, 15, 30, 45, 60, 90, 120, 150, and 180 seconds using a fixed ruler. Foam decay was recorded with a Canon EOS Rebel SL2 camera. Four samples per treatment were averaged to calculate foam values using specified equations. For foam height ( $F$ ) in centimeters for each measuring time ( $t$ ) according to equation 14.

$$F = T - L \quad (14)$$

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 15.

$$\ln(F_t) = a + bt \quad (15)$$

where:  $F$ = foam height;  $t$ = time;  $a$ = intercept;  $b$ = slope.

Other foam attributes were calculated using the coefficients determined by equation 13. These attributes and their corresponding equations 16-22.

Head (after the first minute of pouring), cm

$$\text{Head} = e^{(a+bt)} \quad (16)$$

Half-life (HL), min

$$\text{HL} = \ln(0.5)/b \quad (17)$$

Life, min

$$\text{Life} = -a/b \quad (18)$$

Density (g.mL<sup>-1</sup>)

$$\text{Density} = (L2.5 - L1) / \text{Head}1 - \text{Head}2.5 \quad (19)$$

Quality (Q)

$$Q = 10 \times \text{HL} \times \text{Density} \quad (20)$$

Normalized half-life (NHL-1), min

$$\text{NHL-1} = (10 \times \text{HL}) / \text{Head} \quad (21)$$

Normalized half-life (NHL-0), min

$$\text{NHL-0} = (10 \times \text{HL}) / F_0 = (10 \times \text{HL}) / (e^a) \quad (22)$$

### Biochemical analyses

#### Content of total phenolic compounds (TPC)

Total phenolic compounds were quantified using the Folin-Ciocalteu method as described by Julião et al. (2020). In triplicate, 250  $\mu\text{L}$  of Folin-Ciocalteu reagent was mixed with 50  $\mu\text{L}$  of diluted beer sample (1:10, v/v) and 1 mL of distilled water. After 5 minutes, 750  $\mu\text{L}$  of 20%  $\text{Na}_2\text{CO}_3$  and

2.95 mL of distilled water were added. Absorbance was measured at 798 nm with a spectrophotometer. Phenolic content was determined using a gallic acid standard curve and expressed as mg of gallic acid equivalent per liter of sample ( $\text{mgEq GA L}^{-1}$ ).

#### Total flavonoid content (TFC)

Total flavonoid content was measured using the method from Salgueiro et al. (2014). In triplicate, 2 mL of diluted beer sample (1:10, v/v) was mixed with 2 mL of 2% methanolic  $\text{AlCl}_3$  solution. A blank was prepared with non-alcoholic beer and distilled water. After 30 minutes, absorbance was read at 415 nm. Flavonoid content, expressed as milligrams of quercetin per liter ( $\text{mgEq Q L}^{-1}$ ), was determined using a seven-point calibration curve.

#### Ferric reducing antioxidant power (FRAP)

The antioxidant capacity to reduce ferric ions ( $\text{Fe}^{3+}$ ) was measured using Rufino et al. (2007) method. The test involved reacting 200  $\mu\text{L}$  of diluted beer sample (1:10, v/v) with FRAP reagent. Control samples contained 50  $\mu\text{L}$  of undiluted beer, 150  $\mu\text{L}$  of distilled water, and 4 mL of FRAP reagent. Absorbance at 593 nm was used to determine antioxidant power, with results expressed as millimoles of  $\text{Fe}^{2+}$  per liter ( $\text{mmol Fe}^{2+} \text{ L}^{-1}$ ) using a seven-point  $\text{FeSO}_4$  standard curve.

#### DPPH assay

The scavenging activity against the stable radical DPPH was measured using Al-Sayyed et al. (2022) method. The test involved reacting 200  $\mu\text{L}$  of beer sample with 2.8 mL of DPPH ethanolic solution ( $8 \times 10^{-5} \text{ mol L}^{-1}$ ). Absorbance at 517 nm was recorded, and results were expressed as milligrams of  $\alpha$ -tocopherol per liter of sample ( $\text{mgEq T L}^{-1}$ ) using a  $\alpha$ -tocopherol standard curve.

