

## Article

# Acetic Fermentation of Cagaita Pulp: Technological and Chemical Characteristics

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**Abstract:** The Brazilian Cerrado region has a rich plant diversity, with fruits that have peculiar and unique sensory characteristics. For these reasons, using these fruits for biotechnological production is a promising alternative, mainly to protect this biome from deforestation and degradation. The production of fermented acetic acid is an option to add value to native fruits and offer the market beverages with better nutritional quality and bioactive compounds. This work aimed to characterize fruits and to develop cagaita (*Eugenia dysenterica* DC.) acetic fermented beverage. The fruits were subjected to physical-chemical analyses in the first part. Subsequently, different treatments for fermentation were tested using two types of enzymes (amylase and pectinase), two subspecies of *Saccharomyces cerevisiae* yeast (UFLA CA11 and thermoresistant LNF Angel), and the chaptalization of the must with sucrose (16 °Brix). Alcoholic fermentation was carried out in an incubator with temperature control at 34 ± 1 °C. The pH, total soluble solids, titratable acidity, alcohol content, and density of the fermented products were monitored daily. The chaptalized must with amylase addition and thermoresistant yeast had the best performance during alcoholic fermentation, demonstrating that thermoresistant yeast is an economically advantageous and efficient alternative for the cagaita juice fermentation process. Subsequently, acetic fermentation was carried out using the slow method. Heat-resistant yeast without added enzymes was used to produce cagaita acetic fermented beverages within the parameters of the Brazilian legislation. Furthermore, phenolic compounds and antioxidant activity in the final product were observed. The work demonstrated the possibility of using cagaita fruits in biotechnological processes to produce new food products.

**Keywords:** Cerrado fruits; *Eugenia dysenterica* DC.; vinegar; acetic fermentation; cagaita



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## 1. Introduction

The Cerrado, the second largest biome in South America, covers approximately two million km<sup>2</sup> in central Brazil, standing out for its biodiversity that includes more than 10,000 species of plants, 1200 species of birds, and 800 species of mammals [1]. In addition to its ecological importance as a climate and water quality regulator, the biome is home to several endemic species [2]. However, the Cerrado biome faces severe threats from deforestation and degradation due to the expansion of agriculture, livestock farming, and fires [3]. A strategy to protect the Cerrado biome involves socioeconomic development and technological research into its native species, especially fruits with unique sensory characteristics and high nutritional value. This approach seeks to reconcile environmental

preservation with economic opportunities, promoting the appreciation of the biome's flora [4].

*Eugenia dysenterica* DC., known as cagaita, stands out among the fruit species of the Cerrado, being an exotic fruit (Figure 1) from the Myrtaceae family [5]. Cagaita fruits are characterized by their round shape, light yellow color, and slightly acidic flavor, weighing 14 to 20 g on average and containing three to four seeds each [6]. Furthermore, cagaita is a rich source of bioactive compounds such as quercetin, flavonoids, anthocyanins, and polyphenols, also standing out for its significant content of vitamin C (24.53 mg/100 g of fruit) [7,8]. The diversity of fruits found in the Cerrado, such as cagaita, presents promising economic opportunities for developing fermented beverages while also playing a crucial role in preserving this vital biome. Exploring these fruits for beverage production stimulates local economies and promotes sustainable practices for biodiversity conservation in the region [4,9].



**Figure 1.** Ripe cagaita (*Eugenia dysenterica*) fruits.

Given the nutritional importance and the presence of bioactive compounds in cagaita fruit, it is crucial to explore new ways of integrating this product into popular diets. A viable alternative is the production of acetic fermented beverages using the alcoholic fermentation method followed by acetic fermentation. The acetic fermentation process involves the oxidation of ethanol, previously produced by alcoholic fermentation, using yeasts of the *Saccharomyces* genus, into acetic acid. This process is driven by aerobic bacteria of the *Acetobacter* genus, also known as acetic acid bacteria (AAB) [10,11]. In addition to AAB, vinegar can be produced by inoculating vinegar mother in alcohol samples [12]. This approach offers an innovative way to incorporate cagaita into the diet and presents potential nutritional benefits from fermentation.

The metabolites produced by acetic acid bacteria (AAB) during the fermentation process inhibit the growth of undesirable microorganisms and have nutraceutical properties [13], contributing to the flavor and texture characteristics. Furthermore, several reactions and transformations that occur in the must during vinegar production result in highly flavored beverages [14]. To develop a new technology that promotes the appreciation and protection of a species native to the Cerrado, this study aimed to evaluate cagaita fruits as raw material in the production of cagaita beverages by means of acetic fermentation. This approach sought to explore the beneficial properties of AAB metabolites and contribute to the biome's economic promotion and preservation.

## 2. Materials and Methods

### 2.1. Plant Material

The collection of cagaiteira fruits (*Eugenia dysenterica* DC.) occurred in the natural vegetation of Montes Claros de Goiás (GO) (−16.1634, −51.3208), in October 2018. The fruits were subjected to a sanitization process with hypochlorite sodium at 200 ppm for 15 min. They were then manually pulped, separating the seeds homogenized in an industrial blender (SKYMPSEN, metalúrgica Siemens LTDA, Brusque, Brazil), and the solid residue was separated using around-the-world fabric. Subsequently, the pulp was freeze-dried and stored in a refrigerator.

### 2.2. Pulp Characterization

#### 2.2.1. Determination of Soluble Solids (SS), Titratable Acidity (TA), and pH

The soluble solids (SS) content was determined by means of direct reading using a digital refractometer (Refractometer Reichert, Depew, NY, USA), and the results were expressed in °Brix. The pH value was measured using a digital pH meter (Lucadema, model LUCA-210, São José do Rio Preto, Brazil).

