



OPEN ACCESS

EDITED BY
Athanasia Koliadima,
University of Patras, Greece

REVIEWED BY
Angela Capece,
University of Basilicata, Italy
Laura Pulcini,
Council for Agricultural Research and
Economics – Research Centre for Viticulture
and Enology CREA-VE, Italy

*CORRESPONDENCE
Alberto Luis Rosa
✉ alrosa@unc.edu.ar
María Laura Raymond Eder
✉ mlraymondeder@unc.edu.ar

RECEIVED 09 June 2025
ACCEPTED 28 August 2025
PUBLISHED 18 September 2025

CITATION
Raymond Eder ML, Fariña L, Carrau F and
Rosa AL (2025) Microbial communities
from distinct *Vitis* species shape volatile
profiles of fermenting juices while
preserving varietal typicity.
Front. Fungal Biol. 6:1643880.
doi: 10.3389/ffunb.2025.1643880

COPYRIGHT
© 2025 Raymond Eder, Fariña, Carrau and
Rosa. This is an open-access article distributed
under the terms of the [Creative Commons
Attribution License \(CC BY\)](#). The use,
distribution or reproduction in other forums
is permitted, provided the original author(s)
and the copyright owner(s) are credited and
that the original publication in this journal is
cited, in accordance with accepted academic
practice. No use, distribution or reproduction
is permitted which does not comply with
these terms.

Microbial communities from distinct *Vitis* species shape volatile profiles of fermenting juices while preserving varietal typicity

María Laura Raymond Eder^{1,2*}, Laura Fariña^{3,4},
Francisco Carrau³ and Alberto Luis Rosa^{1,2,5*}

¹Laboratorio de Genética y Biología Celular y Molecular, Departamento de Farmacología Otto Orsingher, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina, ²Departamento de Agroalimentos, Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Córdoba, Argentina, ³Área Enología y Biotecnología de Fermentaciones, Facultad de Química, Universidad de la República, Montevideo, Uruguay, ⁴Laboratorio de Biotecnología de Aromas, Facultad de Química, Universidad de la República, Montevideo, Uruguay, ⁵Instituto de Farmacología Experimental de Córdoba (IFEC-CONICET), Córdoba, Argentina

Spontaneously fermenting grape juices represent complex ecosystems resulting from the dynamic interaction between the unique characteristics of a grape varietal and its indigenous associated microbiota. The extent to which specific grape variety volatile compounds versus microbially derived ones shape wine identity remains incompletely understood. In this work, we explored this issue by characterizing the volatile compound profiles at early stages of fermentation of the highly aromatic Isabella (*V. labrusca* L.) grape juice, conducted by native microbial communities prepared from either Isabella (homologous fermentation) or Malbec (*V. vinifera* L., heterologous fermentation) grapes. Results revealed that microbial starters derived from *V. labrusca* L. and *V. vinifera* L. markedly influenced the volatile profiles of the resulting fermented Isabella grape juices. Joint analysis of volatile profiles from Malbec and Isabella juices fermented with the same set of *Vitis*-specific microbial communities showed that, despite the strong influence of the microbial consortia, the fermented juices retained traits consistent with their original grape varietal identity. Characterization and identification of cultivable yeast species in these homologous and heterologous fermentations of Isabella grape juice showed *H. uvarum*, *H. opuntiae*, and *S. bacillaris* as dominant species in Malbec and Isabella microbial ecosystems. Our results highlight the potential of this innovative experimental approach to examine the relative roles of microbial communities and grape varieties in shaping wine identity. Moreover, they show that different *Vitis*-specific microbiota can distinctly influence the volatile profiles of a fermenting grape juice without altering its varietal identity.

KEYWORDS

terroir, non-*Saccharomyces*, volatile profile, fermentation, Isabella

1 Introduction

Wine fermentation is a complex process where the interplay between grape juice and its associated microbial populations, derived from both the vineyard and winery environments, shapes the chemical and sensory profiles of the resulting wine (Belda et al., 2017; de Celis et al., 2022; García-Izquierdo et al., 2024). Yeasts are particularly important in alcoholic fermentation, where they convert sugars into alcohol and carbon dioxide, while also producing various secondary metabolites that contribute to the wine's flavor and aroma (Maicas and Mateo, 2023; Wang et al., 2023). The indigenous yeast communities that develop during spontaneous grape must fermentations are shaped by annual environmental conditions in the vineyard, agricultural practices, intrinsic factors of the grapevine, —including the physicochemical properties of the grape must, — and winemaking techniques (Varela and Borneman, 2016; Griggs et al., 2021; Liszkowska and Berlowska, 2021; de Celis et al., 2022; Maicas and Mateo, 2023). A consistent pattern of species emerges in the yeast population: non-*Saccharomyces* yeasts dominate the initial phase of fermentation, while *Saccharomyces cerevisiae* becomes the predominant species as fermentation progresses (Bezerra-Bussoli et al., 2013; Drumonde-Neves et al., 2016; Cilião Filho et al., 2017; Raymond Eder et al., 2017, 2018; Borren and Tian, 2021; de Celis et al., 2022; Torres-Guardado et al., 2022; Maicas and Mateo, 2023). Non-*Saccharomyces* yeasts, which are predominant on grape skins, are introduced into the must during grape crushing and play a significant role in the early fermentation stages, producing various secondary metabolites that strongly impact the organoleptic characteristics of wine (Kamilari et al., 2021; Morata et al., 2021; Tufariello et al., 2021; Romano et al., 2022; Torres-Guardado et al., 2022; Maicas and Mateo, 2023; Wang et al., 2023).

Understanding the dynamics of indigenous microbial communities of fermenting grape juices and musts is a central focus in enology, given its significance for both scientific research and industrial applications (Padilla et al., 2016; Varela, 2016; Griggs et al., 2021; de Celis et al., 2022; Maicas and Mateo, 2023; García-Izquierdo et al., 2024). An increasing number of studies point to a relationship between the grape microbiome and *terroir*, which includes factors such as soil composition, climate, and annual precipitation, suggesting that specific microbial populations may contribute to the regional identity of wines (Pretorius, 2017, 2020; Liu et al., 2020; Griggs et al., 2021; Kamilari et al., 2021; Zhang et al., 2023a). Moreover, it has been suggested that particular grape varieties harbor microbiomes involving specific yeast strains or species, influencing the unique characteristics of fermented grape juices (Baffi et al., 2011; Bezerra-Bussoli et al., 2013; Lederer et al., 2013; Raymond Eder et al., 2017, 2018, 2025; Raymond Eder and Rosa, 2019). We have recently suggested that *Vitis*-specific microbial communities play a critical role in shaping the identity of grape juice fermentations (Raymond Eder et al., 2025). However, it remains unclear how strongly a grape varietal-specific indigenous microbial community influences the final organoleptic properties of a given wine, how *terroir*-associated microbial signatures contribute to the wine's identity and profile, and to what extent alternative microbiotas—such as those from different vintages or experimentally

introduced from other *Vitis* species (Raymond Eder et al., 2025)—can alter the fermentation profile of a given grape varietal.

Although the OIV (International Organization of Vine and Wine) primarily recognizes *Vitis vinifera* L. as the species designated for vinification, other *Vitis* species and their hybrids are also used in winemaking, particularly in non-European countries (De la Fuente Lloreda, 2018). These non-*vinifera* *Vitis* species represent underexplored microbial ecosystems in enology and may serve as a potential source of yeasts with unique fermentative properties of both academic and industrial relevance (Drumonde-Neves et al., 2016; Raymond Eder et al., 2017, 2018; Raymond Eder and Rosa, 2019). Moreover, we have recently proposed that they can serve as powerful tools for studying the impact of indigenous microbiota on the aromatic profiles of fermenting grape juices (Raymond Eder et al., 2025). Building on the use of this innovative experimental system, in this study we investigate the impact of the indigenous microbiota of Malbec grapes—a conventional *V. vinifera* L. varietal used in winemaking—versus the native microbiota of Isabella grapes (*V. labrusca* L.), on the volatile profile at early stages of fermentation of Isabella grape juices.