#### ABTS assay

Antioxidant activity was assessed using Rufino et al. (2007) method. The test involved 100  $\mu\text{L}$  of diluted sample (1:10), 200  $\mu\text{L}$  of distilled water, and 2.7 mL of ABTS solution. Absorbance at 734 nm was measured, with results expressed as milligrams of  $\alpha$ -tocopherol per liter of sample ( $\text{mgEq T L}^{-1}$ ) using a  $\alpha$ -tocopherol standard curve.

#### Statistical analysis

Data from physical-chemical analyses, excluding color, phenolic compounds, and antioxidant activity, were subjected to analysis of variance. When significant differences were found ( $P < 0.05$ ), regression analysis was conducted. For the analysis of color, phenolic compounds, and antioxidant activity, Tukey's test was conducted following a significant F-statistic, with a significance level set at  $p \leq 0.05$ . All data analyses were carried out using SISVAR 5.6 statistical software.

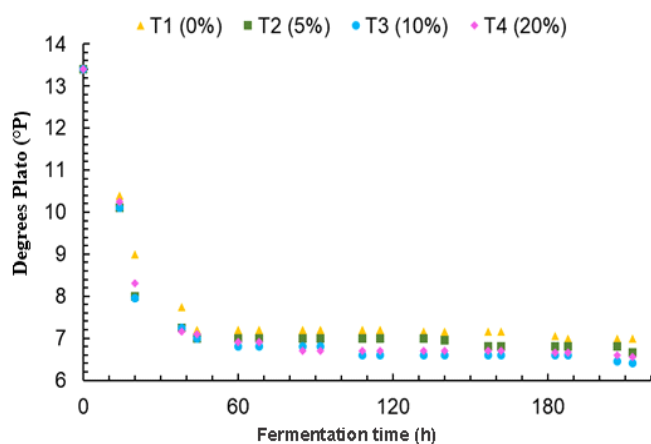
## RESULTS AND DISCUSSION

### Physicochemical analysis

#### Fermentative kinetics of soluble solids content

Figure 2 shows the attenuation profile of the beer wort, represented by the degrees Plato ( $^{\circ}\text{P}$ ), throughout the fermentation of craft beers produced with different concentrations of acerola pulp. During fermentation, the

degrees Plato of the samples decreased from 13.4°P to around 10°P within 14 hours, with a noticeable reduction in soluble solids. The most significant decline in soluble solids occurred during the first 60 hours of fermentation. Among the beers with acerola pulp, sample T3 had the lowest degrees Plato at the end of fermentation compared to T2 and T4, while the control sample retained the highest soluble solids content. Similar findings were reported by Fernandes (2017), who observed that beers with acerola pulp had comparable or lower degrees Plato at the end of fermentation compared to those without pulp. The variation in residual sugars among the acerola-added beers and the control may be attributed to the composition of acerola pulp, which is rich in fermentable sugars and contains bioactive compounds that may influence yeast metabolism during fermentation (CARVALHO, 2022).



**Figure 2.** Degrees Plato (°P) during wort fermentation kinetics of craft beers produced with different concentrations of acerola pulp 0% (T1), 5% (T2), 10% (T3), and 20% (T4).

The Figure 2 shows 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).

Beer T3 demonstrated a higher apparent attenuation of 87% and an alcohol content of 6.17% at

the end of fermentation. According to the Beer Judge Certification Program (BJCP), the typical alcohol range for Witbier is 4.5 to 5.5% ABV, so the results for T3 are outside the standard for this style. This result observed in the acerola beer may be attributed to the bioactive components of acerola, which likely influenced the fermentation performance of the yeasts. In comparison, Costa et al. (2020) reported non-standard alcohol content in Witbier beers with added ginger, with alcohol content values ranging from 6.5% to 6.7%, suggesting that ingredients rich in bioactive compounds can similarly influence fermentation processes. These findings highlight the crucial role of the profile of the raw materials added, thereby justifying future studies to evaluate these interactions in detail.

The addition of acerola pulp caused a significant reduction ( $P < 0.05$ ) in soluble solids (°Bx) by 0.0197% per unit of added pulp. Beers with acerola pulp had lower °Bx values than the control, as most soluble solids in fruit-added beers are fermentable sugars (MUTZ et al., 2021).

Total acidity increased linearly ( $P < 0.05$ ) by 1.1758 mEq L<sup>-1</sup> and 0.1058 g L<sup>-1</sup> of lactic acid per 1% of added acerola pulp. Beer T1 had the lowest acidity, while T4 had the highest, consistent with Pinto (2015), who noted increased acidity with acerola pulp addition. This is due to the organic acids from fermentation and the addition of acerola.