Titrateable acidity (TA) was determined by the volumetric titration method with sodium hydroxide solution (NaOH 0.1 mol/L), with phenolphthalein as the indicator, and expressed in meq/L. The SS/AT ratio indicated the degree of ripeness of the fruits used in pulp production.

#### 2.2.2. Proximal Composition

The proximal composition of the pulp was carried out according to official methods [15] and expressed in g/100 g. The moisture content was determined by means of gravimetry in an oven at 105 °C, with a subsequent determination of ash by means of incineration at 550 °C. Protein content was determined using the Kjeldahl method (chemical digestion, distillation, and titration) and micro Kjeldahl Labconco equipment. Protein content was determined using the conversion factor of 6.25. The lipid content was determined using the method of Bligh and Dyer [16], while the carbohydrate content was calculated by difference (100-moisture-proteins-lipids-ash). To calculate the energy value, the Atwater conversion factor was used (proteins × 4 kcal, lipids × 9 Kcal, and carbohydrates × 4 Kcal).

#### 2.2.3. Determination of Vitamin C

Vitamin C was determined by reducing the sodium salt dye of 2,6-dichlorophenol indophenol by an ascorbic acid solution [15]. The extract was prepared from the sample to quantify total phenolic compounds and antioxidant activity. A total of 2 g of sample was used, and 20 mL of methanol (50%) after 60 min of rest and 20 mL of acetone (70%) were added. After sixty minutes, the extract was filtered and transferred to a 50 mL volumetric flask, and the volume was completed with distilled water.

### 2.3. Alcoholic Fermentation

In fermentation, two separate strains were used: *Saccharomyces cerevisiae*, UFLA CA11, and thermoresistant LNF-ANGEL (LNF, Bento Gonçalves, Brazil), both at a concentration of 10 g/L of dry matter of yeast. The cagaita pulps were thawed and mixed with filtered water to produce the juice, maintaining a water-to-pulp ratio of 1:1 (v:v). The methodology proposed by Oliveira et al. [17], with modifications, was used to prepare the must. Chaptalization, when carried out, adjusted the soluble solids content of the juice to 16 °Brix with the addition of commercial sucrose. Sodium metabisulfite was added to the must at a concentration of 0.1 g/L to reduce the bacterial load without affecting the fermentative activity of the yeasts [18]. Enzymatic treatment with amylase and pectinase was performed at 80 °C in a water bath for 20 min. The inoculated juice underwent anaerobic fermentation in a biochemical oxygen demand incubator at 35 °C, monitored daily for total soluble solids, pH, titrateable acidity, and alcohol content. The process was completed when the soluble

solids content remained stable after three consecutive readings. Details of the treatments studied for the production of alcohol from cagaita juice are presented in Table 1.

**Table 1.** Alcoholic fermentation of cagaita (*Eugenia dysenterica* DC.) juice subjected to different treatments.

Abbreviation	Treatment
NC/SY	Non-chaptalized must + Selected yeast
CM/P/SY	Chaptalized must + Pasteurization <sup>a</sup> + Selected yeast CA11
CM/P/HY	Chaptalized must + Pasteurization <sup>a</sup> + Heat-resistant yeast
CM/A/SY	Chaptalized must + Amylase application+ Selected yeast CA11
CM/A/HY	Chaptalized must + Amylase application + Heat-resistant yeast
CM/PC/SY	Chaptalized must + Pectinase application + Selected yeast CA11
CM/PC/HY	Chaptalized must + Pectinase application + Heat-resistant yeast

<sup>a</sup> 80 °C for 20 min.

#### 2.4. Acetic Fermentation

Acetic fermentation was carried out using the slow method, without stirring, using the cagaita musts obtained in the previous section. The acetic acid bacteria *Acetobacter* sp. (Frings, Piracicaba, SP, Brazil), at a concentration of 2 g/L, was used in the fermentation process. The acetification process occurred in a slow fermentation system at 25 °C, without stirring, with four repetitions. For acetic fermentation, concentrated acetic fermented cagaita with 9% (*v/v*) acidity and alcoholic fermented cagaita at 9% (*v/v*) were used, following a 1:10 ratio. Acetic fermentation lasted 72 days. During this period, samples were collected every 7 days for the analysis of acidity (titration), soluble solids content (refractometer), pH (pHmeter), and alcohol concentration (distillation). The acetic fermentation process was completed after 10 weeks when the cagaita beverages produced using acetic fermentation reached an alcohol content of less than 1.0% (*v/v*).

#### 2.5. Characterization of Alcoholic and Acetic Fermented Products

##### 2.5.1. Determination of Alcohol Concentration

The alcoholic degree of the fermented products was measured from the distilled sample (Alcohol Microdistiller TE-012, Piracicaba, Brazil) by density and alcohol content using a digital hydrometer (DMA35, Anton Paar, Vila Clementino, Brazil).

##### 2.5.2. Colorimetric Determination

The color was determined directly using a Chroma Meter CR-410 colorimeter (Konica Minolta, Tokyo, Japan). The parameters L\* (brightness), a\* (+: red, -: green), b\* (+: yellow, -: blue), C\* (Chroma: saturation), and h\* (hue angle: tone) were obtained through of the CIE (Commission Internationale de l'Éclairage) color space coordinates.

