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Bio-Agronomic Assessment and Quality Evaluation of Sugarcane with Optimized Juice Fermentation in View of Producing Sicilian "Rum Agricole"

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Abstract

Sugarcane (*Saccharum* spp. L.), traditionally cultivated in tropical and subtropical regions, is being explored for its agronomic viability in Mediterranean climates. This study assessed the bio-agronomic performance of seven sugarcane varieties and two accessions grown in Sicily, to enhance the fermentation process to produce rum agricole, a spirit derived from fresh cane juice. Agronomic evaluations revealed significant varietal differences, with juice yields of 5850−14,312 L ha^{-1} and sugar yields of 1.84–5.33 t ha^{-1}. Microbial control was achieved through the addition of lactic acid, which effectively suppressed undesirable bacterial growth and improved fermentation quality. Furthermore, the application of two selected *Saccharomyces cerevisiae* strains (MN113 and SPF21), isolated from high-sugar matrices such as manna and honey byproducts, affected the production of volatile compounds, particularly esters and higher alcohols. Sensory analysis confirmed a more complex aromatic profile in cane wines fermented with these selected yeasts, with overall acceptance scores reaching 7.5. Up to 29 aroma-active compounds were identified, including ethyl esters and higher alcohols. This research represents the first integrated approach combining lactic acid treatment and novel yeast strains for the fermentation of sugarcane juice in a Mediterranean context. The findings highlight the potential for high-quality rum agricole production in Sicily.

Keywords: sugarcane; alcoholic fermentation; rum agricole; volatile organic compounds

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1. Introduction

Sugarcane (*Saccharum* spp. L.) is cultivated mainly in tropical and subtropical regions worldwide. According to the Food and Agriculture Organization of the United Nations, sugarcane is cultivated on 261 million hectares globally, with a total yield of 1.92 billion tons [1]. Among the most economically valuable crops, this species significantly contributes to global food production, supporting the dietary needs of nearly half the world's population [2]. Sugarcane juice is the raw material for many products, including sugar (approximately 70% of production), fruit juice, rum, paper, alcohol, xylitol, chemicals, organic soil improvers, feed, and energy [3].

The growth of sugarcane is highly dependent on weather conditions, especially when cultivated outside tropical or subtropical regions [4]. Sugarcane cannot be grown in areas where the average temperature of the coldest month is below 10 °C [5]. Distinct temperature thresholds have been identified for the four primary developmental stages of sugarcane. Cutting germination and tillering require temperatures above 18 °C, while optimal conditions for the rapid growth phase range between 28 °C and 32 °C. The ripening stage, meanwhile, progresses most efficiently within a range from the basal temperature up to 18 °C [6,7]. The coastal areas of Sicily typically feature a Mediterranean climate with mild winters and hot, sunny summers.

Rum agricole is a type of rum that stands out due to its unique production process and distinctive organoleptic characteristics. Unlike most rums, which are primarily made from molasses, rum agricole is made by fermenting and distilling fresh sugarcane juice (*Saccharum officinarum* L.) [8]. This gives rum agricole its characteristic sugarcane flavor, which is absent in rums made from molasses or syrup [9]. The Caribbean regions, including French overseas departments such as Guadeloupe, Martinique, French Guiana, and Réunion, as well as the autonomous region of Madeira, are renowned for producing this sugarcane spirit [10]. The term "Agricole" can only be used as a geographical indication for the traditional production of this rum in these specific regions of Europe (Regulation EU 2019/787) [11].

The production of rum agricole begins with the harvesting of sugarcane, followed by pressing to extract the juice, which is then filtered and clarified. This juice, rich in fermentable sugars, is the ideal substrate for fermentation [12]. After extraction, the cane juice undergoes fermentation and distillation [13]. Fermentation is a critical stage as it greatly influences the flavor and taste of the final product. *Saccharomyces cerevisiae* yeasts are commonly used, although non-*Saccharomyces* yeasts can also be employed to create a unique sensory profile [8]. During fermentation, yeasts produce not only ethanol but also secondary products such as acids and volatile organic compounds, which affect the final quality of cane wine and rum [13]. The fermentation process is affected not only by the native microbial population present in sugarcane but also by potential contamination introduced through processing equipment [14]. The bacterial communities found on the stalk surface and within the extracted juice comprise aerobic lactic acid bacteria, *Corynebacteria*, *Enterobacteria*, *Micrococcus*, *Bacillus*, and *Pseudomonas*, alongside aerotolerant anaerobes such as *Lactobacillus* spp. and *Leuconostoc* spp. [12]. Microorganisms enter sugarcane through stem cutting and insect attack. These microorganisms proliferate especially if the cut canes are not quickly crushed [15]. Bacterial populations present in the must compete with yeasts for sugars and nutrients that are essential for their growth [8]. Excessive bacterial proliferation has been demonstrated to compromise the quality of cane wine by generating undesirable metabolites such as 2,3-butanedione, acetic acid, and acetoin [16]. To enhance the reproducibility and control of the fermentation process, antimicrobial agents are introduced into the cane juice. This includes the use of sulfuric acid to lower the pH to approximately 3.5–4.5, thereby regulating the microbial community [12,15].

It is widely recognized that lactic acid, sulfuric acid, and metabisulfite can reduce microbial loads due to their antimicrobial properties [17–19]. However, except for sulfuric acid, there is currently no scientific research on the use of these substances in rum production. Despite their strong antimicrobial properties, their potential innovative use in rum production remains untested. In addition, this product is used in non-harmful quantities, ensuring its safety from a food safety perspective. Controlling microbial contamination during sugarcane juice fermentation is particularly important because uncontrolled microflora can compete with selected yeast strains, reducing ethanol yields and generating undesirable metabolites that negatively affect the sensory profile of the final product. Furthermore,

the high sugar content and nutrient-rich composition of sugarcane juice create favorable conditions for rapid microbial proliferation [15]. Therefore, the use of lactic acid, sulfuric acid, and potassium metabisulfite as pre-fermentation treatments may contribute not only to stabilizing the raw material but also to standardizing fermentation performance and ensuring consistent product quality. To ensure better reproducibility and control during fermentation, yeasts with high fermentation yields and the ability to produce significant amounts of volatile compounds are selected [16]. Additional studies have isolated yeasts from high-sugar matrices, such as manna and honey byproducts, to identify osmophilic microorganisms that can survive under the stressful conditions created by osmotic pressure [20,21]. These microorganisms can produce flavors that enhance the final aroma of fermented alcoholic beverages [22]. To our knowledge, this is the first study to apply lactic acid and novel *S. cerevisiae* strains from high-sugar matrices to modulate the microbial and aromatic profile of sugarcane juice fermentation for rum agricole.

Based on these considerations, the objective of this research is to establish a robust foundation for the production of high-quality rum in Sicily, with a view to enhancing the value of sugar cane and providing support for the agricultural and industrial development of the region.

2. Materials and Methods

2.1. Experimental Site and Cultivation Practices

This study was carried out from 2022 to 2023 at the experimental farm "Campo Carboj", University of Palermo (Italy) (37°58′86.0″ N, 12°89′55.7″ E, 39 m a.s.l.). The soil is classified as sandy clay loam. The sugarcane field was established in May 2022 using single-node, one-bud cuttings with a plant distance of 1.0 × 0.5 m. A randomized block design with three replications was used. Prior to transplanting, deep tillage was carried out, followed by organic fertilization (15 t ha^{-1}) and secondary tillage. The experimental plot was 25 m^2. A drip irrigation system was employed to satisfy the crop's evapotranspirative water demands. During the initial growth stages, weed control was carried out manually. The sugarcane genotypes evaluated in this study, along with their respective geographical origins, are presented in Table 1.

Table 1. Plant material.

Variety/Accession	Origin	Centre
PSR 07-334	Filippine	Philsurin
FR 87-83	Guadeloupe	Visacane
KN 07-0037	Sudan	Kenana
CPCL 02-1295	USA—Florida	USDA
CP 06-2495	USA—Florida	USDA
CP 09-1952	USA—Florida	USDA
Mex 69-290	Messico	Visacane
Ananas	Caribbean	-
Baltasià	Caribbean	-

2.2. Meteorological Data

Meteorological data were collected by the Atmos 21 weather station located at the experimental site. The ATMOS 41 station measures 12 meteorological variables, including air temperature, relative humidity, vapor pressure, barometric pressure, wind speed and direction, solar radiation, precipitation, and lightning. The station was connected to a ZL6 datalogger (Meter Group, Pullman, WA, USA) to collect data from environmental sensors. The datalogger transmits the data to the cloud via the Subscriber Identity Module.

2.3. Growing Degree Days

The principal developmental stages of sugarcane were identified using the extended BBCH scale [23]. To track the crop's growth cycle, cumulative growing degree days (CGDD) were computed for the period spanning May 2022 to March 2023, based on the following equation:

$$CGDD = [(Tmax - Tmin)/2] - Tbase$$

where Tmax is the maximum daily temperature, Tmin is the minimum daily temperature, and Tbase is the base temperature below which development stops. A value of 12 °C was used as the base temperature for sugarcane.

2.4. Characteristics of Sugarcane Juice and Production Process

At harvest in March 2023, plant height (cm), millable cane length (cm), number of nodes (n.), average node diameter (mm), number of tillers (n ha^{-1}), and number of millable canes (n ha^{-1}) were recorded. After harvesting, the canes were transported to the laboratory for juice extraction. An electric extractor with three stainless steel rollers (Vevor®, Shangai, China) was used to separate the cane juice from the bagasse. The amount of juice produced was measured for each replicate. The MCP 5500 modular polarimeter (Anton Paar GmbH, Graz, Austria) was used to determine °Brix and sucrose content (g cm^{-3}) of the cane juice. The pH of sugarcane juice was measured with the HI6221-02 pH meter (HANNA® Instruments, Villafranca Padovana, Italy). Commercial cane sugar (CCS) and sugar yield were determined according to Chen & Chou [24]. For each sugarcane accession and replicate, 0.5 L of juice was used for subsequent laboratory analyses related to fermentation tests using new starters.

2.5. Yeast Strains and Media

The yeast strains *Saccharomyces cerevisiae* MN113 and SPF21 utilized in this study were previously isolated from manna and honey byproducts, as reported by Guarcello et al. [21] and Gaglio et al. [20]. These strains were cryopreserved at −80 °C in broth containing 20% (*v*/*v*) glycerol within the microbial collection of the Department of Agricultural, Food and Forest Sciences (SAAF), University of Palermo, Italy. Prior to use, the yeasts were reactivated from cryogenic storage and propagated in accordance with the protocol outlined by Pirrone et al. [25]. The commercial strain *S. cerevisiae* EC1118 (Lallemand Inc., Montreal, QC, Canada) was utilized as the control. All culture media and supplements were obtained from Oxoid (Thermo Fisher, Milan, Italy).