Acerola pulp addition also decreased pH linearly by 0.0290 per 1% added pulp. T4 beer, with the lowest pH of 3.89, exhibited a similar trend to total acidity. Pinto et al. (2015) reported pH values from 4.10 to 4.24 for Pale Ale with acerola and pineapple, and Fernandes (2017) confirmed that fruit reduces pH, preventing contamination and preserving beer quality. Dry matter showed no significant differences ( $P < 0.05$ ), averaging 4.42%, indicating good quality as values above 3.0% are desired (SOUSA and FOGAÇA, 2019). These results are consistent with Souza et al. (2020), who reported similar values in beers containing fruit with a high concentration of solids, such as sugars and structural compounds.

**Table 2.** Physical-chemical analyses of craft beers with the addition of different concentrations of acerola pulp.

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

\*Values without ethanol corrections. 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).

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

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

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).

It was observed a linear increase of 0.0433 in ash content was noted per 1% pulp addition. This increase reflects the mineral contribution of acerola, which contains calcium, magnesium, and potassium, underscores its dietary importance (MARGALHO et al., 2020).

### Color analysis

Regarding the results of the color analysis, Table 3 presents the average values of the color parameters. 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), as 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.

Statistically significant differences were found between the samples in terms of color parameters. Although statistically relevant, these subtle variations in numerical values that may not translate into noticeable changes in the visual appearance of the beers produced. When comparing the a\* and b\* values, all samples exhibited a stronger yellow hue. These color traits, as per BJCP guidelines (STRONG; ENGLAND, 2021), fall within the typical range for Witbier beers, which are characterized by a straw-yellow to very light golden color.

### Foam analysis

Foam is a key appealing feature of beer, closely linked to its quality. Its stability depends on factors such as the right amount of hops, the presence of metallic ions in the wort, and the hydrophobic surface-active proteins (polypeptides) from the albumin class (LUKINAC et al., 2019; BELCAR et al., 2022). Table 4 displays the average values for foam attributes assessed, including foam height, half-life, lifespan, density, and normalized half-life (NHL-1 and NHL-0).

During the first minute after serving, beer T1 had the highest foam height among the samples with acerola. For foam stability and longevity, beer T4 demonstrated the longest half-life and overall foam duration. The half-life is indicative of foam stability, and an increase in acerola pulp content corresponded to a longer foam half-life. Foam density values at 1 minute ranged from 0.34 g mL<sup>-1</sup> for T2 to 0.22 g mL<sup>-1</sup> for T4. Foam density, influenced by components like hops and proteins, affects foam stability; a lower density may suggest fewer of these components (BAMFORTH, 2023). Although the density values are dimensionless, they are presented in g mL<sup>-1</sup>, assuming a beer density of approximately 1 g mL<sup>-1</sup>.

The quality assessment used in this study, as proposed by Nyarko et al. (2021), evaluated foam performance based on density and half-life values. According to this assessment, beer T2 achieved the highest average quality score of 4.44, while beer T1 had the lowest average score of 3.53. These results suggest that the T2 formulation may have presented a more stable, dense and visually appealing foam (CONSTANT, 1992).

**Table 4.** Average and standard deviation for the foam attributes analyzed in control craft beer samples and those with added acerola pulp.

Attributes	Mean ± 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 <sup>a</sup>	2.78±0.64 <sup>b</sup>	3.54±0.32 <sup>a,b</sup>	3.72±0.86 <sup>a,b</sup>
Half-life (min)	1.09±0.90 <sup>b</sup>	1.20±0.22 <sup>b</sup>	1.49±0.07 <sup>a,b</sup>	1.92±0.38 <sup>a</sup>
Life (min)	3.17±0.14 <sup>b</sup>	2.80±0.71 <sup>a,b</sup>	3.70±0.23 <sup>a,b</sup>	4.66±1.30 <sup>a</sup>
Density (g mL <sup>-1</sup> )	0.19±0.04 <sup>a,b</sup>	0.16±0.01 <sup>b</sup>	0.17±0.02 <sup>b</sup>	0.23±0.0 <sup>a</sup>
Quality	2.08±0.45 <sup>b</sup>	1.92±0.46 <sup>b</sup>	2.47±0.30 <sup>b</sup>	4.36±0.97 <sup>a</sup>
NHL-1 (min)	2.72±0.34 <sup>c</sup>	4.38±0.37 <sup>a,b</sup>	4.23±0.47 <sup>b</sup>	5.24±0.60 <sup>a</sup>
NHL-0 (min)	1.44±0.26 <sup>c</sup>	2.42±0.19 <sup>b</sup>	2.65±0.33 <sup>b</sup>	3.60±0.38 <sup>a</sup>

\*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).

