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Aroma profile of wine-based beverages produced by co-fermentation of white grape must, apple and orange juices

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ABSTRACT

This study evaluated the effect of co-fermenting grape must with apple and/or orange juices using two inoculation strategies, single inoculation with *S. cerevisiae* and sequential inoculation with *T. delbrueckii* followed by *S. cerevisiae*, on fermentation kinetics, conventional oenological parameters, and the volatile and sensory profiles of the resulting beverages. Both juice composition and fermentation strategy significantly influenced fermentation dynamics and the chemical profile of the resulting beverages. Blends containing non-grape juices, particularly orange, and sequential inoculation showed prolonged lag phases and modified CO_2 production. The co-ferments achieved reduced alcohol content, higher acidity, and a balanced pH compared to control wines. Sequential inoculation significantly enhanced acetate ester production, like isoamyl acetate and 2-phenylethyl acetate, contributing fruity and floral aromas. Orange juice introduced high terpene levels, while apple juice enriched C13-norisoprenoids, enhancing the aromatic complexity of the co-ferments. Overall, co-fermentation with "non-grape" juices offers a promising strategy to create wine-base beverages with unique sensory profiles, reduced alcohol content, and greater aromatic diversity, aligning with consumer demand for innovative and healthier products.

1. Introduction

There is an increasing demand for natural, healthy, and eco-friendly products, along with a desire for new aromas, tastes and textures. This has led to the exploration of innovative raw materials. In the wine industry, these trends have prompted a recovery of traditional production methods, and the creation of wine-based beverages that offer new tastes and lower alcohol content.

Co-fermentation is a technique that involves blending different grape varieties or mixing grapes with other fruits in a single fermentation process. The aims of this approach include enhancing the sensory profile of the product, stabilizing colour, managing acidity, introducing innovation, or adjusting the alcohol content of the final beverage.

Several authors have studied co-fermentation using different grape varieties or different "non-grape" fruits to produce distillates, liqueurs, and fruit wines (Alonso González et al., 2010; Japtap and Bapat, 2015; Márquez-Lemus et al., 2019). Co-fermentation has also been applied to grapes and cereals, resulting in wine-based beverages with increased fibre content (Yong et al., 2013). However, co-fermenting grapes with other fruits remains new and less researched practice. Some studies have tested combinations like Chardonnay grapes with pear, peach, or banana juice, focusing mainly on the volatile composition of the final product (Patel & Shibamoto, 2003). More recently, Fracassetti et al. (2019) co-fermented Cabernet Sauvignon grapes with kiwi, producing a beverage with low alcohol content; and Martín-Gómez et al. (2021) examined the phenolic composition, antioxidant activity, and colour of beverages obtained by co-fermenting Cabernet Sauvignon grapes with blueberries.

Co-ferments align with current trends in sustainable by enabling the use of surplus or less marketable fruits, thereby reducing waste and creating value-added products. Also, the integration of diverse fruits allows for more versatile products that appeal to consumers seeking innovative beverages.

The selection of the right "non-grape" fruits for the winemaking

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process is a significant challenge and depends on both their availability and the desired outcomes. The aroma profile plays a crucial role in the sensory quality of the co-ferments and will significantly influence their overall acceptance. In Mediterranean countries, apples are among the most important "non-citrus" fruits, while oranges are a key citrus option. Apples contain over 300 identified volatile compounds, but only a few significantly influence their sensory quality (Espino-Díaz et al., 2016; Lavilla et al., 1999). Esters are dominant, accounting for up to 88 % of the total volatiles, along with a variety of alcohols and aldehydes (Espino-Díaz et al., 2016; Lavilla et al., 1999). In the case of oranges, their flavour profile is influenced by a blend of more than 200 compounds, including esters, aldehydes, ketones, terpenes, and alcohols (Perez Cacho & Rouseff, 2008).

Another key factor in co-ferments is the fermentation process, mainly the initial sugar content, pH and total acidity, and yeast strains. In recent years, there has been a growing demand for lower-alcohol wines, driven by health and economic factors, as many countries impose higher taxes on beverages with higher ethanol content. Both apples and oranges have low sugar content and high acidity, making them ideal candidates for reducing alcohol levels and adjusting the pH of the final product, potentially resulting in a fresher beverage. Regarding yeast strains, grape-fruit co-ferments can involve typical wine yeast species, such as *Saccharomyces cerevisiae*, or non-*Saccharomyces* strains. Recent trials with non-*Saccharomyces* yeasts have aimed to lower alcohol content, decrease pH, or enhance the aromatic complexity of wines (Izquierdo-Cañas et al., 2025; Martín-Gómez et al., 2021; Morata et al., 2020; Varela et al., 2017). Among the non-*Saccharomyces* yeasts, *Torulaspora delbrueckii* in sequential inoculation with *Saccharomyces cerevisiae* has showed significant oenological potential for producing beverages with lower alcohol content and an improved aroma profile. (Morata et al., 2020).

This study investigates the influence of incorporating apple and/or orange juice into white grape must on fermentation kinetics and on the volatile and sensory attributes of the resulting wine-based beverages. The effects of single yeast inoculation (*S. cerevisiae*) and sequential inoculation (*T. delbrueckii* followed by *S. cerevisiae*) are evaluated with respect to key oenological parameters and to the formation of relevant aroma compounds. The work provides insight into how "non-grape" fruits modulate fermentative behaviour and drive sensory differentiation in wine-based products.

2. Material and methods

2.1. Fruits and juices

For white grape must, it was worked with the "Instituto de la Vid y el Vino de Castilla-La Mancha" (IVICAM). White *Vitis vinifera* L. Cv. Cayetana grapes were harvested and destemmed, crushed and pressed to obtain the must. This must was sulfited with 3 g of potassium metabisulfite by each 100 kg of grape and 30 g/hL of glutathione were also added. Then, a pectolytic enzyme (Lallizyme,Lallemand Inc, Montreal, Canada) was added using a low dose of 0.5 g/hL. Relative to orange juice, *Citrus sinensis Navelina* oranges were acquired, squeezed and bottled directly in the supermarket. All bottles were homogenized into a bigger container and 20 mg/L of potassium metabisulfite were added to avoid oxidation. About apple juice, *Granny smith* apples were acquired in a local market and then submerged in sulfited water (40 mg/L), dried and cut. Apple juice was obtained with a blender Amzchef Slow Juicer SJ-036 (Amzchef Co Ltd, New York, U.S.A.). Then, 0.2 g/hL of Lallizyme (Lallemand Inc, Montreal, Canada) and 40 mg/L of potassium metabisulfite were added into the apple juice.