2.6. Experimental Plan and Sample Collection for Sugarcane Fermentation

Juices extracted from nine sugarcane varieties were then mixed in equal proportions to obtain a single batch of 24 L of juice, with the aim of ensuring chemical and physical homogeneity between fermentation treatments. The juice was divided into 16 experimental treatments (SC1-SC16), with each treatment being conducted in three independent 500 mL fermentation units.

Trials from SC1 to SC4 did not include any chemical treatment, trials from SC5 to SC8 included the addition of 5 g per hectoliter (g/hL) potassium metabisulfite, trials from SC9 to SC12 were acidified with sulfuric acid to pH 3.5 [15], while trials from SC13 to SC16 were acidified with lactic acid to pH 3.5. The different treatments were applied to three different strains of inocula and a spontaneous fermentation. Specifically, the strains used were *S. cerevisiae* SPF21, *S. cerevisiae* MN113, and the commercial control *S. cerevisiae* EC1118. Finally, sixteen experimental trials were set up as shown in Figure 1.

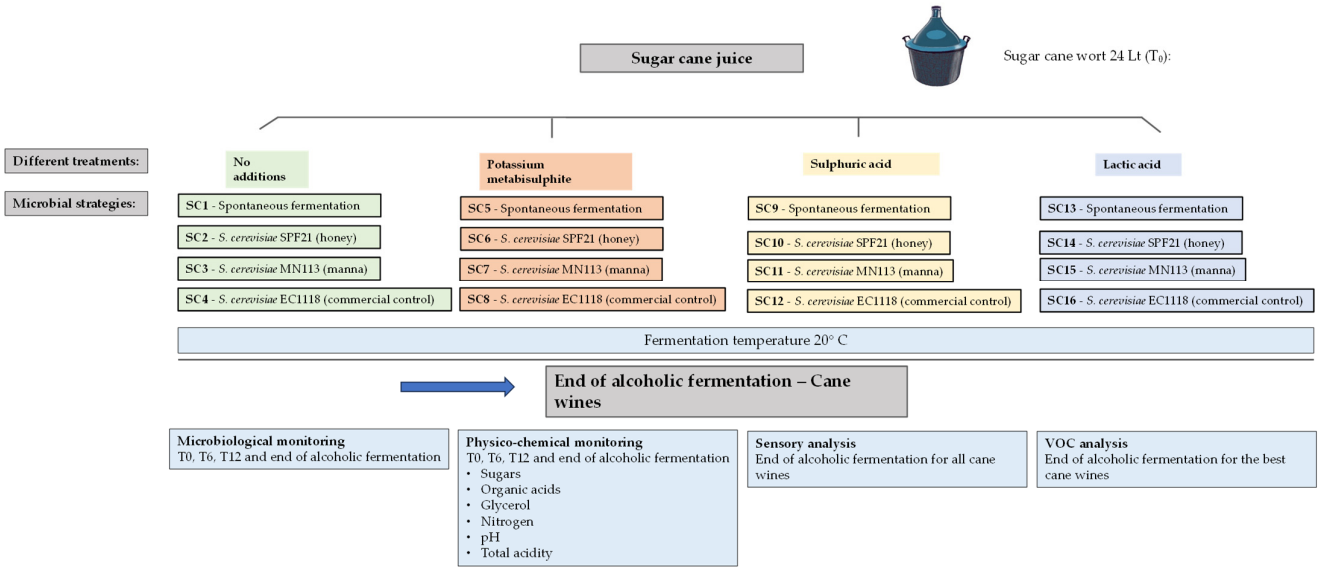

Figure 1. Experimental plan to produce fermented sugarcane juice.

The quality parameters of the wort before the treatments were pH 5.30 and 20.80 °Bx (Brix degrees). These parameters were measured as described in Section 2.9. The chemical parameters of the wort are shown in Table 2.

Table 2. Chemical parameters identified in the sugarcane wort before treatments.

Chemical Parameters	Wort
D-fructose (g/L)	13.19 ± 0.12
D-glucose (g/L)	23.01 ± 0.22
D-sucrose (g/L)	168.50 ± 1.35
Glycerol (g/L)	0.32 ± 0.05
Acetic acid (g/L)	0.09 ± 0.06
Lactic acid (g/L)	0.06 ± 0.05
Malic acid (g/L)	0.02 ± 0.02
Tartaric acid (g/L)	0.03 ± 0.01
Ammoniacal nitrogen (mg/L)	0.00 ± 0.00
Alpha-amino nitrogen (mg/L)	74.08 ± 0.26
pH	5.11 ± 0.12
Total acidity (TA; g/L tartaric acid)	2.85 ± 0.10

Values are expressed as an average of three measurements.

Prior to yeast inoculation, all trials were supplemented with 80 g/hL diammonium phosphate (Chimica Noto s.r.l., Partinico, Italy) and the respective treatment as described above. Each strain was cultured in YPD broth (yeast extract 10 g/L, peptone 20 g/L, dextrose 20 g/L) in 250 mL Erlenmeyer flasks containing 100 mL of medium. Cultures were incubated at 28 °C for 48 h under orbital shaking at 120 rpm. After incubation, cells were harvested by centrifugation at 4000 rpm for 10 min, washed twice with sterile saline solution (0.9% NaCl), and resuspended in the same solution. The concentration of the resulting suspension was estimated by OD_{600} and adjusted to approximately 2×10^8 CFU/mL. A volume of 5 mL of this suspension was inoculated into each 500 mL fermentation unit, resulting in a final concentration of approximately 2×10^6 CFU/mL in the sugarcane juice. The cell count was confirmed by plate counting on WL nutrient agar [26]. Fermentation was performed at 20 °C in hermetically sealed glass fermenters (total capacity: 750 mL), each containing 500 mL of sugarcane juice and equipped with a sluice valve. At each sampling point (day 0, 6, 12, and at the end of fermentation),

10 mL of fermented juice was aseptically collected from each fermenter for microbiological and physicochemical analyses. Sugar concentration was monitored daily throughout the process to precisely assess sugar consumption and fermentation dynamics. The process of fermentation was considered to have reached its conclusion when no variation in residual sugar levels was observed over the course of two consecutive days. After this, all samples were subjected to immediate analysis.

2.7. Monitoring Sugarcane Juice Fermentation

Microbiological analyses were conducted on each sample using plate count methods. Specifically, yeast and bacterial populations were assessed at various fermentation stages. Wort samples were serially diluted in Ringer's solution (Sigma–Aldrich, Milan, Italy) and plated on selective media: total mesophilic microorganisms on Plate Count Agar (PCA) [27]; *Saccharomyces* spp. on Wallerstein Laboratory (WL) nutrient agar [28]; non-*Saccharomyces* yeasts on Lysine Agar (LA) [29]; mesophilic rod-shaped lactic acid bacteria (LAB) on de Man–Rogosa–Sharpe (MRS) agar [30]; coccus-shaped LAB on Glucose M17 agar [31]; and acetic acid bacteria (AAB) on Kneifel agar, as described by Francesca et al. [31]. Presumptive identification of colonies was based on morphological traits, confirmed by microscopic examination of cell structure [32]. All analyses were performed in triplicate. Culture media and supplements were obtained from Oxoid (ThermoFisher, Milan, Italy).

2.8. Yeast Isolation, Molecular Identification, and Strain Typing

The dominance of the inoculated yeast strain during alcoholic fermentation was assessed in accordance with the methodology outlined by Francesca et al. [33]. Confirmation was achieved by comparing the interdelta profiles of isolates obtained from the highest cell dilution of cane wort with those of the reference pure culture. The interdelta analysis was conducted in accordance with the protocol described by Legras and Karst (2003) [34], and the PCR products were visualized and compared as detailed by Alfonzo et al. [35].

2.9. Determination of Physicochemical Parameters

pH measurements were conducted using a pH meter (Model Mod.70 XS/50010162, Cheimika, Pellezzano, Italy), while soluble solids (°Bx) were estimated with a refractometer (Model DBR Salt, Zetalab srl, Padova, Italy). Total acidity was determined following the OIV-MAAS313-01 protocol [36]. The quantification of sucrose, glucose, fructose, glycerol, ammonia nitrogen, alpha-amino nitrogen, tartaric acid, acetic acid, malic acid, and lactic acid was carried out according to the method described by Matraxia et al. [37]. Ethanol concentration in the fermented juice was assessed via distillation, following the procedure outlined by Chawafambira [38]. All analyses were performed in triplicate.

2.10. Sensory Analysis

Fifteen judges (aged between 28 and 47, 9 men and 6 women) were selected by the University of Palermo and trained to evaluate the odor of cane wines. The sensory analysis in this study adhered to ethical standards for sensory research. All participants provided voluntary consent and were informed about this study. Participant information and privacy were protected through anonymization and appropriate measures. Prior to the publication of experimental data, informed consent was obtained from all participants. Moreover, no ethical permission is required from the institution and/or country for this study.

All the panelists had experience in the production of fermented alcoholic beverages and had acted as judges in several rum agricole tastings. The judges were trained in advance to focus on the sensory characteristics that describe cane wine. A sensory analysis is necessary for this fermented product as it permits the identification of differences between the protocols used and potential aromas that may be present in the future rum after

distillation. The evaluation procedure was carried out according to Pirrone et al. [25] using the same descriptors as Freitas Schwan et al. [39]: odor (intensity, complexity, apple, caramel, vanilla, melon, alcohol, citrus, spicy, vinegar, sulphury, and grassy). The average of the three assessments was used to obtain the final scores.

2.11. Analysis of VOCs of Fermented Sugarcane Samples

Esters and alcohols were analyzed, identified, and quantified in cane wine samples that exhibited regular fermentation dynamics, achieved the highest sensory analysis scores, and exhibited no sensory or physicochemical defects. The control samples were the respective spontaneously fermented cane wines. This was accomplished using solid-phase headspace microextraction (HS-SPME) in combination with gas chromatography-mass spectrometry (GC-MS). This method is a straightforward and eco-friendly extraction and concentration analytical procedure. To identify and quantify each compound, a sequence of standard organic compounds was injected using the same extraction procedure. The identification of each compound was further supported by Kovats Indices (KI), which are derived from the retention time normalized to the adjacent eluting n-alkanes. KI values are independent of the analytical conditions, making them useful for identifying unknown compounds. Gas chromatographic analyses were conducted using an Agilent 5890 GC system coupled with an HP 5973 quadrupole mass spectrometer. An HP5-MS column (5% diphenyl-95% dimethylpolysiloxane, 30 m $\times$ 0.2 mm, 0.25 µm film, J & W Scientific, Folsom, CA, USA) was utilized. Ultra-high-purity helium was used as carrier gas, with water and oxygen traps (Supelco®, Sigma-Aldrich Italia S.r.l., Milan, Italy) installed on the carrier gas lines. The oven temperature was initially set at 40 °C for 5 min, then increased at a rate of 5 °C/min to 220 °C and subsequently by 10 °C/min to 280 °C. The temperature was then held at these levels for a period of 10 min. The carrier gas (He) was utilized at 1 mL/min. The mass spectrum was recorded at an ionization voltage of 70 eV and an ion source temperature of 220 °C [40]. Samples were analyzed using the HS-SPME-GC-MS method with a PDMS-CAR-DVB fiber (Supelco, ITA). The extraction and purification procedure involved exposing the fiber to 5 mL of cane wine in a 40 mL vial sealed with a silica septum at 70 °C for 20 min. The fiber was then manually inserted into a GC inlet port that was equipped with a specific glass liner for SPME injection (0.75 mm i.d.). The desorption of the fibers was then conducted in the gas chromatograph inlet port for a period of 3 min at a temperature of 250 °C, utilizing the splitless injection mode. The compounds identified were then verified by comparing the mass spectrum profile with those of authentic reference compounds. In instances where reference standards were not available, the identification of the organic compound, using a mass spectrometry profile, was carried out by the comparison of the experimental mass spectrum with the NIST11 mass spectra library collection (minimum match quality $\geq$ 90%) and considering the KI, also.

