



Review

Non-Conventional Grape Varieties and Yeast Starters for First and Second Fermentation in Sparkling Wine Production Using the Traditional Method

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Abstract: Sparkling wine production using the traditional method involves a second fermentation of still wines in bottles, followed by prolonged aging on lees. The key factors affecting the organoleptic profiles of these wines are the grape varieties, the chemical and sensory attributes of the base wines elaborated, the yeast strains used for first and second fermentation, and the winery practices. While Chardonnay and Pinot noir are gold standard grape varieties in sparkling wine production, other valuable grape cultivars are used worldwide to elaborate highly reputable sparkling wines. Fundamental research on the chemical and sensory profiles of innovative sparkling wines produced by the traditional method, using non-conventional grape varieties and novel yeast strains for first and/or second fermentation, is accompanying their market diversification. In this review, we summarize relevant aspects of sparkling wine production using the traditional method and non-conventional grape varieties and yeast starters.

Keywords: sparkling wine; non-conventional grape varieties; second fermentation; yeast



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1. Introduction

The traditional method for sparkling wine production (i.e., *méthode champenoise*) consists of the fermentation of a base wine following the addition of a *liqueur de tirage* (i.e., wine, sucrose, ethanol-adapted yeast cells, nutrients, and a clarifying agent), in characteristic bottles [1] (Figure 1). The development of this second fermentation in sealed bottles greatly contributes to the sensory complexity of the resulting wine [2,3]. Among other factors, the *in bottle* fermentation method contributes to a refined development of foam and bubbles, which are key properties identifying *champenoise* sparkling wines [3,4].

Conventional grape varieties used in this industry are linked to prestigious sparkling wines produced in France (e.g., Chardonnay, Pinot noir, and Pinot meunier), Italy (e.g., Chardonnay, Pinot nero, Pinot bianco, and Lambrusco), and Spain (e.g., Macabeo, Xarel.lo, Parellada, Pinot noir, and Chardonnay) (Table 1). Each country has different officially authorized grape varieties for their production. These varieties are linked to various Protected Designations of Origin [3,4] (Table 1).

Accompanying the global expansion of sparkling wine production, emerging grape varieties for the elaboration of fine quality sparkling wines are being explored worldwide [3,4] (Table 2). Non-conventional grape varieties challenge the wine industry to recognize and market products with novel sensory profiles, and to anticipate potential consumer acceptance [2,5]. Adding complexity to the scenario of innovation in sparkling wine production, first and second fermentation strategies are also being explored, based on novel and/or indigenous yeast starters [2]. In this review, we summarize relevant studies of sparkling wine production, innovating both in grape varieties and in yeast starters, and using the traditional method.

