*fermentation*

MDPI

*Article*

# The Impact of Microbial Activity on the Chemical Composition and Aroma Profile of Traditional Sparkling Wines

**Stephan Sommer [1,*], Stella J. Sommer [2], Connie Liu [1], Olivia Burken [1] and Andrea Faeth Anderson [2]**

[1] Grape and Wine Institute, University of Missouri, 223 Eckles Hall, Columbia, MO 65211, USA
[2] Viticulture and Enology Research Center, California State University, 2360 E. Barstow Ave, Fresno, CA 93740, USA
* Correspondence: ssommer@missouri.edu

**Abstract:** Traditional sparkling wines are produced in a two-step sequence of alcoholic fermentations, followed by extended aging which is an influential factor for the final aroma profile. Traditionally, the second fermentation and aging are conducted in bottles over a minimum of 18 months, resulting in an aroma profile which is shaped by oxidative secondary metabolites like aldehydes, acids and fatty acid esters. In this study, a total of 29 traditional commercial sparkling wines from the categories Champagne, Cava, California Champagne, and others (Prosecco and Cremant) were analyzed. The objective was to determine the impact of microbial activity on the stylistic characteristics of traditional sparkling wines and allow winemakers to reproduce the specific fermentation conditions. The results indicate that malolactic fermentation plays an important role in Champagne and some Cavas, but not in the other sparkling wine categories. The metabolic activity of lactic acid bacteria results in an altered acid profile, amino acid utilization, and aroma production. While primary fermentation esters like phenylethyl acetate and isoamyl acetate are significantly reduced in Champagne and Cava, aroma compounds from secondary microbial activity like ethyl lactate and 2-acetyl-1-pyrroline are increased. This underlines the importance of diverse microbial activity of the characteristic style of traditional sparkling wines.

**Keywords:** Champagne; Cava; malolactic fermentation; proline; lactic acid bacteria; acetyl pyrroline



**Citation:** Sommer, S.; Sommer, S.J.; Liu, C.; Burken, O.; Anderson, A.F. The Impact of Microbial Activity on the Chemical Composition and Aroma Profile of Traditional Sparkling Wines. *Fermentation* **2024**, *10*, 212. https://doi.org/10.3390/fermentation10040212

Academic Editor: Antonio Morata

Received: 17 February 2024
Revised: 19 March 2024
Accepted: 10 April 2024
Published: 13 April 2024



## 1. Introduction

Sparkling wine is traditionally produced by primary fermentation at a larger tank scale and secondary fermentation in single bottles [1,2]. In many cases, the bottles in which the product is fermented are also the vessels for aging and final sales, making traditional sparkling wine very unique in the food and beverage market. Bottle to bottle variability and flavor consistency are the biggest challenges for producers, which led to the development of alternative fermentation strategies such as the Transversage Method where the sparkling wine is removed from the fermentation bottle, clarified in a tank, and rebottled [3]. However, consumers prefer Méthode Champenoise or Methode Tradionelle sparkling wines over tank methods [2], making traditional bottle fermentation the logical choice for producers in the mid to high price market.

Grapes designated for sparkling wine are harvested at lower sugar and higher acid levels compared to grapes for still wine production [4]. This strategy ensures reasonable alcohol concentrations after secondary fermentation, as well as microbial stability during the, on average, 18 to 24 months of bottle aging [3]. The long contact with the yeast during that time is believed to be responsible for the unique flavor profile of traditional sparkling wine [5–7], but the use of specific cultivars like Chardonnay, Pinot Meunier, and Pinot Noir in Champagne or Macabeo, Xarel-lo, and Parellada in Cava [4] also contribute to the niche character of these products. Replicating a "Champagne character" in a sparkling wine using non-traditional cultivars like French–American hybrids could be an interesting

marketing strategy for wineries who cannot grow *Vitis vinifera* and still need to serve a market which demands traditional sparkling wine styles.

The aroma profile of traditional sparkling wines is influenced by the primary aroma from the grapes that were used, the secondary aroma from the first alcoholic fermentation, and the tertiary aroma from the second fermentation and aging on the lees [7]. Due to the early harvest, primary and secondary aroma characters are mostly subtle, creating a product that is influenced by secondary metabolites like alcohols and oxidation products such as aldehydes and acids [6,8,9]. Traditionally, the impact of yeast has been studied thoroughly; however, the contribution of other organisms like lactic acid bacteria has been mostly overlooked. Most producers of traditional sparkling wine do not inoculate malolactic fermentation [10], but it has been shown that lactic acid bacteria (LAB) naturally occur on grapes [11], and can only be suppressed but not eliminated during the winemaking process [12]. It is therefore plausible that lactic acid bacteria may also influence the aroma profile of sparkling wine, given the long fermentation and aging time without the protective effect of sulfur dioxide. Secondary metabolites of LABs include organic acids, amines, and degradation products of other wine components [11], making their contribution potentially impactful for the flavor of the final product.

The objective of this study was to screen a variety of commercial sparkling wines from different traditional and non-traditional regions and determine the contribution of lactic acid bacteria to the aroma profiles. Using specific indicators for microbial activity, the aim was to demonstrate the impact of bacteria on the desirable Champagne character.

## 2. Materials and Methods

### *2.1. Selection of Commercial Sparkling Wines*

For this study, a total of 29 commercial sparkling wines were selected based their origin and production methods as shown on the labels. The products can be categorized into 9 Champagnes, 10 Cavas, 6 California Champagnes, and 4 others (Cremant and Prosecco Spumante). Countries of origin were France, Spain, USA, and Italy. All sparkling wines were pre-screened by a panel of industry professionals to ensure that sensory expectations were met and the wines were a representation of their respective style. The samples were anonymized and randomized after that to remove any bias for further testing, and protect the identity of the producers.

### *2.2. Chemical Analyses*

All sparkling wines were analyzed for their malic acid, L-lactic acid, D-lactic acid, α-amino nitrogen (NOPA assay), and total polyphenol (Folin–Ciocalteu assay) concentrations using the respective enzymatic and colorimetric method kits on a discrete analyzer (Gallery, Thermo Scientific, Waltham, MA, USA). Since proline is not an α-amino acid and is not included in the NOPA assay, it was analyzed separately using a spectrophotometric method described by Elliott and Gardner 1976 [13]. Using the same spectrophotometer (Genesys 150, Thermo Scientific, Waltham, MA, USA), yellow/brown color was determined by reading the absorbance at 420 nm. The pH was analyzed using a HI2222 multi- meter (Hanna Instruments, Smithfield, RI, USA). Acetaldehyde was measured with a commercial enzymatic kit on a SPICA Automated Analyzer (Admeo, Inc., Napa, CA, USA).

### *2.3. Aroma Analysis via GC-MS*

In a 20 mL amber glass vial, 5 mL of sample was spiked with 50 µL of the internal standard (>98.8% 3-Octanone, Sigma-Aldrich, St. Louis, MO, USA) to a final internal concentration of 0.05 mg/L. To the sample vials, 2 g of NaCl (Fisher Chemicals, Fair Lawn, NJ, USA) was added. All samples were run in duplicate.

Qualitative and semi-quantitative analyses were conducted using the triple quadrupole in a scan or using an MRM mode as needed. The fiber (85 µm, 1 cm SPME fiber, 23 gauge, coated with carboxen-polydimethylsiloxane Carboxen/PDMS; Supelco) was conditioned before use according to the manufacturer's recommendations. Sample vials were pre-

incubated for 5 min at 45 °C. The fiber was exposed for 30 min at 45 °C in the headspace for volatile extraction. Samples were agitated in an autosampler incubator at 350 rpm during extraction.

The HS-SPME GC-MS/MS system consisted of a MicroCal autosampler (MicroCal, LLC, Northampton, MA, USA) mounted on an Agilent 7890A gas chromatograph (Santa Clara, CA, USA) coupled with an Agilent 7000 Triple Quadrupole detector (Santa Clara, CA, USA). The SPME fiber was desorbed in the inlet at 250 °C for 2 min in splitless mode, after which the split flow was turned on to 50 mL/min for the remainder of the run. The fiber was re-conditioned in the inlet for 14 min prior to the next sample. A DB-5MS column (30 m × 0.25 mm ID., 0.25 µm film thickness; Agilent Santa Clara, CA, USA) and helium carrier gas (flow rate: 1.0 mL/min) were used for all analyses. The initial GC temperature was 40 °C for 3.0 min, and then was increased to 90 °C at 10 °C/min, followed by an increase to 200 °C at 5 °C/min for 10.0 min, and another increase at 20 °C/min to the final temperature of 250 °C, which was held for 5 min. For GC-MS/MS, the temperature of the transfer line was 240 °C, and nitrogen (1.5 mL/min) was used as the collision gas.