Normalized half-life values reflect the adjusted expected duration for foam samples with an initial height (NHL-1) or foam volume (NHL-0) of 10 cm, calculated as ten times the single-point slope of the half-life for foam height. In the analysis of normalized half-life values, T4 exhibited the highest average compared to the other samples. While sample T1 generated more foam, the foam from sample T4 demonstrated superior stability, which may be related to the composition of the acerola pulp added. The bioactive compounds and sugars present in acerola may have contributed to the formation of more stable structures in the foam, increasing its resistance to degradation.

### Biochemical analyses

The results of the biochemical analysis, summarized in Table 5, indicated significant variations in the total phenolic compounds and antioxidant activity, as assessed by the FRAP, DPPH, and ABTS methods, among the samples. However, no significant differences were observed in the total flavonoid content.

Each 1% increase in acerola pulp led to a linear increase of 105.16 in the total phenolic content of the beer ( $P < 0.05$ ). Beers with acerola pulp had higher phenolic concentrations compared to the control, with T4 having the highest average. These results surpass those reported by Zhao et al. (2010), who found phenolic contents between 152.01 and 339.12 mg GAE L<sup>-1</sup> in commercial beers. The significant increase in phenolic compounds due to acerola pulp highlights its efficacy as an adjunct in beer production to enhance phenolic content.

Similar results were found by Dalla Santa et al. (2020), where the addition of red pitaya pulp in Fruit Beers increased the total phenolic content. According to Silva (2018), phenolic compounds are important antioxidant sources in beers, preventing degradation and oxidation, and contributing to colloidal stability, flavor, and sensory properties. Callemien and Collin (2009)

state that phenolic compounds in beer offer various health benefits, acting as cardioprotective agents with antioxidant properties, inhibiting platelet activity, and promoting vasodilation. These compounds also have anticancer and anti-inflammatory effects, estrogenic activity, and may help prevent neurodegenerative diseases such as Alzheimer's and Parkinson's (CALLEMIEN; COLLIN, 2009).

Silva et al. (2022) analyzed the total phenolic content and antioxidant capacity of craft beers with fruits produced in Portugal. These beers showed high levels of total phenolic compounds, ranging from 343.8 to 2,172.5 mg GAE L<sup>-1</sup>, similar to those found in this study, which ranged from 399.2 to 2,486.4 mg GAE L<sup>-1</sup>, with the highest values obtained in the beer with the most acerola pulp.

Significant differences ( $P < 0.05$ ) in antioxidant activity were observed among samples using the FRAP and DPPH methods. Adding acerola pulp resulted in a linear increase in antioxidant activity, with values of 449.9 (FRAP) and 12.1 (DPPH) for each 1% pulp added. There was a positive correlation between total phenolic levels and antioxidant activity measured by these methods. Sorbo and Broetto (2019) produced craft Pilsner beers with passion fruit pulp, and Hübner (2019) produced Catharina Sour craft beers with pitaya and ginger, obtaining similar results with increased total phenolic content and antioxidant activity. Using the ABTS radical scavenging method, the antioxidant activity of the beers, expressed in  $\alpha$ -tocopherol equivalents per liter, ranged from 90.76 to 777.71 mgEq T L<sup>-1</sup>. Among the tested beers, T3 had the highest average antioxidant activity, while the control had the lowest. These results differed from those obtained by the FRAP and DPPH methods, which had higher correlation coefficients of  $r^2 = 99.4$  and  $r^2 = 96.9$ , respectively, compared to the ABTS method ( $r^2 = 74.6$ ). Thus, the FRAP and DPPH methods are more closely related to the phenolic compound analysis results.