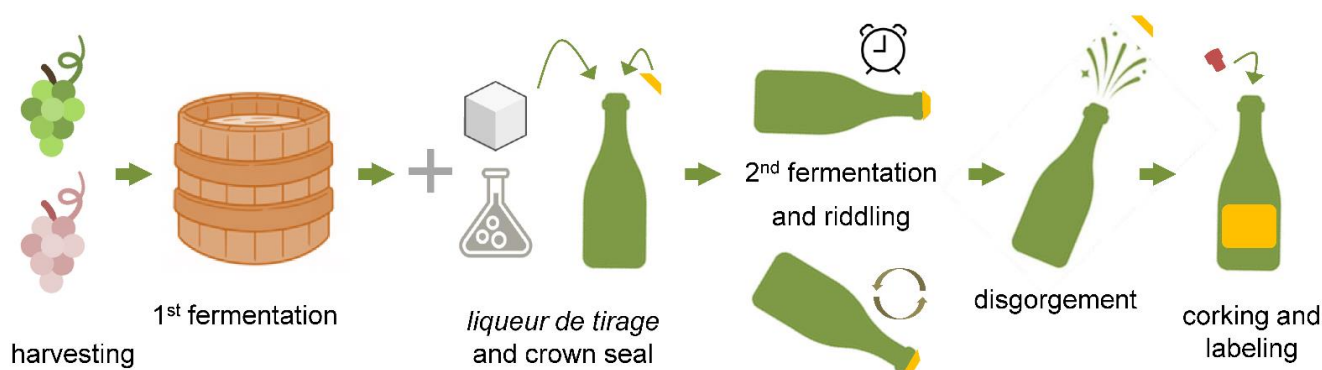


Figure 1. Schematic representation of sparkling wine production using the traditional method. During the first fermentation, the grape juice is converted into a base wine, which is then subjected to a second fermentation following the addition of wine, sucrose, ethanol-adapted yeast cells, nutrients, and a clarifying agent, in characteristic bottles. After the second fermentation and aging, riddling is performed to allow accumulation of the lees at the bottle neck, facilitating their mechanical elimination. Disgorging (i.e., lees removal) is followed by the addition of a *liqueur de expedition*, bottle corking, and labelling.

Table 1. Conventional grape varieties used for sparkling wine production by the traditional method.

Country	Sparkling Wine	Color(s)	Grape Varieties
France	Champagne	White, rosé	Chardonnay, Pinot noir, and Pinot meunier
France	Crémant	White, rosé	Chardonnay, Pinot noir, Chenin blanc, Cabernet franc, Mauzac, and Pinot blanc
Spain	Cava	White, rosé	Macabeo, Xarel.lo, Parellada, Pinot noir, and Chardonnay
Italy	Talento	White, rosé	Chardonnay, Pinot nero, and Pinot bianco
Italy	Lambrusco	Red	Lambrusco Grasparossa, Lambrusco Maestri, Lambrusco Marani, Lambrusco Montericco, Lambrusco Salamino, and Lambrusco Sorbara
Australia	Shiraz	Red	Shiraz
(*)	New sparkling	White, rosé	Chardonnay and Pinot noir

(*) Argentina, Australia, Brazil, Chile, New Zealand, South Africa, Uruguay, USA.

Table 2. Non-conventional grape varieties used for sparkling wine production by the traditional method.

Country	Grape Variety	Reference
Argentina	Torrontés Riojano	[6]
Brazil	Goethe	[7,8]
	Manzoni	[7,8]
	Moscatel	[8,9]
	Niagara	[7,8]
	Villenave	[7,8]
	Moscato Giallo	[10]
	Syrah	[11]
Chile	País	[12]
	Sauvignon blanc	[13]

Table 2. Cont.

Country	Grape Variety	Reference
Italy	Bombino bianco	[14]
	Grillo	[15]
	Malvasia di Candia	[16]
	Nero di Troia	[14]
	Maresco	[17]
	Verdicchio	[18]
	Pinot gris	[19]
Portugal	Pignoletto	[19]
	Baga	[20,21]
Romania	Fernão-Pires	[20,21]
	Muscat Ottonel	[22]
Spain	Feteasca Neagra	[23]
	Airén	[24]
	Albarín	[25–28]
	Bobal	[29]
	Garnacha	[25–28,30]
	Godello	[25–28]
	Malvasía	[25–28]
	Monastrell	[30–32]
	Pedro Ximénez	[33]
	Pietro Picudo	[25–28]
	Tempranillo	[24,28,34–36]
Turkey	Verdejo	[25–28]
	Viura	[25–28]
	Dimrit	[37]
	Emir	[38]

2. Non-Conventional Grape Varieties for Sparkling Wine Production Using the Traditional Method

Sugar content (i.e., 16–21 °Brix), titratable acidity (i.e., >8 g/L tartaric acid) and pH (i.e., 2.9–3.2) of grape musts are significant chemical parameters when harvesting grapes destined for sparkling wine production [39,40]. This “ripened” stage is dramatically influenced by the grape variety, and by the soil and climate (i.e., *terroir*) [39]. Organoleptic analyses of grape musts and base wines produced with various non-conventional grape varieties (Table 2) have shown highly valuable chemical and sensory profiles for the production of white, rosé, and red sparkling wines (Table 3), comparable to those obtained by using the gold standard grape varieties (Table 1) [3,4,41]. In addition to market innovation, the use of alternative grape cultivars would enable, for example, development of this industry in geographic areas where climatic conditions do not allow more conventional grape cultivars to reach optimal grape maturity [15].