The mass spectrometer was operated in electron ionization mode at 70 eV with multiple reaction monitoring (MRM) for quantification. Data acquisition and qualitative analyses were performed using the MassHunter Workstation software version B.07.00 (Agilent Technologies, Santa Clara, CA, USA). The compounds were tentatively identified using the NIST MS Search v2.2, NIST 14 Mass Spectral Library database (Scientific Instrument Services, Ringoes, NJ, USA) by matching the mass spectral data with that of the compound. Additionally, linear retention indices (RI) were calculated using Kovats' equation from a sequence of linear hydrocarbons (C5-C30 hydrocarbon mixture, Beck Flavors, Maryland Heights, MO, USA). Semi-quantitative analysis was done by assuming a response factor equal to 3-Octanone equivalents.

### *2.4. Statistical Analysis*

Data handling, statistical analysis, and some visualization were performed using SigmaPlot 15.0 (Systat Software Inc., San Jose, CA, USA). Other Visualizations and Principal Component Analysis was performed with XLStat 2022.3.1 (Addinsoft, New York, NY, USA).

## 3. Results and Discussion

The initial screening of all commercial sparkling wines reveals some interesting details about the different styles and microbial activity during production. The main attributes of interest related to overall wine composition are shown in Figure 1.

While there are obvious trends between the different types of sparkling wine, the variability within the groups is also relatively large, leading to only a few statistically significant differences between styles. There are no significant differences in total polyphenolic content (Figure 1b) or yellow color (Figure 1c), even though a larger number of Champagne and Cava samples showed higher numbers in both categories. There is a moderate positive correlation between total polyphenols and color (correlation coefficient 0.65), indicating that a deeper yellow color may be based on phenolic oxidation. However, there are also Champagne and California Champagne samples with elevated phenolic concentrations and low absorbance at 420 nm, making these two attributes an interesting selection for stylistic choices. Even though malic acid (Figure 1a) and the pH value (Figure 1d) are not correlated (correlation coefficient 0.02), they follow a similar trend and show statistically significant differences between styles. Interestingly, all Champagne samples finished malolactic fermentation, showing a significantly lower malic acid concentration than all the other styles. However, Champagne pH values are on average also among the lowest in this study. This reveals an interesting stylistic characteristic of Champagne where lactic acid bacteria contribute to the final product, but the pH remains in the very low range that is characteristic of sparkling wine [14].

While all sparkling wines in this study stayed within that pH range, there are significant differences between styles as well as the use of malolactic fermentation during

production. All Cavas underwent at least a partial malolactic fermentation, and retain some of the lowest pH values in this sample set. California Champagnes, on the other hand, are exclusively made without malolactic fermentation and still have a significantly higher pH than the Cavas. The lack of malic acid in some of the California-made wines is most likely caused by an increase in respiratory activity in the grapes [15] rather than by microbial activity, so the acid composition in the sparkling wine differs based on growing location. The sparkling wines in the "others" category are all from France or Italy, showing similar characteristics to the other European products, with the exception of the use of malolactic fermentation. None of the sparkling wines in that category show signs of lactic acid bacteria activity during or after fermentation, since the malic acid concentration is high in all products. This also indicates that Champagne- and Cava-style sparkling wines are developing their unique styles at least partially due to the activity of lactic acid bacteria.

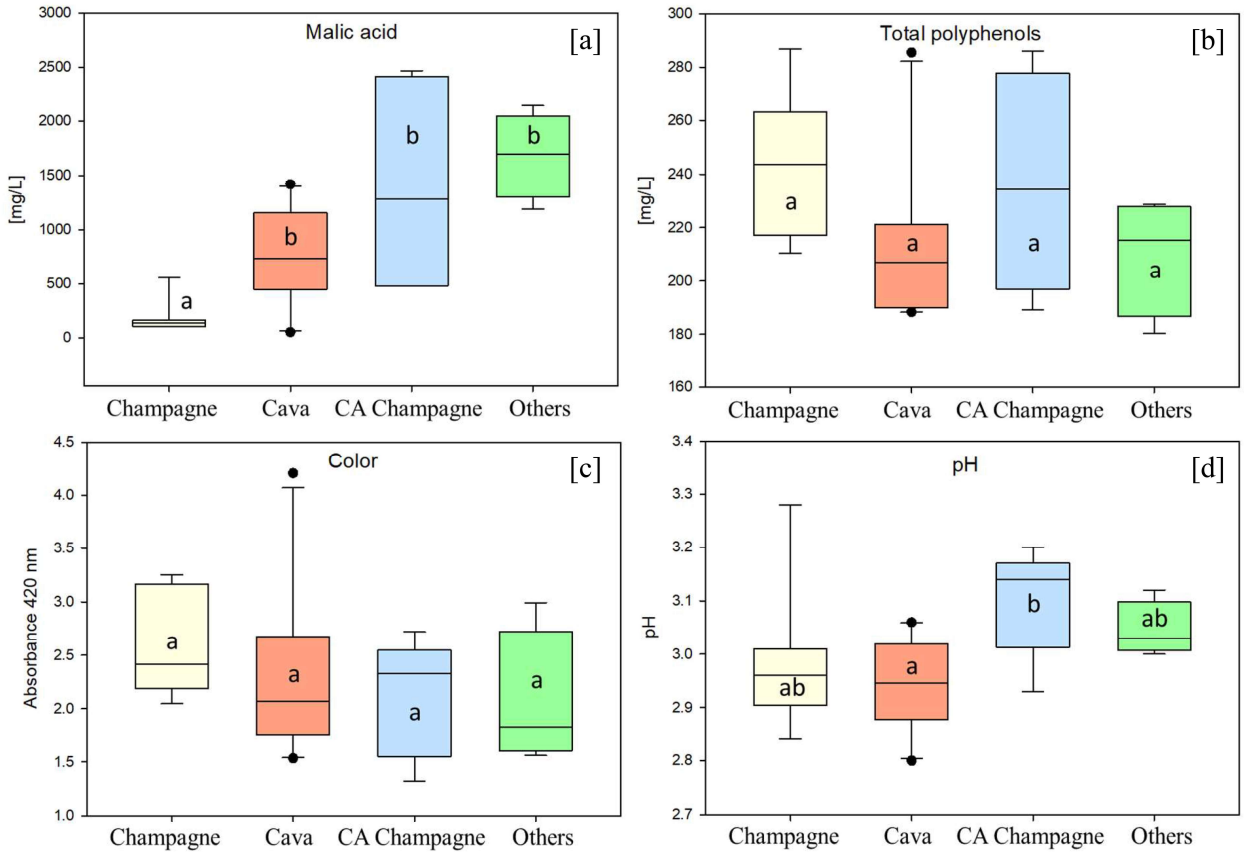


**Figure 1.** Distribution of analytical attributes in sparkling wines grouped by origin ((**a**): malic acid, (**b**): total polyphenols, (**c**): color, (**d**): pH). Statistically significant differences between groups are indicated by a,b letters within the box plots.

In order to clarify the impact of bacterial activity on the overall product characteristics, Figure 2 shows selected marker compounds that are known for being relevant to the activity of lactic acid bacteria. While L-lactic acid is produced by decarboxylation of malic acid, D-lactic acid is the main product of microbial sugar metabolism [16]. Elevated levels indicate metabolic activity before or during alcoholic fermentation, which, in the case of sparkling wines, could include secondary fermentation in the bottle. The D-lactic acid concentrations in the final sparkling wines (Figure 2a) indicate that there is a wider range of lactic acid bacteria activity in Champagnes than in any other category. The timing of that activity, however, seems to vary between commercial Champagnes. While all Champagnes have elevated levels of L-lactic acid due to malolactic fermentation (data not shown), only two out of nine products showed D-lactic acid concentrations above average. This indicates that bacterial activity in the majority of Champagnes only occurred after the second alcoholic

fermentation during aging in the absence of sugar. This is supported by the acetaldehyde concentrations (Figure 2b), since LABs, depending on the strain, are known to lower the acetaldehyde levels during malolactic fermentation [17].