2.12. Statistical Analysis

Data related to biometric, yield, and quality traits of the sugarcane genotypes, as well as the chemical-physical and microbiological characteristics and volatile organic compounds (VOCs) of the cane juice, alongside the sensory evaluation of cane wine aromas, were subjected to an analysis of variance (ANOVA). Pairwise comparisons were conducted using Tukey's test ($p \leq 0.001$). Subsequently, hierarchical cluster analysis (HCA) was performed to classify the cane wines based on dissimilarity, calculated using Euclidean distance and Ward's linkage method. All statistical analyses and graphical outputs were generated using XLStat software, version 2020.3.1 (Addinsoft, New York, NY, USA), integrated with Microsoft Excel.

3. Results and Discussion

3.1. Analysis of Rainfall and Air Temperature Trends at the Experimental Site

The weather is one of the crucial aspects for sugarcane yields. These parameters are significantly influenced by rainfall, temperature, solar radiation, and relative humidity [41]. Rainfall and air temperature trends are shown in Figure S1. During the test (April 2022–March 2023), a total rainfall of 444 mm was observed. The abundant rainfall observed in May (54 mm) has allowed the regular settlement of the sugarcane. The highest monthly rainfall was observed in September (88 mm) and November (95 mm). Between December and February, few rainfall events were observed.

The temperature trend was in line with the average temperature of the test site. Temperatures gradually increased in the spring-summer period, with an average peak of 36 °C in July. Since the end of the summer period, temperatures have gradually fallen. The lowest average maximum (15 °C) and average minimum (7 °C) temperatures have been recorded in February. The plants have survived the winter without any damage, despite values below 10 °C being recorded for more than a month.

3.2. Plant Growth Phases and Cumulative Growing Degree Days

As demonstrated in [42], thermal time is a pivotal factor in the regulation of the phenological development of sugarcane. During the trial, the evaluated genotypes exhibited comparable growth cycle durations. The complete cycle, from transplanting to harvest, lasted 306 days, which is consistent with the findings of Zhou et al. [43]. According to these authors, the transplantation period typically extends from March to early June. The stem elongation phase extends from early June to late September, followed by sugar accumulation in October, maturation from November to December, and harvesting from late December to March of the subsequent year. Growing degree days (GDDs) have been shown to provide a quantitative measure of the heat accumulation required for sugarcane development. The cumulative GDDs recorded during this study are illustrated in Figure 2.

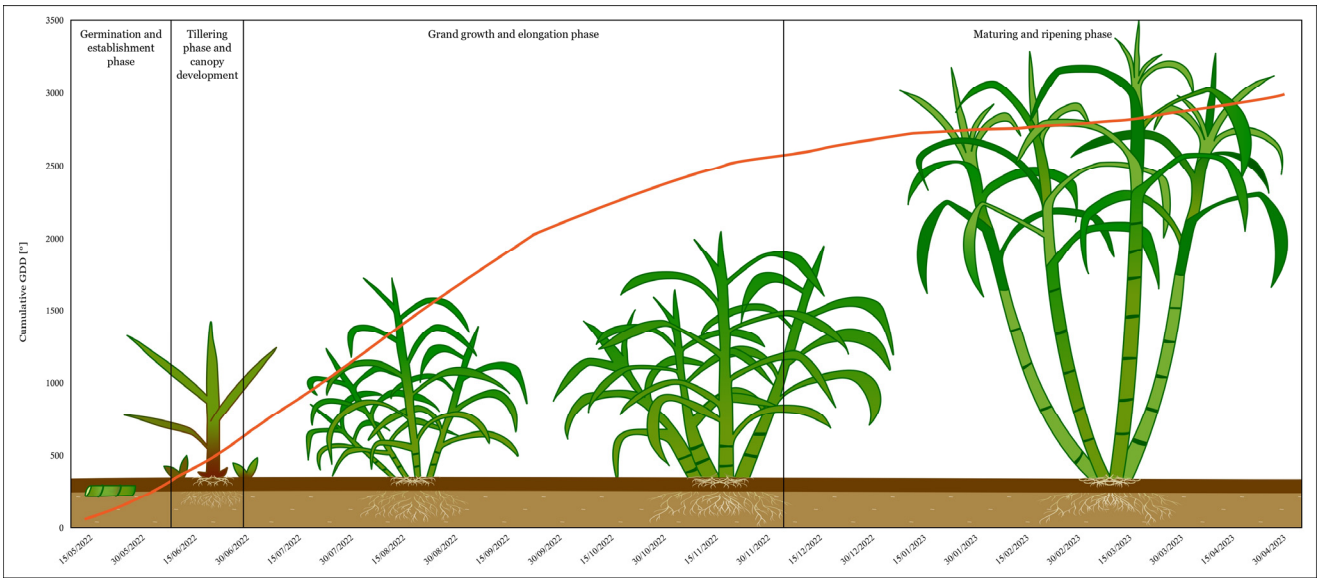

Figure 2. Cumulative growing degree days for sugarcane plants grown in Sicily.

At the experimental location, the cumulative growing degree days (GDD) recorded from transplanting to harvest amounted to 2800 °C, a value considered fully adequate for optimal sugarcane development, as noted by Som-Ard et al. [7]. The initial phase, which is associated with the sprouting of cuttings, corresponds to a GDD accumulation between 0 and 250 °C [44]. The subsequent vegetative state requires cumulative GDD values ranging from 250 to 600 °C [42]. The third developmental phase, encompassing tillering, stem elongation, and biomass accumulation, occurs between 600 and 900 °C [45]. The maturation stage, during which sucrose accumulation occurs, is characterized by GDD values ranging from 900 to 1400 °C [46].

3.3. Field Evaluation of Sugarcane Genotypes

The analysis of variance showed highly significant differences for all morphological parameters (Table 3). FR 87-83 showed the highest plant height, 212.16 cm.

Table 3. Biometric parameters of sugarcane genotypes.

Variety/ Accession	Plant Height (cm)	Millable Canes Length (cm)	Number of Nodes (n)	Average Node Diameter (mm)	Number of Tillers (n ha^{-1})	Number of Millable Canes (n ha^{-1})
PSR 07-334	190.93 ± 33.60 ^{ab}	155.04 ± 3.24 ^{abc}	14.91 ± 0.58 ^{a}	28.08 ± 1.76 ^{ab}	247,000.00 ± 1414.00 ^{ab}	189,333.00 ± 3771.00 ^{b}
FR 87-83	212.16 ± 4.48 ^{a}	164.47 ± 1.03 ^{ab}	12.60 ± 0.56 ^{bc}	28.31 ± 2.15 ^{ab}	237,500.00 ± 3536.00 ^{b}	160,000.00 ± 0.00 ^{c}
KN 07-0037	173.25 ± 4.60 ^{bc}	143.50 ± 2.12 ^{bc}	10.80 ± 0.28 ^{c}	28.79 ± 2.14 ^{ab}	188,350.00 ± 1180.00 ^{d}	180,833.00 ± 8250.00 ^{b}
CPCL 02-1295	130.73 ± 2.92 ^{d}	98.04 ± 6.66 ^{d}	11.50 ± 0.35 ^{c}	25.06 ± 1.14 ^{ab}	118,000.00 ± 2828.00 ^{f}	117,333.00 ± 1508.00 ^{e}
CP 06-2495	170.62 ± 17.10 ^{bc}	167.95 ± 18.50 ^{a}	13.90 ± 0.35 ^{ab}	22.70 ± 0.73 ^{b}	175,000.00 ± 0.00 ^{e}	157,500.00 ± 1550.00 ^{c}
CP 09-1952	177.50 ± 0.70 ^{bc}	134.57 ± 0.24 ^{c}	11.45 ± 1.34 ^{c}	20.96 ± 4.20 ^{b}	183,333.00 ± 4714.00 ^{de}	152,333.00 ± 6128.00 ^{cd}
Mex 69-290	196.83 ± 1.18 ^{ab}	157.00 ± 3.77 ^{abc}	11.50 ± 0.23 ^{c}	26.42 ± 2.72 ^{ab}	253,333.00 ± 9428.00 ^{a}	206,667.00 ± 9428.00 ^{a}
Ananas	142.57 ± 6.60 ^{cd}	139.00 ±11.64 ^{bc}	12.23 ± 0.22 ^{bc}	27.90 ± 2.28 ^{ab}	180,000.00 ± 1733.00 ^{de}	138,333.00 ± 2357.00 ^{d}
Baltasià	189.60 ± 39.00 ^{ab}	156.50 ± 27.90 ^{abc}	12.50 ± 0.35 ^{bc}	31.98 ± 0.06 ^{a}	217,000.00 ± 4243.00 ^{c}	138,667.00 ± 7542.00 ^{d}
p-values	***	***	***	*	***	***

Results indicate the mean value ± standard deviation of three determinations. Means followed by the same letter are not significantly different according to Tukey's test. The letters in the same column suggest homogeneous groups. *p*-values: ***, $p < 0.001$; *, $p < 0.05$.

Other accessions that showed high plant heights are PR 07334, Mex 69-29, and Baltasià, with values between 190 and 197 cm. CPCL 02-1295 and Ananas showed the lowest plant height (under 150 cm). The other accessions have reached values of about 170 cm. As reported by Silva et al. [47], the different growth may depend on the genetic pool. The highest millable cane length (167.95 cm) has been found in variety CP 06-2495, followed by FR 87-83 and Baltasià, in accordance with Singh et al. [48], although it is not among the varieties that have shown the highest height. As noted in our and other studies, higher plant height does not always correspond to a longer millable cane length [49]. This can be attributed not only to genetic characteristics, but also to the different adaptability of the varieties to soil and climate conditions.

The highest number of nodes (14.9) has been found in the variety PSR 07-334, followed by CP 06-2495 (13.9). The lowest numbers of nodes (between 10.8 and 11.5) have been measured in KN 07-0037, CP 09-1952, Mex 69-290, and CPCL 02-1295. The number of nodes is a very important parameter both to produce propagation material and for the processing industry. A higher number of nodes corresponds to a higher amount of juice accumulated in the respective internodes [50].