After sulfiting, orange juice, apple juice, and grape must were clarified under static conditions at 5 °C for 24 h. Once clarification was completed, the juices were mixed at a 1:1 ratio for the must/apple and must/orange mixtures, and at a 2:1:1 ratio for the must/apple/orange blend. The mixtures were thoroughly homogenized before fermentation, and the desired pH, total acidity, and °Brix values were achieved simply by adjusting the proportions of grape must and juices.

2.2. Fermentation assays

All controls (only must) and juice blends were fermented in triplicate under two conditions: (i) inoculation with *Saccharomyces cerevisiae* (Safoeno UCLM S235, Lesaffre Ibérica, Valladolid, Spain) and (ii) sequential inoculation with *Torulaspora delbrueckii* (Zymaflore Alpha TD, Laffort, France) followed by *S. cerevisiae*. Both microorganisms were commercial strains, and the manufacturer's rehydration and inoculation protocols were carefully followed. Dry yeasts were rehydrated in water at 25–30 °C prior to use. For single inoculation, 20 g/hL of rehydrated *S. cerevisiae* was added. For the sequential strategy, 30 g/hL of *T. delbrueckii* was first inoculated, and after 72 h of fermentation, 20 g/hL of *S. cerevisiae* was subsequently added. Fermentations were carried out at 18 °C in 3 L vessels. The fermentation kinetics were monitored daily by measuring CO_2 loss through weighing of the vessels, and fermentations were considered complete when the weight remained constant. After fermentation, the beverages were decanted at 18 °C, and glucose and fructose contents were enzymatically determined. Finally, all samples were filtered through 0.45 μm membranes and bottled.

2.3. Conventional analysis

Conventional parameters as º Brix, pH, total acidity, yeast assimilable nitrogen (YAN), malic acid, residual sugars (glucose and fructose (G + F)), alcohol, glycerol and volatile acidity, were determined using the official analytical methods established by the International Vine and Wine Organization (OIV).

2.4. Major volatile compound analysis

On-hundred microliters (100 μL) of fermented beverage was mixed with 100 μL of 4-methyl-2-pentanol (internal standard, 60.35 mg/L) and 1 mL of Milli-Q water. An aliquot (1 μL) of this mixture was injected in split mode into a Focus-ISQ gas chromatograph (Thermo Scientific, Milan, Italy). The chromatographic separation was performed on a BP21 column (60 m x 0.32 mm×0.25 μm) with an FFAP phase (polyethylene glycol modified with nitroterephthalic acid). Helium was used as carrier gas at a constant flow of 1.2 mL/min. The injector temperature was set to 195 °C, and the oven temperature was programmed as follows: 32 °C for 2 min, followed by a ramp of 5 °C/min to 120 °C, then increased by 75 °C/min to 190 °C, where it was held for 18 min. The mass spectrometer operated in electron impact mode at 70 eV, with the ion source temperature at 250 °C. Compound identification was performed by comparison with commercial standards (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), and quantification was based on calibration curves for each standard.

2.5. Minor volatile compound analysis

Minor volatile compounds were isolated from samples by SPE on 0.5 g Supelclean Envi-Chrom P cartridges (Sigma-Aldrich, Missouri, U.S.A.) which were activated following fabricant instructions. Before the isolation, 4-nonanol (1 g/L) was added as an internal standard into 100 mL of the samples: 40 μL for fermented beverages and 20 μL for juices. The cartridge extraction was made under vacuum conditions. Hydrophilic compounds were eliminated with Mili-Q water and volatile compounds were extracted by 10 mL of dichloromethane. The extract was concentrated using a nitrogen stream till an approximate volume of 200 μL. They were stored under freezing conditions (-18 °C) until their analysis.

Minor volatile compounds were analyzed in a CG-MS Agilent 6890GC / Agilent 5973 MD (Agilent Technologies, Santa Clara CA, U.S.A.). One microliter (1 μL) of extract was injected in split mode (1/3) into a ultra inert polar column DB-WAX (60 m × 0.25 mm × 0.25 μm) (Agilent Technologies, Santa Clara, CA, U.S.A.). Helium was used as carrier gas (constant flow 1.0 mL/min). Injector temperature was 250 °C and the oven temperature was programmed as follows: 70 °C during 5 min, ramped 1 °C/min to 90 °C, maintaining during 10 min, and then increasing 2 °C/min to 210 °C, that it was maintained during 40 min. The MS operated in the electron impact mode (70 eV), ion source temperature 230 °C.

Identification was carried out by comparison with commercial standards (Sigma-Aldrich Chemie GmbH, Steinhein, Germany); in some cases, commercial standards were not found so the tentative identification was carried out by comparing their spectrums with data libraries (Wiley G 1035 A and NBS 75 K). Quantification was based on calibration curves for each standard. Compounds without available standards were quantified in relation to those with a similar chemical structure.

2.6. Descriptive sensory analysis

As our institutions do not have a research ethics committee for sensory analysis involving humans or a formal documentation process, panelists were selected from the community of the two participating institutions. The evaluation included eight expert assessors with experience in wine sensory analysis, consisting of five females and three males, aged between 22 and 59 years. Before the sensory evaluation test, all panelists gave their consent to participate and for the use of the collected data. The study was conducted in accordance with ethical standards, and the rights and privacy of all participants were protected.

All samples were analyzed using quantitative descriptive analysis (QDA). Assessment took place in a standard sensory analysis room (ISO 8589:2010). Samples were prepared into the sensory analysis room's kitchen and were served into a transparent, colorless crystalline wine-tasting glass, with a regular, smooth and rounded rim, at 10 – 12 °C (UNE 87022:1992). Glasses were identified by three-character codes and covered to minimized volatiles' scape.

Expert assessors worked with a tasting sheet of 8 attributes: citric fruit flavor, green apple flavor, floral flavor, acidity, body, aftertaste intensity, aftertaste quality and global quality. Control wines and co-ferments were sniffed and tasted in duplicate, and the descriptors were scored on a 10 cm unstructured scale to rate the intensity of each attribute (0: absence of a descriptor; 10: maximum intensity).

2.7. Statistical analysis

Statistical analysis was carried out by using the IBM SPSS software, version 24.0. A one-way analysis of variance (ANOVA) was applied to evaluate differences among the samples in both chemical and sensory data. To identify specific differences among the samples, the Student–Newman–Keuls (SNK) test was applied as a post hoc analysis. In addition, principal component analysis (PCA) was applied to the major and minor volatile compounds to summarize and visualize the relationships and variability among the samples.

3. Results and discussion

3.1. Fermentation kinetics and conventional oenological parameters

Fig. 1 shows the fermentation kinetics of the control wine (grape must only) and three juice blends: 1:1 grape must/apple juice, 1:1 grape must/orange juice, and 2:1:1 grape must/apple/orange juice. Fermentations were conducted either by single inoculation with *S. cerevisiae* or sequential inoculation with *T. delbrueckii* followed by *S. cerevisiae*.