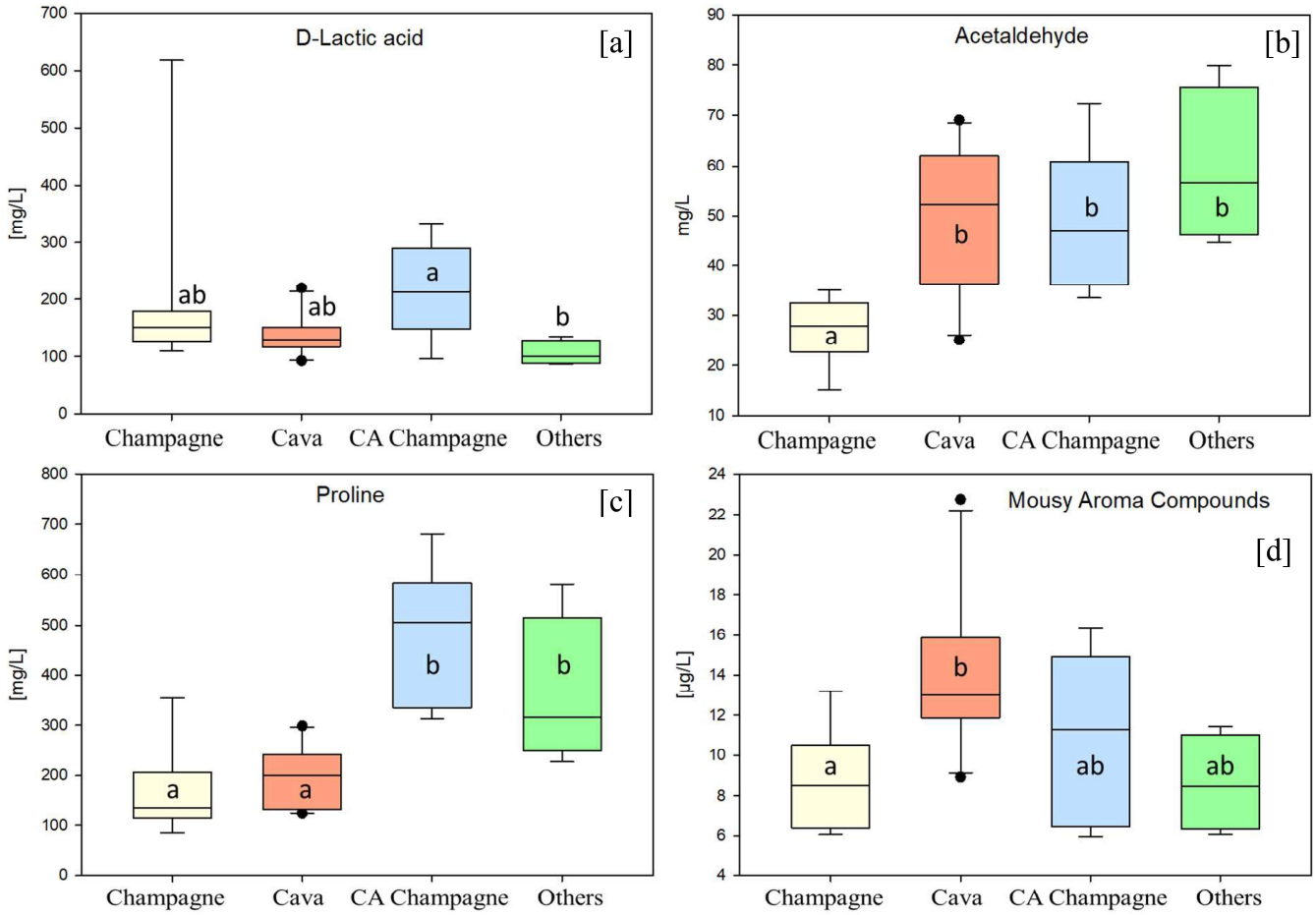


**Figure 2.** Distribution of analytical attributes in sparkling wines grouped by origin ((**a**): D-Lactic acid, (**b**): acetaldehyde, (**c**): proline, (**d**): sum of mousy aroma compounds). Statistically significant differences between groups are indicated by a,b letters within the box plots.

Most *Saccharomyces* yeast only metabolizes α-amino acids under anaerobic conditions [18], leaving most of the proline unused. This leads to an accumulation of proline in the finished wine, specifically if cultivars like Chardonnay are used, which are known to have higher proline concentrations in the grapes [19], while others like Pinot Noir accumulate more arginine [20]. None of the typical cultivars used for Cava has been reported to develop very high proline levels [21]. It is therefore surprising to see proline concentrations in Champagne that are significantly lower than the other product groups like CA Champagne (Figure 2c), which is known to use the traditional Champagne cultivars like Chardonnay as well. Lactic acid bacteria have been reported to be able to utilize β-amino acids such as proline [22], implying active LABs in the process. In fact, there is a moderate negative correlation between the occurrence of malolactic fermentation and high proline levels in this dataset (correlation coefficient −0.58), indicating that LABs are using proline in their metabolism. It remains unclear what the metabolic pathways of proline utilization under sparkling wine conditions could be; however, some studies suggest a correlation between proline metabolism and the occurrence of heterocyclic aroma compounds such as 2-acetyl-1-pyrroline (AcPy) [23,24], which can be described as bready or mousy [25,26]. The Cavas in this study show significantly elevated levels of mousy aroma compounds, indicating that such metabolic activity could be relevant for the production of sparkling

wines. It is important to note that there is no correlation between the proline concentration and the level of mousy aroma (correlation coefficient −0.12). This can be explained by the magnitude of concentration difference where proline is present in mg/L and AcPy is present and aroma-active in the low µg/L range. A small quantity of conversion would not lead to a statistical correlation; however, the data suggests that proline is utilized by LABs during sparkling wine fermentation and aging.

In order to compare the full aroma spectrum between induvial samples but also the groups of sparkling wine products, Table 1 shows the results of the most important compounds.

**Table 1.** Aroma compounds analyzed in the sparkling wine samples. The bottom of the table shows averages and standard deviations within each sparkling wine style (statistically significant differences are indicated by letters a,b,c at $\alpha = 0.05$). All concentrations are expressed in µg/L. (nd: not detected).