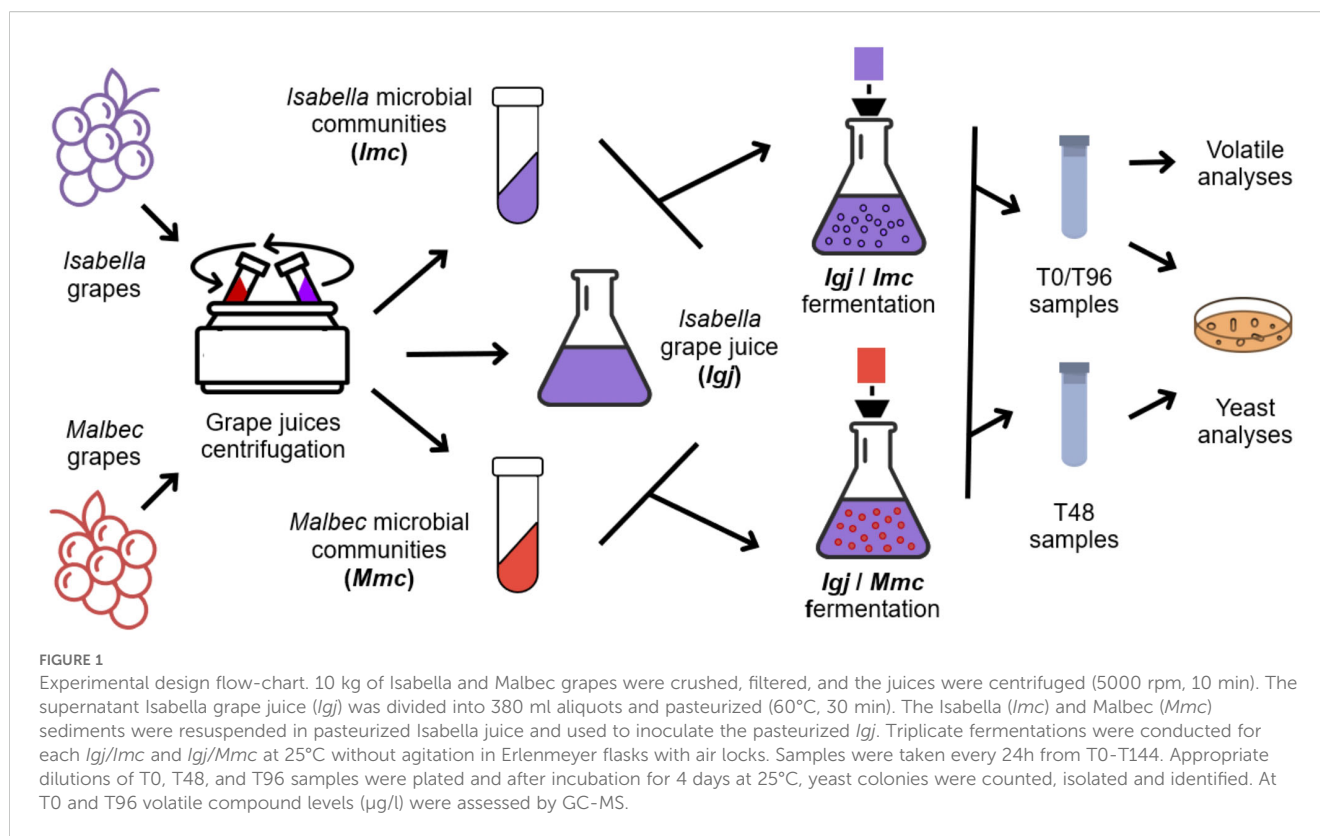
2 Materials and methods

2.1 Grape juice fermentations

Grapes from Isabella (*Vitis labrusca* L.) and Malbec (*Vitis vinifera* L.) were harvested from two closely located, small vineyards (i.e., ~1.5 Ha each), with intermixed rows of both varieties (Raymond Eder et al., 2018), in Colonia Caroya, Argentina, during the 2021 vintage. Isabella and Malbec grape juice supernatants (~3.5 l each), collected after centrifugation of filtered musts (5000 rpm for 10 min at 20°C), were pasteurized (60°C, 30 min) (Malletroit et al., 1991; Englezos et al., 2018; Raymond Eder et al., 2025). The corresponding Isabella (I) and Malbec (M) sedimented fractions, which contain their native microbial communities (i.e., *Imc* and *Mmc*, respectively) were each suspended into 400 ml of pasteurized Isabella grape juice (*Igi*) to reconstitute homologous (*Igi/Imc*) or heterologous (*Igi/Mmc*) fermenting ecosystems (Raymond Eder et al., 2025). Fermentations were performed in triplicate for each condition (*Igi/Imc* and *Igi/Mmc*) in 500 ml Erlenmeyer flasks, sealed with air locks, without agitation, at 25°C. Aliquots were collected from 0 to 96 hours (T0 to T96) for volatile, physicochemical, and microbial analyses, as previously described (Raymond Eder et al., 2025). Figure 1 presents a schematic overview of the experimental design.

2.2 Volatile compounds identification and quantification

Volatile compounds were extracted by HS-SPME-GC-MS using an automatic injector AOAC-6000 Shimadzu, according to the methodology exposed by Garde-Cerdán et al. (2018). GC-MS analyses were conducted using a Shimadzu GC-20 plus gas chromatograph coupled to a Shimadzu QP 2020 mass spectrometer with a DW-Wax 30 (Agilent Technologies J&W,



Santa Clara, CA, USA) bonded fused silica capillary column, coated with poly(ethylene glycol). The experimental conditions were performed according to Dellacassa et al. (2017) as follows: column temperature, 40°C for 8 min, rising to 180°C at 3°C/min, then to 230°C at 20°C/min; injector temperature, 250°C; detector temperature, 250°C; injection mode: splitless (2 min); carrier gas, hydrogen, 30 kPa. GC-MS instrumental procedures, using an internal standard (1-heptanol), were applied for quantitative purposes (Dellacassa et al., 2017). Volatile compounds were identified by comparison of Kovats indices (KI, Table 1; Supplementary Table S1).

An ANOVA was conducted on the chemical and volatile compounds data obtained for the different treatments and replicas. Principal Component Analysis (PCA) was performed using InfoStat (InfoStat, FCA, Universidad Nacional de Córdoba, Argentina) to differentiate between samples and identify contributing compounds. A Hierarchical Cluster Analysis (HCA) was performed using Euclidean distances calculated from the average volatile profiles of each fermenting condition. Clustering was performed using the complete linkage method. The resulting dendrogram was constructed using base R functions (dist and hclust) and visualized with the *dendextend* package for enhanced customization.

2.3 Yeast identification from Isabella fermentations

Yeasts were isolated from Isabella homologous and heterologous fermentations at different time points (T0, T48, and

T96 h) by plating pooled samples on YPD-Cm [yeast extract 1.0% (w/v), peptone 2.0% (w/v), glucose 2.0% (w/v), agar 2.0% (w/v), chloramphenicol 10 μg/ml] and YPD-Cm-Cx agar [yeast extract 1% (w/v), peptone 2% (w/v), glucose 2% (w/v), agar 2% (w/v), chloramphenicol 10 μg/ml, cycloheximide 0.5 μg/ml] to estimate total and non-*Saccharomyces* populations, respectively (Raymond Eder et al., 2025). Predominant yeast species were randomly selected from high-dilution YPD-Cm plates using a grid-based method to ensure unbiased isolation (Raymond Eder et al., 2017). Colonies were purified, stored in glycerol stocks, and subsequently identified by PCR-RFLP and/or sequencing of the 5.8S-ITS rDNA region. Restriction profiles were generated using *Hinf I* and *CfoI*, and representative isolates were confirmed by Sanger sequencing, with species assignment based on ≥99% identity to reference sequences in NCBI BLAST. Identified yeast strains were deposited in GenBank (NCBI) under the accession numbers OP584257, OP584258, OP584259, OP584260, OP584261, OP584262, OP584263, and OP584265.

3 Results

3.1 Physicochemical and volatile analyses of Isabella grape juice

High nitrogen compounds levels, such as α Amino (171.1 ± 4.3 mg/l), NH₄⁺ (77.8 ± 4.2 mg/l), and yeast assimilable nitrogen-YAN- (235.5 ± 7.8 mg/l) were found in the *Igj*, as well as a total acidity value of 2.9 ± 0.0 g/l, a density of 1.083 ± 0.001 g/ml, and 19.1 ± 0.4°

TABLE 1 Volatile compounds in *Igj* at T0 and in *Igj/lmc* and *Igj/Mmc* at T96.

Compounds	KI	lgj			lgj/lmc			lgj/Mmc		
		Mean	SD	Group	Mean	SD	Group	Mean	SD	Group
Higher alcohols										
Isoamyl alcohol	1221	13,79	0,86	a	198,12	104,82	b	93,56	31,79	b
3-etoxy-1-propanol	1378	0,00	0,00		0,53	0,22		0,67	0,59	
2-Ethyl-1-hexanol	1490	1,07	0,22		0,69	0,30		0,43	0,17	
2-nonanol	1530	0,00	0,00	a	3,84	1,46	b	5,82	0,60	b
1-octanol	1566	1,08	0,17		1,26	0,58		0,74	0,21	
2,3-butanediol	1590	0,27	0,01		1,28	1,84		0,99	0,62	
1-nonanol	1694	4,58	1,43		6,21	2,50		3,54	0,97	
Phenethyl alcohol	1918	14,91	2,86	a	77,48	14,20	c	44,45	6,46	b
Total higher alcohols		35,69	3,81	a	289,40	119,37	b	150,19	38,06	ab
Acetate esters										
Isoamyl acetate	1125	4,57	0,59	a	81,37	19,72	b	102,78	21,15	b
Hexyl acetate	1271	10,77	3,54		17,05	3,93		19,72	17,07	
(E)-3-Hexenyl acetate	1306	0,72	0,11	b	0,28	0,08	a	0,23	0,07	a
(Z)-3-Hexenyl acetate	1314	0,60	0,03		0,59	0,17		0,81	0,21	
Heptyl acetate	1382	1,93	0,47	b	0,62	0,20	a	0,94	0,30	a
Ethyl phenylacetate	1823	1,93	0,47	a	4,50	0,48	b	2,12	0,34	a
Phenethyl acetate	1822	3,10	0,87	a	56,49	9,57	b	20,76	6,43	a
Total acetate esters		23,61	2,24	a	160,91	33,05	b	147,36	27,83	b
Ethyl esters										
Ethyl 2-butenoate	1158	11,09	0,75	b	5,69	1,47	a	4,44	0,65	a
Ethyl hexanoate	1237	9,58	0,11		24,01	17,77		11,14	2,35	
Ethyl heptanoate	1323	0,46	0,04		1,36	0,97		0,78	0,25	
Ethyl lactate	1353	0,00	0,00	a	5,15	1,54	b	7,05	2,11	b
Ethyl 2-hexenoate	1360	8,14	0,68	b	3,45	0,87	a	2,95	0,33	a
Ethyl octanoate	1436	2,43	0,23	a	88,84	4,79	c	47,74	9,89	b
Ethyl 3-hydroxybutyrate	1530	0,85	0,06		0,62	0,10		0,62	0,24	
Ethyl nonanoate	1534	0,59	0,24	a	8,10	3,42	b	3,67	1,26	ab
Ethyl decanoate	1684	0,77	0,32	a	141,41	8,83	c	65,11	29,10	b
Ethyl dodecanoate	1822	0,16	0,08		119,10	89,29		43,69	21,73	
Ethyl hexadecanoate	2270	0,00	0,00	a	7,37	3,44	b	3,11	1,11	ab
Total ethyl esters		34,06	2,44	a	405,09	117,50	b	190,30	62,85	ab