Both juice composition and fermentation strategy significantly influenced fermentation dynamics and the conventional chemical profile of the resulting beverages. Samples containing non-grape juices exhibited longer lag phases, particularly those with orange juice, likely due to their lower sugar content and higher total acidity. Sequentially

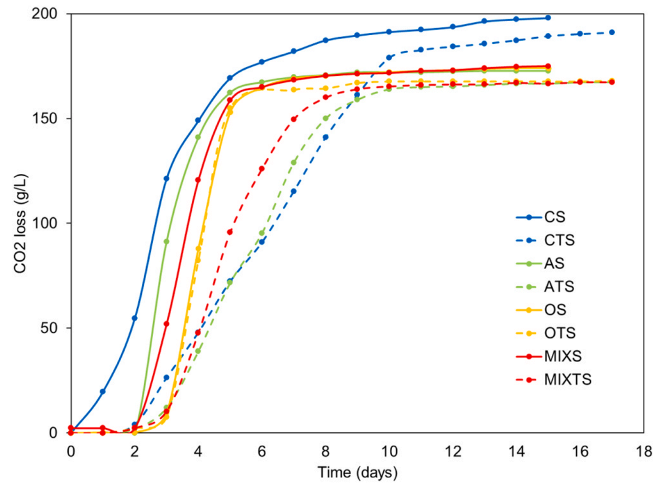

Fig. 1. Fermentation kinetics of control wines and co-ferments. CS: control wine *S. cerevisiae*; CTS: control wine *T. delbruekii* + *S. cerevisiae*; AS: grape-apple co-ferment *S. cerevisiae*; ATS: grape-apple co-ferment *T. delbruekii* + *S. cerevisiae*; OS: grape-orange co-ferment *S. cerevisiae*; OTS: grape-orange co-ferment *T. delbruekii* + *S. cerevisiae*; MIXS: grape-apple-orange co-ferment *S. cerevisiae*; MIXTS: grape-apple-orange co-ferment *T. delbruekii* + *S. cerevisiae*.

inoculated samples also showed extended lag phases. *T. delbrueckii* displayed lower fermentative vigour than *S. cerevisiae*, with a slightly slower exponential growth phase, consistent with Belda et al. (2015). This difference was not observed in the grape/orange blend, where both inoculation strategies produced similar kinetics.

Fermentation lasted longer in control wines due to the higher sugar content of grape must, resulting in greater CO_2 production. Samples inoculated with *S. cerevisiae* alone exhausted sugars approximately two days earlier than sequentially inoculated samples, leading to higher CO_2 release. Specifically, in control wines, single inoculation required 15 days with 66.0 g/L CO_2 produced, while sequential inoculation lasted 17 days with 63.7 g/L CO_2. In the juice blends, adding apple juice or combining all three juices did not significantly affect fermentation duration. However, including orange juice shortened fermentation by 3–4 days, with CO_2 production of 58 g/L and 56 g/L for single and sequential inoculations, respectively.

Conventional oenological parameters measured in the individual juices and blends helped explain these observations. Orange juice had the highest total acidity (13.2 g/L), followed by apple juice (7.5 g/L) and grape must (2.5 g/L), with pH values of 3.0, 3.0, and 3.7, respectively. In contrast, grape must was richer in sugars (19.7 ºBrix) than orange or apple juices (12.4 ºBrix each). These properties guided juice blends, as one of our goals was to produce co-ferments with reduced alcohol content while increasing the acidity and lowering the pH of the grape must. As a result, all blends achieved a pH of 3.2, with the must-orange juice blend showing 8.2 g/L total acidity and 15.8 ºBrix, the must-apple juice blend 5.2 g/L and 15.3 ºBrix, and the triple blend 6.5 g/L and 15.2 ºBrix.

On the other hand, to ensure a good development of the alcoholic fermentation, the easily assimilable nitrogen (YAN) was determined. The grape must had a YAN value of 329 mg/L, the must-orange juice blend 252 mg/L, the must-apple juice blend 254 mg/L, and the triple blend 285 mg/L. All YAN values exceeded the minimum concentration required for successful alcoholic fermentation, as levels below 140 mg/L can increase the risk of sluggish fermentation (Martínez-Moreno et al., 2012).

As can be seen in Table 1, co-fermented samples exhibited lower alcohol content and higher total acidity than control wines, particularly in grape–orange and triple blends due to the high citric acid content of orange juice. Sugar depletion was nearly complete in co-ferments, whereas residual sugars in control wines ranged from 2.56 to 3.24 g/

Table 1
Conventional oenological parameters in control wines and co-ferments (n = 3).

	CS Mean ± SD	CTS Mean ± SD	AS Mean ± SD	ATS Mean ± SD	OS Mean ± SD	OTS Mean ± SD	MIXS Mean ± SD	MIXTS Mean ± SD
Alcohol (% v/v)	9.26 ± 0.10^{b}	9.47 ± 0.14^{c}	8.23 ± 0.04^{a}	8.15 ± 0.01^{a}	8.08 ± 0.03^{a}	8.19 ± 0.05^{a}	8.17 ± 0.03^{a}	8.13 ± 0.01^{a}
pH	3.86 ± 0.09^{b}	3.85 ± 0.10^{b}	3.72 ± 0.05^{b}	3.71 ± 0.01^{b}	3.53 ± 0.16^{a}	3.50 ± 0.03^{a}	3.52 ± 0.07^{a}	3.41 ± 0.10^{a}
Total acidity (g/L)	2.83 ± 0.16^{a}	3.20 ± 0.16^{b}	4.70 ± 0.09^{c}	4.83 ± 0.04^{c}	8.05 ± 0.19^{e}	7.80 ± 0.23^{e}	6.18 ± 0.19^{d}	6.38 ± 0.08^{d}
Volatile acidity (g/L)	0.34 ± 0.09 ^{cd}	0.32 ± 0.04 ^{cd}	0.11 ± 0.01^{a}	0.14 ± 0.01^{a}	0.39 ± 0.03^{d}	0.19 ± 0.03^{ab}	0.34 ± 0.02 ^{cd}	0.26 ± 0.02^{bc}
G + F (g/L)	3.24 ± 1.21^{b}	2.56 ± 0.45^{b}	0.29 ± 0.17^{a}	0.48 ± 0.05^{a}	0.62 ± 0.47^{a}	0.03 ± 0.00^{a}	0.93 ± 0.09^{a}	0.20 ± 0.04^{a}
Glycerol	3.32 ± 0.14^{c}	4.74 ± 0.21^{e}	2.69 ± 0.07^{a}	2.66 ± 0.04^{a}	2.96 ± 0.07^{b}	3.94 ± 0.09^{d}	3.04 ± 0.16^{b}	3.75 ± 0.20^{d}

Different superscripts (a-e) in the same row mean significant differences (α = 0.05) according to the test of Student–Newman–Keuls. CS: control wine *S. cerevisiae*; CTS: control wine *T. delbruekii* + *S. cerevisiae*; AS: grape-apple co-ferment *S. cerevisiae*; ATS: grape-apple co-ferment *T. delbruekii* + *S. cerevisiae*; OS: grape-orange co-ferment *S. cerevisiae*; OTS: grape-orange co-ferment *T. delbruekii* + *S. cerevisiae*; MIXS: grape-apple-orange co-ferment *S. cerevisiae*; MIXTS: grape-apple-orange co-ferment *T. delbruekii* + *S. cerevisiae*.