| Sample ID | 2-Methyl-1-Butanol | Ethyl Butanoate | Ethyl Lactate | Furfural | Ethyl Isovalerate | Isoamyl Acetate | 2-Ethyltetrahydro Pyridine |
|---|---|---|---|---|---|---|---|
| #1 | 58 ± 23.7 | 31 ± 7.6 | 140 ± 43.9 | 14 ± 4.0 | 6 ± 0.9 | 11 ± 1.4 | 1 ± 0.1 |
| #2 | 69 ± 28.7 | 41 ± 8.9 | 98 ± 45.1 | 7 ± 0.6 | 6 ± 0.9 | 15 ± 1.2 | 1 ± 1.2 |
| #3 | 92 ± 47.1 | 23 ± 1.8 | 6 ± 1.6 | 9 ± 2.2 | 6 ± 0.4 | 34 ± 4.8 | 1 ± 0.2 |
| #4 | 63 ± 7.5 | 28 ± 11.2 | 6 ± 1.3 | 16 ± 0.0 | nd | 45 ± 13.5 | 1 ± 0.1 |
| #5 | 55 ± 16.3 | 18 ± 1.2 | 62 ± 5.1 | 7 ± 0.1 | nd | 60 ± 5.7 | 1 ± 0.0 |
| #6 | 63 ± 21.5 | 22 ± 0.2 | 8 ± 2.5 | 4 ± 1.6 | nd | 68 ± 3.8 | 1 ± 0.2 |
| #7 | 59 ± 7.1 | 17 ± 4.0 | 12 ± 1.6 | 12 ± 4.4 | nd | 41 ± 10.8 | 1 ± 0.1 |
| #8 | 58 ± 6.3 | 26 ± 5.9 | 104 ± 4.9 | 26 ± 1.6 | 6 ± 0.9 | 8 ± 1.3 | 1 ± 0.1 |
| #9 | 62 ± 15.8 | 24 ± 5.4 | 48 ± 58.0 | 11 ± 4.2 | 4 ± 1.6 | 13 ± 2.6 | nd |
| #10 | 52 ± 1.0 | 20 ± 4.1 | 51 ± 66.5 | 33 ± 1.2 | 6 ± 0.7 | 11 ± 2.4 | 1 ± 0.3 |
| #11 | 75 ± 33.5 | 30 ± 2.4 | 141 ± 60.4 | 22 ± 9.6 | 7 ± 0.3 | 15 ± 1.8 | 1 ± 0.3 |
| #12 | 72 ± 27.6 | 24 ± 3.9 | nd | 15 ± 8.9 | nd | 156 ± 24.5 | 2 ± 1.2 |
| #13 | 49 ± 11.7 | 19 ± 4.6 | nd | 11 ± 6.8 | nd | 44 ± 14.2 | 2 ± 1.6 |
| #14 | 70 ± 7.3 | 24 ± 0.7 | 11 ± 0.9 | 50 ± 4.4 | nd | 7 ± 2.2 | 1 ± 0.2 |
| #15 | 65 ± 16.2 | 28 ± 6.0 | 11 ± 1.7 | 43 ± 10.9 | 7 ± 0.7 | 11 ± 1.9 | 1 ± 0.1 |
| #16 | 70 ± 20.0 | 30 ± 10.9 | 44 ± 10.4 | 29 ± 5.2 | 8 ± 1.8 | 10 ± 0.6 | 2 ± 0.0 |
| #17 | 53 ± 13.1 | 24 ± 7.7 | 16 ± 1.4 | nd | nd | 194 ± 45.2 | 3 ± 0.5 |
| #18 | 62 ± 0.9 | 27 ± 7.9 | 16 ± 1.7 | nd | nd | 177 ± 18.7 | 3 ± 0.3 |
| #19 | 65 ± 13.7 | 25 ± 3.9 | nd | 18 ± 2.7 | 6 ± 1.3 | 18 ± 2.9 | 1 ± 0.1 |
| #20 | 62 ± 11.9 | 28 ± 7.7 | 24 ± 2.7 | 41 ± 16.3 | 8 ± 1.9 | 8 ± 1.8 | 1 ± 0.0 |
| #21 | 66 ± 17.3 | 23 ± 7.8 | 121 ± 10.0 | 90 ± 4.1 | 8 ± 2.8 | 11 ± 2.9 | 2 ± 0.3 |
| #22 | 72 ± 27.5 | 25 ± 6.5 | 115 ± 7.9 | 74 ± 0.9 | 7 ± 2.4 | 9 ± 2.2 | 1 ± 0.0 |
| #23 | 65 ± 10.5 | 31 ± 6.1 | 34 ± 37.9 | 29 ± 9.2 | nd | 10 ± 1.9 | 1 ± 0.3 |
| #24 | 84 ± 23.2 | 35 ± 12.2 | 150 ± 0.2 | 17 ± 0.8 | 6 ± 1.0 | 9 ± 2.7 | 1 ± 0.3 |
| #25 | 72 ± 9.2 | 27 ± 5.4 | 102 ± 25.8 | 57 ± 11.3 | 5 ± 1.0 | 10 ± 2.0 | 1 ± 0.2 |
| #26 | 106 ± 1.0 | 23 ± 4.7 | 86 ± 20.1 | 136 ± 12.3 | 10 ± 3.2 | 20 ± 6.0 | 1 ± 0.7 |
| #27 | 129 ± 52.3 | 37 ± 14.8 | nd | nd | 10 ± 3.3 | 12 ± 3.3 | 2 ± 0.1 |
| #28 | 89 ± 1.9 | 33 ± 7.9 | 58 ± 6.7 | 23 ± 15.9 | nd | 21 ± 5.6 | 1 ± 0.1 |
| #29 | 128 ± 78.5 | 29 ± 0.9 | 4 ± 5.2 | 25 ± 5.3 | nd | 10 ± 0.3 | 3 ± 0.8 |
| Champagne | 66 ± 10.2 a | 27 ± 4.5 ab | 108 ± 37.1 a | 38 ± 28.6 a | 6 ± 1.4 a | 11 ± 2.2 a | 1 ± 0.4 a |
| Cava | 85 ± 26.5 a | 30 ± 5.5 b | 37 ± 34.4 b | 38 ± 37.5 ab | 5 ± 4.4 a | 12 ± 4.8 a | 1 ± 0.7 a |
| CA Champagne | 59 ± 8.3 a | 23 ± 4.2 a | 17 ± 23.4 b | 8 ± 7.0 b | nd | 113 ± 70.3 b | 2 ± 1.0 a |
| Others | 70 ± 15.2 a | 22 ± 3.2 a | 7 ± 5.0 b | 11 ± 5.9 ab | 3 ± 3.5 a | 40 ± 20.7 ab | 1 ± 0.2 a |

| Sample ID | Benzaldehyde | 2-Acetyl-1-pyrroline | Ethyl hexanoate | Hexyl acetate | Ethyl furoate | Methyl benzaldehyde | Phenylethanol |
|---|---|---|---|---|---|---|---|
| #1 | 5 ± 1.6 | 2 ± 0.1 | 351 ± 9.3 | nd | 6 ± 2.0 | 143 ± 27.6 | 89 ± 22.2 |
| #2 | nd | 3 ± 0.0 | nd | nd | 7 ± 1.5 | 273 ± 25.8 | 104 ± 17.8 |
| #3 | nd | 2 ± 0.1 | 321 ± 13.8 | nd | 6 ± 1.8 | 208 ± 44.1 | 132 ± 38.6 |
| #4 | nd | 2 ± 0.6 | 270 ± 8.1 | 6 ± 1.7 | 4 ± 1.1 | 199 ± 30.1 | 98 ± 13.4 |
| #5 | nd | 2 ± 0.1 | 212 ± 29.0 | 8 ± 1.0 | nd | 245 ± 1.9 | 86 ± 8.6 |
| #6 | 4 ± 1.3 | 3 ± 1.3 | 312 ± 17.4 | 9 ± 4.0 | nd | 365 ± 75.3 | 193 ± 55.6 |
| #7 | nd | 2 ± 0.5 | 282 ± 7.1 | nd | nd | 182 ± 6.2 | 171 ± 8.5 |
| #8 | 37 ± 4.8 | 3 ± 0.2 | 316 ± 20.9 | nd | 7 ± 1.4 | 145 ± 15.4 | 125 ± 6.4 |
| #9 | 15 ± 0.4 | 2 ± 0.2 | 355 ± 37.2 | nd | 6 ± 0.6 | 171 ± 5.5 | 141 ± 2.3 |
| #10 | 60 ± 2.7 | 2 ± 0.5 | 267 ± 6.3 | nd | 7 ± 0.3 | 96 ± 4.8 | 104 ± 0.2 |
| #11 | 10 ± 4.9 | 3 ± 1.1 | 410 ± 64.4 | nd | 10 ± 4.8 | 155 ± 41.2 | 211 ± 63.1 |
| #12 | 6 ± 2.1 | 3 ± 1.4 | 330 ± 8.8 | 25 ± 4.1 | nd | 322 ± 99.1 | 153 ± 48.2 |
| #13 | 6 ± 0.9 | 2 ± 0.8 | 284 ± 105.4 | 19 ± 12.6 | nd | 345 ± 59.3 | 140 ± 38.2 |
| #14 | nd | 4 ± 0.7 | 384 ± 55.0 | nd | 13 ± 2.1 | 414 ± 57.9 | 221 ± 1.8 |
| #15 | 5 ± 0.1 | 4 ± 1.0 | 325 ± 19.3 | nd | 18 ± 3.8 | 353 ± 56.0 | 191 ± 7.9 |
| #16 | nd | 6 ± 0.9 | 338 ± 26.3 | nd | 12 ± 2.6 | 398 ± 71.2 | 197 ± 12.5 |
| #17 | nd | 2 ± 0.3 | 309 ± 92.1 | 19 ± 7.5 | nd | 455 ± 96.9 | 151 ± 61.1 |
| #18 | 6 ± 2.0 | 2 ± 0.1 | 323 ± 23.7 | 16 ± 2.3 | nd | 371 ± 25.1 | 176 ± 50.2 |
| #19 | nd | 2 ± 0.5 | 344 ± 22.5 | nd | 10 ± 1.5 | 297 ± 15.6 | 183 ± 11.8 |
| #20 | nd | 3 ± 0.7 | 299 ± 30.4 | nd | 15 ± 1.7 | 215 ± 6.5 | 198 ± 13.1 |