(Continued)

TABLE 1 Continued

Compounds	KI	lgj			lgj/lmc			lgj/Mmc		
		Mean	SD	Group	Mean	SD	Group	Mean	SD	Group
C6 compounds										
Hexanol	1368	46,64	3,01		38,50	12,00		30,73	4,65	
(E)-3-hexen-1-ol	1372	0,67	0,00		0,54	0,23		0,44	0,07	
(Z)-3-hexen-1-ol	1388	0,86	0,10	b	0,59	0,12	ab	0,33	0,05	a
Total C6 compounds		48,17	2,91	a	39,64	12,34	a	31,50	4,77	a
Terpenes										
limonene	1190	0,66	0,24		1,17	0,49		1,05	0,23	
Linalol	1558	1,20	0,35		2,03	0,64		1,22	0,18	
4-terpineol	1630	0,59	0,11		0,79	0,24		0,68	0,11	
alpha terpineol	1718	2,30	0,65		3,68	1,58		2,31	0,63	
Nerol	1810	0,14	0,08		0,98	0,52		0,49	0,04	
(E,E)-Farnesol	2350	0,00	0,00		0,77	0,53		0,22	0,10	
Total terpens		4,89	1,43	a	9,42	3,94	a	5,96	1,05	a
Miscellaneous										
2-heptanone	1180	0,00	0,00	a	0,83	0,53	ab	1,57	0,56	b
Acetoin	1266	0,00	0,00		3,03	2,15		2,02	1,24	
2-nonanone	1395	0,28	0,03	a	3,44	1,72	a	12,08	3,08	b
Benzaldehyde	1540	6,98	2,59	b	1,01	0,36	a	0,98	0,45	a
Acetophenone	1670	0,40	0,38		1,15	0,23		1,02	0,38	
Methyl salicylate	1735	0,58	0,10		0,97	0,19		1,06	0,63	
(Z)-Methyl cinnamate	2080	0,64	0,29		1,19	0,58		0,48	0,11	
Methyl antranilate	2255	0,06	0,00	a	0,25	0,03	b	0,05	0,01	a
Total miscellaneous		8,93	3,33	a	11,87	3,37	ab	19,25	2,29	b
Total volatile compounds		155,35	16,16	a	916,34	289,56	b	544,55	136,85	ab

*Kovats Index. Identities confirmed by comparing mass spectra and linear retention indices with those of authentic standards supplied by Aldrich (Milwaukee, WI, USA) and Fluka (Buchs, Switzerland), or reported in the literature. Values (µg/l) correspond to the mean of two (*Igj* T0) or three (*Igj/Imc* and *Igj/Mmc*) replicas ± SD. Values with a common letter are not significantly different (p >0.05).

Brix. Volatile compounds in the *Igj* at T0 were analyzed using GC-MS (Table 1; Supplementary Table S1). The detected compounds exclude bound volatiles and potential artifacts arising from enzymatic treatments used for their release, thus accurately reflecting the aroma-active profile of a fresh grape juice (Raymond Eder et al., 2025). The recognized profile of *Igj* is distinguished by the presence of the two correlated aroma terpenes α-terpineol/linalool, a characteristic marker of the Isabella varietal, along with a high proportion of alcohols, particularly hexanol, phenethyl alcohol, isoamyl alcohol, and 1-nonanol, followed by some ethyl esters of medium chain fatty acids (Table 1; Supplementary Table S1). Ethyl 3-hydroxybutyrate and methyl anthranilate, previously identified as free volatile aroma compounds in Isabella grape samples (Ghaste et al., 2015), were also

recognized in the analyzed grape juices. In particular, methyl anthranilate is associated with the perception of foxiness in *V. labrusca* L. grapes (Raymond Eder and Rosa, 2019).

3.2 Isabella grape juice fermentations

Indigenous microbial communities from Isabella (*Imc*) and Malbec (*Mmc*) grapes were prepared as indicated (Raymond Eder et al., 2025) (see also Figure 1). As recently reported, the centrifugation process enabled effective collection of the cultivable yeast community; additionally, the pasteurized grape juices showed no detectable presence of cultivable yeasts (i.e., <10 CFU/ml) (Raymond Eder et al., 2025). The mild pasteurization applied (i.e.,

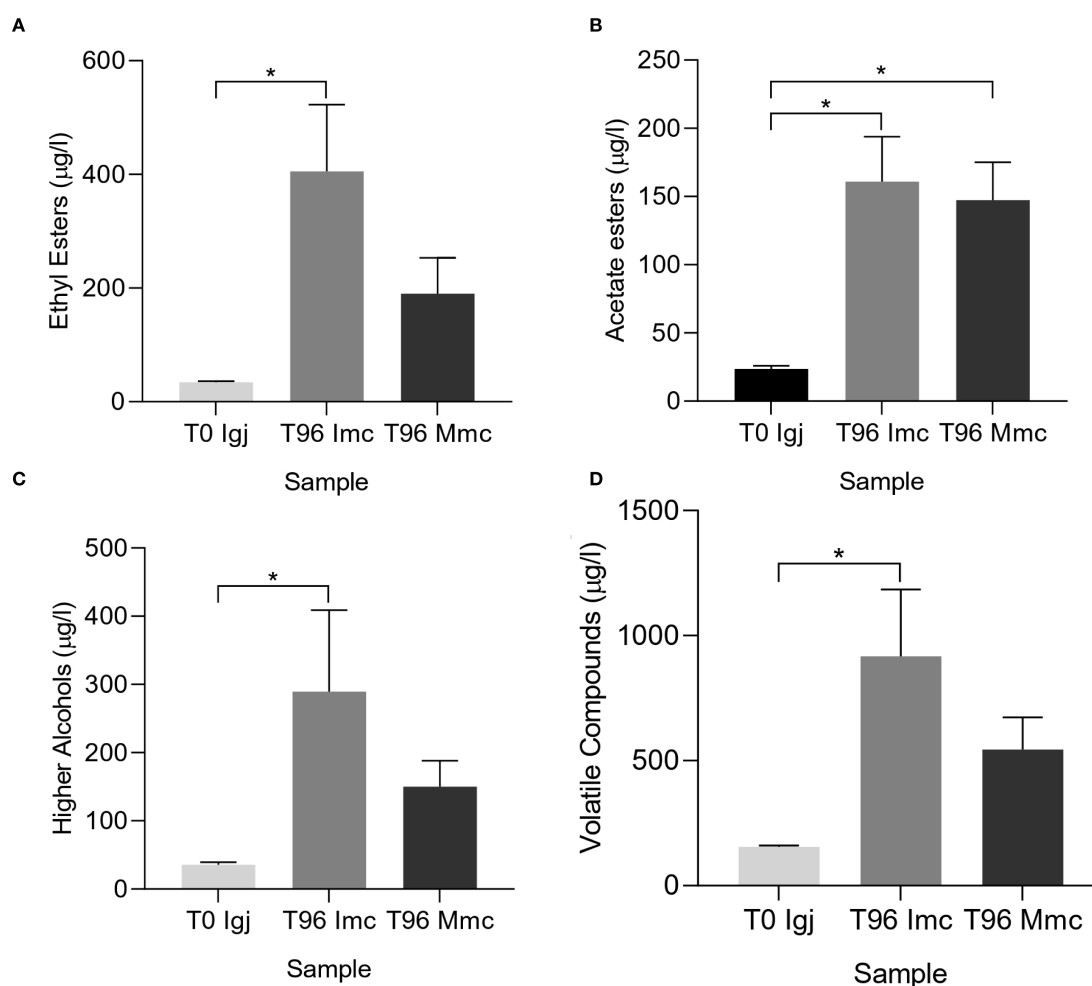


FIGURE 2

Concentration of volatile compounds (µg/l) determined by GC-MS in Isabella grape juice (T0) and after 96 h of fermentation using *Imc* (T96 *Imc*) and *Mmc* (T96 *Mmc*) as inocula. Total ethyl ester (A), acetate ester (B), higher alcohol (C), and volatile (D) compound concentrations (see Table 1). Values are means $\pm$ SD (Table 1; Supplementary Table S1). Significant differences ($p < 0.05$) are marked with an asterisk *.

60°C for 30 minutes) is much less intense than other processes studied (i.e., $81.5 \pm 0.5^\circ\text{C}$ for 450 min) (Malletroit et al., 1991), preserving the sensory profile of the grape juice (Malletroit et al., 1991; Raymond Eder et al., 2025).