The highest average node diameter was also recorded in the Baltasià accession with 31.98 mm, values in line with those reported by Batista et al. [51] on other irrigated accessions. The lowest average diameter was found in the two varieties from Canal Point: CP 06-2495 (22.7 mm) and CP 09-1952 (20.96 mm). These values are consistent with those measured by Singh et al. [48], between 21 and 22.9 mm, which, as in this study, showed the smallest diameters among the varieties studied. Davidson et al. [52] reported a higher average node diameter than those measured in CP 09-1952.

The highest number of tillers has been recorded in the varieties Mex 69-290 (253,333 n ha^{-1}) and PSR 07334 (247,000 n ha^{-1}). The highest number of tillers and millable canes of the Mex 69-290 and PSR 07-334 varieties might suggest a greater adaptability of these than the other varieties studied. Sood et al. [53] reported an inverse relationship between the number of tillers and the average diameter of the nodes.

Considering the number of millable canes, Mex 69-290 produced the highest value (206,667 n ha^{-1}), followed by PSR 07-334 (189,333 n ha^{-1}) and KN 07-0037 (180,833 n ha^{-1}).

3.4. Juice Quality and Yield

The qualitative parameters of sugarcane juice are affected by the interaction of the genotype with the environment, the harvest period of sugarcane, and the juice extraction process [54]. The analysis of variance of qualitative parameters, juice yield, and sugar yield is reported in Table 4.

Table 4. Production and quality parameters of sugarcane genotypes.

Variety/Accession.	Juice Yield (L ha^{-1})	°Brix of Juice (°)	pH of Juice	Sucrose (g/cm^3)	CCS (%)	Sugar Yield (t ha^{-1})
PSR 07-334	11,995.00 ± 1351.00 ^{abc}	22.90 ± 1.17 ^{a}	5.28 ± 0.08 ^{d}	19.76 ± 0.04 ^{b}	13.51 ± 0.39 ^{b}	4.11 ± 0.04 ^{ab}
FR 87-83	11,292.50 ± 293.00 ^{abc}	19.82 ± 0.00 ^{b}	5.24 ± 0.05 ^{d}	15.35 ± 1.55 ^{d}	9.02 ± 2.82 ^{c}	3.04 ± 0.01 ^{ab}
KN 07-0037	12,623.30 ± 3003.00 ^{ab}	14.74 ± 1.24 ^{c}	5.40 ± 0.03 ^{ab}	12.33 ± 2.61 ^{e}	8.30 ± 2.31 ^{c}	2.31 ± 0.03 ^{b}
CPCL 02-1295	5850.00 ± 1344.00 ^{d}	24.10 ± 0.60 ^{a}	5.42 ± 0.04 ^{a}	16.73 ± 1.17 ^{cd}	10.06 ± 1.02 ^{c}	1.84 ± 0.03 ^{b}
CP 06-2495	7871.50 ± 1419.00 ^{c}	23.87 ± 0.24 ^{a}	5.30 ± 0.02 ^{bc}	18.98 ± 2.73 ^{bc}	13.00 ± 2.00 ^{b}	2.78 ± 0.05 ^{ab}
CP 09-1952	5932.50 ± 470.00 ^{d}	23.82 ± 0.81 ^{a}	5.44 ± 0.00 ^{a}	23.11 ± 0.34 ^{a}	16.66 ± 0.11 ^{a}	3.03 ± 0.03 ^{ab}
Mex 69-290	9876.70 ± 2814.00 ^{bc}	20.69 ± 0.28 ^{b}	5.29 ± 0.08 ^{cd}	19.61 ± 0.48 ^{bc}	14.00 ± 0.57 ^{ab}	3.55 ± 0.06 ^{ab}
Ananas	9586.70 ± 2480.00 ^{bc}	20.65 ± 1.39 ^{b}	5.36 ± 0.09 ^{bc}	19.54 ± 1.57 ^{bc}	13.94 ± 1.19 ^{ab}	3.86 ± 0.10 ^{ab}
Baltasià	14,312.50 ± 3624.00 ^{a}	20.24 ± 0.21 ^{b}	5.42 ± 0.04 ^{a}	19.56 ± 0.33 ^{bc}	14.08 ± 0.40 ^{ab}	5.33 ± 0.07 ^{a}
p-values	***	***	***	***	***	*

Results indicate the mean value ± standard deviation of three determinations. Abbreviations: CCS, commercial cane sugar. Means followed by the same letter are not significantly different according to Tukey's test. The letters in the same column suggest homogeneous groups. *p*-values: ***, $p < 0.001$; *, $p < 0.05$.

All parameters showed some statistically significant differences ($p < 0.05$). The variety Baltasià produced the highest juice yield (14,312.5 l ha^{-1}). Similar statistical values have been recorded in KN 07-0037 (12,623.3 l ha^{-1}), PSR 07-334 (11,995 l ha^{-1}), and FR 87-83 (11,292.5 l ha^{-1}).

The lowest juice yield has been observed in CPCL 02-1295 (5850 l ha^{-1}) and CP 09-1952 (5932.5 l ha^{-1}). Knowledge of °Brix content is an important parameter for determining the appropriate time for sugarcane harvest. As reported by Bomdespacho et al. [55], this parameter depends on the cultivar, the physiological conditions of the plant, and the degree of ripeness. The highest °Brix values were recorded in the variety PSR 07-334 and in the three varieties from Florida (CPCL 02-1295, CP 06-2495, CP 09-1952) with values ranging from 22.9°Brix to 24.1°Brix. The lowest values were highlighted in the variety KN 07-0037 (14.74 °Brix). All °Brix values (from 18.5°Brix to 25.3 °Brix), except those observed in the variety KN 07-0037, were in line with the study by Pimenta et al. [56].

In the literature, pH values between 4.6 and 6.0 are reported for raw juice [24]. The genotype, type of soil, fertilization, climatic conditions, degree of ripening, harvesting, and extraction methods are important factors to consider in the difference of juice pH [57]. The highest pH has been recorded in the varieties CPCL 02-1295 (5.42), CP 09-1952 (5.44), and Baltasià (5.42), while PSR 07-334 and FR 87-83 produced the lowest values (5.28 and 5.24). The highest content of sucrose (23.11 g cm^{-3}) has been measured in the CP 09-1952 variety, while the lowest (12.33 g cm^{-3}) was in KN 07-0037. As reported by Bonnet et al. [58], the genotype, number of nodes, and environmental temperature affect the sucrose accumulation within the cane, and a higher number of nodes would result in lower sucrose content. This can be seen in our work, where the variety with the highest sucrose content (CP 09-1952) also generated the lowest number of nodes. The commercial cane sugar (CCS) is influenced by the genotype, the length of the growing period, and the harvesting period [6]. The highest CCS percentages have been recorded in CP 09-1952 (16.66%), Baltasià (14.08%), Mex 69-290 (14.0%), and Ananas (13.94%). These varieties with a CCS content equal to or greater than 14% are, in our environments, the most efficient in terms of quality [59]. The lowest CCS percentage has been obtained in KN 07-0037 (8.3%), FR 97-83 (9.02%), and CPCL 02-1295 (10.06%). The highest sugar yield (5.33 t ha^{-1}) was obtained in the accession Baltasià, while the lowest was in the varieties CPCL 02-1295 (1.84 t ha^{-1}) and KN 07-0037 (2.31 t ha^{-1}). The other genotypes produced sugar yields from 3 to 4 t ha^{-1}, not statistically different from the accession Baltasià.

3.5. Wort Fermentation of Sugarcane Samples

The populations of different microbial groups during fermentation are reported in Figures 3–6. Before inoculation and chemical treatments, the microbial levels in sugarcane juice were 5.20 log CFU/mL for total mesophilic microorganism, 4.70 log CFU/mL for *Saccharomyces* spp., 4.35 for non-*Saccharomyces* yeasts, 3.60 log CFU/mL for LAB cocci, 5.20 log CFU/mL for LAB rods, and 2.40 log CFU/mL for AAB.

The stems of sugarcane, and consequently the extracted juice, host a diverse bacterial community comprising both aerobic lactic acid bacteria and microaerophilic species [12]. The juice also contained yeasts belonging to both *Saccharomyces* and non-*Saccharomyces* genera, with the former being predominant. These yeast population levels are consistent with the observations reported by Martini et al. [60]. Following yeast inoculation and the addition of various chemical adjuncts, the total mesophilic microbial load ranged from 4.01 to 5.91 log CFU/mL, as illustrated in Figure 3. These values increased at the next sampling point. Overall, trials without chemical treatment showed higher values. Specifically, the highest cell counts were registered at the inoculum for *S. cerevisiae* EC1118 in the trial SC4 (5.91 log CFU/mL), on day 6 for spontaneous fermentation in SC1 and for EC1118 in SC4 (7.89 log CFU/mL), on day 12 for *S. cerevisiae* MN113 in trial SC3 (8.02 log CFU/mL), and at the end of alcoholic fermentation for *S. cerevisiae* MN113 in SC3 (7.61 log CFU/mL). Trials with lactic acid, sulfuric acid, and metabisulfite showed lower microbial loads compared to the trials without these additions, due to their antimicrobial activity [17–19]. Although the yeast inoculum was prepared at a concentration of approximately 2×10^6 CFU/mL, the viable cell count measured immediately after inoculation was 5.91 log CFU/mL (approximately 8.1×10^5 CFU/mL). This indicates a viability of around 59.5%. While this level of viability was slightly lower than expected, it was sufficient to ensure rapid yeast dominance and successful fermentation, as confirmed by the microbial dynamics and sugar consumption profiles. The reduced viability may be attributed to osmotic stress or handling during inoculum preparation.

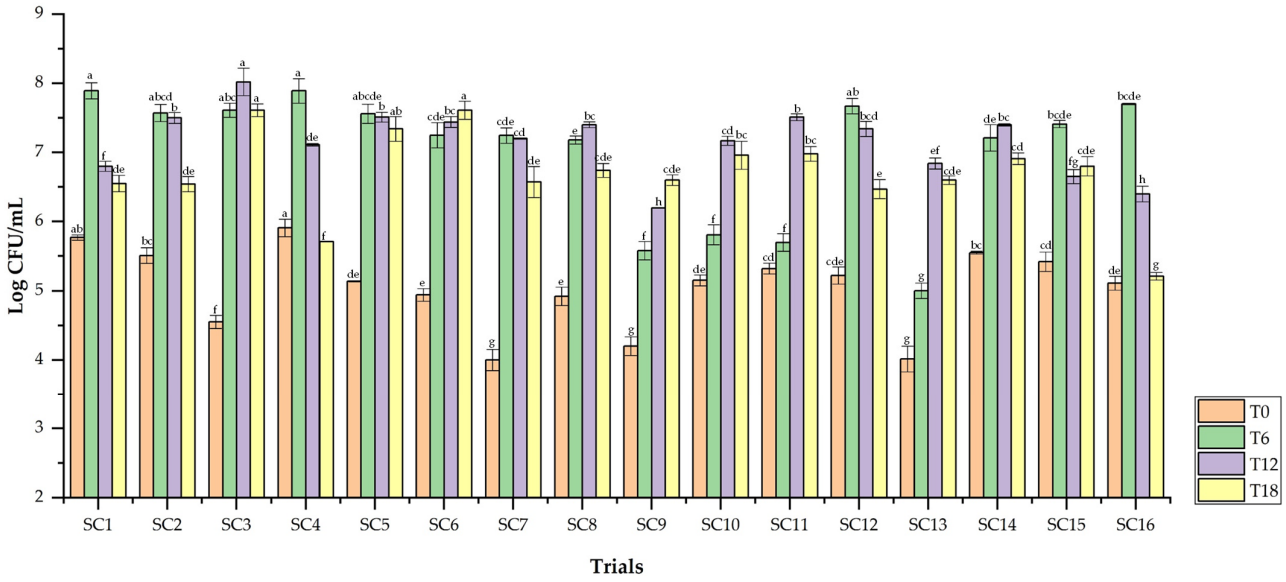

Figure 3. Results of microbiological analyses for total mesophilic microorganisms of different samples. T0, T6, T12, and T18 represent the sampling times (in days) during alcoholic fermentation. Lowercase letters indicate statistically significant differences among treatments according to Tukey's test ($p \leq 0.001$); treatments sharing the same letter are not significantly different.