**Table 1.** *Cont.*

| Sample ID | Benzaldehyde | 2-Acetyl-1-pyrroline | Ethyl hexanoate | Hexyl acetate | Ethyl furoate | Methyl benzaldehyde | Phenylethanol |
|---|---|---|---|---|---|---|---|
| #21 | 12 ± 0.6 | 5 ± 1.0 | 332 ± 33.6 | nd | 11 ± 1.3 | 219 ± 9.2 | 216 ± 24.7 |
| #22 | 22 ± 1.2 | 3 ± 0.1 | 289 ± 20.6 | nd | 9 ± 0.5 | 182 ± 23.1 | 174 ± 38.9 |
| #23 | 8 ± 1.1 | 3 ± 0.2 | 355 ± 7.2 | nd | 14 ± 0.1 | 290 ± 21.9 | 221 ± 72.1 |
| #24 | nd | 4 ± 0.2 | 454 ± 55.7 | nd | 8 ± 0.0 | 219 ± 17.6 | 293 ± 63.8 |
| #25 | 13 ± 0.8 | 3 ± 0.2 | 306 ± 14.9 | nd | 9 ± 1.2 | 138 ± 34.5 | 191 ± 81.0 |
| #26 | 18 ± 2.2 | 5 ± 1.1 | 274 ± 21.5 | nd | 15 ± 2.3 | 215 ± 36.2 | 252 ± 89.9 |
| #27 | nd | 5 ± 1.6 | 376 ± 1.0 | nd | 24 ± 3.0 | 358 ± 46.3 | 327 ± 103.7 |
| #28 | 16 ± 0.2 | 4 ± 0.6 | 398 ± 31.2 | nd | 11 ± 1.1 | 492 ± 29.4 | 264 ± 41.4 |
| #29 | 7 ± 2.4 | 8 ± 2.3 | 375 ± 49.8 | nd | 13 ± 0.6 | 383 ± 60.5 | 344 ± 43.7 |
| Champagne | 19 ± 18.6 a | 3 ± 1.0 ab | 342 ± 59.1 a | nd | 8 ± 1.9 a | 163 ± 39.6 a | 172 ± 64.3 ab |
| Cava | 5 ± 6.8 ab | 5 ± 1.6 b | 312 ± 116.6 a | nd | 14 ± 4.4 b | 339 ± 89.7 b | 232 ± 69.7 a |
| CA Champagne | 3 ± 3.4 a | 2 ± 0.5 a | 288 ± 43.7 a | 15 ± 7.3 a | 1 ± 1.7 c | 323 ± 91.3 b | 134 ± 34.7 b |
| Others | 1 ± 2.1 b | 2 ± 0.6 a | 315 ± 25.4 a | 2 ± 4.3 b | 4 ± 5.0 ac | 263 ± 84.0 ab | 170 ± 26.7 ab |

| Sample ID | 2-Acetyl-3,4,5,6-tetrahydropyridine | Ethyl succinate | Ethyl octanoate | 2-Acetyl-1,4,5,6-tetrahydropyridine | Phenylethyl acetate | TDN (1,1,6,-trimethyl-1,2-dihydronaphthalene) | β-Damascenone | Ethyl decanoate |
|---|---|---|---|---|---|---|---|---|
| #1 | 3 ± 0.1 | 126 ± 21.7 | 194 ± 11.2 | 1 ± 0.1 | nd | 42 ± 50.9 | 6 ± 2.6 | 5 ± 0.4 |
| #2 | 5 ± 0.7 | 132 ± 23.0 | 388 ± 21.3 | 2 ± 0.2 | nd | 201 ± 152.9 | 5 ± 1.9 | 13 ± 2.4 |
| #3 | 3 ± 0.1 | 164 ± 32.3 | 212 ± 29.9 | 1 ± 0.0 | nd | 32 ± 25.9 | 7 ± 3.0 | 5 ± 0.3 |
| #4 | 3 ± 0.6 | 90 ± 15.4 | 192 ± 42.2 | 1 ± 0.1 | nd | 36 ± 19.3 | 10 ± 4.3 | 6 ± 1.1 |
| #5 | 3 ± 0.4 | 123 ± 3.8 | 201 ± 30.9 | 1 ± 0.1 | 4 ± 0.9 | 18 ± 7.4 | 6 ± 2.7 | 4 ± 0.1 |
| #6 | 6 ± 2.6 | 90 ± 26.2 | 404 ± 141.7 | 2 ± 0.8 | 8 ± 5.5 | 66 ± 45.9 | 3 ± 1.8 | 6 ± 3.3 |
| #7 | 4 ± 1.2 | 158 ± 15.0 | 247 ± 39.8 | 1 ± 0.3 | 6 ± 1.4 | 56 ± 29.3 | 6 ± 2.0 | 5 ± 2.6 |
| #8 | 2 ± 0.6 | 171 ± 16.5 | 173 ± 19.5 | 1 ± 0.2 | nd | 16 ± 8.2 | 5 ± 2.4 | 3 ± 0.6 |
| #9 | 5 ± 2.2 | 175 ± 10.4 | 373 ± 133.4 | 1 ± 0.5 | nd | 25 ± 9.5 | 9 ± 2.5 | 11 ± 7.8 |
| #10 | 2 ± 0.8 | 160 ± 1.3 | 172 ± 22.4 | 1 ± 0.2 | nd | 37 ± 16.5 | 10 ± 3.4 | 6 ± 0.8 |
| #11 | 5 ± 1.2 | 217 ± 38.6 | 310 ± 44.9 | 1 ± 0.2 | nd | 40 ± 25.3 | 11 ± 6.7 | 8 ± 3.0 |
| #12 | 7 ± 1.1 | 62 ± 25.6 | 439 ± 46.4 | 2 ± 0.3 | 11 ± 3.8 | 217 ± 190.2 | 33 ± 17.3 | 16 ± 6.8 |
| #13 | 4 ± 1.4 | 61 ± 8.7 | 346 ± 178.4 | 1 ± 0.4 | 5 ± 0.4 | 202 ± 123.7 | 26 ± 10.0 | 16 ± 5.9 |
| #14 | 6 ± 1.6 | 203 ± 40.8 | 416 ± 58.9 | 2 ± 0.4 | nd | 509 ± 416.9 | 5 ± 1.6 | 15 ± 4.6 |
| #15 | 6 ± 0.1 | 144 ± 118.3 | 434 ± 6.0 | 1 ± 0.2 | nd | 610 ± 493.0 | 7 ± 1.8 | 15 ± 2.1 |
| #16 | 6 ± 1.5 | 241 ± 34.9 | 432 ± 10.5 | 1 ± 0.1 | nd | 937 ± 714.4 | 3 ± 0.9 | 12 ± 2.7 |
| #17 | 7 ± 1.8 | 128 ± 28.6 | 468 ± 177.6 | 2 ± 0.5 | 38 ± 3.3 | 215 ± 119.9 | 5 ± 1.8 | 19 ± 0.6 |
| #18 | 8 ± 0.3 | 131 ± 11.6 | 530 ± 40.0 | 2 ± 0.1 | 43 ± 2.3 | 241 ± 138.2 | 16 ± 4.7 | 23 ± 6.9 |
| #19 | 5 ± 0.9 | 221 ± 11.7 | 349 ± 41.3 | 1 ± 0.4 | 6 ± 1.6 | 90 ± 68.0 | 4 ± 1.4 | 17 ± 6.5 |
| #20 | 4 ± 0.6 | 225 ± 17.5 | 263 ± 32.9 | 1 ± 0.1 | nd | 345 ± 214.5 | 8 ± 1.9 | 11 ± 3.6 |
| #21 | 3 ± 0.5 | 271 ± 2.2 | 249 ± 14.0 | 1 ± 0.1 | nd | 63 ± 31.6 | 8 ± 2.4 | 6 ± 2.4 |
| #22 | 3 ± 0.4 | 198 ± 23.3 | 201 ± 33.5 | 1 ± 0.0 | nd | 54 ± 22.9 | 8 ± 1.2 | 5 ± 1.8 |
| #23 | 8 ± 1.3 | 208 ± 27.3 | 541 ± 58.8 | 2 ± 0.2 | nd | 1023 ± 529.8 | 9 ± 0.6 | 24 ± 4.9 |
| #24 | 7 ± 1.3 | 330 ± 26.0 | 534 ± 97.2 | 2 ± 0.4 | nd | 127 ± 76.2 | 11 ± 1.2 | 18 ± 6.3 |
| #25 | 4 ± 0.8 | 167 ± 40.9 | 329 ± 49.5 | 1 ± 0.2 | nd | 60 ± 5.3 | 13 ± 1.7 | 15 ± 6.4 |
| #26 | 5 ± 2.2 | 282 ± 62.0 | 271 ± 34.6 | 1 ± 0.0 | nd | 546 ± 284.0 | 6 ± 0.2 | 8 ± 3.9 |
| #27 | 7 ± 0.0 | 318 ± 38.4 | 549 ± 16.8 | 2 ± 0.1 | nd | 1130 ± 325.5 | 11 ± 0.4 | 23 ± 5.2 |
| #28 | 8 ± 1.7 | 224 ± 11.5 | 601 ± 148.4 | 2 ± 0.4 | nd | 907 ± 521.5 | 3 ± 0.7 | 24 ± 9.6 |
| #29 | 9 ± 2.7 | 286 ± 9.4 | 592 ± 9.3 | 2 ± 0.5 | nd | 1577 ± 994.3 | 10 ± 1.8 | 25 ± 3.1 |
| Champagne | 4 ± 1.5 a | 202 ± 63.1 a | 282 ± 119.3 a | 1 ± 0.4 a | nd | 51 ± 32.1 ab | 9 ± 2.5 a | 8 ± 5.2 a |
| Cava | 7 ± 1.7 b | 226 ± 59.4 a | 449 ± 121.9 b | 2 ± 0.5 a | nd | 779 ± 413.5 b | 7 ± 2.7 a | 17 ± 6.3 b |
| CA Champagne | 5 ± 2.5 ab | 99 ± 32.5 b | 363 ± 141.8 ab | 1 ± 0.7 a | 17 ± 18.7 a | 155 ± 100.0 ab | 16 ± 11.6 a | 14 ± 7.5 ab |
| Others | 4 ± 1.4 ab | 158 ± 53.6 ab | 303 ± 88.9 ab | 1 ± 0.4 a | 5 ± 3.3 a | 61 ± 24.3 a | 5 ± 2.0 a | 8 ± 5.7 ab |