L. Volatile acidity remained low in all samples, although *T. delbrueckii* tended to produce slightly less acetic acid than *S. cerevisiae* (Izquierdo Cañas et al., 2011; Tondini et al., 2019).

Significant differences in glycerol content were observed among the samples. Control wines had the highest values, followed by grape-orange co-ferments, grape-apple-orange co-ferments, and grape-apple co-ferments. This is likely due to the higher concentration of fermentable sugars in the grape must. Also, except for the grape-apple co-ferments, higher glycerol values were observed in samples obtained through sequential inoculation of *T. delbrueckii* and S. cerevisiae. This effect has been previously reported during both sequential inoculation and co-inoculation fermentation (Belda et al., 2015; Izquierdo Cañas et al., 2011; Lu et al., 2016). The increase in glycerol during fermentations involving *T. delbrueckii* has been associated with lower final ethanol content or a slight increase in pyruvic acid (Belda et al., 2015; Lu et al., 2016). No correlation was found between glycerol and ethanol levels in the samples, thus, the increase in glycerol may be linked to an enhanced development of the glycerol-pyruvate pathway in Torulaspora, as suggested by Belda et al. (2015).

3.2. Volatile compounds

The co-fermentation of grape must with orange and apple juices added intense aroma compounds to the hybrid beverages obtained, fulfilling with one of the main aims of the present study. Tables 2 and 3 show the major and minor volatile compounds identified in control wines and co-ferments, respectively.

Major volatile compounds primarily form during alcoholic fermentation, with limited contribution from the fruit juices used, except for 2-phenylethanol, which is present in low concentrations in grapes, oranges, and apples. Consequently, the main differences were found between samples fermented by single or sequential inoculation.

Sequential inoculation with *T. delbrueckii* and *S. cerevisiae* led to a significant increase in the concentrations of ethyl acetate, isobutanol, isoamyl alcohols, and 2-phenylethanol. Ethyl acetate is formed naturally through the reaction of ethanol and acetic acid. At concentrations above 200 mg/L, it can cause a pronounced sensory defect due to its solvent-like aroma (Etiévant, 1991). However, Ribéreau-Gayon (1978) demonstrated that at concentrations of 80 mg/L or lower, it can positively contribute to the wine's aroma. Also, previous studies have reported higher ethyl lactate levels in sequential *T. delbrueckii/S. cerevisiae* fermentations compared to single *S. cerevisiae* fermentations, however, this effect was observed only in grape–orange co-ferments (Loira et al., 2014).

Additionally, the increase in isoamyl alcohols and 2-phenylethanol may enhance the sensory profile of the samples. Isoamyl alcohol combines with acetic acid to form isoamyl acetate, which has a desirable banana-like aroma, while 2-phenylethanol gets a pleasant rose odour (Etiévant, 1991). Several studies have reported increases in both compounds in wines produced by sequential inoculation of *T. delbrueckii* and *S. cerevisiae* compared to those inoculated only with *S. cerevisiae* (Azzolini et al., 2015; Renault et al., 2015).

Regardless of the influence of "non-grape" fruits, only minor differences were observed between the control wine and the co-ferments. The total concentration of higher alcohols in the hybrid beverages was significantly lower than the 400 mg/L threshold generally established as the sensory defect limit for these compounds (Longo et al., 2021). Slightly elevated but safe methanol levels were detected in the grape-orange co-ferments, likely attributed to the higher pectin content and pectinolytic enzyme activity of oranges (Hou et al., 2008).

Among the minor volatile compounds, 32 were identified and grouped into five chemical families: terpenes and norisoprenoids, benzenic compounds, ethyl esters, acetate esters, and alcohols (Table 3).

Terpenes and norisoprenoids, with floral and fruity notes, appeared at high concentrations in hybrid beverages, as they mainly originate from "non-grape" fruits, such as oranges and apples. These compounds

Table 2
Major volatile components (mg/L) in control wines and co-ferments (n = 3).

	CS Mean ± SD	CTS Mean ± SD	AS Mean ± SD	ATS Mean ± SD	OS Mean ± SD	OTS Mean ± SD	MIXS Mean ± SD	MIXTS Mean ± SD
Acetaldehyde	31.9 ± 2.7^{ab}	38.2 ± 3.0^{b}	32.7 ± 1.0^{ab}	29.5 ± 3.3^{ab}	27.2 ± 3.8^{a}	36.8 ± 1.2^{b}	36.8 ± 2.1^{b}	36.3 ± 7.1^{b}
Ethyl acetate	31.7 ± 2.3^{bc}	78.2 ± 5.0 ^{f}	28.2 ± 1.4^{b}	44.2 ± 2.0^{d}	21.6 ± 1.4^{a}	35.5 ± 5.8^{c}	30.8 ± 2.1^{bc}	50.7 ± 1.0^{e}
Methanol	33.9 ± 2.9^{ab}	31.4 ± 0.9^{ab}	32.1 ± 1.2^{ab}	30.7 ± 2.4^{a}	39.8 ± 1.3^{d}	38.2 ± 0.8^{cd}	35.7 ± 1.2^{bc}	34.2 ± 1.9^{ab}
Propanol	21.3 ± 0.7^{bc}	19.7 ± 1.9^{b}	15.4 ± 0.1^{a}	22.2 ± 0.8^{c}	19.1 ± 1.2^{b}	16.9 ± 0.4^{a}	15.9 ± 1.2^{a}	19.5 ± 1.0^{b}
Isobutanol	11.1 ± 1.2^{a}	26.6 ± 0.3^{c}	17.9 ± 1.0^{b}	26.6 ± 0.8^{c}	16.7 ± 0.8^{b}	16.9 ± 1.1^{b}	24.9 ± 2.3^{c}	31.1 ± 2.0^{d}
1-Butanol	0.6 ± 0.1^{c}	0.3 ± 0.0^{a}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.4 ± 0.0^{b}	0.3 ± 0.1^{a}	0.3 ± 0.1^{a}	0.3 ± 0.0^{a}
Isoamyl alcohols	99.6 ± 4.1^{a}	141.1 ± 5.7^{e}	131.2 ± 7.1^{d}	184.9 ± 5.6 ^{g}	110.3 ± 3.4^{b}	123.4 ± 0.7^{c}	133.1 ± 2.0^{d}	172.6 ± 3.9 ^{f}
Ethyl lactate	0.7 ± 0.0^{a}	0.7 ± 0.1^{a}	1.1 ± 0.0^{b}	1.0 ± 0.1^{b}	1.0 ± 0.1^{b}	1.6 ± 0.2^{d}	1.4 ± 0.1^{c}	1.4 ± 0.1^{c}
2-Phenylethanol	10.3 ± 0.6^{ab}	17.2 ± 1.1^{d}	9.6 ± 1.2^{a}	14.1 ± 0.9^{c}	8.9 ± 0.8^{a}	11.1 ± 0.6^{b}	9.2 ± 0.6^{a}	14.0 ± 0.4^{c}
TOTAL	241.1 ± 6.2^{a}	353.4 ± 13.8^{d}	268.4 ± 8.7^{b}	353.4 ± 1.9^{d}	245.1 ± 4.2^{a}	280.8 ± 5.8^{bc}	287.9 ± 6.7^{c}	360.0 ± 2.5^{d}