3.5.1. Yeasts

The population dynamics of yeasts, both *Saccharomyces* and non-*Saccharomyces*, are shown in Figure 4. After inoculation, the plate count of *Saccharomyces* populations (Figure 4a) ranged from 6.28 to 7.00 log CFU/mL, with significant differences observed between trials. The values refer to the initial viable cell counts of the inoculated *S. cerevisiae* strains (MN113, SPF21, EC1118), which were introduced at a standardized concentration of approximately 2×10^6 cells/mL. This observation is indicative of higher values when compared to the indigenous *Saccharomyces* population detected in untreated juice (4.70 log CFU/mL). The observed counts are consistent with expected plating efficiency and standard inoculation practices in controlled fermentations. Spontaneous fermentation trials (SC1, SC5, SC9, and SC13) had significantly lower values, ranging from 3.23 to 3.81 log CFU/mL, compared to the inoculated trials. In these cases, the untreated trials exhibited higher values than those with chemical treatments. Specifically, trials treated with potassium metabisulfite showed lower values than others. From the next sampling point, the microbial load of *Saccharomyces* yeasts increased, while the population of non-*Saccharomyces* yeasts (Figure 4b) began to decrease, with most becoming undetectable. Additionally, a slight decrease was observed in the uninoculated and untreated trials, except for SC10 and SC11 treated with sulfuric acid, which had a value of 3.48 log CFU/mL. The decrease in non-*Saccharomyces* species populations can be attributed to several factors, including the presence of ethanol and chemical compounds, competition for nutrients, and metabolites produced by *S. cerevisiae* that inhibit the growth of other yeasts [61].

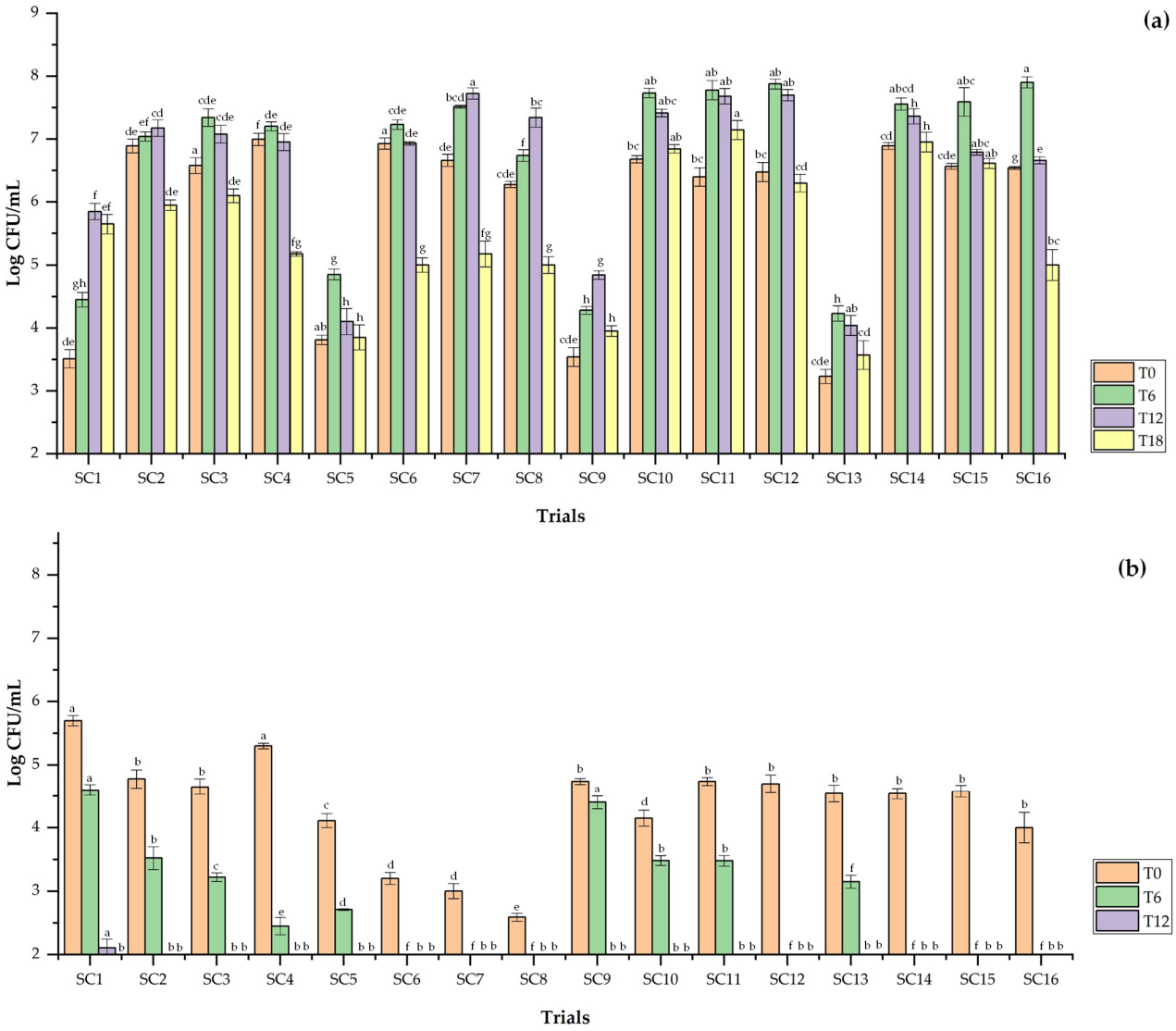

Figure 4. Results of microbiological analyses for *Saccharomyces* yeast (**a**) and non-*Saccharomyces* yeasts (**b**) of different samples. T0, T6, T12, and T18 represent the sampling times (in days) during alcoholic fermentation. Lowercase letters indicate statistically significant differences among treatments according to Tukey's test ($p \leq 0.001$); treatments sharing the same letter are not significantly different.

The *Saccharomyces* spp. population was found to be most responsive to treatments involving sulfuric and lactic acids, as evidenced by the higher cell counts recorded in these trials when compared to other treatments. It is worth noting that on the sixth day of fermentation, *S. cerevisiae* EC1118 in the SC16 trial attained the highest recorded value of 7.90 log CFU/mL. A decline in yeast count was observed as the process of alcoholic fermentation concluded. In general, treatments involving acidification with sulfuric and lactic acids have been shown to promote increased yeast proliferation. The yeast growth dynamics observed during sugarcane juice fermentation were consistent with those previously reported by Tzeng et al. [62].

3.5.2. Lactic Acid Bacteria

The population dynamics of mesophilic rod- and coccus-shaped LAB are shown in Figure 5. At the first sampling, coccus-shaped LAB cell densities (Figure 5a) ranged from 2.87 to 4.58 log CFU/mL across the trials. In the untreated trials (SC1 to SC4), microbial levels increased until day 12. The highest cell counts were recorded on day

12 for spontaneous fermentation in SC1 (8.45 log CFU/mL). Overall, the trials treated with lactic acid (SC13, SC14, SC15, and SC16) showed better results. Starting from day 6, they did not contain any coccus-shaped LAB, except for the uninoculated SC13 trial, which had undetectable values from day 12. Similar results were obtained for rod-shaped LAB (Figure 5b). Microbial levels increased in the untreated trials (SC1 to SC4) up to day 6. The highest cell count was recorded on day 6 for spontaneous fermentation in SC1 (8.68 log CFU/mL). Again, the trials treated with lactic acid (SC13 to SC16) showed better results. From day 6, they did not contain any rod-shaped bacteria, except for the uninoculated SC13 trial, which had undetectable values from day 12.