Since the samples are randomized to remove potential bias from the study, the average and standard deviation for each group were calculated and will be used to discuss styles rather than induvial commercial products. Interestingly, some aroma compounds only appear in samples that did not undergo malolactic fermentation, implying an influence of lactic acid bacteria on the stability of these molecules. It is unlikely that esters like phenylethyl acetate or isoamyl acetate were not produced in all base wines during primary fermentation; however, Champagne and Cava sparkling wines show none or significantly fewer of these compounds, respectively. The influence of LABs on aroma development was studied before [11,27], and it is apparent that their metabolic activity reaches far beyond the decarboxylation of acids. Table 2 summarizes the perceived aroma of each compound and the sensory threshold if it has been established in the literature before.

**Table 2.** Thresholds and aroma descriptors of all compounds analyzed in this study.

| Compound | Threshold [µg/L] | Sensory Impression | References |
|---|---|---|---|
| 2-Methyl-1-butanol | not reported | Sherry | Muños et al., 2006 [28] |
| Ethyl butanoate | 20 | Pineapple | Guth 1997 [29] |
| Ethyl lactate | 110,000 | Sweet, creamy | Radler 1986 [30] |
| Furfural | 14,100 | Almond | Ferreira et al., 2000 [31] |
| Ethyl isovalerate | 3 | Pineapple | Ferreira et al., 2000 [31] |
| Isoamyl acetate | 30 | Banana | Guth 1997 [29] |
| 2-Ethyltetrahydropyridine | 150 | Brioche, mousy | Snowdon et al., 2006 [32] |
| Benzaldehyde | 2000 | Honey | Arias-Perez et al., 2021 [33] |
| 2-Acetyl-1-pyrroline | 0.1 | Bread crust, mousy | Herderich et al., 1995 [25] |
| Ethyl hexanoate | 14 | Pineapple | Ferreira et al., 2000 [31] |
| Hexyl acetate | not reported | Pear | Dennis et al., 2012 [34] |
| Ethyl furoate | 16,000 | Balsamic | Ferreira et al., 2000 [31] |
| Methylbenzaldehyde | not reported | Cherry | Chávez-Márquez et al., 2022 [35] |
| Phenylethanol | 14,000 | Rose | Ferreira et al., 2000 [31] |
| 2-Acetyl-3,4,5,6-tetrahydropyridine | 1.6 | Popcorn, mousy | Snowdon et al., 2006 [32] |
| Ethyl succinate | not reported | Cooked apple | Dachery et al., 2023 [36] |
| Ethyl octanoate | 5 | Waxy, musty | Ferreira et al., 2000 [31] |
| 2-Acetyl-1,4,5,6-tetrahydropyridine | 1.6 | Popcorn, mousy | Snowdon et al., 2006 [32] |
| Phenylethyl acetate | 250 | Rose | Guth 1997 [29] |
| TDN (1,1,6,-trimethyl-1,2-dihydronapthalene) | 2 | Kerosene | Sacks et al., 2012 [37] |
| β-Damascenone | 0.05 | Floral | Guth 1997 [29] |
| Ethyl decanoate | 200 | waxy, Brandy | Ferreira et al., 2000 [31] |

While some of the aldehydes and esters are only found in concentrations below their threshold and are therefore not contributing to the overall sensory impression, some molecules of interest appear to have an impact on the perception of sparkling wines. Especially ethyl esters of fatty acids, which contribute fruity and musty characters, are present in high enough concentrations to be aroma-active. This is in agreement with previous studies [4,6,9], and was described as "Champagne character" before. However, our data suggests that aroma compounds previously described as mousy flavor in wine, such as 2-acetyl-1-pyrroline and acteyl tetrahydropyridine, have a significant aroma impact as well. In other foods, these molecules have been described as "bread crust" and "popcorn" [38], which are aroma descriptors also frequently found in traditional sparkling wines. Only ethyltetrahydropyridine does not reach the concentration threshold in this study. The volatility and sensory perception are dependent on pH and other matrix attributes, so it can be hypothesized that their aroma impact is different in sparkling wine compared to still wine and might contribute to the overall "Champagne character". Other descriptors of aroma compounds like furfural and benzaldehyde match the stylistic expectations of traditional sparkling wine, since they contribute nutty and creamy attributes to the product. However, the concentrations of some of these molecules in this study were well below the sensory threshold, so it seems unlikely that they are of any specific importance to the aroma quality. Exceptions might exist where the combination of several compounds enhances the perception of each in a cumulative fashion. This has been shown to be true for β-damascenone, which enhances the perception of fruity notes in red wines [39] and could have a similar effect in other wine matrices. In this study, β-damascenone concentrations exceed the sensory threshold in most samples, implying a direct impact on the floral character of the sparkling wines [29], as well as an indirect enhancing effect on other aroma impressions.

Figure 3a shows a discriminant analysis using pH, malic acid, and mousy compounds as variables. It is possible to differentiate Champagne and Cava from the other two product groups, which indicates that the regulation of pH during the winemaking process and the activity of lactic acid bacteria are useful tools in creating these unique styles of sparkling wine. The Principal Component Analysis (Figure 3b) summarizes the findings by showing

correlations between attributes and sparkling wine samples. Champagnes group together in the bottom right quadrant and are characterized by L-lactic acid and the absence of acetaldehyde and proline. The majority of Cavas can be found in the bottom left quadrant, characterized by the presence of mousy compounds and acetaldehyde, as well as the absence of polyphenols. The other sparkling wines are mostly described by higher malic acid and proline concentrations and the absence of LAB indicators.

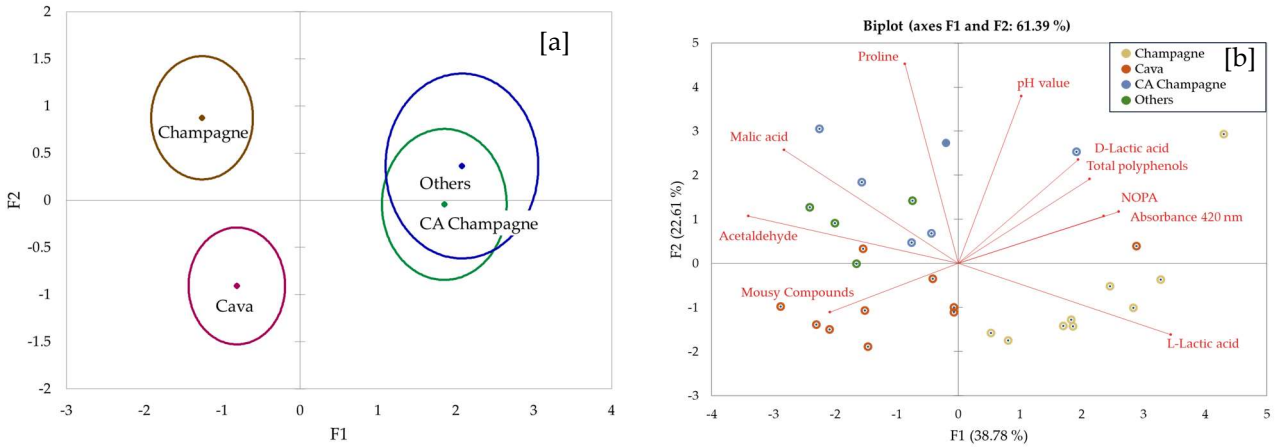


**Figure 3.** Statistical evaluation of similarities and differences in traditional sparkling wine using (**a**) Discriminant Analysis and (**b**) Principal Component Analysis.