The pasteurized *Igj* was inoculated with *Imc* and *Mmc* and their effect on the volatile profiles of fermenting *Igj* were evaluated from both homologous (*Igj/Imc*) and heterologous (*Igj/Mmc*) fermentations. After four days of fermentation (i.e., T96), a total of forty-three compounds, including acetates, alcohols, esters, and terpenes, were identified and quantified in *Igj* (Table 1; Supplementary Table S1). Significant differences in the overall volatile compound concentrations were observed between *Igj/Imc* T0 and T96 fermentations (Figure 2; Table 1; Supplementary Table S1). The varietal compounds (E)-3-hexenyl acetate, heptyl acetate, ethyl 2-butenate, ethyl 2-hexenoate, identified in *Igj* T0 samples, were found in lower concentrations at T96. At this fermenting time, the most abundant compounds in both conditions were isoamyl alcohol, phenethyl alcohol, isoamyl acetate, phenethyl acetate, ethyl octanoate, ethyl decanoate, and ethyl dodecanoate (Table 1). The

compounds ethyl lactate, ethyl hexadecanoate, 2-nonanol, (E,E)-farnesol, 2-heptanone, and acetoin were identified only at T96. The terpenic and C6 compounds identified in Isabella grape juices showed no significant differences between fermenting times (i.e., T0 and T96; Figure 2). Significant differences were found for ethyl octanoate, ethyl decanoate, phenethyl alcohol, phenethyl acetate, and ethyl phenylacetate, which were quantified in higher concentrations in the homologous fermentations (Table 1; Supplementary Table S1; Figure 2).

The volatile compound profiles of fermented *Igj* using the alternative *Imc* and *Mmc* starters were further analyzed through Principal Component Analysis (PCA). Figure 3 illustrates that PC1 accounted for 49.0% of the variance, while PC2 accounted for 27.4%. This analysis demonstrated a distinct separation between fermented (i.e., T96) and non-fermented (i.e., T0) grape juices. Moreover, among the fermented grape juices, the analysis revealed clustering based on the starter used for fermentation (Figure 3). In Figure 3, *Igj/Mmc* fermentations are located in the negative values of PC1, whereas *Igj/Imc* samples cluster together in the positive

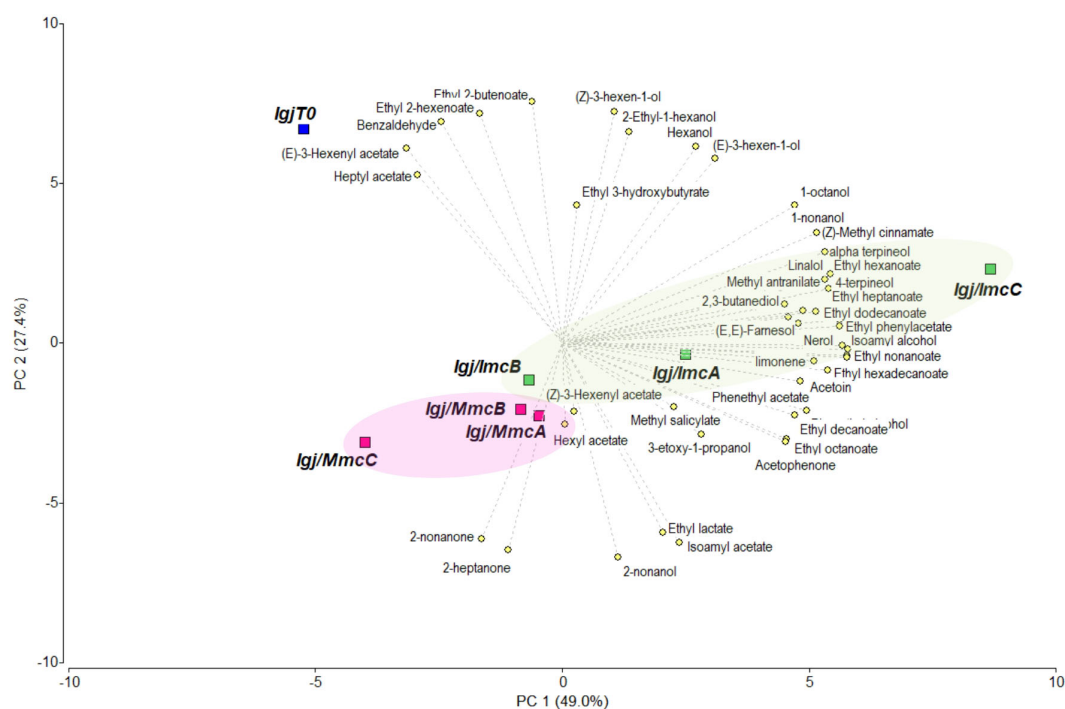


FIGURE 3

Principal Component Analysis (PCA) performed on the concentration of the volatile compounds identified in the Isabella grape juice at T0 and the *Igi/Imc* and *Igi/Mmc* fermenting conditions at T96 (Table 1). Representation of the volatile compounds, the Isabella grape juice at T0 (i.e., *Igi/T0*), and three replicates (i.e., A, B, C) of each fermenting condition using *Imc* (i.e., *Igi/ImcA*, *Igi/ImcB*, *Igi/ImcC*) or *Mmc* (i.e., *Igi/MmcA*, *Igi/MmcB*, *Igi/MmcC*) at T96 in the two first components (PC1 and PC2).

values of PC1, primarily associated with ethyl esters, which are volatile aroma compounds found in significantly higher quantities under this fermenting condition.

3.3 Impact of homologous and heterologous microbial starters on the volatile profiles of Malbec and Isabella grape juice

To evaluate the relative influence of the fermentation inoculum and the fermenting grape juice on volatile profiles, we conducted a PCA integrating the volatile profile data obtained in this study from *Igi/Imc* and *Igi/Mmc* fermentations at T96 with previously published data from Malbec grape juices (*Mgi*) (Raymond Eder et al., 2025), fermented using the identical *Imc* and *Mmc* inocula as in the present work. All Isabella and Malbec samples were prepared from grapes harvested in the same vintage and processed and fermented in parallel, which enables proper comparison of previous and current datasets (see Materials and Methods section) (Raymond Eder et al., 2025). Figure 4 shows that PC1 counted for 46.2%, while PC2 for the 29.9% of the variance. The PC1 showed a separation between the grape juice used for fermentation, with *Igi* (this work) and *Mgi* (Raymond Eder et al., 2025) locating at positive and negative values of the graph, respectively. An ANOVA performed on the miscellaneous varietal compounds (i.e., acetophenone, methyl salicylate, 3,5-dimethyl benzaldehyde, (z)-methyl cinnamate, and methyl

anthranilate; Supplementary Table S2), revealed significant differences among the homologous fermentations (i.e., *Igi/Imc* and *Mgi/Mmc*). However, similar concentrations of these compounds were found in Isabella and Malbec fermentations performed with the heterologous microbial communities (i.e., *Igi/Mmc* and *Mgi/Imc*; Supplementary Table S2), suggesting that these communities reduce the varietal variability in these fermentations. Regarding the impact of the microbial communities in the volatile profiles, a differentiation among the microbial communities, although to a limited extent in the *Igi* fermentations, is observed in the PC2. In general, the *Imc* replicas were positioned in positive values, while *Mmc* were positioned in negative values in the analysis. To further analyze these results, a hierarchical cluster analysis (HCA) based on Euclidean distances between the average volatile profiles of each fermenting condition was performed and plotted (Supplementary Table S3; Supplementary Figure S1). The resulting dendrogram shows that the volatile profiles of fermenting grape juices cluster primarily according to the microbial community used as inoculum. Specifically, samples inoculated with the Malbec microbial community (*Mmc*) cluster together (*Mgi/Mmc* and *Igi/Mmc*), regardless of grape variety, indicating a strong shaping effect of this community. In contrast, the two samples inoculated with the Isabella microbial community (*Imc*) are more distant from each other, with *Mgi/Imc* appearing as the most distinct condition overall. These results suggest that the microbial community has a dominant influence on the volatile profile, but the interaction with grape varieties also contributes to the observed variability.

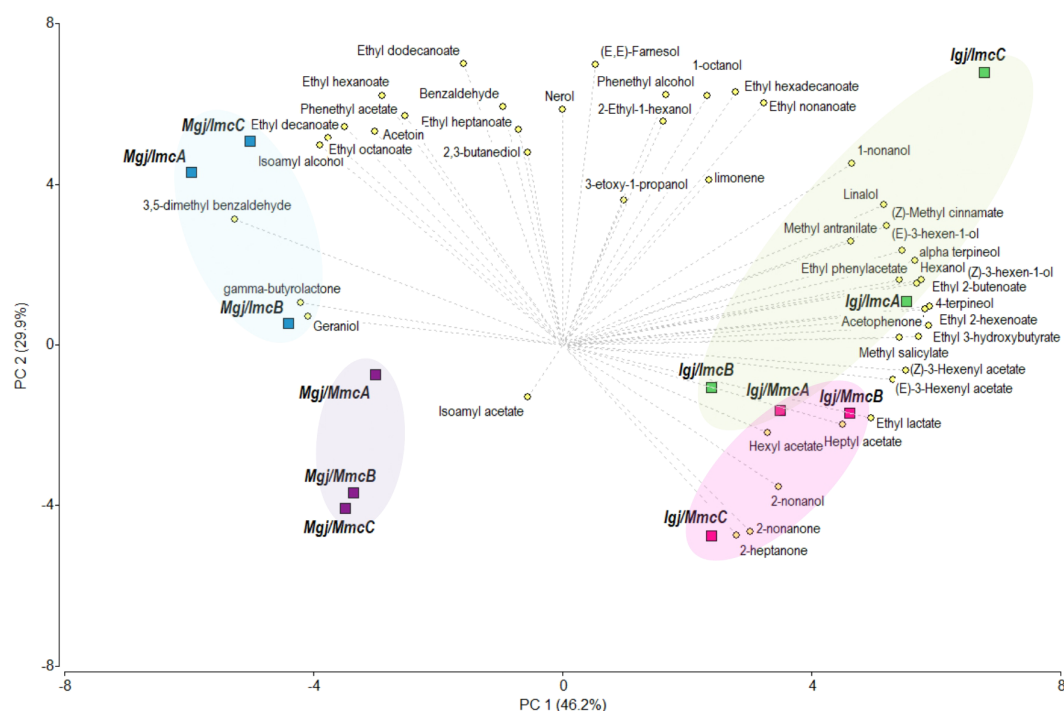


FIGURE 4

Principal Component Analysis (PCA) performed on the concentration of the volatile compounds identified in the *Igj/Imc* and *Igj/Mmc* fermenting conditions at T96 and the *Mgi/Mmc* and *Mgi/Imc* fermenting conditions at T96 reported in (Raymond Eder et al., 2025) (Supplementary Table S2). Representation of the volatile compounds and the three replicas (i.e., A, B, C) of each fermenting condition at T96 in the two first components (PC1 and PC2). Compounds that were not identified at T96 and compounds that did not increase their concentration from T0 to T96 were not considered in this analysis.