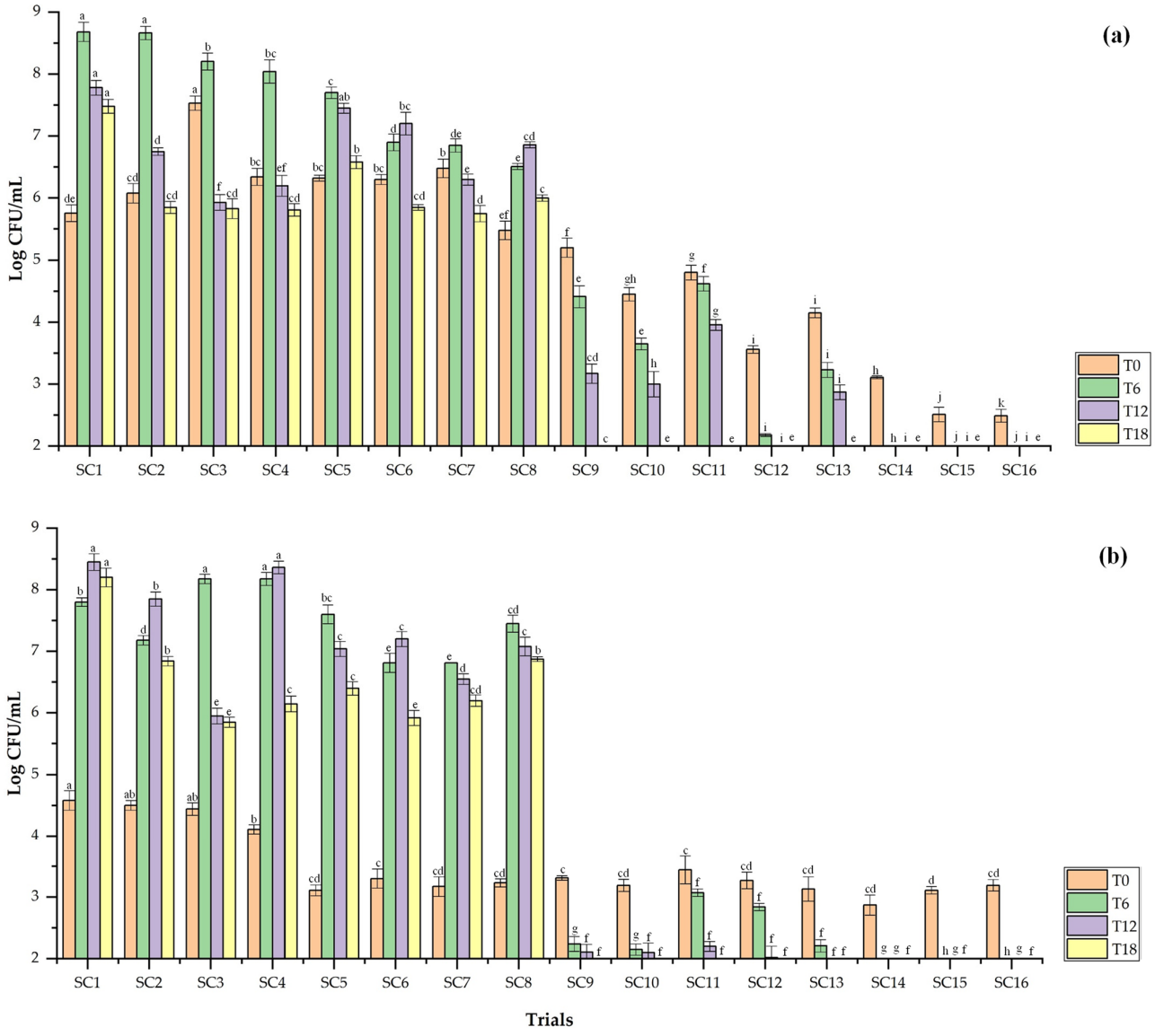

Figure 5. Results of microbiological analyses for mesophilic cocci (**a**) and rod-shaped LAB (**b**) of different samples. T0, T6, T12, and T18 represent the sampling times (in days) during alcoholic fermentation. Lowercase letters indicate statistically significant differences among treatments according to Tukey's test ($p \leq 0.001$); treatments sharing the same letter are not significantly different.

The presence of LAB during sugarcane juice fermentation is generally considered undesirable, as these microorganisms compete with yeasts for essential sugars and nutrients [16,39]. LAB species such as *Lactobacillus* are particularly well adapted to fermentation environments with pH values below 5.0, owing to their rapid growth rates and high ethanol tolerance [63]. The activity of lactic acid bacteria has been demonstrated to significantly

influence the quality of fermentation, as lactic fermentation has been observed to reduce pH levels and inhibit the proliferation of spoilage bacteria [64]. This effect is especially relevant in uninoculated trials, where microbial competition is more pronounced.

The concentration of AAB during the alcoholic fermentation process is shown in Figure 6, as the results follow a similar trend to LAB.

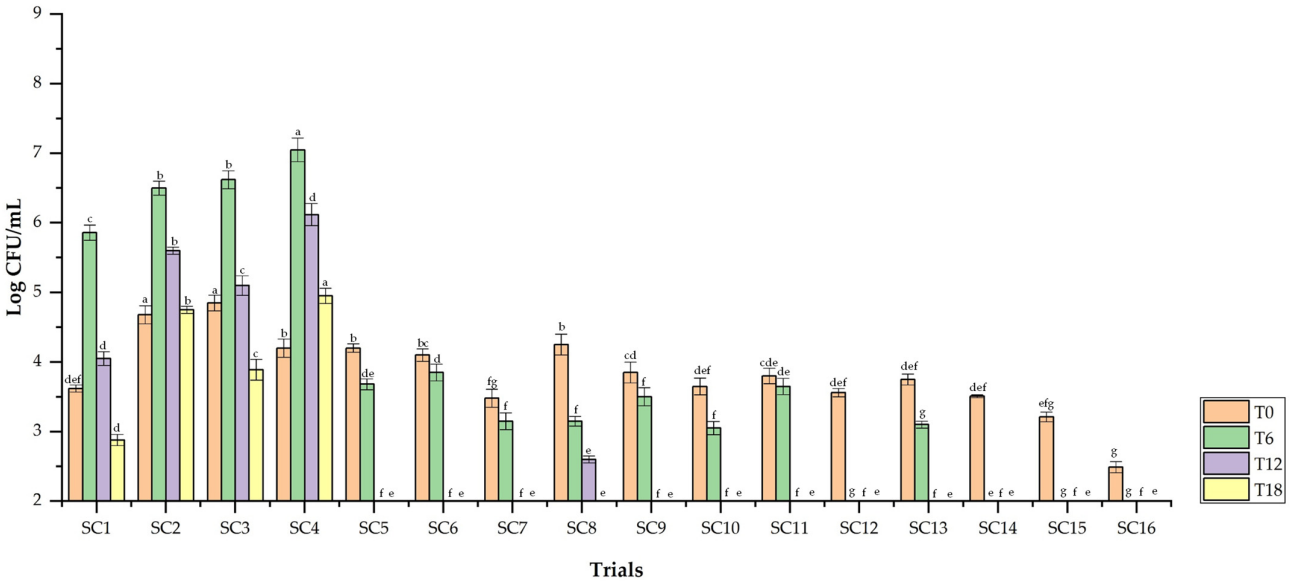

Figure 6. Results of microbiological analyses for acetic acid bacteria of different samples. T0, T6, T12, and T18 represent the sampling times (in days) during alcoholic fermentation. Lowercase letters indicate statistically significant differences among treatments according to Tukey's test ($p \leq 0.001$); treatments sharing the same letter are not significantly different.

On the first day of sampling, AAB densities in the different trials ranged from 2.49 to 4.85 log CFU/mL. Notably, microbial levels only increased in the untreated trials (SC1 to SC4) until day 6, after which they began to decrease. The trials inoculated with lactic acid (SC13 to SC16) yielded superior results, except for the uninoculated SC13 trial, where the concentration was undetectable by day 6.

High cell densities of AAB can lead to the formation of undesirable organic compounds, including acetaldehyde, ethyl acetate, acetic acid, copper, and ethyl carbamate [65]. Overall, excessive activity of LAB and AAB negatively affects the quality of the fermented product due to the significant production of 2,3-butanedione, acids (lactic, acetic, and formic), and acetoin [8]. These bacterial species are typically considered contaminants in industrial fermentations of sugarcane ethanol [66]. Therefore, the results shown by the lactic acid-treated samples are encouraging, as they showed a total reduction in the microbial load of LAB and AAB, which could otherwise negatively affect the final product.

3.6. Dominance of Inoculated Yeasts

A total of 540 colonies were isolated on WL, sequentially re-cultured on WL, and examined for colony color, colony topography, and microscopic characteristics [32]. All isolates were identified as presumptive *S. cerevisiae* based on their macroscopic and microscopic traits. This identification was supported by analysis of the 5.8 S-ITS region, which produced bands of a size (880 bp) comparable to *S. cerevisiae* [67]. The classification of these isolates as *S. cerevisiae* was confirmed by comparing the restriction fragment profiles with C*fo*I, H*ae*III, and H*inf*I to those reported by Guillamón et al. [68]. The interdelta profiles of the isolates were compared, revealing a high dominance of the starter strains inoculated in the different trials, with dominance ranging from 93.3% to 94.8%. The interdelta pro-

files of the three starter yeasts were found exclusively in the respective trials where they were inoculated.

3.7. Physicochemical Parameters

The principal chemical characteristics of sugarcane juice, including sugars, organic acids, nitrogen content, pH, total acidity, and glycerol, are summarized in Table 1. The juice exhibited a pH of 5.11 and a soluble solids content of 20.80°Bx, values consistent with those reported by Kaavya et al. [69] and Tzeng et al. [62]. The slightly acidic pH is a crucial factor in determining the freshness of the juice. It has been established that lower pH values are typically associated with ongoing fermentation and microbial activity [70]. Following the conclusion of alcoholic fermentation, the pH levels of the resulting cane wines ranged from 3.21 to 3.98. The addition of lactic or sulfuric acid not only directly lowered the initial pH of the sugarcane juice but also limited the proliferation of spoilage microorganisms, including AAB and LAB, which are known to affect fermentation dynamics and acid production. Specifically, in the trials acidified with sulfuric acid, the final pH ranged from 3.13 to 3.39, while in those treated with lactic acid, it was from 3.42 to 3.57. Overall, these values were lower compared to the trials treated with potassium metabisulfite, which showed pH values ranging from 3.36 to 3.98. In untreated trials, the absence of treatment resulted in uncontrolled microbial proliferation and higher levels of lactic and acetic acid accumulation, leading to more variable final pH values. The physicochemical composition of the cane wines following 18 days of fermentation is detailed in Table 5.

Residual sugars in the inoculated trials ranged between 4.29 (SC16) and 1.52 (SC6) g/L, with no statistical differences between trials. In contrast, the uninoculated trials (SC1, SC5, SC9, and SC13) were unable to consume all the sugars present, resulting in residual sugar values ranging from 91.11 to 126.95 g/L.

Regarding acetic acid, the untreated trials (SC1 to SC4) yielded values between 4.01 and 4.73 g/L, due to the absence of chemical treatment that allowed the proliferation of AAB. In contrast, the commercial control *S. cerevisiae* EC1118 in SC16, treated with lactic acid, showed the lowest acetic acid levels, likely due to the killer activity of this strain inhibiting AAB growth [71]. However, the trials treated with lactic acid (SC13 to SC16) had higher lactic acid values than all other trials, specifically between 5.10 and 5.37 g/L. Apart from these, trials SC1 and SC5 showed the highest lactic acid values, 2.86 and 2.09 g/L, respectively, due to the proliferation of LAB without competition from inoculated yeasts [72].

No significant differences were found between the various trials for malic acid, tartaric acid, and total acidity. However, the trials inoculated without chemical treatment (SC2, SC3, and SC4) exhibited higher levels of glycerol, ranging from 9.14 to 9.80 g/L. The trials inoculated with the control strain *S. cerevisiae* EC1118 (SC4, SC8, SC12, and SC16) produced higher glycerol values than the other yeast strains. The concentration of glycerol produced by *S. cerevisiae* in wine typically ranges from 4 to 9 g/L [73], and glycerol yield is significantly affected by different yeast strains [74]. In this study, differences were observed among the strains, with the control strain of *S. cerevisiae* EC1118 producing more glycerol than the others. However, no significant differences were found between the different chemical treatments.

Table 5. Physicochemical parameters identified in the different trials of sugarcane wine at the end of alcoholic fermentation.