Different superscripts (a-g) in the same row mean significant differences (α = 0.05) according to the test of Student–Newman–Keuls. CS: control wine *S. cerevisiae*; CTS: control wine *T. delbruekii* + *S. cerevisiae*; AS: grape-apple co-ferment *S. cerevisiae*; ATS: grape-apple co-ferment *T. delbruekii* + *S. cerevisiae*; OS: grape-orange co-ferment *S. cerevisiae*; OTS: grape-orange co-ferment *T. delbruekii* + *S. cerevisiae*; MIXS: grape-apple-orange co-ferment *S. cerevisiae*; MIXTS: grape-apple-orange co-ferment *T. delbruekii* + *S. cerevisiae*.

Table 3
Minor volatile components (μg/L) in control wines and co-ferments (n = 3).

	CS Mean ± SD	CTS Mean ± SD	AS Mean ± SD	ATS Mean ± SD	OS Mean ± SD	OTS Mean ± SD	MIXS Mean ± SD	MIXTS Mean ± SD
TERPENES AND NORISOPRENOIDS								
Linalool	Nd	Nd	0.3 ± 0.1^{a}	0.1 ± 0.1^{a}	687.1 ± 21.5^{c}	726.9 ± 29.1^{d}	357.7 ± 6.5^{b}	320.5 ± 38.2^{b}
1,4-Terpineol	Nd	Nd	Nd	Nd	819.7 ± 18.2^{c}	862.0 ± 38.4^{d}	412.3 ± 5.5^{b}	374.3 ± 40.4^{a}
Citronellyl acetate	Nd	Nd	Nd	Nd	4.7 ± 1.7^{a}	11.1 ± 3.8^{b}	6.8 ± 1.1^{a}	5.6 ± 1.5^{a}
α-Terpineol	Nd	Nd	Nd	Nd	572.5 ± 20.9^{d}	501.8 ± 26.6^{c}	268.0 ± 32.1^{b}	231.0 ± 5.3^{a}
Neryl acetate	Nd	Nd	Nd	Nd	15.4 ± 1.8^{b}	19.9 ± 2.7^{c}	12.0 ± 2.1^{a}	12.2 ± 1.4^{a}
Geranyl acetate	Nd	Nd	Nd	Nd	3.2 ± 0.9^{a}	6.6 ± 1.8^{b}	5.2 ± 1.3^{b}	4.7 ± 1.3^{ab}
β-Citronellol	Nd	Nd	Nd	Nd	30.4 ± 6.1^{c}	27.3 ± 3.4^{c}	20.5 ± 2.6^{b}	15.7 ± 0.7^{a}
Nerol	Nd	Nd	Nd	Nd	35.2 ± 2.8^{c}	29.0 ± 2.3^{b}	18.3 ± 4.4^{a}	16.1 ± 1.1^{a}
Isopiperitone	Nd	Nd	Nd	Nd	21.6 ± 0.5^{b}	23.4 ± 1.9^{b}	11.9 ± 1.4^{a}	12.5 ± 2.9^{a}
trans-Carveol	Nd	Nd	Nd	Nd	15.0 ± 1.3^{b}	14.6 ± 1.4^{b}	9.2 ± 0.9^{a}	8.6 ± 1.7^{a}
Geraniol	4.3 ± 0.1^{a}	4.4 ± 1.0^{a}	3.7 ± 0.3^{a}	7.6 ± 0.8^{b}	43.1 ± 0.9^{e}	38.3 ± 2.6^{d}	26.8 ± 0.8^{c}	25.3 ± 3.1^{c}
p-Mentha-7,8,10-dien-9-ol	Nd	Nd	Nd	Nd	28.7 ± 0.9^{c}	26.2 ± 2.4^{b}	13.1 ± 1.4^{a}	12.3 ± 1.2^{a}
Farnesol	15.4 ± 2.6^{c}	14.2 ± 2.2^{bc}	11.1 ± 1.5^{abc}	13.5 ± 1.9^{bc}	14.9 ± 0.7^{bc}	7.3 ± 2.6^{a}	9.6 ± 0.9^{ab}	11.8 ± 3.0^{abc}
3-OH-β-Damascone	Nd	Nd	28.0 ± 1,0^{b}	33.8 ± 5.5^{c}	Nd	Nd	6.3 ± 0.5^{a}	8.4 ± 1.0^{a}
3-Oxo-α-ionol	Nd	Nd	13.4 ± 1.9^{c}	23.2 ± 2.2^{d}	Nd	Nd	5.6 ± 1.4^{a}	9.7 ± 0.7^{b}
Nootkatone	Nd	Nd	Nd	Nd	77.9 ± 22.4^{b}	88.3 ± 33.4^{b}	39.6 ± 8.4^{a}	43.0 ± 6.4^{a}
TOTAL	19.7 ± 2.6^{a}	18.6 ± 3.2^{a}	56.6 ± 2.0^{a}	78.3 ± 7.1^{a}	2369.4 ± 91.5^{c}	2382.8 ± 149.0^{c}	1223.1 ± 38.0^{b}	1111.8 ± 95.8^{b}
BENZENIC COMPOUNDS								
Guaiacol	42.2 ± 2.3^{bc}	47.6 ± 11.3^{c}	16.8 ± 1.6^{a}	14.6 ± 3.3^{a}	15.8 ± 1.3^{a}	22.8 ± 5.0^{a}	33.1 ± 4.0^{b}	34.9 ± 2.8^{b}
Benzyl alcohol	12.4 ± 3.0^{bc}	14.5 ± 4.7^{c}	7.1 ± 1.9^{ab}	6.1 ± 0.6^{a}	6.8 ± 0.6^{ab}	8.1 ± 0.2^{ab}	11.2 ± 1.1^{abc}	9.2 ± 1.1^{ab}
4-Vinylguaiacol	339.5 ± 25.2^{a}	362.6 ± 93.2^{a}	443.9 ± 12.4^{a}	401.8 ± 41.4^{a}	1903.5 ± 116.1^{c}	1593.1 ± 376.0^{b}	1308.2 ± 34.7^{b}	1395.9 ± 205.2^{b}
TOTAL	394.1 ± 29.8^{a}	424.7 ± 107.4^{a}	467.8 ± 15.8^{a}	422.5 ± 38.4^{a}	1926.1 ± 116.3^{c}	1624.1 ± 381.0^{b}	1352.6 ± 36.6^{b}	1440.0 ± 206.1^{b}
ETHYL ESTERS								