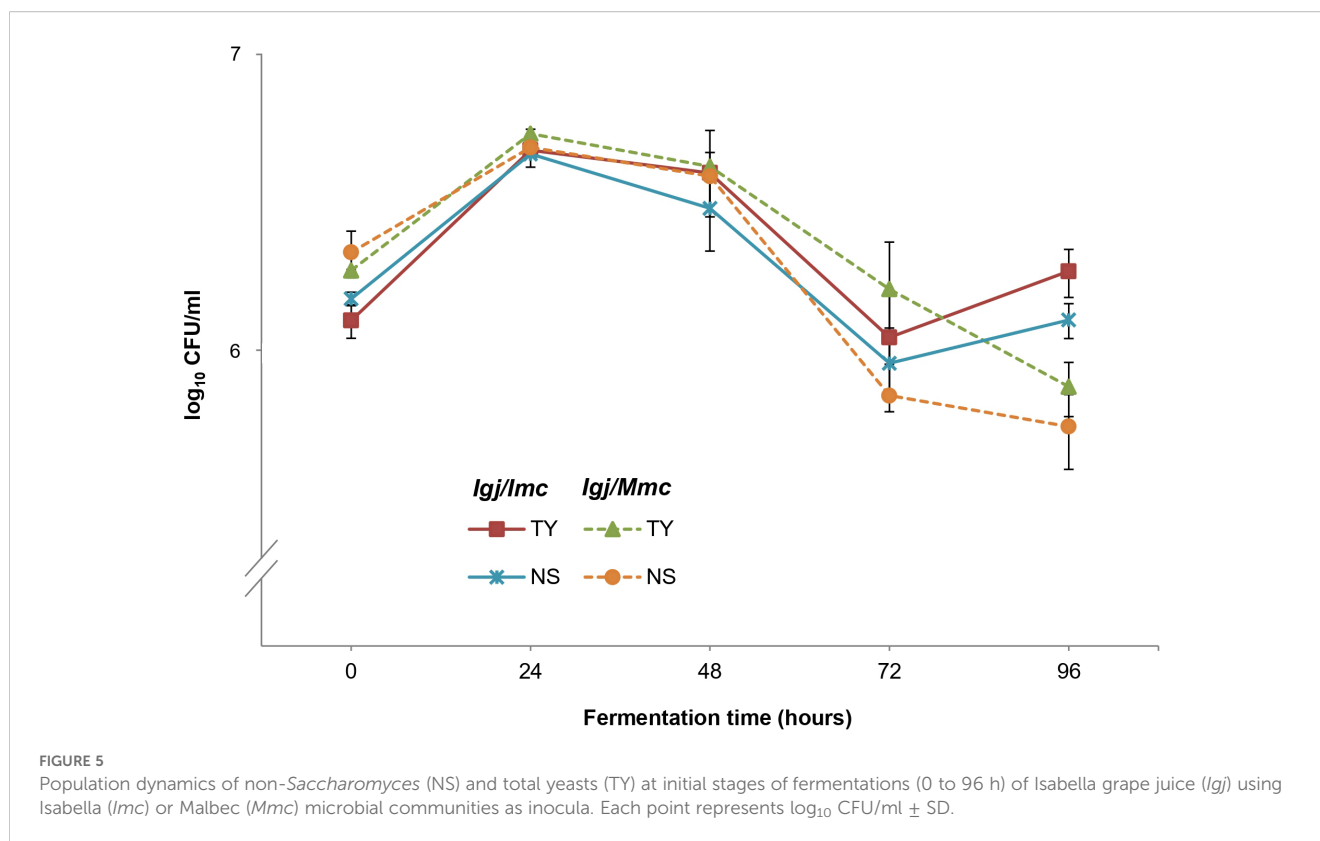
A factorial ANOVA was performed in order to evaluate the impact of the analyzed factors (i.e., grape juice -either *Igj* or *Mgi*- and inoculum -either *Imc* or *Mmc*-) and their interactions in the volatile profile variabilities. This analysis revealed that there were no significant effects from the interactions between the two factors for any of the volatile compounds identified (Supplementary Table S2). The grape juice and inoculum, however, significantly impacted 30 and 16 volatile compounds, respectively. The compounds phenethyl alcohol, phenethyl acetate, ethyl nonanoate, ethyl decanoate, ethyl hexadecanoate, and (E,E)-farnesol were the only compounds significantly impacted only by the inoculum. Interestingly, phenethyl acetate, ethyl decanoate, and ethyl hexadecanoate were present in significantly higher concentrations in fermentations performed using *Imc* (Supplementary Table S2).

These analyses reveal that alternative microbial communities (i.e., *Imc* or *Mmc*) can shape the profile of volatile compounds in ways that allow differentiation between fermentation conditions, while the fermenting grape juices preserve the aromatic typicity and varietal identity of each cultivar (Figure 4).

3.4 Yeast communities in fermenting Isabella grape juices

Differential CFU/ml counts obtained from YPD-Cm and YPD-Cm-Cx agar plates (Raymond Eder et al., 2017, 2018) were used to

assess the contributions of total yeasts and non-*Saccharomyces* yeasts, respectively. Results from this analysis revealed that both conditions (i.e., *Igj/Imc* and *Igj/Mmc*) started with similar counts ($\sim 1-2 \times 10^6$ CFU/ml), with non-*Saccharomyces* species predominating during the early stages of fermentation (T0 to T96) (Figure 5; Supplementary Figure S2). *S. cerevisiae* started to become dominant at middle stages of fermentation (i.e., T144; not shown). Based on these results, we focused our analyses of non-*Saccharomyces* species present at early stages of fermentations (i.e., T0 to T96). A total of 140 isolates from *Igj/Imc* and *Igj/Mmc* were isolated and identified (Supplementary Figure S2). *Hanseniaspora opuntiae* and *Hanseniaspora uvarum* were the most common species at early stages of *Igj/Imc* and *Igj/Mmc* fermentations (Supplementary Figure S2), followed by *Starmerella bacillaris*. Similar results have been obtained when analyzing grape-associated indigenous yeast communities from this ecosystem (Raymond Eder et al., 2017, 2018, 2025; Raymond Eder and Rosa, 2019). Even if similarities were found among the main recognized yeast species in the homologous and heterologous fermenting samples (i.e., *Igj/Imc* and *Mgi/Imc*, respectively), differences in the relative proportion of these yeasts were observed at the analyzed times (Figure 3). Also, *Igj/Imc* fermentations at T96 revealed the presence of *Torulaspora delbrueckii*, *Hanseniaspora vineae*, and *Pichia terricola* as representative isolates (Figure 6). This greater yeast biodiversity, as well as changes in the relative contribution of the main yeast species identified in the fermentations, could help



explain the variations observed in the evolving volatile profiles of *Igj/Imc* replicates (Figure 3).

4 Discussion

Varietal or primary wine flavors originate from the grape variety itself. In addition to the volatile compounds naturally present in grape juice, secondary flavors are developed through alcoholic fermentation via yeast metabolism, which produces higher alcohols, esters, and fatty acids (Carrau et al., 2020; Borren and Tian, 2021; Zilelidou and Nisiotou, 2021; Romano et al., 2022; Liang et al., 2023; Wang et al., 2023; Zhang et al., 2023a). In spontaneous fermentations, the complex native microbial community associated to grapes contributes to the final volatile profiles of wines (Padilla et al., 2016; Belda et al., 2017). Indigenous grape-associated yeasts species are particularly relevant contributors to the volatile profiles of fermenting juices at early times of fermentation (Jolly et al., 2014; Pretorius, 2017; Raymond Eder et al., 2025). In addition to yeast, lactic-acid and acetic-acid bacteria, even when present at low concentrations at early times of fermentations, can also contribute to the final volatile compositions of spontaneous fermentations (Barata et al., 2012; Andorrà et al., 2019; Shimizu et al., 2023; Raymond Eder et al., 2025).