##### 2.5.3. Total Phenolic Compounds

To quantify total phenolic compounds, 200 µL of the extract was added to 1.9 mL of freshly prepared Follin–Ciocalteu reagent 1:9 in distilled water. The same volume (1.9 mL) of aqueous sodium carbonate solution (60 g/L) was used to neutralize the mixture. After 120 min of reaction without light and at room temperature, the absorbance was measured at 725 nm. The calculation was performed using the standard curve ( $R^2 = 0.998$ ), and the results were expressed in g gallic acid equivalents (GAE) per 100 g of sample.

##### 2.5.4. Antioxidant Capacity

The antioxidant capacity was measured using two methods: ABTS ((2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) and FRAP (iron reduction). For the ABTS method, an aliquot of 30 µL of the extract with 3 mL of the ABTS<sup>+</sup> radical and the reading was taken at 734 nm after 6 min, using ethyl alcohol to calibrate the spectrophotometer, as described by Pulido, Bravo, and Saura-Calixto [19]. The percentage of discoloration was calculated

according to Equation (1). The FRAP method was carried out as described by Pulido, Bravo, and Saura-Calixto [19], using 100  $\mu$ L of the extract and 3 mL of the FRAP reagent. Ferrous sulfate was used as the standard, and the absorbance reading was performed at 595 nm.

$$\% \text{ Discoloration} = \left[ 1 - \left( \frac{\text{Sample absorbance}}{\text{Control absorbance}} \right) \right] \times 100 \quad (1)$$

### 3. Results and Discussion

#### 3.1. Characterization of Cagaita Pulp

Table 2 presents the results of chemical analyses of cagaita fruits. The results found for the SS, pH, and TA parameters of cagaita fruits (Table 2) were superior compared to those reported by Araújo et al. [20] (5.2 °Brix, 2.2, and 0.64 g of citric acid/100 g, respectively) and the results obtained by Oliveira et al. [17] for SS and AT (8.3° Brix and 0.73 g/100 g, respectively).

The moisture content found in this work (85.61 g/100 g) was close to the values reported by Araújo et al. [20] (88.87%) and by the authors of [17] (88.32 g/100 g) for cagaita fruits. This parameter is associated with the quality of the fruit, as well as storage conditions. High moisture makes fruits highly susceptible to enzymatic and microbial deterioration, making their conservation difficult [21].

The ash content (Table 2), which refers to the mineral content, was twice as high as that found by Schiassi et al. [22] (0.30%) for fruits of the same species. The protein content in this study (1.37 g/100 g) exceeded the amounts reported by Morais et al. [21] (0.63 g/100 g) and Araújo et al. [20] (0.63 g/100 g).

**Table 2.** Chemical characterization of cagaita (*Eugenia dysenterica*) fruits.

Variables <sup>a</sup>	Mean <sup>b</sup> $\pm$ SE	Reference <sup>1</sup>	Reference <sup>2</sup>
Yield (%)	74.35 $\pm$ 0.34	-	-
Total soluble solids (°Brix)	8.8 $\pm$ 0.07	8.00 $\pm$ 0.15	9.12
pH	3.47 $\pm$ 0.01	3.84 $\pm$ 0.02	3.3
Titrateable acidity (g citric acid/100 g)	0.90 $\pm$ 0.02	0.64 $\pm$ 0.01	0.73
Maturation index (SST/ATT)	9.71 $\pm$ 0.13	12.64	12.49
Moisture (g/100 g)	85.61 $\pm$ 0.64	89.74 $\pm$ 0.10	91.56
Ash (g/100 g)	0.70 $\pm$ 0.1	0.30 $\pm$ 0.09	0.18 $\pm$ 0.02
Proteins (g/100 g)	1.37 $\pm$ 0.07	0.77 $\pm$ 0.01	0.63 $\pm$ 0.09
Lipids (g/100 g)	0.30 $\pm$ 0.007	0.49 $\pm$ 0.28	0.57 $\pm$ 0.05
Carbohydrates (g/100 g)	12.48 $\pm$ 0.42	8.09 $\pm$ 0.54	5.54 $\pm$ 0.65
Energetic value (kcal/100 g)	119.27 $\pm$ 3.5	39.87 $\pm$ 0.44	29.83 $\pm$ 3.43
Vitamin C (mg ascorbic acid/100 g)	26.38 $\pm$ 0.01	31.95 $\pm$ 0.77	34.11 $\pm$ 1.48

SD Standard deviation. <sup>a</sup> Values expressed in fresh matter. <sup>b</sup> Mean of three repetitions; <sup>1</sup> [22], <sup>2</sup> [21].

The lipid content in this work was lower than that reported by Morais et al. [21] (0.57 g/100 g), Araújo et al. [20] (0.57 g/100 g), and Schiassi et al. [22] (0.49 g/100 g) for cagaita fruits. Meanwhile, the carbohydrate content and, consequently, the caloric value were higher than those reported by Morais et al. [21] (5.54 g/100 g and 29.83 kcal/100 g), Araújo et al. [20] (29.83 kcal/100 g), and Schiassi et al. [22] (8.09 and 39.87 kcal/100 g) for cagaita fruits, respectively.