These observations allow for a reasonably accurate characterization of conditions that need to be met in order to recreate a traditional sparkling wine style. Producers in areas that do not support the growth of European winegrapes (*Vitis vinifera*) and rely on interspecific cultivars could reproduce the styles by mimicking these conditions. While Champagne is using Chardonnay, for example, the interspecific French–American hybrid equivalent is Chardonel. While most hybrid grapes naturally maintain lower sugar [40] and higher acid levels compared to *V. vinifera* [41], which makes them good candidates for sparkling wine production, the pH-to-acidity balance could be different. Hybrid grapes like Chardonel were found to develop high acid and high pH conditions in some vintages. Other studies suggest that the wines can have medium pH levels around 3.3 [42], which would still be above the Champagne and Cava range. Our data suggest that malolactic fermentation is an important factor in recreating the traditional Champagne style, which means that the pH of the sparkling wine after the degradation of malic acid should ideally still be between 2.9 and 3.1. It can be assumed that a titratable acidity reduction of one gram per liter of wine leads to a pH increase of 0.1 units [43]. In order to achieve the target pH after malolactic fermentation, the beginning juice pH should not exceed 2.8, which could be the main challenge when using interspecific cultivars.

Inducing malolactic fermentation at a low pH and following the appropriate bottle aging protocol should then lead to a comparable aroma profile as seen in this study. The production of acetyl-1-pyrroline and acteyl tetrahydropyridine is suspected to have a significant influence on the desired aroma profile, and should be considered desirable when producing sparkling wines from interspecific cultivars.

The overall "Champagne character" has been mostly described using aroma compounds that derived from yeast autolysis. Fruity esters and primary grape aroma play a lesser role in the traditional style that can be described as toasty, creamy, and waxy [9]. These descriptors are caused by organic acids like decanoic acid and their ethyl esters [31]. Most white interspecific cultivars like Seyval Blanc or Vidal Blanc do not have a distinct primary aroma profile, and while this might lower their value for still wine production, it presents an opportunity for sparkling wines. Naturally low sugar levels, high acid concentrations, and a neutral aroma profile potentially allow producers to shape these

grape cultivars into sparkling wines that resemble traditional styles like Champagne or Cava.

## 4. Conclusions

Traditional sparkling wines are produced using similar methods; however, the final product characteristics may vary significantly. The impact of lactic acid bacteria on product composition and aroma profile was demonstrated using a variety of commercial products. Malolactic fermentation and the production of related aroma compounds can be used to characterize Champagne and Cava, which represent a unique style of bottle fermented and aged sparkling wine. Mousy flavor compounds that are also responsible for pleasant aroma in bread and rice could be partially responsible for the traditional "Champagne character", and should be further investigated. We suggest that proline could be a substrate in sparkling wine fermentation and aging; however, the pathway still needs to be clarified. While cultivars like Chardonnay have the potential to result in higher concentrations of acetyl-1-pyrroline due to much higher proline levels after primary fermentation, other traditional Champagne grapes like Pinot Noir accumulate arginine instead. That should result in a significantly different production of mousy flavors depending on the cultivar composition of the Champagne base wine. Aldehydes and organic acids are also responsible for the unique character of traditional sparkling wines; however, the impact of microbial activity on these molecules can be significant. Especially in the case of lactic acid bacteria, the metabolic activity during prolonged periods of bottle aging can be significant. With the analysis of finished commercial products, the timing of that activity can only be estimated, but it appears to take place during bottle aging as can be deducted from the lower acetaldehyde concentrations in Champagne. This would have implications for the production strategies when recreating a Champagne-style sparkling wine from non-traditional grape cultivars. Based on the results of this study, pH adjustment prior to primary alcoholic fermentation and the timing of lactic acid bacteria inoculation seem to be the major stylistic tools.

**Author Contributions:** Conceptualization, S.S. and S.J.S.; methodology, S.S. and A.F.A.; formal analysis, S.J.S., O.B., A.F.A. and C.L.; investigation, S.S.; data curation, S.S., S.J.S., and C.L.; writing—original draft preparation, S.S.; writing—review and editing, S.S.; visualization, S.S. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Data are contained within the article.

**Conflicts of Interest:** The authors declare no conflicts of interest.

## References

1. Caliari, V.; Panceri, C.P.; Rosier, J.P.; Bordignon-Luiz, M.T. Effect of the Traditional, Charmat and Asti method production on the volatile composition of Moscato Giallo sparkling wines. *LWT-Food Sci. Technol.* **2015**, *61*, 393–400. [CrossRef]
2. Culbert, J.A.; McRae, J.M.; Condé, B.C.; Schmidtke, L.M.; Nicholson, E.L.; Smith, P.A.; Howell, K.S.; Boss, P.K.; Wilkinson, K.L. Influence of production method on the chemical composition, foaming properties, and quality of Australian carbonated and sparkling white wines. *J. Agric. Food Chem.* **2017**, *65*, 1378–1386. [CrossRef] [PubMed]
3. Todd, B. *Microbiological and Chemical Changes during the Fermentation and Ageing of Sparkling Wines*; UNSW Sydney: Kensington, Australia, 1995.
4. Ubeda, C.; Kania-Zelada, I.; del Barrio-Galán, R.; Medel-Marabolí, M.; Gil, M.; Peña-Neira, Á. Study of the changes in volatile compounds, aroma and sensory attributes during the production process of sparkling wine by traditional method. *Food Res. Int.* **2019**, *119*, 554–563. [CrossRef] [PubMed]
5. Gnoinski, G.B.; Schmidt, S.A.; Close, D.C.; Goemann, K.; Pinfold, T.L.; Kerslake, F.L. Novel Methods to Manipulate Autolysis in Sparkling Wine: Effects on Yeast. *Molecules* **2021**, *26*, 387. [CrossRef] [PubMed]
6. Martínez-García, R.; Mauricio, J.C.; García-Martínez, T.; Peinado, R.A.; Moreno, J. Towards a better understanding of the evolution of odour-active compounds and the aroma perception of sparkling wines during ageing. *Food Chem.* **2021**, *357*, 129784. [CrossRef] [PubMed]