We have recently demonstrated that microbial communities associated to different *Vitis* species condition the volatile profiles of early-stage Malbec grape juice fermentations (Raymond Eder et al., 2025). In this work, we have extended these studies to analyze the volatile profiles of Isabella grape juices fermented using

homologous (Isabella; *V. labrusca* L.) or heterologous (i.e., Malbec; *V. vinifera* L.) native microbial communities. Our analyses consider the profiles of free volatile compounds in grape juices, excluding glycoside-bound volatiles (Raymond Eder et al., 2025). Although bound volatiles contribute to the full aromatic potential of a grape juice—upon enzymatic or fermentative release—the analyzed free fraction offers a representative expression of varietal identity at the juice stage. Moreover, this approach avoids potential artifacts introduced by exogenous enzymes, yields reproducible data under our standardized conditions, and reveals the aromatic identity of fresh varietal grape juice. This experimental strategy allowed us to perform a joint analysis of the volatile compound datasets obtained from homologous and heterologous fermentations of Malbec (Raymond Eder et al., 2025) and Isabella (this work) grape juices, conducted with the same starter microbial communities and grape juices samples prepared from grapes harvested in vintage of year 2021 (Raymond Eder et al., 2025). Taken together, the results presented in this work show that the microbiota shapes the volatile profiles of the fermented Malbec and Isabella juices, which nonetheless retain the identity of their original grape varietal.

It has been reported that Isabella, a varietal from the American-originated *Vitis labrusca* L., is rich in aroma compounds both qualitatively and quantitatively (Ghaste et al., 2015). Key alcohols and terpenes, including methyl anthranilate, β-phenyl ethanol, ethyl-3-hydroxybutyrate, ethyl-β-hydroxy hexanoate, fureneol, phenylacetaldehyde, tryptophol, and 2-hexenol, have been detected in Isabella grape juices and crushed grapes originating from Brazil and Italy, respectively (Ghaste et al., 2015; Gomez et al.,

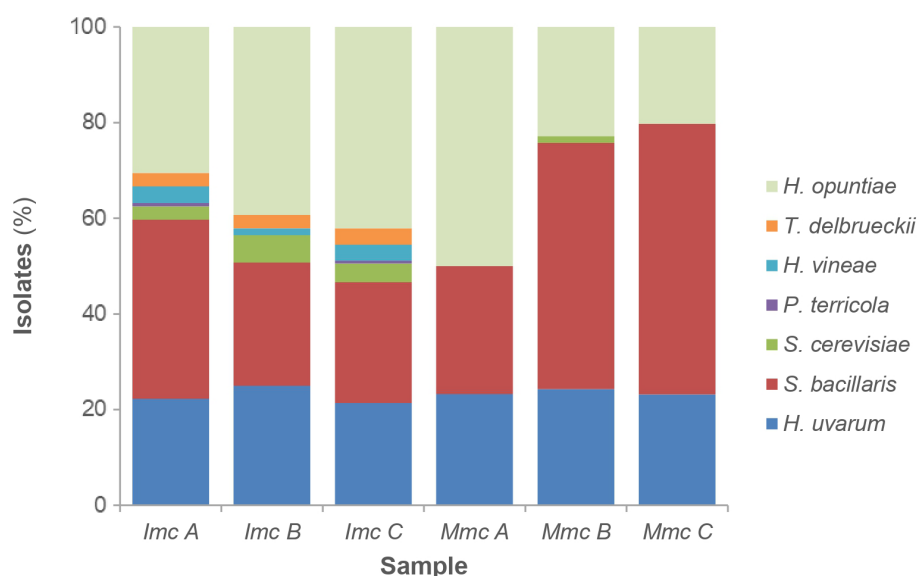


FIGURE 6

Main represented yeast species at T96 of the three replicas from each fermenting condition. Appropriate dilutions of replicas A, B, and C from *Imc* (left; columns 2–4) and *Mmc* (right; columns 6–8) conditions were plated individually, and percentages represent the relative contribution of the indicated yeast species.

2024). Our analysis revealed that Isabella juice from grapes harvested in the geographic region of Córdoba (Argentina) has a substantial proportion of alcohols, esters, and terpenes, including the previously reported methyl anthranilate, β -phenyl ethanol, ethyl-3-hydroxybutyrate, and methyl salicylate (Ghaste et al., 2015; Gomez et al., 2024). Although different techniques of extraction of volatile compounds were used, Isabella juice from Córdoba was found to contain eleven ethyl esters, whereas only two (i.e., ethyl-butanoate and ethyl-metacrylate) and four (i.e., methyl salicylate, methyl anthranilate, methyl- β -hydroxybutyrate, ethyl-3-hydroxyhexanoate) ethyl esters were detected in the Brazilian and Italian studies, respectively (Ghaste et al., 2015; Gomez et al., 2024). Previous research on the volatile profiles of Isabella and Ives commercial wines also emphasized the prominent role of ethyl acetate and esters, contributing to their characteristic fruity aroma descriptors (Arcanjo et al., 2018). In the present study, isoamyl alcohol, phenethyl alcohol, isoamyl acetate, phenethyl acetate, ethyl octanoate, ethyl decanoate, and ethyl dodecanoate emerged as the dominant volatile compounds during the initial stages of fermentation. Interestingly, as it was observed in our recent study of homologous and heterologous fermentation of Malbec grape juice (Raymond Eder et al., 2025), differences were observed in the volatile profiles of the Isabella fermenting grape juice when using the alternative either Malbec or Isabella associated microbial communities as starters. Homologous fermentations (*Igj/Imc*) show higher concentrations of ethyl octanoate, ethyl decanoate, phenethyl alcohol, phenethyl acetate, and ethyl phenylacetate than the heterologous conditions (*Igj/Mmc*) (Raymond Eder et al., 2025).

The evolution of culturable yeast populations in homologous and heterologous Isabella grape juice fermentations was studied using culture-dependent methods. Although his strategy limits our study to the identification of only cultivable yeast species from the overall fungal, yeast, and bacterial biodiversity in the samples, it is known that cultivable non-*Saccharomyces* yeast species significantly contribute to aromatic profiles in early fermentation stages (Andorrà et al., 2010; Borren and Tian, 2021; Zilelidou and Nisiotou, 2021; Romano et al., 2022; de Celis et al., 2024). At T0, *Hanseniaspora* spp. (*H. opuntiae* and *H. uvarum*) and, to a lesser extent, *S. bacillaris*, predominated in the fermented juices, consistent with previous studies on yeast communities from this region's spontaneously fermenting musts and reconstituted grape juices (Raymond Eder et al., 2017, 2018, 2025). As fermentation progressed, from T0 to T96, *Hanseniaspora* spp. dominated the fermentations in *Imc*, while *S. bacillaris* increased its contribution in *Mmc*. It has been recently reported that the dominating yeast species in a fermentation defines its performance and metabolite profile of the resulting wines (de Celis et al., 2024). In this sense, members of the *Hanseniaspora* genus have been reported to play a significant role in the production of volatile compounds in wine, particularly acetate esters, in a strain-dependent manner (Jolly et al., 2014; Martin et al., 2018). Specifically, *H. uvarum* significantly increased ethyl and isoamyl esters and reduced acetate ester concentrations in co-fermentations with *S. cerevisiae* in a strain-specific way (Tristezza et al., 2016; Hu et al., 2018b, 2018a; Zhang et al., 2023b). *H. opuntiae* has been shown to positively influence volatile profiles and sensory characteristics of fermentations,

significantly increasing acetate esters, mainly 2-phenylethyl acetate, and ethyl esters levels (Hu et al., 2020; del Fresno et al., 2022, 2023; Filippousi et al., 2024). Moreover, fermentation studies where *Hanseniaspora* spp. were the dominating yeasts, were characterized by higher fusel alcohol acetates production (de Celis et al., 2024). (i.e., phenethyl alcohol). On the other hand, *S. bacillaris* has been reported to overproduce ethyl and other acetate esters, as well as terpenes (Andorrà et al., 2010; Englezos et al., 2015; Raymond Eder and Rosa, 2021). However, elevated levels of these compounds were not detected in the *Igj/Mmc* fermentation, where this yeast was dominant at T96. Four other non-*Saccharomyces* species previously recognized in this *terroir* (i.e., *Candida diversa*, *T. delbrueckii*, *H. vineae*, and *P. terricola*) were also isolated at these fermentations. Proposed signature *V. labrusca* L. non-*Saccharomyces* species (i.e., *Candida azymoides*, *Pichia cecembensis*, and *Candida californica*) (Drumonde-Neves et al., 2016; Raymond Eder et al., 2017, 2018) were not identified among the limited number of isolates characterized in this study. In addition to the contribution to the volatile profiles of the main yeast species, increased yeast diversity has been linked to greater flavor complexity (Carrau and Henschke, 2021). In this context, the minor yeast species present at lower abundances in *Igj/Imc* may have also contributed to the elevated concentrations of certain volatile compounds. *T. delbrueckii*, for instance, enhances succinic acid, linalool, acetate esters, medium chain fatty acids, and terpenes in aromatic grapes (Jolly et al., 2014; Varela and Borneman, 2016). Similarly, *P. terricola* has been reported to release precursors from grape juice, boosting free monoterpenes and norisoprenoids (Jolly et al., 2014). The apiculate yeast *H. vineae* has been reported to produce terpenes, sesquiterpenes, and high amounts of acetate esters, such as 2-phenylethyl acetate and ethyl acetate, which influence fermentations by producing flavors and increasing sensory complexity (Martin et al., 2018).

Our results show that microbial communities assembled in different *Vitis* species influence the volatile profiles of early-stage fermentations of different grape juices. Additional data from fermentations using other microbial communities and grape juice varieties would strengthen the conclusion that alternative yeast populations shape the volatile profiles of fermented juice while preserving grape varietal identity. Also, grape fermentations using pure cultures of selected non-*Saccharomyces* strains, and/or pools of selected strains that resemble native microbial communities, could further clarify the specific contributions of individual yeast species and strains to the final volatile profiles of fermented grape juices. Emerging evidence suggest that non-conventional *Vitis* ecosystems may carry unique yeast species or strains not found in *V. vinifera* L., presenting opportunities for the isolation of valuable *Saccharomyces* and non-*Saccharomyces* strains of potential relevance for the winemaking industry.