Ethyl butanoate	279.9 ± 46.9^{c}	195.2 ± 18.4^{b}	115.6 ± 29.1^{a}	70.5 ± 18.9^{a}	300.6 ± 23.4^{c}	381.6 ± 28.6^{d}	294.2 ± 71.6^{c}	77.3 ± 19.2^{a}
Ethyl hexanoate	768.8 ± 59.7^{b}	522.9 ± 67.6^{a}	782.2 ± 69.4^{b}	589.5 ± 42.5^{a}	840.5 ± 82.8^{b}	1066.6 ± 45.0^{c}	750.2 ± 95.5^{b}	619.6 ± 32.6^{a}
Ethyl octanoate	1241.0 ± 48.6^{e}	481.1 ± 56.0^{a}	969.2 ± 45.1^{d}	534.1 ± 66.4^{ab}	954.2 ± 19.3^{d}	686.1 ± 71.2^{bc}	767.9 ± 187.6^{c}	617.9 ± 53.4^{abc}
Ethyl decanoate	302.6 ± 5.3^{b}	197.6 ± 17.7^{a}	258.1 ± 23.1^{b}	172.1 ± 39.9^{a}	259.0 ± 38.8^{b}	188.5 ± 30.0^{a}	272.3 ± 6.0^{b}	174.7 ± 42.0^{a}
TOTAL	2592.3 ± 154.9^{c}	1396.8 ± 151.7^{a}	2125.0 ± 142.2^{b}	1366.1 ± 123.0^{a}	2354.3 ± 106.0^{bc}	2322.7 ± 123.0^{b,c}	2084.6 ± 211.4^{b}	1489.5 ± 80.8^{a}
ACETATE ESTERS								
Isoamyl acetate	2957.6 ± 575.6^{a}	4810.2 ± 97.7 ^{cd}	3843.7 ± 220.7^{b}	5455.0 ± 99,5^{d}	2863.9 ± 443.8^{a}	4638.4 ± 147.6^{c}	3158.64 ± 159.4^{a}	5453.3 ± 357.8^{d}
Hexyl acetate	175.8 ± 13.3^{b}	174.0 ± 15.7^{b}	247.9 ± 21.8^{c}	237.9 ± 3.6^{c}	100.4 ± 27.4^{a}	110.5 ± 0.3^{a}	163.4 ± 41.3^{b}	156.5 ± 40.7^{b}
2-Phenylethyl acetate	615.6 ± 56.2^{a}	1647.9 ± 219.2^{c}	566.5 ± 40.5^{a}	1060.5 ± 86.2^{b}	453.6 ± 130.3^{a}	606.2 ± 37.3^{a}	485.1 ± 19.4^{a}	1060.4 ± 46.4^{b}
TOTAL	3749.1 ± 613.5^{a}	6632.1 ± 331.8^{d}	4658.1 ± 280.9^{b}	6753.5 ± 149.8^{d}	3417.8 ± 597.0^{a}	5355.1 ± 114.3^{c}	3807.2 ± 152.9^{a}	6670.2 ± 413.7^{d}
ALCOHOLS								
1-Hexanol	570.4 ± 23.8 ^{cd}	474.6 ± 46.0^{b}	884.0 ± 14.6 ^{f}	766.6 ± 9.7^{e}	399.2 ± 16.6^{a}	356.1 ± 7.5^{a}	622.1 ± 52.5^{d}	518.0 ± 46.6^{bc}
cis-3-Hexen-1-ol	81.8 ± 4.2^{ab}	56.8 ± 6.3^{a}	148.2 ± 1.5^{c}	79.1 ± 3.3^{ab}	120.5 ± 4.8^{bc}	80.0 ± 4.3^{ab}	129.2 ± 41.1^{c}	90.0 ± 21.3^{ab}
trans-3-Hexen-1-ol	525.2 ± 25.5^{c}	520.4 ± 7.5^{c}	633.0 ± 18.0^{d}	633.1 ± 30.8^{d}	249.5 ± 16.1^{a}	289.1 ± 6.2^{a}	429.5 ± 45.2^{b}	380.4 ± 64.3^{b}
1-Octanol	26.6 ± 5.1^{a}	15.1 ± 4.2^{a}	3.3 ± 1.3^{a}	12.4 ± 2.7^{a}	610.2 ± 98.9^{c}	584.0 ± 46.9^{c}	322.6 ± 82.2^{b}	262.1 ± 39.9^{b}
TOTAL	1203.9 ± 54.4^{b}	1066.9 ± 55.3^{a}	1668.5 ± 9.6^{d}	1491.3 ± 34.5^{c}	1379.5 ± 93.9^{bc}	1309.2 ± 57.1^{b}	1503.5 ± 157.2^{c}	1250.5 ± 63.6^{b}

Different superscripts (a-g) in the same row mean significant differences (α = 0.05) according to the test of Student–Newman–Keuls. CS: control wine *S. cerevisiae*; CTS: control wine *T. delbruekii* + *S. cerevisiae*; AS: grape-apple co-ferment *S. cerevisiae*; ATS: grape-apple co-ferment *T. delbruekii* + *S. cerevisiae*; OS: grape-orange co-ferment *S. cerevisiae*; OTS: grape-orange co-ferment *T. delbruekii* + *S. cerevisiae*; MIXS: grape-apple-orange co-ferment *S. cerevisiae*; MIXTS: grape-apple-orange co-ferment *T. delbruekii* + *S. cerevisiae*.. Nd: no detected.

can enhance the aromatic complexity of hybrid beverages, as they are practically absent in the control wines. The grape-orange co-ferments were characterized by a high terpene content, while the grape-apple co-ferments exhibited higher concentrations of the two identified C13-norisoprenoids, 3-OH-β-damascone and 3-oxo-α-ionol. This resulted in hybrid mix beverages with a more balanced content of terpenes and norisoprenoids.