7. Alexandre, H.; Guilloux-Benatier, M. Yeast autolysis in sparkling wine—A review. *Aust. J. Grape Wine Res.* **2006**, *12*, 119–127. [CrossRef]
8. Webber, V.; Dutra, S.V.; Spinelli, F.R.; Marcon, Â.R.; Carnieli, G.J.; Vanderlinde, R. Effect of glutathione addition in sparkling wine. *Food Chem.* **2014**, *159*, 391–398. [CrossRef] [PubMed]
9. Sawyer, S.; Longo, R.; Solomon, M.; Nicolotti, L.; Westmore, H.; Merry, A.; Gnoinski, G.; Ylia, A.; Dambergs, R.; Kerslake, F. Autolysis and the duration of ageing on lees independently influence the aroma composition of traditional method sparkling wine. *Aust. J. Grape Wine Res.* **2022**, *28*, 146–159. [CrossRef]
10. Garofalo, C.; Arena, M.P.; Laddomada, B.; Cappello, M.S.; Bleve, G.; Grieco, F.; Beneduce, L.; Berbegal, C.; Spano, G.; Capozzi, V. Starter cultures for sparkling wine. *Fermentation* **2016**, *2*, 21. [CrossRef]
11. Cappello, M.S.; Zapparoli, G.; Logrieco, A.; Bartowsky, E.J. Linking wine lactic acid bacteria diversity with wine aroma and flavour. *Int. J. Food Microbiol.* **2017**, *243*, 16–27. [CrossRef]
12. Davis, C.R.; Wibowo, D.; Fleet, G.H.; Lee, T.H. Properties of Wine Lactic Acid Bacteria: Their Potential Enological Significance. *Am. J. Enol. Vitic.* **1988**, *39*, 137–142. [CrossRef]
13. Elliott, R.J.; Gardner, D.L. Proline determination with isatin, in the presence of amino acids. *Anal. Biochem.* **1976**, *70*, 268–273. [CrossRef] [PubMed]
14. Scheiber, E. Schaumwein. In *Handbuch für die Getränkeindustrie: Ein kaufmännisches Lehr- und Informationswerk für die Getränkewirtschaft*; Gabler Verlag: Wiesbaden, Germany, 1968; pp. 80–90.
15. Ruffner, H.P.; Possner, D.; Brem, S.; Rast, D.M. The physiological role of malic enzyme in grape ripening. *Planta* **1984**, *160*, 444–448. [CrossRef] [PubMed]
16. Fugelsang, K.C. The Lactic Acid Bacteria. In *Wine Microbiology*; Springer US: Boston, MA, USA, 1997; pp. 3–47.
17. Durner, D.; Weber, F.; Neddermeyer, J.; Koopmann, K.; Winterhalter, P.; Fischer, U. Sensory and Color Changes Induced by Microoxygenation Treatments of Pinot noir before and after Malolactic Fermentation. *Am. J. Enol. Vitic.* **2010**, *61*, 474–485. [CrossRef]
18. Long, D.; Wilkinson, K.L.; Taylor, D.K.; Jiranek, V. Novel wine yeast for improved utilisation of proline during fermentation. *Fermentation* **2018**, *4*, 10. [CrossRef]
19. Sommer, S.; Wegmann-Herr, P.; Wacker, M.; Fischer, U. Rationale for a stronger disposition of Chardonnay wines for stuck and sluggish fermentation. *S. Afr. J. Enol. Vitic.* **2015**, *36*, 180–190. [CrossRef]
20. Bell, S.-J.; Henschke, P.A. Implications of nitrogen nutrition for grapes, fermentation and wine. *Aust. J. Grape Wine Res.* **2005**, *11*, 242–295. [CrossRef]
21. Presa-Owens, C.D.L.; Lamuela-Raventos, R.M.; Buxaderas, S.; Torre-Boronat, M.C.D.L. Differentiation and Grouping Characteristics of Varietal Grape Musts from Penedès Region (I). *Am. J. Enol. Vitic.* **1995**, *46*, 283–291. [CrossRef]
22. Di Martino, C.; Testa, B.; Letizia, F.; Iorizzo, M.; Lombardi, S.J.; Ianiro, M.; Di Renzo, M.; Strollo, D.; Coppola, R. Effect of exogenous proline on the ethanolic tolerance and malolactic performance of *Oenococcus oeni*. *J. Food Sci. Technol.* **2020**, *57*, 3973–3979. [CrossRef]
23. Yoshihashi, T.; Huong, N.T.T.; Inatomi, H. Precursors of 2-Acetyl-1-pyrroline, a Potent Flavor Compound of an Aromatic Rice Variety. *J. Agric. Food Chem.* **2002**, *50*, 2001–2004. [CrossRef]
24. Wu, M.-L.; Chou, K.-L.; Wu, C.-R.; Chen, J.-K.; Huang, T.-C. Characterization and the Possible Formation Mechanism of 2-Acetyl-1-Pyrroline in Aromatic Vegetable Soybean (*Glycine max* L.). *J. Food Sci.* **2009**, *74*, S192–S197. [CrossRef] [PubMed]
25. Herderich, M.; Costello, P.J.; Grbin, P.R.; Henschke, P.A. Occurrence of 2-acetyl-1-pyrroline in mousy wines. *Nat. Prod. Lett.* **1995**, *7*, 129–132. [CrossRef]
26. Cho, I.H.; Peterson, D.G. Chemistry of bread aroma: A review. *Food Sci. Biotechnol.* **2010**, *19*, 575–582. [CrossRef]
27. Antalick, G.; Perello, M.-C.; de Revel, G. Characterization of Fruity Aroma Modifications in Red Wines during Malolactic Fermentation. *J. Agric. Food Chem.* **2012**, *60*, 12371–12383. [CrossRef] [PubMed]
28. Muñoz, D.; Peinado, R.A.; Medina, M.; Moreno, J. Higher alcohols concentration and its relation with the biological aging evolution. *Eur. Food Res. Technol.* **2006**, *222*, 629–635. [CrossRef]
29. Guth, H. Quantitation and sensory studies of character impact odorants of different white wine varieties. *J. Agric. Food Chem.* **1997**, *45*, 3027–3032. [CrossRef]
30. Radler, F. Microbial biochemistry. *Experientia* **1986**, *42*, 884–893. [CrossRef]
31. Ferreira, V.; López, R.; Cacho, J.F. Quantitative determination of the odorants of young red wines from different grape varieties. *J. Sci. Food Agric.* **2000**, *80*, 1659–1667. [CrossRef]
32. Snowdon, E.M.; Bowyer, M.C.; Grbin, P.R.; Bowyer, P.K. Mousy Off-Flavor: A Review. *J. Agric. Food Chem.* **2006**, *54*, 6465–6474. [CrossRef]
33. Arias-Pérez, I.; Sáenz-Navajas, M.P.; de-la-Fuente-Blanco, A.; Ferreira, V.; Escudero, A. Insights on the role of acetaldehyde and other aldehydes in the odour and tactile nasal perception of red wine. *Food Chem.* **2021**, *361*, 130081. [CrossRef]
34. Dennis, E.G.; Keyzers, R.A.; Kalua, C.M.; Maffei, S.M.; Nicholson, E.L.; Boss, P.K. Grape Contribution to Wine Aroma: Production of Hexyl Acetate, Octyl Acetate, and Benzyl Acetate during Yeast Fermentation Is Dependent upon Precursors in the Must. *J. Agric. Food Chem.* **2012**, *60*, 2638–2646. [CrossRef]
35. Chávez-Márquez, A.; Gardea, A.A.; González-Rios, H.; Vazquez-Moreno, L. Characterization of Cabernet Sauvignon Wines by Untargeted HS-SPME GC-QTOF-MS. *Molecules* **2022**, *27*, 1726. [CrossRef] [PubMed]

36. Dachery, B.; Hernandes, K.C.; Zini, C.A.; Welke, J.E.; Manfroi, V. Volatile and sensory profile of sparkling wines produced by faster and alternative methods (Ancestral and Single Tank Fermentation) compared to the usual methods (Charmat and Traditional). *Eur. Food Res. Technol.* **2023**, *249*, 2363–2376. [CrossRef]
37. Sacks, G.L.; Gates, M.J.; Ferry, F.X.; Lavin, E.H.; Kurtz, A.J.; Acree, T.E. Sensory Threshold of 1,1,6-Trimethyl-1,2-dihydronaphthalene (TDN) and Concentrations in Young Riesling and Non-Riesling Wines. *J. Agric. Food Chem.* **2012**, *60*, 2998–3004. [CrossRef] [PubMed]
38. Hofmann, T.; Schieberle, P. New and Convenient Synthesis of the Important Roasty, Popcorn-like Smelling Food Aroma Compounds 2-Acetyl-1-pyrroline and 2-Acetyltetrahydropyridine from their Corresponding Cyclic α-Amino Acids. *J. Agric. Food Chem.* **1998**, *46*, 616–619. [CrossRef] [PubMed]
39. Pineau, B.; Barbe, J.-C.; Van Leeuwen, C.; Dubourdieu, D. Which Impact for β-Damascenone on Red Wines Aroma? *J. Agric. Food Chem.* **2007**, *55*, 4103–4108. [CrossRef] [PubMed]
40. Pedastsaar, P.; Vaher, M.; Helmja, K.; Kulp, M.; Kaljurand, M.; Karp, K.; Raal, A.; Karathanos, V.; Püssa, T. Chemical composition of red wines made from hybrid grape and common grape (*Vitis vinifera* L.) cultivars. *Proc. Est. Acad. Sci.* **2014**, *63*, 444. [CrossRef]
41. Homich, L.J.; Scheinberg, J.A.; Elias, R.J.; Gardner, D.M. Effects of co-inoculation on wine-quality attributes of the high-acid, red hybrid variety chambourcin. *Am. J. Enol. Vitic.* **2016**, *67*, 245–250. [CrossRef]
42. Cadwallader, K.R.; Lopez, J.R.; Menke, S.D.; Surakarnkul, R. Aroma Components of Wines from Chardonel: A French—American Hybrid Grape. In *Nutraceutical Beverages*; ACS Symposium Series; American Chemical Society: Washington, DC, USA, 2003; Volume 871, pp. 365–378.
43. Boulton, R. The relationships between total acidity, titratable acidity and pH in grape tissue. *Vitis* **1980**, *19*, 113–120.