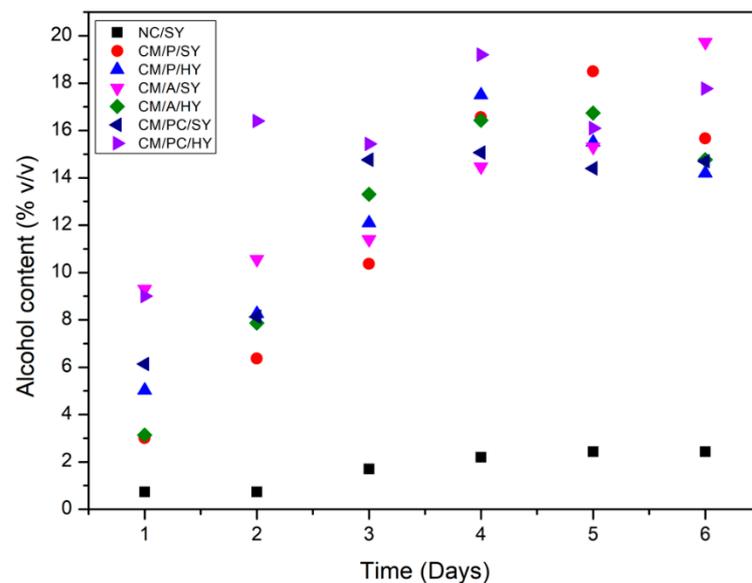
The vitamin C content found (26.38 mg of ascorbic acid/100 g) was lower than the value found by Araújo et al. [20] (34.11 mg/100 g) and higher than the value found by Alves et al. [23] (10.63 mg/100 g). Cagaita may be important for dietary and nutritional factors, as it contributes to meeting daily vitamin C recommendations ranging from 85 to 110 mg/day [21,24]. Furthermore, there are reports in the literature linking ascorbic acid's antioxidant properties to combating illnesses such as cardiovascular diseases, cancer, and infections [25].

Compared to the literature, the differences observed between the physical-chemical characteristics of the fruits can be attributed to several factors, such as soil, climate, the

time of year, and genetic variability, which significantly impact the physical-chemical characteristics of the fruits [22].

### 3.2. Assessment of Alcoholic Fermentation

Figure 1 shows the alcohol content of the treatments over six days of fermentation. Notably, the NC/SY treatment demonstrated the lowest alcohol content compared to the other treatments (Figure 2). This observation can be attributed to the fact that this treatment was not subjected to the chaptalization process. The initial soluble solids content was low (2.5 °Brix). It is worth mentioning that the soluble solids content is a measurement that reflects the amount of sugars and other soluble compounds in a liquid. Therefore, higher soluble solids content implies a greater quantity of sugars available for fermentation [26].



**Figure 2.** Alcohol content (% *v/v*) during alcoholic fermentation in the different treatments.

The CM/P/SY and CM/P/HY treatments, which used selected yeast and thermoresistant yeast, respectively, without pre-treatment with exogenous enzymes, demonstrated that the use of thermoresistant yeast allowed a more continuous fermentation, resulting in a higher alcoholic content, which was elevated during the fermentation process (Figure 2). This characteristic is due to the resistance of this strain to increased temperature, providing higher stability during the fermentation process [27].

The CM/A/SY and CM/A/HY treatments showed that the addition of amylase, an enzyme responsible for converting starch into simple sugars [28], increased alcohol content. Especially in the CM/A/HY treatment, which used thermoresistant yeast, the highest alcohol content was observed among all treatments on the sixth day of fermentation (Figure 2). These results indicate that the enzyme amylase played a significant role in increasing the availability of sugars for fermentation. A study by Favaretto et al. [29] also observed an increase in ethanol production using pre-treatment with amylase in banana, apple, mango, and papaya waste.

In the CM/PC/SY and CM/PC/HY treatments, pre-treatment was carried out with pectinase, an enzyme known for hydrolyzing pectic substances responsible for turbidity and high viscosity in fruit juices [30]. The smaller difference between these treatments and those where only pasteurization was performed (CM/P/SY and CM/P/HY) suggests that pectin did not significantly limit alcoholic fermentation in this context. Although pectinase reduces viscosity and turbidity, it does not appear to have facilitated the fermentation process, indicating that pectin was not a restrictive factor in this scenario.

In general, thermoresistant yeast proved to be more efficient in the alcoholic fermentation of cagaita juice. Enzymes may be unnecessary since thermoresistant yeast can break

down the carbohydrate macromolecules in the juice. This simplified approach highlights the effectiveness of thermoresistant yeast as an economically advantageous and efficient alternative for the cagaita juice fermentation process.

Table 3 presents the results of alcoholic fermented products. The samples presented  $L^*$  values closer to 0, indicating a dark color (Table 3). The lowest  $L^*$  values were observed for samples pre-treated with pectinase (CM/PC/SY and MC/PC/TR). Studies by Khandare et al. [31] found that enzyme-assisted processing reduced the luminosity ( $L$ ) of black carrot (*Daucus carota ssp. sativus*) juice and attributed this result to the intense color produced by anthocyanins, which increase with processing, assisted by the enzyme pectinase, a substance this is also present in cagaita [21].

**Table 3.** Colorimetric analysis of different alcoholic fermentation treatments.

Treatments	$L^*$	$^{\circ}h$	$C^*$
NC/SY	$30.83 \pm 0.04^b$	$139.37 \pm 0.45^b$	$2.40 \pm 0.04^c$
CM/P/SY	$29.39 \pm 0.09^b$	$119.23 \pm 1.01^e$	$0.85 \pm 0.05^e$
CM/P/HY	$29.21 \pm 0.02^b$	$130.49 \pm 0.05^c$	$2.21 \pm 0.02^d$
CM/A/SY	$28.72 \pm 0.11^c$	$124.05 \pm 0.79^d$	$2.68 \pm 0.02^b$
CM/A/HY	$35.53 \pm 0.02^a$	$119.30 \pm 0.03^e$	$8.36 \pm 0.03^a$
CM/PC/SY	$24.98 \pm 0.02^e$	$152.47 \pm 1.14^a$	$0.24 \pm 0.01^g$
CM/PC/HY	$26.59 \pm 0.04^d$	$139.28 \pm 2.17^b$	$0.44 \pm 0.05^f$

Values in the same column followed by at least one common letter (or not followed by any letter) are not significantly different according to Tukey's test ( $p < 0.05$ ).