Trials	D-Sucrose [1]	D-Glucose [1]	D-Fructose [1]	Glycerol [1]	L-Malic Acid [1]	Lactic Acid [1]	Acetic Acid [1]	Tartatic Acid [1]	pH	TA
SC1	109.00 ± 0.94 ^{a}	5.75 ± 0.15 ^{c}	12.20 ± 0.14 ^{a}	2.14 ± 0.07 ^{h}	0.07 ± 0.00 ^{f}	2.86 ± 0.15 ^{c}	4.01 ± 0.08 ^{c}	0.03 ± 0.00 ^{h}	3.32 ± 0.10 ^{fgh}	16.10 ± 0.22 ^{a}
SC2	1.85 ± 0.10 ^{d}	0.34 ± 0.04 ^{ef}	1.20 ± 0.08 ^{e}	9.80 ± 0.24 ^{a}	0.08 ± 0.01 ^{h}	1.20 ± 0.00 ^{e}	4.73 ± 0.12 ^{a}	0.23 ± 0.01 ^{de}	3.76 ± 0.09 ^{bc}	13.87 ± 0.09 ^{b}
SC3	1.74 ± 0.05 ^{d}	1.55 ± 0.03 ^{d}	0.87 ± 0.07 ^{f}	9.14 ± 0.16 ^{b}	0.09 ± 0.01 ^{def}	0.90 ± 0.00 ^{f}	4.28 ± 0.11 ^{b}	0.35 ± 0.01 ^{b}	3.92 ± 0.10 ^{b}	11.25 ± 0.07 ^{d}
SC4	0.88 ± 0.07 ^{d}	1.24 ± 0.07 ^{de}	1.22 ± 0.06 ^{e}	9.80 ± 0.24 ^{a}	0.08 ± 0.00 ^{ef}	0.70 ± 0.00 ^{f}	4.62 ± 0.10 ^{a}	0.26 ± 0.01 ^{d}	3.79 ± 0.08 ^{bc}	12.37 ± 0.08 ^{c}
SC5	78.40 ± 1.20 ^{c}	1.21 ± 0.06 ^{de}	11.50 ± 0.24 ^{b}	3.84 ± 0.12 ^{g}	0.09 ± 0.02 ^{def}	2.09 ± 0.06 ^{d}	0.53 ± 0.02 ^{ef}	0.06 ± 0.00 ^{gh}	3.36 ± 0.07 ^{efgh}	13.50 ± 0.17 ^{b}
SC6	0.75 ± 0.08 ^{d}	0.22 ± 0.04 ^{f}	0.55 ± 0.11 ^{gh}	5.17 ± 0.09 ^{f}	0.07 ± 0.01 ^{f}	1.17 ± 0.10 ^{e}	0.55 ± 0.03 ^{e}	0.14 ± 0.00 ^{f}	3.65 ± 0.06 ^{cd}	10.12 ± 0.18 ^{e}
SC7	0.85 ± 0.06 ^{d}	0.55 ± 0.03 ^{ef}	1.20 ± 0.07 ^{e}	5.96 ± 0.09 ^{e}	0.07 ± 0.01 ^{f}	0.71 ± 0.02 ^{f}	0.55 ± 0.04 ^{e}	0.26 ± 0.02 ^{d}	3.65 ± 0.10 ^{cd}	6.00 ±0.07 ^{h}
SC8	1.10 ± 0.07 ^{d}	1.67 ± 0.05 ^{d}	0.78 ± 0.04 ^{fg}	7.40 ± 0.12 ^{c}	0.07 ± 0.01 ^{f}	0.83 ± 0.01 ^{f}	0.73 ± 0.03 ^{ef}	0.26 ± 0.01 ^{d}	3.98 ± 0.08 ^{a}	7.65 ± 0.13 ^{f}
SC9	97.60 ± 1.32 ^{b}	20.75 ± 0.84 ^{a}	4.71 ± 0.15 ^{d}	1.12 ± 0.05 ^{i}	0.09 ± 0.02 ^{def}	0.15 ± 0.09 ^{g}	0.44 ± 0.02 ^{ef}	0.39 ± 0.01 ^{a}	3.13 ± 0.09 ^{h}	10.87 ± 0.20 ^{d}
SC10	1.95 ± 0.09 ^{d}	1.14 ± 0.12 ^{def}	0.25 ± 0.11 ^{h}	6.51 ± 0.13 ^{d}	0.11 ± 0.00 ^{cde}	0.07 ± 0.00 ^{g}	0.46 ± 0.01 ^{ef}	0.26 ± 0.01 ^{d}	3.37 ± 0.07 ^{efgh}	7.12 ± 0.17 ^{g}
SC11	0.85 ± 0.11 ^{d}	0.97 ±0.04 ^{def}	0.30 ± 0.02 ^{h}	6.52 ± 0.11 ^{d}	0.19 ± 0.02 ^{a}	0.06 ± 0.00 ^{g}	0.45 ± 0.01 ^{ef}	0.31 ± 0.02 ^{c}	3.39 ± 0.08 ^{efg}	7.14 ± 0.16 ^{g}
SC12	0.65 ± 0.13 ^{d}	1.15 ± 0.07 ^{def}	0.63 ± 0.03 ^{fg}	7.33 ± 0.12 ^{c}	0.15 ± 0.01 ^{b}	0.05 ± 0.01 ^{g}	0.26 ± 0.01 ^{gh}	0.20 ± 0.01 ^{e}	3.21 ± 0.10 ^{gh}	6.90 ± 0.09 ^{g}
SC13	99.20 ± 1.28 ^{b}	19.37 ± 0.92 ^{b}	5.39 ± 0.12 ^{c}	4.24 ± 0.09 ^{g}	0.15 ± 0.01 ^{b}	5.37 ± 0.11 ^{a}	0.42 ± 0.03 ^{efg}	0.13 ± 0.01 ^{f}	3.46 ± 0.05 ^{def}	10.87 ± 0.21 ^{d}
SC14	0.88 ± 0.10 ^{d}	0.77 ± 0.07 ^{def}	0.55 ± 0.07 ^{gh}	5.95 ± 0.15 ^{e}	0.12 ± 0.01 ^{bcd}	5.18 ± 0.10 ^{ab}	0.37 ± 0.02 ^{fg}	0.16 ± 0.01 ^{f}	3.47 ± 0.09 ^{def}	9.75 ± 0.08 ^{e}
SC15	1.79 ± 0.10 ^{d}	0.55 ± 0.08 ^{def}	0.68 ± 0.04 ^{gh}	6.08 ± 0.12 ^{e}	0.20 ± 0.01 ^{a}	5.10 ± 0.09 ^{b}	0.40 ± 0.01 ^{efg}	0.08 ± 0.01 ^{g}	3.57 ± 0.06 ^{cde}	9.75 ± 0.12 ^{e}
SC16	1.52 ± 0.15 ^{d}	1.10 ± 0.12 ^{ef}	1.67 ± 0.05 ^{fg}	7.44 ± 0.20 ^{c}	0.14 ± 0.00 ^{bc}	5.21 ± 0.07 ^{ab}	0.19 ± 0.00 ^{h}	0.07 ± 0.00 ^{g}	3.42 ± 0.04 ^{defg}	9.97 ± 0.08 ^{e}
p-values	***	***	***	***	***	***	***	***	***	***

Results indicate the mean value ± standard deviation of three determinations from two replicates. Data within a line followed by the same letter are not significantly different according to Tukey's test. [1] Expressed in g/L. Abbreviations: TA, total titratable acidity (as tartaric acid). *p*-values: ***, $p < 0.001$.

3.8. Sensory Evaluation of Aroma

Regarding the fermentation protocol used for each trial, there was a noticeable variability in the olfactory perceptions of the cane wines (Table S1). The intensity of the odors varied significantly across the different treatments: SC15 had the highest value (6.5) and SC2 the lowest (2.3). Odor complexity was also highest in SC15 (7.2) and lowest in SC2 (1.5). The fermentation protocol in SC15 seemed to promote the generation of a wider range of active aroma compounds, such as esters and higher alcohols, contributing to a more complex aroma profile [67].

Among the specific attributes, the apple odor was more pronounced in SC14 (5.6), while the floral odor was stronger in SC15 (4.5). These attributes, including apple, apple-like, and cooked apple, and floral odors, are commonly associated with different rums undergoing sensory analysis [75]. The vanilla odor was mainly detected in SC11 (3.5). This aroma, as noted by Ickes and Cadwallader [76], becomes more prominent as the rum ageing process in barrels progresses. The melon odor reached its peak in SC12 (5.1). Melon is recognized in the characterization of fruity aromas in many rums, though it is more pronounced in cachaça, a rum-like spirit [77]. The alcohol odor was most intense in SC4, with citrus and spices being the most prominent odors in SC4 (3.4) and SC15 (7.6), respectively. These attributes are typical of rum, and, together with herbaceous notes, define its main aromatic component [78]. The vinegar odor was most pronounced in SC1 (7.0), while the sulfurous and herbaceous odors were generally low in all trials. In trials SC1–SC5, the presence of AAB, which remained until the end of alcoholic fermentation, oxidizing ethanol into acetic acid, contributed to the increased perception of vinegar odor [79]. The overall acceptance levels were highest in SC15 (7.5) and lowest in SC9 (2.3). These results indicate a significant variability in the olfactory perceptions of the different cane wine samples.

The agglomerative hierarchical clustering analysis (AHC) of the sensory data of the cane wines grouped the 16 cane wines into four distinct clusters (Figure 7), each characterized by specific olfactory attributes. Cluster 1 included trials SC1 to SC4, characterized by a high perception of vinegar odor and low aromatic intensity and complexity. Cluster 2 included eight treatments (SC5–SC10, SC13, and SC14), characterized by a dominance of apple and sulfurous odors. Cluster 3 consisted of the SC11 and SC15 trials, characterized by high complexity and floral, alcohol, citrus, spicy, and vanilla odors, and high overall acceptance. Finally, cluster 4 consisted of the SC12 and SC16 trials, characterized mainly by the smell of melon. The total variance was 27.54% within the cluster and 72.46% between clusters, suggesting an optimal separation between the groups.

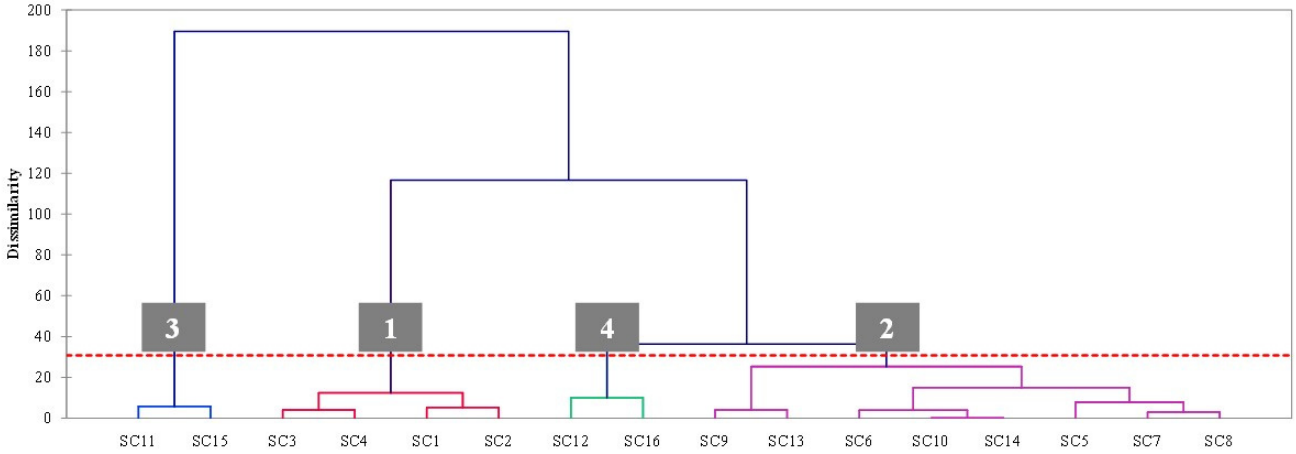

Figure 7. Agglomerative hierarchical clustering obtained by the olfactory analysis of cane wines after the end of alcoholic fermentation.