Data availability statement

The original contributions presented in the study are publicly available. This data can be found here: NCBI GenBank, accession OP584257, OP584258, OP584259, OP584260, OP584261, OP584262, OP584263, and OP584265.

Author contributions

MR: Investigation, Writing – original draft, Conceptualization, Writing – review & editing, Methodology, Validation, Formal analysis, Project administration, Supervision. LF: Software, Investigation, Writing – review & editing, Formal Analysis. FC: Supervision, Writing – review & editing, Conceptualization. AR: Formal analysis, Validation, Methodology, Project administration, Resources, Supervision, Investigation, Writing – review & editing, Conceptualization, Funding acquisition, Writing – original draft.

Funding

The author(s) declare that no financial support was received for the research and/or publication of this article.

Acknowledgments

We thank S. Lauret (Colonia Caroya, Argentina) for assisting us in obtaining Malbec and Isabella grape samples, and H.E. Martínez Beladelli for providing technical assistance during the initial stages of this project. M.L.R.E. held a postdoctoral fellowship of the Consejo Nacional de Investigaciones Científicas y Técnicas de Argentina (CONICET). A.L.R. is Principal Investigator of CONICET.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/ffunb.2025.1643880/full#supplementary-material>

SUPPLEMENTARY FIGURE 1

Dendrogram resulting from hierarchical cluster analysis based on Euclidean distances calculated from the average profiles of volatile compounds in each

fermenting condition (i.e., *Igj/Imc*, *Igj/Mmc*, *Mgj/Mmc*, and *Mgj/Imc*) at 96 hours (Supplementary Table S3). Each point of the dendrogram represents the centroid of three biological replicates performed in the study.

SUPPLEMENTARY FIGURE 2

Main represented yeast species at the initial stages of fermentations (0 to 96 h) of Isabella grape juice using Isabella (*Imc*) or Malbec (*Mmc*) microbial communities as inocula. Percentages represent the relative contribution of the indicated yeast species among the colonies obtained at the indicated times of fermentation.

References

- Andorrà, I., Berradre, M., Rozès, N., Mas, A., Guilmón, J. M., and Esteve-Zarzoso, B. (2010). Effect of pure and mixed cultures of the main wine yeast species on grape must fermentations. *Eur. Food Res. Technol.* 231, 215–224. doi: 10.1007/s00217-010-1272-0
- Andorrà, I., Miró, G., Espligares, E., Mislata, A. M., Puxeu, M., and Ferrer-Gallego, R. (2019). "Wild Yeast and Lactic Acid Bacteria of Wine," in *Yeasts in Biotechnology*. Ed. T. Peixoto Basso (London, England: IntechOpen).
- Arcanjo, N. M. D. O., Oliveira, M. E. D. S., Araújo, Í.B.D.S., da Silva, F. L. H., and Madruga, M. S. (2018). Red wine produced from the Isabella and Ives cultivar (*Vitis Labrusca*): Profile of volatiles and aroma descriptors. *Food Sci. Technol. (Brazil)* 38, 271–279. doi: 10.1590/1678-457X.04717
- Baffi, M. A., Dos Santos Bezerra, C., Arévalo-Villena, M., Isabel Briones-Pérez, A., Gomes, E., and Da -Silva, R. (2011). Isolation and molecular identification of wine yeasts from a Brazilian vineyard. *Ann. Microbiol.* 61, 75–78. doi: 10.1007/s13213-010-0099-z
- Barata, A., Malfeito-Ferreira, M., and Loureiro, V. (2012). The microbial ecology of wine grape berries. *Int. J. Food Microbiol.* 153, 243–259. doi: 10.1016/j.jfoodmicro.2011.11.025
- Belda, I., Ruiz, J., Esteban-fernández, A., Navascués, E., Marquina, D., Santos, A., et al. (2017). Microbial contribution to wine aroma and its intended use for wine quality improvement. *Molecules* 22 (2), 189. doi: 10.3390/molecules22020189
- Bezerra-Bussoli, C., Baffi, M. A., Gomes, E., and Da-Silva, R. (2013). Yeast diversity isolated from grape musts during spontaneous fermentation from a Brazilian winery. *Curr. Microbiol.* 67, 356–361. doi: 10.1007/s00284-013-0375-9
- Borren, E., and Tian, B. (2021). The important contribution of non-Saccharomyces yeasts to the aroma complexity of wine: A review. *Foods* 10(1), 13. doi: 10.3390/foods10010013
- Carrau, F., Boido, E., and Ramey, D. (2020). *Yeasts for low input winemaking: Microbial terroir and flavor differentiation. 1st Edn* (Amsterdam, Netherlands: Elsevier Inc). doi: 10.1016/bs.aambs.2020.02.001
- Carrau, F., and Henschke, P. A. (2021). *Hanseniaspora vineae* and the concept of friendly yeasts to increase autochthonous wine flavor diversity. *Front. Microbiol.* 12. doi: 10.3389/fmicb.2021.702093
- Cilião Filho, M., Bertélli, M. B. D., Valle, J. S., Paccola-Meirelles, L. D., Linde, G. A., Barcellos, F. G., et al. (2017). Genetic diversity and pectinolytic activity of epiphytic yeasts from grape carposphere. *Genet. Mol. Res.* 16, 1–13. doi: 10.4238/gmr16029698
- de Celis, M., Ruiz, J., Benitez-Dominguez, B., Vicente, J., Tomasi, S., Izquierdo-Gea, S., et al. (2024). Multi-omics framework to reveal the molecular determinants of fermentation performance in wine yeast populations. *Microbiome* 12, 203. doi: 10.1186/s40168-024-01930-w
- de Celis, M., Ruiz, J., Vicente, J., Acedo, A., Marquina, D., Santos, A., et al. (2022). Expectable diversity patterns in wine yeast communities. *FEMS Yeast Res.* 22, foac034. doi: 10.1093/femsyr/foac034
- De la Fuente Lloreda, M. (2018). Use of hybrids in viticulture. A challenge for the OIV. *OENO One* 52, 231–234. doi: 10.20870/oeno-one.oeno-one.2018.52.3.2312
- del Fresno, J. M., Carrau, F., Escott, C., Vaquero, C., González, C., and Morata, A. (2023). Use of *Hanseniaspora* spp. in sequential fermentation with *Saccharomyces cerevisiae* to improve the aromatic complexity of Alballo Mayor white wines. *Bio Web Conferences*. doi: 10.1051/bioconf/20236802029
- del Fresno, J. M., Escott, C., Carrau, F., Herbert-Pucheta, J. E., Vaquero, C., González, C., et al. (2022). Improving aroma complexity with *hanseniaspora* spp: terpenes, acetate esters, and safranal. *Fermentation* 8, 654. doi: 10.3390/fermentation8110654
- Dellacassa, E., Trenchs, O., Fariña, L., Debernardis, F., Perez, G., Boido, E., et al. (2017). Pineapple (*Ananas comosus* L. Merr.) wine production in Angola: Characterisation of volatile aroma compounds and yeast native flora. *Int. J. Food Microbiol.* 241, 161–167. doi: 10.1016/j.jfoodmicro.2016.10.014
- Drumonde-Neves, J., Franco-Duarte, R., Lima, T., Schuller, D., and Pais, C. (2016). Yeast biodiversity in vineyard environments is increased by human intervention. *PloS One* 11, 1–13. doi: 10.1371/journal.pone.0160579
- Englezos, V., Rantsiou, K., Cravero, F., Torchio, F., Giacosa, S., Ortiz-Julien, A., et al. (2018). Volatile profiles and chromatic characteristics of red wines produced with *Starmerella bacillaris* and *Saccharomyces cerevisiae*. *Food Res. Int.* 109, 298–309. doi: 10.1016/j.foodres.2018.04.027
- Englezos, V., Rantsiou, K., Torchio, F., Rolle, L., Gerbi, V., and Cocolin, L. (2015). Exploitation of the non-*Saccharomyces* yeast *Starmerella bacillaris* (synonym *Candida zemplinina*) in wine fermentation: Physiological and molecular characterizations. *Int. J. Food Microbiol.* 199, 33–40. doi: 10.1016/j.ijfoodmicro.2015.01.009
- Filippousi, M. E., Chalvanti, I., Mallouchos, A., Marmaras, I., Banilas, G., and Nisiotou, A. (2024). The use of *hanseniaspora opuntiae* to improve 'Sideritis' Wine quality, a late-ripening greek grape variety. *Foods* 13, 1061. doi: 10.3390/foods13071061
- García-Izquierdo, I., Colino-Rabanal, V. J., Tamame, M., and Rodríguez-López, F. (2024). Microbiota ecosystem services in vineyards and wine: A review. *Agronomy* 14, 131. doi: 10.3390/agronomy14010131
- Garde-Cerdán, T., Gutiérrez-Gamboa, G., Baroja, E., Rubio-Bretón, P., and Pérez-Álvarez, E. P. (2018). Influence of methyl jasmonate foliar application to vineyard on grape volatile composition over three consecutive vintages. *Food Res. Int.* 112, 274–283. doi: 10.1016/j.foodres.2018.06.048
- Ghaste, M., Narduzzi, L., Carlin, S., Vrhovsek, U., Shulaev, V., and Mattivi, F. (2015). Chemical composition of volatile aroma metabolites and their glycosylated precursors that can uniquely differentiate individual grape cultivars. *Food Chem.* 188, 309–319. doi: 10.1016/j.foodchem.2015.04.056
- Gomez, H. A. G., Niederauer, G. F., Minatel, I. O., Antunes, E. R. M., Carneiro, M. J., Sawaya, A. C. H. F., et al. (2024). Metabolite profiling reveals the influence of grapevine genetic distance on the chemical signature of juices. *J. Sci. Food Agric.* 104 (4), 2383–2397. doi: 10.1002/jsfa.13124
- Griggs, R. G., Steenwerth, K. L., Mills, D. A., Cantu, D., and Bokulich, N. A. (2021). Sources and assembly of microbial communities in vineyards as a functional component of winegrowing. *Front. Microbiol.* 12. doi: 10.3389/fmicb.2021.673810
- Hu, K., Jin, G. J., Mei, W. C., Li, T., and Tao, Y. S. (2018a). Increase of medium-chain fatty acid ethyl ester content in mixed *H. uvarum*/*S. cerevisiae* fermentation leads to wine fruity aroma enhancement. *Food Chem.* 239, 495–501. doi: 10.1016/j.foodchem.2017.06.151
- Hu, K., Jin, G. J., Xu, Y. H., and Tao, Y. S. (2018b). Wine aroma response to different participation of selected *Hanseniaspora uvarum* in mixed fermentation with *Saccharomyces cerevisiae*. *Food Res. Int.* 108, 119–127. doi: 10.1016/j.foodres.2018.03.037
- Hu, L., Liu, R., Wang, X., and Zhang, X. (2020). The sensory quality improvement of citrus wine through co-fermentations with selected non-saccharomyces yeast strains and *Saccharomyces cerevisiae*. *Microorganisms* 8, 323. doi: 10.3390/microorganisms8030323
- Jolly, N. P., Varela, C., and Pretorius, I. S. (2014). Not your ordinary yeast: Non-*Saccharomyces* yeasts in wine production uncovered. *FEMS Yeast Res.* 14, 215–237. doi: 10.1111/1567-1364.12111
- Kamilari, E., Mina, M., Karallis, C., and Tsaltas, D. (2021). Metataxonomic analysis of grape microbiota during wine fermentation reveals the distinction of Cyprus regional terroirs. *Front. Microbiol.* 12. doi: 10.3389/fmicb.2021.726483
- Lederer, M. A., Nielsen, D., Toldam-Andersen, T. B., Herrmann, J. V., and Arneborg, N. (2013). Yeast species associated with different wine grape varieties in Denmark. *Acta Agric. Scand. B Soil Plant Sci.* 63, 89–96. doi: 10.1080/09064710.2012.723738
- Liang, L., Ma, Y., Jiang, Z., Eudes, F., and Peng, S. (2023). Dynamic analysis of microbial communities and flavor properties in Merlot wines produced from inoculation and spontaneous fermentation. *Food Res. Int.* 164, 112379. doi: 10.1016/j.foodres.2022.112379
- Liszkowska, W., and Berłowska, J. (2021). Yeast fermentation at low temperatures: Adaptation to changing environmental conditions and formation of volatile compounds. *Molecules* 26, 1035. doi: 10.3390/molecules26041035
- Liu, D., Chen, Q., Zhang, P., Chen, D., and Howell, K. S. (2020). The fungal microbiome is an important component of vineyard ecosystems and correlates with regional distinctiveness of wine. *mSphere* 5, 1–15. doi: 10.1128/msphere.00534-20