Fruits contain not only free volatile compounds but also many aroma precursors. These precursors can be transformed into free aromatic compounds through different biochemical reactions during alcoholic fermentation, leading to diverse flavours. Sequential inoculation resulted in higher concentrations of various terpenes, including linalool, citronellyl acetate, neryl acetate, geranyl acetate, 1,4-terpineol and β-citronellol, in the grape-orange co-ferments, as well as higher levels of C13-norisoprenoids, such as 3-OH-β-damascone and 3-oxo-α-ionol, in the grape-apple and grape-apple-orange co-ferments. These results could be due to the greater glycosidase activity of *T. delbrueckii* and to the impact of different interactions between *T. delbrueckii* and *S. cerevisiae* on aroma profiles in mixed fermentations (Chen & Liu, 2016; Englezos et al., 2022; Qiu et al., 2022).

The content of 4-vinylguaiacol was particularly high in the grape-orange and grape-apple-orange co-ferments. This benzenic compound is related to the phenolic and clove-like aroma found in orange juices stored at high temperature or made from concentrates (Averbeck & Schieberle, 2011). In this work, fresh juice was used, with 4-vinylguaiacol levels measured at 116.4 μg/L in the grape must and orange juice mixture, and 52.2 μg/L in the triple mixture. However, this compound is formed during alcoholic fermentation due to the degradation of phenolic acids present in the juices via substituted cinnamate carboxy-lyase activity (Chatonnet et al., 1993). As a result, its concentration in the fermented beverages is significantly higher and could influence their aroma. That enzymatic activity is rapidly inhibited by condensed tannins (Chatonnet et al., 1993), which are present in higher concentrations in grapes and apples than in oranges (results not shown). This could explain the higher levels of 4-vinylguaiacol observed in the co-ferments made with orange juice.

Esters, the main contributors to the fruity aroma of fermented beverages (Gürbüz et al., 2006), were divided into two groups, ethyl esters and acetate esters. All samples showed significant concentrations of ethyl esters, whose formation is associated with various factors such as fruit variety, fermentation conditions, nutrients, and yeast strain (Ferreira et al., 2000; Miller et al., 2007; Vilanova et al., 2007). The major ethyl esters in all samples were ethyl hexanoate and ethyl octanoate and may contribute to their fruity aroma with apple or pear notes. Among the acetate esters, isoamyl acetate, characterized by its banana odour, was the most abundant, followed by 2-phenylethyl acetate, described as floral, and hexyl acetate, with herbaceous and fruity notes (Antalick et al., 2010; Sumby et al., 2010).

Ethyl and acetate esters behaved differently depending on the yeast used. Both the control wines and the hybrid beverages produced through sequential inoculation of *T. delbrueckii* and *S. cerevisiae* showed lower concentrations of ethyl esters and higher concentrations of acetate esters compared to samples made through single inoculation with *S. cerevisiae*. Renault et al. (2015) observed an increase in overall esters when *T. delbrueckii* is used with *S. cerevisiae*, either sequentially or simultaneously; however, in our study, this behaviour was only observed in acetate esters.

Finally, four alcohols were identified, three C6-alcohols and 1-octanol. The C6-alcohols, characterized by herbaceous aromas, are formed during alcoholic fermentation from their corresponding C6-aldehydes, which originate from the degradation of fruit polyunsaturated fatty acids (Etievant, 1991). These compounds were found in higher concentrations in the grape-apple co-ferments due to the elevated C6-aldehyde content in apple juice, whereas their levels in grape-orange co-ferments were very low. Although these compounds can be perceived as off-notes at high concentrations, no sensory defects were detected in the hybrid beverages produced by co-fermentation of grape must and apple juice. On the other hand, the grape-orange and grape-apple-orange co-ferments showed high concentrations of 1-octanol. This alcohol is associated with herbaceous and citrus notes and has been positively correlated with the aroma of fresh orange juice (Cuevas et al., 2017).

Application of principal component analysis (PCA) to the major and minor volatile compounds allowed visualizing the main differences among samples in the first two principal components (PC1 and PC2), which together accounted for 66.3 % of the total variance (Fig. 2). PC1 (47.3 %) mainly separated beverages obtained by co-fermentation of grape must and orange juice, with or without apple juice. This component was primarily associated with terpenes, mainly provided by orange juice, as well as with 1-octanol (0.969) and 4-vinylguaiacol (0.960). PC2 explained 19.0 % of the variance and separated the samples according to the yeast strain used. It showed positive correlations with isoamyl acetate (0.908) and 2-phenylethanol (0.850), and negative correlations with ethyl octanoate (–0.915) and ethyl decanoate (–0.882). Overall, these results support the differences previously discussed in the volatile composition of the samples, highlighting the distinct behaviour of the yeasts in the formation of ethyl esters and acetates.

3.3. Sensory analysis

The sensory profiles of the control wines and co-ferments are shown in Fig. 3 as a spider web graph. The control wines presented light citrus, green apple, and floral flavours. However, in the grape-orange co-ferments, citrus notes became significantly more dominant with a slightly

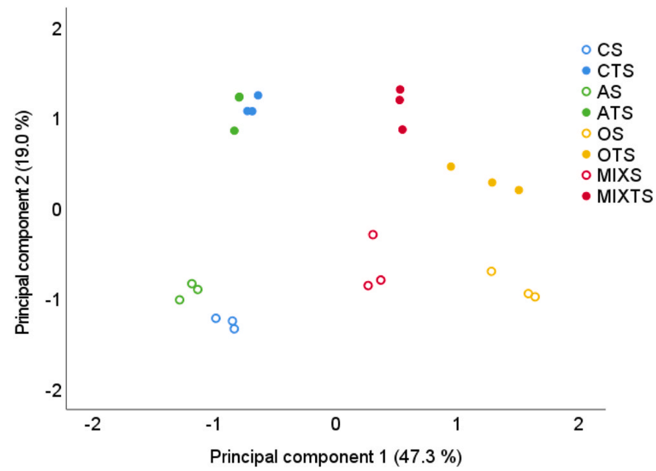

Fig. 2. Principal component analysis of control wines and co-ferments. CS: control wine *S. cerevisiae*; CTS: control wine *T. delbrueckii* + *S. cerevisiae*; AS: grape-apple co-ferment *S. cerevisiae*; ATS: grape-apple co-ferment *T. delbrueckii* + *S. cerevisiae*; OS: grape-orange co-ferment *S. cerevisiae*; OTS: grape-orange co-ferment *T. delbrueckii* + *S. cerevisiae*; MIXS: grape-apple-orange co-ferment *S. cerevisiae*; MIXTS: grape-apple-orange co-ferment *T. delbrueckii* + *S. cerevisiae*.

bitter taste, overshadowing the green apple and floral flavours, despite their high terpene content. This enhanced citrus character may be attributed to compounds such as nootkatone, which was found at higher concentrations in these samples and is known for its intense citrus flavour and bitter notes (Ramachanderan & Schaefer, 2024). Additionally, the grape-orange co-ferments had a slightly fermentation aroma. Liu et al. (2023) correlated the high content of 4-vinylguaiacol found in navel orange wines with that aromatic note, so this compound could be associated with the fermentation-like aroma described by tasters in grape-orange co-ferments.