3.9. Volatile Organic Compounds of Cane Wines

Chromatographic analysis of the headspace of the cane wines identified a total of 29 compounds, with their distribution varying by sample (Table 6).

Nineteen compounds were detected in the SC15 trial, 16 in SC16, 15 in SC12, and 13 in SC13. The lowest number of VOCs was recorded in the spontaneously fermented trials, nine in SC9 and ten in SC11. Among the esters, decanoic acid, ethyl ester had the highest relative peak area values in SC11 (29.38%) and SC16 (28.79%), while dodecanoic acid, ethyl ester had the highest in SC13 (52.31%). Ethyl ester is one of the most abundant esters in commercial rums [80], as dodecanoic acid [81]. Fruity and floral notes in some commercial rums are linked to decanoic acid, ethyl ester [75], while sweet and waxy odors are emitted by dodecanoic acid, ethyl ester in some unaged rums [74,82]. Notably, hexadecanoic acid, ethyl ester was only detected in the inoculated trials (SC12, SC13, SC15, and SC16), and octadecanoic acid, ethyl ester was only present in SC15 and SC16. Both esters, derived from fatty acids, enhance the complexity and depth of the rum's aromatic bouquet, imparting waxy and sweet notes [81].

1-butanol, 3-methyl-, acetate, and octanoic acid, ethyl ester were detected in all cane wines, with the highest relative peak area values in SC9 and SC11, respectively. The positive aromatic impact of these esters in rum has been identified in rum distillates [10,83]. Octanoic acid, ethyl ester, butanoic acid, ethyl ester, and hexanoic acid, ethyl ester play key roles in the overall aromatic expression of rum. 1-butanol, 3-methyl-, acetate is present in various alcoholic beverages, including rum, but its role in defining the overall flavor profile is secondary and varies by the geographical origin of the rum [79,84].

Phenylethyl alcohol was only observed in the SC9 trial, and it is important in defining the aroma of rums aged in oak barrels [83]. Acetic acid, 2-phenylethyl ester, identified in SC9 and SC13, is also present in high quantities in aged rums [79,83]. These results confirm the importance of specific esters and alcohols in defining the flavor profile of rum. However, variations in fermentation protocols can significantly influence the VOC composition and, consequently, the flavor profile of the respective cane wines.

Table 6. Analysis of volatile organic compounds emitted by cane wines at the end of alcoholic fermentation.

RT [1]	KI [2]	Compounds [3]	CAS	SC9 [4]	SC11 [4]	SC12 [4]	SC13 [4]	SC15 [4]	SC16 [4]	*p*-Values
9.123	858	1-Butanol, 3-methyl-, acetate	000123-92-2	27.15 ± 1.05 ^{a}	6.25 ± 0.45 ^{b}	1.53 ± 0.09 ^{de}	4.33 ± 0.25 ^{c}	0.88 ± 0.08 ^{e}	2.40 ± 0.12 ^{d}	***
10.831	899	Oxime-, methoxy-phenyl-_	1000222-86-6	5.92 ± 0.25 ^{b}	3.27 ± 0.24 ^{d}	1.26 ± 0.11 ^{e}	6.93 ± 0.33 ^{a}	4.30 ± 0.18 ^{c}	1.74 ± 0.11 ^{e}	***
14.481	989	Hexanoic acid, ethyl ester	000123-66-0	n.d.	3.88 ± 0.31 ^{a}	1.61 ± 0.08 ^{b}	n.d.	0.41 ± 0.03 ^{c}	1.61 ± 0.12 ^{c}	***
18.559	1060	Phenylethyl Alcohol	000060-12-8	2.81 ± 0.15 ^{a}	n.d.	n.d.	n.d.	n.d.	n.d.	***
20.407	1179	Octanoic acid	000124-07-2	n.d.	6.34 ± 0.18 ^{a}	3.70 ± 0.17 ^{b}	n.d.	1.31 ± 0.15 ^{d}	2.88 ± 0.21 ^{c}	***
21.222	1195	Octanoic acid, ethyl ester	000106-32-1	2.83 ± 0.12 ^{c}	22.79 ± 0.74 ^{a}	10.32 ± 0.37 ^{b}	1.05 ± 0.09 ^{d}	3.37 ± 0.25^{c}	9.81 ± 0.15 ^{b}	***
22.972	1248	Propanoic acid, 2-methyl-, 2-phenylethyl ester	000103-48-0	n.d.	n.d.	n.d.	n.d.	n.d.	0.04 ± 0.01 ^{a}	***
22.986	1256	Acetic acid, 2-phenylethyl ester	000103-45-7	26.74 ± 0.80 ^{a}	n.d.	n.d.	8.71 ± 0.41 ^{b}	n.d.	n.d.	***
25.668	1311	1H-Indene, 2,3-dihydro-1,1,5,6-tetramethyl	000942-43-8	n.d.	n.d.	n.d.	1.01 ± 0.05 ^{a}	n.d.	n.d.	***
26.082	1387	n-Decanoic acid	000334-48-5	1.55 ± 0.08 ^{e}	6.06 ± 0.31 ^{a}	3.79 ± 0.15 ^{c}	4.69 ± 0.41 ^{b}	2.53 ± 0.11 ^{d}	4.04 ± 0.34 ^{bc}	***
26.52	1388	2-Buten-1-one, 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-	023696-85-7	1.20 ± 0.09 ^{a}	n.d.	n.d.	0.85 ± 0.06 ^{b}	n.d.	n.d.	***
26.655	1389	Ethyl 9-decenoate	067233-91-4	n.d.	4.04 ± 0.15 ^{a}	0.49 ± 0.03 ^{d}	n.d.	2.03 ± 0.10 ^{e}	1.07 ± 0.09^{c}	***
26.892	1391	Decanoic acid, ethyl ester	000110-38-3	13.16 ± 0.75 ^{d}	29.38 ± 0.64 ^{a}	27.46 ± 0.61^{b}	13.01 ± 0.65 ^{d}	15.78 ± 0.55 ^{c}	28.79 ± 0.64 ^{ab}	***
28.242	1471	3-Methylbutyl 2-ethylhexanoate	1000099-99-3	n.d.	n.d.	0.47 ± 0.08 ^{a}	n.d.	n.d.	n.d.	***
28.247	1450	Octanoic acid, 3-methylbutyl ester	002035-99-6	n.d.	n.d.	n.d.	n.d.	n.d.	0.41 ± 0.02 ^{a}	***
31.524	1580	Dodecanoic acid	000143-07-7	n.d.	n.d.	0.85 ± 0.05 ^{ab}	n.d.	0.94 ± 0.05 ^{a}	0.79 ± 0.04 ^{b}	***
32.088	1581	Dodecanoic acid, ethyl ester	000106-33-2	18.64 ± 0.95 ^{c}	9.89 ± 0.35 ^{d}	21.54 ± 0.55 ^{b}	52.31 ± 1.05 ^{a}	7.47 ± 0.31 ^{e}	21.22 ± 0.71 ^{b}	***
33.298	1615	Pentadecanoic acid, 3-methylbutylester	002306-91-4	n.d.	n.d.	1.14 ± 0.10 ^{b}	n.d.	0.97 ± 0.06 ^{b}	1.57 ± 0.15 ^{a}	***
37.185	1687	Ethyl tridecanoate	028267-29-0	n.d.	n.d.	1.74 ± 0.11 ^{b}	2.34 ± 0.12 ^{a}	n.d.	n.d.	***
37.195		Octadecanoic acid, ethyl ester	000111-61-5	n.d.	n.d.	n.d.	n.d.	1.09 ± 0.07 ^{b}	1.65 ± 0.11 ^{a}	***
37.204	1880	Pentadecanoic acid, ethyl ester	041114-00-5	n.d.	n.d.	n.d.	1.89 ± 0.24 ^{a}	n.d.	n.d.	***
43.893	1978	Hexadecanoic acid, ethyl ester	000628-97-7	n.d.	n.d.	18.32 ± 0.47 ^{b}	1.44 ± 0.10 ^{e}	27.54 ± 0.84 ^{a}	15.67 ± 0.31 ^{c}	***
43.973	1980	Ethyl 9-hexadecenoate	054546-22-4	n.d.	8.1 ± 0.41 ^{b}	5.78 ± 0.34 ^{c}	n.d.	29.78 ± 0.67 ^{a}	6.31 ± 0.34 ^{c}	***
50.206	2144	Linoleic acid ethyl ester	000544-35-4	n.d.	n.d.	n.d.	n.d.	0.39 ± 0.02 ^{a}	n.d.	***
50.383	2153	9,12-Octadecadienoic acid, ethyl ester	007619-08-1	n.d.	n.d.	n.d.	n.d.	0.39 ± 0.03 ^{a}	n.d.	***
50.96	2162	cis-Vaccenic acid	000506-17-2	n.d.	n.d.	n.d.	n.d.	0.24 ± 0.01 ^{a}	n.d.	***
51.081	2173	9,12-Octadecadien-1-ol, (Z,Z)-	000506-43-4	n.d.	n.d.	n.d.	n.d.	0.48 ± 0.02 ^{a}	n.d.	***

[1] Retention time (minutes) [2] Kovats Indices represent retention times normalized to the retention times of adjacent eluting n-alkanes [3] Compounds are classified in order of retention time. [4] The contents of the volatile compounds were expressed as relative peak areas percentage (peak area of each compound/total area) × l00% ± standard deviation. Abbreviations: n.d., not detected. Data in the same line followed by the same letter are not significantly different according to Tukey's test. *p*-values: ***, $p < 0.001$.

4. Conclusions

This study demonstrates the feasibility of cultivating sugarcane in Mediterranean environments and presents an innovative fermentation strategy to produce high-quality Sicilian rum agricole. Through the integration of varietal selection, microbial control measures, and the application of novel *S. cerevisiae* strains derived from sugar-rich matrices, the research offers a reproducible and effective approach to enhancing both the aromatic complexity and microbiological stability of cane wine. These findings provide a robust foundation for the establishment of a local rum agricole supply chain in Sicily, thereby contributing to the diversification and valorization of regional agri-food resources.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/app15147696/s1: Figure S1. Temperature and rainfall trends at the experimental site; Table S1. Sensory analysis of the odor of cane wines.

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