**Table 5.** Results of biochemical analysis, average values of total phenolics, FRAP, Flavonoids, DPPH, and ABTS of craft beers added with acerola pulp with different levels of inclusion

Variables	Mean±SD			
	0% of acerola pulp (T1)	5% of acerola pulp (T2)	10% of acerola pulp (T3)	20% of acerola pulp (T4)
Total phenolic compounds (mgEq GA L <sup>-1</sup> )	399.6±24.64 <sup>d</sup>	908.1±97.49 <sup>c</sup>	1,549.2±184.72 <sup>b</sup>	2,486.4±266.06 <sup>a</sup>
Total flavonoids (mgEq Q L <sup>-1</sup> )	463.57±5.88 <sup>a</sup>	466.68±14.17 <sup>a</sup>	407.04±96.08 <sup>a</sup>	477.25±33.48 <sup>a</sup>
FRAP (mmol Fe <sup>2+</sup> L <sup>-1</sup> )	1.01±0.05 <sup>b</sup>	0.84±0.19 <sup>b</sup>	2.23±0.16 <sup>b</sup>	5.34±1.32 <sup>a</sup>
DPPH (mgEq GA L <sup>-1</sup> )	129.10±22.25 <sup>c</sup>	168.53±22.05 <sup>bc</sup>	210.13±13.95 <sup>b</sup>	368.38±12.91 <sup>a</sup>
ABTS (mgEq T L <sup>-1</sup> )	90.76±42.57 <sup>b</sup>	212.89±6.54 <sup>b</sup>	777.71±9.99 <sup>a</sup>	765.98±163.43 <sup>a</sup>

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).

Dietary antioxidants, such as phenolic compounds, help prevent diseases related to oxidative stress. The antioxidant activity and low alcohol content of beer

enhance its nutritional value (PIAZZON et al., 2010). Flavonoid analysis showed no significant differences ( $P < 0.05$ ) and averaged 453.64 mg QE L<sup>-1</sup>, indicating that

base ingredients primarily influence flavonoid levels rather than acerola pulp. Flavonoids contribute to beer's astringency and bitterness. The flavonoid levels in this study were higher than those in a craft Pilsner with acerola pulp (68.9 mg QE L<sup>-1</sup>) (SOUZA et al., 2020). The higher phenolic concentration in samples with pulp is likely due to other phenolic compounds like carotenoids, anthocyanins, and phenylpropanoids, not just flavonoids. In this research, the reducing activity, as determined by the FRAP method, showed little variation between the control beer (without fruit) and the beer containing 5% pulp. However, a significant increase in reducing activity was observed when the pulp content was increased to between 10% and 20%, with values rising from 0.84 (with 5% pulp) to 5.34 mmol Fe<sup>2+</sup> L<sup>-1</sup> (with 20% pulp). This rise is attributed to the higher concentration of acerola pulp, which is rich in vitamins A and C, thiamine, riboflavin, niacin, rutin, quercetin, cyanidin, catechin, kaempferol, anthocyanins, and flavonoids (PIAZZON et al., 2010; MEZADRI et al., 2008; BATAGLION et al., 2015).

Ditrych et al. (2015) evaluated the antioxidant properties of sixteen beers produced in Poland and found that dark beers had higher reducing potentials, ranging from 5.0 to 8.8 mmol Fe<sup>2+</sup> L<sup>-1</sup>. In contrast, the reducing powers of craft lager beers ranged from 2.9 to 3.8 mmol Fe<sup>2+</sup> L<sup>-1</sup>, while large-scale lager beers ranged from 2.0 to 2.4 mmol Fe<sup>2+</sup> L<sup>-1</sup>. Additionally, the study highlighted that dark beers (such as Bock and Porter) require a higher malt load, contributing to their antioxidant content.

## CONCLUSION

Beers containing acerola pulp exhibited higher acidity, lower pH, reduced levels of soluble solids, and increased ash content in comparison to the control samples. The fermentation process with acerola pulp led to decreased soluble solids at the conclusion and elevated levels of phenolic compounds and antioxidant capacity, highlighting its potential as a differentiated option in the craft beer market. All the beers maintained a consistent straw yellow color with slight visual variations. Additionally, the inclusion of acerola pulp enhanced the stability and quality of the foam compared to the control, thus contributing to a higher quality of the final product.

## CONFLICTS OF INTEREST

There are no conflicts to declare.

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