The hue values ( $^{\circ}h$ ), which define red at  $0^{\circ}$ , yellow at  $90^{\circ}$ , green at  $180^{\circ}$ , and blue at  $270^{\circ}$ , varied between 119.23 and 152.47, indicating a greenish-yellow hue. The presence of fat-soluble compounds, such as carotenoids, can explain this color. Carotenoids are natural pigments that give the product a yellow color [32].

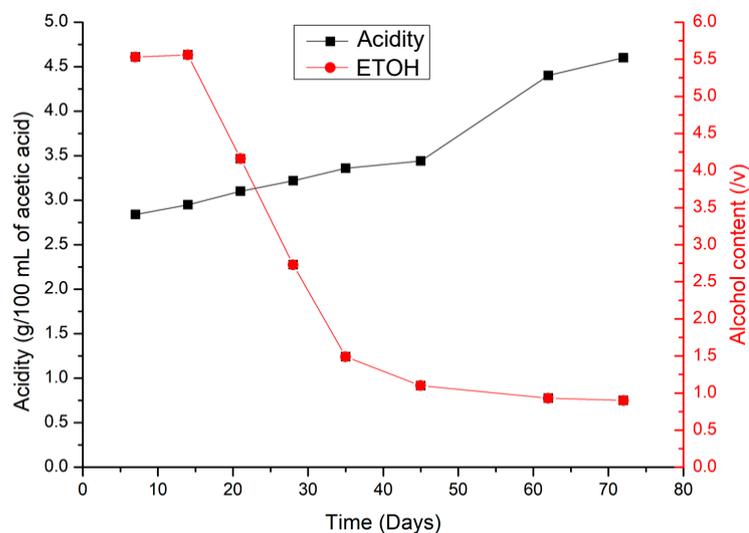
The highest  $C^*$  values were observed for treatments CM/A/HY (8.36), CM/A/SY (2.68), and NC/SY (2.40), indicating that the coloring of these samples was more saturated. Among the treatments, CM/PC/SY presented the lowest value in the  $L^*$  parameter and the highest in  $^{\circ}h$ . The macromolecule degradation can explain the difference in colorimetric parameter values between fermented samples during fermentation, where yeasts and bacteria break down macromolecules, such as starch, proteins, and pectin. This results in the formation of smaller molecules, which are more easily perceived by the human eye. Thus, the decrease in the values of colorimetric parameters in fermented samples can be interpreted as a reduction in luminosity, yellow tone, and color saturation.

After verifying that CM/P/HY presented more efficient results (the strain used presents better performance due to its greater tolerance to ethanol, as it produces more alcohol in a shorter time when compared to the other treatments), a triplicate assay was carried out using the thermoresistant yeast with chaptalized juice and soluble solids at  $11^{\circ}$  Brix. It took four days to stabilize this variable, resulting in an alcohol content of  $9 \pm 0.1$  (%  $v/v$ ). This demonstrated that it is unnecessary to ferment until the sixth day to increase the desired alcohol content.

### 3.3. Assessment of Acetic Fermentation

The production of fermented acetic beverages was monitored for 10 weeks, and over this period, the concentration of organic acids, mainly acetic acid, significantly increased (Figure 2). This increase in acetic acid gives the fermented acetic acid a characteristic flavor and inhibits the growth of undesirable or pathogenic microorganisms due to its high acidity [33]. During the fermentation process, a reduction in alcohol content was observed from  $5.9 \pm 0.3$  (%  $v/v$ ) to  $0.46 \pm 0.05$  (%  $v/v$ ). This type of acetic fermentation, known as the slow method, occurs when acetic bacteria convert ethanol into acetic acid, which is catalyzed by an enzyme family called acetobacteraceae that catalyzes the oxidation reaction of ethanol into acetic acid [34].

The results presented in Figure 3, which show an acetic acid concentration greater than 4 g/100 mL and an alcohol content less than 1% *v/v*, indicate that the acetic fermentation carried out in this study was more efficient than that described by Isham et al. [12], who obtained acetic acid concentrations of 2.05 to 2.41 g/100 mL using mushrooms and yeast as a starter culture for vinegar production.



**Figure 3.** Titratable acidity and alcohol content during the acetic fermentation of cagaita (*Eugenia dysenterica* DC.).

According to Guerreiro et al. [35], the most common acetification system is slow. This system is preferred for producing quality vinegar, as it provides a product with a higher acetic acid content and a lower content of undesirable volatile compounds. Another factor to consider is the raw material, as vinegar from fruits, for example, tend to be more aromatic and less acidic than vinegar from cereals [36].

### 3.4. Chemical Changes in the Development of Acetic Fermented Cagaita

The results of the analysis of bioactive compounds (Table 4) reveal that the amounts of total phenolic compounds (TPC) in cagaita pulp ( $1287.59 \pm 33.45$  mg gallic acid equivalents (GAE)/L) reduced after fermentations ( $730.88 \pm 12.82$  mg GAE/L for alcoholic fermentation and  $728.64 \pm 14.61$  mg GAE/L for acetic fermentation). This result is in line with other research that reported a reduction in the concentrations of phenolic compounds throughout the production of prickly pear [37] and pomegranate vinegar [38,39].