Table 3. Chemical profiles of base wines obtained using non-conventional grape varieties in sparkling wine production by the traditional method.

Grape Variety	Wine	pH	Titrateable Acidity (g/L)	Volatile Acidity (g/L)	SO ₂ Total (mg/L)	Ethanol (% v/v)	Sugars (g/L)	Ref.
Grillo	White	3.07 ± 0.04	17.03 ± 0.07	0.39 ± 0.05	42.00 ± 2.00	10.05 ± 0.08	1.75 ± 0.04	[15]
Albarin	White	2.79	9.5	0.28	56	11.1	ND	[25]
Viura	White	2.95	8.2	0.16	53	10.7	ND	[25]
Godello	White	2.84	7.6	0.31	64	11.7	ND	[25]
Malvasía	White	2.99	8.0	0.27	65	10.6	ND	[25]
Verdejo	White	2.93	8.2	0.21	56	10.3	ND	[25]
Torrontés Riojano	White	3.3	5.1	ND	ND	10.7	1.83	[6]
Villenave	White	2.69 ± 0.1	9.13 ± 0.23	0.18 ± 0.01	24.72 ± 0.08	10.03 ± 0.05	ND	[7]
Niagara	White	3.11 ± 0.1	5.24 ± 0.09	0.19 ± 0.01	11.28 ± 0.08	10.07 ± 0.02	ND	[7]
Manzoni	White	3.07 ± 0.1	7.59 ± 0.09	0.09 ± 0.02	61.92 ± 0.08	12.07 ± 0.06	ND	[7]
Goethe	White	3.32 ± 0.1	6.03 ± 0.08	0.17 ± 0.02	48.08 ± 0.08	10.47 ± 0.04	ND	[7]
Pedro Ximénez	White	3.24	5.67	0.39	ND	12.4	2.4	[33]
Airén	White	3.36	5.40	0.31	ND	9.49	0.36	[24]
Muscat Ottonel	White	2.90 ± 0.02	6.3 ± 0.07	0.30 ± 0.02	72 ± 0.47	12.5 ± 0.03	3.4 ± 0.13	[22]
Garnacha	Rosé	3.03	7.5	0.18	63	11.8	ND	[25]
Pietro Picudo	Rosé	3.08	8.5	0.17	30	11.5	ND	[25]
Tempranillo	Red	3.7 ± 0.1	4.6 ± 0.2	0.39 ± 0.05	ND	13.0 ± 0.2	ND	[34]
Tempranillo	Red	3.45 ± 0.1	5.5 ± 0.2	0.55 ± 0.06	ND	12.3 ± 0.2	ND	[36]
Tempranillo ¹	Red	3.47	5.10	0.32	ND	11.1	ND	[35]
Tempranillo ²	Red	3.71	4.80	0.20	ND	13.0	ND	[35]
Tempranillo	Red	3.36	5.20	0.48	ND	9.74	2.21	[24]
Chardonnay	White	2.80 ± 0.00	7.5 ± 0.0	ND	ND	11.0 ± 0.1	6.1 ± 0.5	[30]
Pinot meunier	White	2.85 ± 0.01	7.6 ± 0.0	ND	ND	10.9 ± 0.1	ND	[30]
Riesling	White	3.3	6.9	0.26	ND	10.5	ND	[42]

Conventional grape cultivars for sparkling wine production are shown in gray; ¹ Premature and ² mature Tempranillo grapes; ND: not determined.