- Maicas, S., and Mateo, J. J. (2023). The life of saccharomyces and non-saccharomyces yeasts in drinking wine. *Microorganisms* 11, 1178. doi: 10.3390/microorganisms11051178
- Malletroit, V., Guinard, J.-X.-X., Kunkee, R. E., and Lewis, M. J. (1991). Effect of pasteurization on microbiological and sensory quality of white grape juice and wine. *J. Food Process Preserv* 15, 19–29. doi: 10.1111/j.1745-4549.1991.tb00151.x
- Martin, V., Jose Valera, M., Medina, K., Boido, E., and Carrau, F. (2018). Oenological impact of the *Hanseniaspora/Kloeckera* yeast genus on wines — A review. *Fermentation* 4, 76. doi: 10.3390/fermentation4030076
- Morata, A., Loira, I., González, C., and Escott, C. (2021). Non-saccharomyces as biotools to control the production of off-flavors in wines. *Molecules* 26, 4571. doi: 10.3390/molecules26154571
- Padilla, B., Gil, J. V., and Manzanera, P. (2016). Past and future of non-*Saccharomyces* yeasts: From spoilage microorganisms to biotechnological tools for improving wine aroma complexity. *Front. Microbiol.* 7. doi: 10.3389/fmicb.2016.00411
- Pretorius, I. S. (2017). Solving yeast jigsaw puzzles over a glass of wine. *EMBO Rep.* 18, 1875–1884. doi: 10.15252/embr.201745231
- Pretorius, I. S. (2020). Tasting the terroir of wine yeast innovation. *FEMS Yeast Res.* 20(1), foz084. doi: 10.1093/femsyr/foz084
- Raymond Eder, M. L., Conti, F., and Rosa, A. L. (2018). Differences between indigenous yeast populations in spontaneously fermenting musts from *V. vinifera* L. and *V. labrusca* L. Grapes harvested in the same geographic location. *Front. Microbiol.* 9. doi: 10.3389/fmicb.2018.01320
- Raymond Eder, M. L., Fariña, L., Carrau, F., and Rosa, A. L. (2025). Grape-specific native microbial communities influence the volatile compound profiles in fermenting grape juices. *Food Chem.* 466, 142155. doi: 10.1016/j.foodchem.2024.142155
- Raymond Eder, M. L., Reynoso, C., Lauret, S. C., and Rosa, A. L. (2017). Isolation and identification of the indigenous yeast population during spontaneous fermentation of Isabella (*Vitis labrusca* L.) grape must. *Front. Microbiol.* 8. doi: 10.3389/fmicb.2017.00532
- Raymond Eder, M. L., and Rosa, A. L. (2019). Yeast diversity in *Vitis non-vinifera* ecosystems. *Rev. Argent Microbiol.* 51, 278–283. doi: 10.1016/j.ram.2018.09.004
- Raymond Eder, M. L., and Rosa, A. L. (2021). Genetic, physiological, and industrial aspects of the fructophilic non-saccharomyces yeast species, *starmerella bacillaris*. *Fermentation* 7, 87. doi: 10.3390/fermentation7020087
- Romano, P., Braschi, G., Siesto, G., Patrignani, F., and Lanciotti, R. (2022). Role of yeasts on the sensory component of wines. *Foods* 11, 1921. doi: 10.3390/foods11131921
- Shimizu, H., Kamada, A., Koyama, K., Iwashita, K., and Goto-Yamamoto, N. (2023). Yeast diversity during the spontaneous fermentation of wine with only the microbiota on grapes cultivated in Japan. *J. Biosci. Bioeng* 136, 35–43. doi: 10.1016/j.jbiosc.2023.03.013
- Torres-Guardado, R., Esteve-Zarzoso, B., Reguant, C., and Bordons, A. (2022). Microbial interactions in alcoholic beverages. *Int. Microbiol.* 25, 1–15. doi: 10.1007/s10123-021-00200-1
- Tristezza, M., Tufariello, M., Capozzi, V., Spano, G., Mita, G., and Grieco, F. (2016). The oenological potential of *hanseniaspora uvarum* in simultaneous and sequential co-fermentation with *Saccharomyces cerevisiae* for industrial wine production. *Front. Microbiol.* 7. doi: 10.3389/fmicb.2016.00670
- Tufariello, M., Fragasso, M., Pico, J., Panighel, A., Castellarin, S. D., Flamini, R., et al. (2021). Influence of non-saccharomyces on wine chemistry: A focus on aroma-related compounds. *Molecules* 26, 644. doi: 10.3390/molecules26030644
- Varela, C. (2016). The impact of non-*Saccharomyces* yeasts in the production of alcoholic beverages. *Appl. Microbiol. Biotechnol.* 100, 9861–9874. doi: 10.1007/s00253-016-7941-6
- Varela, C., and Borneman, A. R. (2016). Yeasts found in vineyards and wineries. *Yeast* 34 (3), 111–128. doi: 10.1002/yea.3219
- Wang, X., Fan, G., Peng, Y., Xu, N., Xie, Y., Zhou, H., et al. (2023). Mechanisms and effects of non-*Saccharomyces* yeast fermentation on the aromatic profile of wine. *J. Food Composition Anal.* 124, 1–20. doi: 10.1016/j.jfca.2023.105660
- Zhang, L., Liu, Q., Li, Y., Liu, S., Tu, Q., and Yuan, C. (2023a). Characterization of wine volatile compounds from different regions and varieties by HS-SPME/GC-MS coupled with chemometrics. *Curr. Res. Food Sci.* 6, 100418. doi: 10.1016/j.crf.2022.100418
- Zhang, Z., Wang, H., Xia, H., Sun, L., Zhang, Q., Yang, H., et al. (2023b). Wine aroma modification by *Hanseniaspora uvarum*: A multiple-step strategy for screening potential mixed starters. *Food Chem. X* 20, 100930. doi: 10.1016/j.fochx.2023.100930
- Zilelidou, E. A., and Nisiotou, A. (2021). Understanding wine through yeast interactions. *Microorganisms* 9, 1620. doi: 10.3390/microorganisms9081620