**Table 4.** Phenolic compounds and antioxidant capacity of pulp, and alcoholic and acetic fermented beverage of cagaita (*Eugenia dysenterica* DC.).

Composition	Pulp	Alcoholic Fermented Beverage	Acetic Fermented Beverage
TPC (mg GAE/L)	$1287.59 \pm 33.45^a$	$730.88 \pm 12.82^b$	$728.64 \pm 14.61^b$
ABTS (% decoloration)	$16.97 \pm 0.49^a$	$8.81 \pm 0.08^b$	$6.32 \pm 0.73^c$
FRAP ( $\mu\text{M FeSO}_4/\text{mL}$ )	$809.60 \pm 1.76^a$	$149.97 \pm 1.76^c$	$243.28 \pm 1.52^b$

Mean  $\pm$  SE. GAE: gallic acid equivalents;  $\text{FeSO}_4$ : ferrous sulfate. Values in the same line followed by at least one common letter (or not followed by any letter) are not significantly different according to Tukey's test ( $p < 0.05$ ).

The reduction in TPC after fermentative processes may be associated with condensation and polymerization reactions and adsorption in yeast cells [39]. According to Davies et al. [40], factors such as temperature, fermentation time, and the type of yeast

quantitatively and qualitatively affect the phenolic composition due to the oxidative processes that occur during these procedures.

The antioxidant activity of the pulp, alcoholic fermented beverage, and acetic fermented beverage was investigated using the antioxidant systems: 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) by discoloration and iron-reducing antioxidant power (FRAP). Regardless of the method, the antioxidant capacity of alcoholic and acetic fermented products decreased in relation to cagaita pulp (Table 3). These results are similar to those found by Ubeda et al. [41], Kharchoufi et al. [38], and Leonés et al. [42] for the production of strawberry, pomegranate, and lemon vinegar, respectively. The reduction in antioxidant activity may be related to the reduction in total phenolic compound content, as previously reported (Table 4).

#### 4. Conclusions

Cagaita beverages produced by means of acetic fermentation add value to this native Cerrado fruit and are an innovative product. The study in question proved the feasibility of using cagaita in developing fermented acetic acid for the food industry, which is justified by the presence of bioactive compounds that benefit human health. The treatments efficiently identified the viability of producing alcoholic fermented beverages, resulting in a drink with a satisfactory alcoholic content for acetic fermentation. The last stage also efficiently produced quality acetic fermented drinks. Therefore, the acetic fermented cagaita produced can be used as a beverage or applied to foods such as salads, thus offering a versatile and nutritious option that taps into the rich potential of this native fruit from the Cerrado region.

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#### References

1. Colli, G.R.; Vieira, C.R.; Dianese, J.C. Biodiversity and conservation of the Cerrado: Recent advances and old challenges. *Biodivers. Conserv.* **2020**, *29*, 1465–1475. [[CrossRef](#)]
2. Lewis, K.; de V. Barros, F.; Cure, M.B.; Davies, C.A.; Furtado, M.N.; Hill, T.C.; Hirota, M.; Martins, D.L.; Mazzochini, G.G.; Mitchard, E.T. Mapping native and non-native vegetation in the Brazilian Cerrado using freely available satellite products. *Sci. Rep.* **2022**, *12*, 1588. [[CrossRef](#)] [[PubMed](#)]
3. Hofmann, G.S.; Cardoso, M.F.; Alves, R.J.; Weber, E.J.; Barbosa, A.A.; de Toledo, P.M.; Pontual, F.B.; Salles, L.d.O.; Hasenack, H.; Cordeiro, J.L. The Brazilian Cerrado is becoming hotter and drier. *Glob. Change Biol.* **2021**, *27*, 4060–4073. [[CrossRef](#)] [[PubMed](#)]
4. Lima, D.C.; da Rocha Alves, M.; Nogueira, N.H.; do Nascimento, R.d.P. A review on Brazilian baru plant (*Dipteryx alata* Vogel): Morphology, chemical composition, health effects, and technological potential. *Future Foods* **2022**, *5*, 100146. [[CrossRef](#)]
5. Santana, L.F.; Sasso, S.; Aquino, D.F.S.; de Cássia Freitas, K.; de Cássia Avellaneda Guimarães, R.; Pott, A.; do Nascimento, V.A.; Bogo, D.; de Oliveira Figueiredo, P.; Hiane, P.A. Nutraceutical potential of bioactive compounds of *Eugenia dysenterica* DC in metabolic alterations. *Molecules* **2022**, *27*, 2477. [[CrossRef](#)] [[PubMed](#)]
6. da Silva, M.M.M.; da Silva, E.P.; da Silva, F.A.; Ogando, F.I.B.; de Aguiar, C.L.; Damiani, C. Physiological development of cagaita (*Eugenia dysenterica*). *Food Chem.* **2017**, *217*, 74–80. [[CrossRef](#)] [[PubMed](#)]
7. Daza, L.D.; Fujita, A.; Granato, D.; Fávares-Trindade, C.S.; Genovese, M.I. Functional properties of encapsulated Cagaita (*Eugenia dysenterica* DC.) fruit extract. *Food Biosci.* **2017**, *18*, 15–21. [[CrossRef](#)]