When apple was used, the co-ferments were characterized by intense green apple and floral notes, with reduced citrus character. Co-ferments combining grape, orange, and apple achieved a well-balanced flavour profile, featuring noticeable contributions from all three fruits. Additionally, the bitter note detected in grape-orange co-ferments disappeared, and the fermentation aroma was significantly reduced, no longer perceived as a defect.

Co-fermenting grape must with orange and/or apple juice enhanced the acidity and body of the hybrid beverages, which were notably lacking in the control wines. Additionally, the co-fermented beverages exhibited a stronger aftertaste intensity compared to the controls, with grape-apple co-ferments and grape-apple-orange co-ferments standing out for their particularly high-quality aftertaste. These two samples also received the highest overall quality ratings from the tasters.

Significant differences between samples were observed based on the yeasts used as starters. Beverages fermented by sequential inoculation with *T. delbrueckii* and *S. cerevisiae* showed less pronounced citrus fruit flavours but exhibited stronger green apple and floral notes compared to those fermented only with *S. cerevisiae*. Different authors have reported higher fruity and floral flavours and an enhanced aroma quality in white wines with *T. delbrueckii* sequential fermentations (Cordero-Bueso et al., 2013; Renault et al., 2015). The enhancement in the aroma of these wines has been linked to an increase in ester content, a trend that was also observed in the case of acetate esters.

Although there were minimal differences in acidity, body, and aftertaste intensity, a slight increase in body was noted in the Torulaspora-fermented beverages, likely due to increased glycerol production (Belda et al., 2015; Lu et al., 2016). Finally, the enhanced green apple and floral flavours in these last co-ferments contributed to a higher aftertaste and overall qualities.

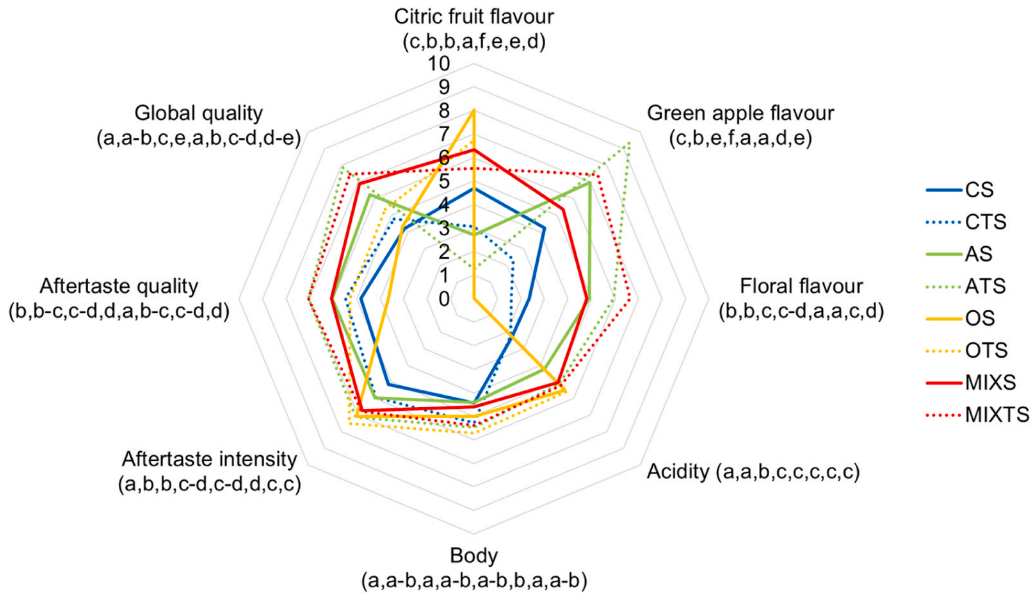

Fig. 3. Sensory profile of the control wines and co-ferments. Different letters (a-f) within the same descriptor indicate significant differences (α = 0.05) between samples, following the order presented in the legend, according to the test of Student–Newman–Keuls. CS: control wine *S. cerevisiae*; CTS: control wine *T. delbruekii* + *S. cerevisiae*; AS: grape-apple co-ferment *S. cerevisiae*; ATS: grape-apple co-ferment *T. delbruekii* + *S. cerevisiae*; OS: grape-orange co-ferment *S. cerevisiae*; OTS: grape-orange co-ferment *T. delbruekii* + *S. cerevisiae*; MIXS: grape-apple-orange co-ferment *S. cerevisiae*; MIXTS: grape-apple-orange co-ferment *T. delbruekii* + *S. cerevisiae*.

4. Conclusions

Co-fermentation of grape must with orange and/or apple juices offers a promising approach to creating innovative wine-based beverages with reduced alcohol content, complex aromas, and improved sensory characteristics. By combining juices with different chemical composition, such as the high acidity of orange and apple juices and the sugar richness of grape must, the wine-based beverages achieved a balanced pH, reduced alcohol content, and increased acidity compared to control wines, resulting in beverages with improved structure and freshness.

Sequential inoculation with *T. delbrueckii* and *S. cerevisiae* significantly influenced the chemical and sensory attributes of the hybrid beverages. This fermentation strategy led to higher concentrations of acetate esters and specific terpenes and norisoprenoids, enhancing the fruity and floral aromas, particularly in grape-apple and grape-apple-orange co-ferments. Also, sensory analysis highlighted the distinct contributions of each fruit. Grape-orange co-ferments featured pronounced citrus notes, while grape-apple co-ferments emphasized green apple and floral aromas. The triple blend achieved a harmonious integration of citrus, apple, and floral notes. These findings underline the importance of juice composition and yeast selection in optimizing the final product.

CRediT authorship contribution statement

S. Parra-Cadenas: Formal Analysis, Methodology, Investigation. **V. Molero-Gutiérrez:** Formal Analysis. **E. Romero-Bercebal:** Formal Analysis. **E. García-Romero:** Writing-review & editing, Methodology, Supervision, Funding acquisition. **M.S. Pérez-Coello:** Writing-review & editing, Investigation, Project administration, Funding acquisition. **M.C. Díaz-Maroto:** Writing-original draft, Writing-review & editing, Methodology, Investigation, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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