8. Santos, D.d.; Oliveira Filho, J.d.; Sousa, T.d.; Ribeiro, C.; Egea, M. Ameliorating effects of metabolic syndrome with the consumption of rich-bioactive compounds fruits from Brazilian Cerrado: A narrative review. *Crit. Rev. Food Sci. Nutr.* **2021**, *62*, 7632–7649. [[CrossRef](#)] [[PubMed](#)]
9. Araujo, R.L.d.; Tomás-Barberán, F.A.; Santos, R.F.d.; Martínez-Blazquez, J.A.; Genovese, M.I. Postprandial glucose-lowering effect of cagaita (*Eugenia dysenterica* DC) fruit juice in dysglycemic subjects with metabolic syndrome: An exploratory study. *Food Res. Int.* **2021**, *142*, 110209. [[CrossRef](#)]
10. Girardi Piva, G.; Casalta, E.; Legras, J.-L.; Tesnière, C.; Sablayrolles, J.-M.; Ferreira, D.; Ortiz-Julien, A.; Galeote, V.; Mouret, J.-R. Characterization and role of sterols in *Saccharomyces cerevisiae* during white wine alcoholic fermentation. *Fermentation* **2022**, *8*, 90. [[CrossRef](#)]
11. Song, J.; Wang, J.; Wang, X.; Zhao, H.; Hu, T.; Feng, Z.; Lei, Z.; Li, W.; Zheng, Y.; Wang, M. Improving the acetic acid fermentation of *Acetobacter pasteurianus* by enhancing the energy metabolism. *Front. Bioeng. Biotechnol.* **2022**, *10*, 815614. [[CrossRef](#)] [[PubMed](#)]
12. Isham, N.K.M.; Mokhtar, N.; Fazry, S.; Lim, S.J. The development of an alternative fermentation model system for vinegar production. *LWT* **2019**, *100*, 322–327. [[CrossRef](#)]
13. Aulitto, M.; Alfano, A.; Maresca, E.; Avolio, R.; Errico, M.E.; Gentile, G.; Cozzolino, F.; Monti, M.; Pirozzi, A.; Donsì, F.; et al. Thermophilic biocatalysts for one-step conversion of citrus waste into lactic acid. *Appl. Microbiol. Biotechnol.* **2024**, *108*, 155. [[CrossRef](#)] [[PubMed](#)]
14. Gomes, R.J.; de Fatima Borges, M.; de Freitas Rosa, M.; Castro-Gómez, R.J.H.; Spinosa, W.A. Acetic acid bacteria in the food industry: Systematics, characteristics and applications. *Food Technol. Biotechnol.* **2018**, *56*, 139. [[CrossRef](#)] [[PubMed](#)]
15. Horwitz, W.; Chichilo, P.; Reynolds, H. *Official Methods of Analysis of the Association of Official Analytical Chemists*; Association of Official Analytical Chemists: Rockville, MD, USA, 1970.
16. Bligh, E.G.; Dyer, W.J. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **1959**, *37*, 911–917. [[CrossRef](#)] [[PubMed](#)]
17. Oliveira, M.; Pantoja, L.; Duarte, W.; Collela, C.; Valarelli, L.; Schwan, R.; Dias, D. Fruit wine produced from cagaita (*Eugenia dysenterica* DC) by both free and immobilised yeast cell fermentation. *Food Res. Int.* **2011**, *44*, 2391–2400. [[CrossRef](#)]
18. Dias, D.R.; Schwan, R.F.; Freire, E.S.; Serôdio, R.d.S. Elaboration of a fruit wine from cocoa (*Theobroma cacao* L.) pulp. *Int. J. Food Sci. Technol.* **2007**, *42*, 319–329. [[CrossRef](#)]
19. Pulido, R.; Bravo, L.; Saura-Calixto, F. Antioxidant activity of dietary as determined by a modified ferric reducing/ antioxidant power assay. *J. Agric. Food Chem.* **2000**, *48*, 3396–3402. [[CrossRef](#)] [[PubMed](#)]
20. Araújo, F.F.d.; Neri-Numa, I.A.; Paulo Farias, D.d.; Cunha, G.R.M.C.d.; Pastore, G.M. Wild Brazilian species of *Eugenia* genera (Myrtaceae) as an innovation hotspot for food and pharmacological purposes. *Food Res. Int.* **2019**, *121*, 57–72. [[CrossRef](#)]
21. de Moraes Cardoso, L.; Martino, H.S.D.; Moreira, A.V.B.; Ribeiro, S.M.R.; Pinheiro-Sant’Ana, H.M. Cagaita (*Eugenia dysenterica* DC.) of the Cerrado of Minas Gerais, Brazil: Physical and chemical characterization, carotenoids and vitamins. *Food Res. Int.* **2011**, *44*, 2151–2154. [[CrossRef](#)]
22. Schiassi, M.C.E.V.; de Souza, V.R.; Lago, A.M.T.; Campos, L.G.; Queiroz, F. Fruits from the Brazilian Cerrado region: Physico-chemical characterization, bioactive compounds, antioxidant activities, and sensory evaluation. *Food Chem.* **2018**, *245*, 305–311. [[CrossRef](#)] [[PubMed](#)]
23. Alves, A.M.; Dias, T.; Hassimotto, N.M.A.; Naves, M.M.V. Ascorbic acid and phenolic contents, antioxidant capacity and flavonoids composition of Brazilian Savannah native fruits. *Food Sci. Technol.* **2017**, *37*, 564–569. [[CrossRef](#)]
24. Mason, S.A.; Parker, L.; van der Pligt, P.; Wadley, G.D. Vitamin C supplementation for diabetes management: A comprehensive narrative review. *Free Radic. Biol. Med.* **2023**, *194*, 255–283. [[CrossRef](#)]
25. Doseděl, M.; Jirkovský, E.; Macáková, K.; Krčmová, L.K.; Javorská, L.; Pourová, J.; Mercolini, L.; Remião, F.; Nováková, L.; Mladěnka, P. Vitamin C—Sources, physiological role, kinetics, deficiency, use, toxicity, and determination. *Nutrients* **2021**, *13*, 615. [[CrossRef](#)]
26. Resende Oliveira, É.; Caliari, M.; Soares Soares Júnior, M.; Ribeiro Oliveira, A.; Cristina Marques Duarte, R.; Valério de Barros Vilas Boas, E. Assessment of chemical and sensory quality of sugarcane alcoholic fermented beverage. *J. Food Sci. Technol.* **2018**, *55*, 72–81. [[CrossRef](#)]
27. Hasunuma, T.; Kondo, A. Consolidated bioprocessing and simultaneous saccharification and fermentation of lignocellulose to ethanol with thermotolerant yeast strains. *Process Biochem.* **2012**, *47*, 1287–1294. [[CrossRef](#)]
28. De Souza, P.M.; de Oliveira Magalhães, P. Application of microbial  $\alpha$ -amylase in industry—A review. *Braz. J. Microbiol.* **2010**, *41*, 850. [[CrossRef](#)] [[PubMed](#)]
29. Favaretto, D.P.C.; Rempel, A.; Lanzini, J.R.; Silva, A.C.M.; Lazzari, T.; Barbizan, L.D.; Brião, V.B.; Colla, L.M.; Treichel, H. Fruit residues as biomass for bioethanol production using enzymatic hydrolysis as pretreatment. *World J. Microbiol. Biotechnol.* **2023**, *39*, 144. [[CrossRef](#)] [[PubMed](#)]
30. Patel, V.B.; Chatterjee, S.; Dhoble, A.S. A review on pectinase properties, application in juice clarification, and membranes as immobilization support. *J. Food Sci.* **2022**, *87*, 3338–3354. [[CrossRef](#)]
31. Khandare, V.; Walia, S.; Singh, M.; Kaur, C. Black carrot (*Daucus carota* ssp. sativus) juice: Processing effects on antioxidant composition and color. *Food Bioprod. Process.* **2011**, *89*, 482–486. [[CrossRef](#)]
32. Bicanic, D.D. On the photoacoustic, photothermal and colorimetric quantification of carotenoids and other phytonutrients in some foods: A review. *J. Mol. Struct.* **2011**, *993*, 9–14. [[CrossRef](#)]

33. Viana, R.O.; Magalhães-Guedes, K.T.; Braga, R.A.; Dias, D.R.; Schwan, R.F. Fermentation process for production of apple-based kefir vinegar: Microbiological, chemical and sensory analysis. *Braz. J. Microbiol.* **2017**, *48*, 592–601. [[CrossRef](#)] [[PubMed](#)]
34. Gullo, M.; Verzelloni, E.; Canonico, M. Aerobic submerged fermentation by acetic acid bacteria for vinegar production: Process and biotechnological aspects. *Process Biochem.* **2014**, *49*, 1571–1579. [[CrossRef](#)]
35. Guerreiro, T.M.; de Oliveira, D.N.; Ferreira, M.S.; Catharino, R.R. High-throughput analysis by SP-LDI-MS for fast identification of adulterations in commercial balsamic vinegars. *Anal. Chim. Acta* **2014**, *838*, 86–92. [[CrossRef](#)] [[PubMed](#)]
36. Luzón-Quintana, L.M.; Castro, R.; Durán-Guerrero, E. Biotechnological processes in fruit vinegar production. *Foods* **2021**, *10*, 945. [[CrossRef](#)] [[PubMed](#)]
37. Hammouda, M.B.; Azabou, S.; Durán-Guerrero, E.; Attia, H.; Castro, R. Investigation of phenolic and volatile compounds changes during vinegar production using spontaneous fermentation of different prickly pear (*Opuntia ficus-indica*)-based matrices. *J. Food Compos. Anal.* **2024**, *126*, 105855. [[CrossRef](#)]
38. Kharchoufi, S.; Gomez, J.; Lasanta, C.; Castro, R.; Sainz, F.; Hamdi, M. Benchmarking laboratory-scale pomegranate vinegar against commercial wine vinegars: Antioxidant activity and chemical composition. *J. Sci. Food Agric.* **2018**, *98*, 4749–4758. [[CrossRef](#)] [[PubMed](#)]
39. Ordoudi, S.A.; Mantzouridou, F.; Daftsiou, E.; Malo, C.; Hatzidimitriou, E.; Nenadis, N.; Tsimidou, M.Z. Pomegranate juice functional constituents after alcoholic and acetic acid fermentation. *J. Funct. Foods* **2014**, *8*, 161–168. [[CrossRef](#)]
40. Davies, C.V.; Gerard, L.M.; Ferreyra, M.M.; Schvab, M.d.C.; Solda, C.A. Bioactive compounds and antioxidant activity analysis during orange vinegar production. *Food Sci. Technol.* **2017**, *37*, 449–455. [[CrossRef](#)]
41. Ubeda, C.; Callejón, R.; Hidalgo, C.; Torija, M.; Troncoso, A.; Morales, M. Employment of different processes for the production of strawberry vinegars: Effects on antioxidant activity, total phenols and monomeric anthocyanins. *LWT-Food Sci. Technol.* **2013**, *52*, 139–145. [[CrossRef](#)]
42. Leonés, A.; Durán-Guerrero, E.; Carbú, M.; Cantoral, J.M.; Barroso, C.G.; Castro, R. Development of vinegar obtained from lemon juice: Optimization and chemical characterization of the process. *LWT* **2019**, *100*, 314–321. [[CrossRef](#)]

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