2.1. White Sparkling Wines

White and/or red grape varieties can be used in the production of white sparkling wines [3,39]. Golden yellow sparkling wines are typically manufactured using white grapes (i.e., *blanc de blanc*) [3,39,40]. On the other hand, when red grape varieties are used, they are vinified as base white wines by eliminating or greatly reducing must maceration, to produce sparkling wines known as *blanc de noirs* [3,39,40]. In addition to traditional grape cultivars (Table 1), several non-conventional varieties are used for the production of white sparkling wine (Tables 2 and 4). Interestingly, Muscat-related sparkling wines have a worldwide distribution [9,10,22], and the volatile profile of these wines has revealed a homogeneous signature for this grape family [9,10,22]. Major classes of volatile compounds in these sparkling wines are terpenes, a predominant class of compounds associated with their floral profiles, and higher alcohols and esters, which contribute to their fruity aromas [9,10,22]. In particular, Moscato Giallo sparkling wines from Brazil have shown high concentrations of 2-phenylethanol, ethyl octanoate, linalool, and α -terpineol [10] (Table 5). Similarly, Romanian Muscat Ottonel sparkling wines show high levels of ethyl octanoate and ethyl decanoate, as well as high levels of linalool, regardless of the yeast used for second fermentation [22] (Table 5). In Argentina, Torrontés Riojano (Listan Prieto × Moscatel de Alejandría), an aromatic cultivar with rich and variegated profiles comparable to that of Muscat in terms of intensity and bouquet [43], is also used for sparkling wine production [6]. The volatile fraction of Torrontés Riojano sparkling wines revealed a high concentration of volatile compounds associated with fruit aromas, as well as terpene compounds associated with the floral Muscat-like aromas [6] (Table 5). In fact, the final sensory properties of Muscat, Moscato Giallo, Muscat Ottonel, and Torrontés Riojano sparkling wines appears to depend on their unique terpenic profiles [5,7,22,44,45]. As shown in Table 5, even if differences in total terpene concentrations are evident between the various Muscat-type varieties, their

total contribution is higher (above 0.5 mg/L) than in other varieties used for sparkling wine production, such as Chardonnay and Riesling [46,47]. In the last sparkling wines, the composition of volatile compounds show a relevant contribution of esters, alcohols, and acetates [46,47].

Other cultivars, such as the white grapes Albarín, Fernão-Pires, Godello, Malvasía, Verdejo, and Viura, as well as the red grapes Bobal, País, and Baga, which are traditional varieties used to produce still wines, have also been employed for the production of high-quality white sparkling wines [12,13,19,25,29] (Tables 3 and 4). Studies on the organoleptic profiles of these grape varieties, their various ripening stages, the influence of the alternative soils, as well as other factors, have been conducted for the production of Spanish (i.e., Albarín, Viura, Malvasía, Verdejo, and Godello), German (i.e., Sauvignon blanc), Chilean (i.e., País), and Portuguese (i.e., Fernão-Pires and Baga) sparkling wines [12,20,21,25–27,51]. The grape variety and/or soil have been shown to significantly influence the relative contribution of various volatile and phenolic compounds in these wines [12,21,25,51]. These parameters influenced their organoleptic profiles more than shorter or longer aging times on lees [12,27]. Although sparkling wines made with Verdejo, Viura, Malvasía, Albarín, and Godello presented excellent physicochemical parameters (Table 4), Albarín and Verdejo wines showed more color and olfactory intensity than those elaborated with the other three grape varieties [25]. Albarín also had the highest contents of catechins, proanthocyanidins, and, together with Viura, hydroxycinnamates, all of which contribute to their phenolic profile [25]. Differences in the abundance and glycosylation profiles of grape glycoproteins could influence foam production and foam quality in non-conventional Sauvignon blanc Sekt versus conventional Riesling Sekt [13]. Portuguese Fernão-Pires sparkling wines show higher contents of volatile compounds that contribute to their varietal character, including linalool, hotrienol, α -terpineol, geraniol, and nerol, than Baga sparkling wines [21]. Baga wines, however, showed a higher maximum foam height than Fernão-Pires [20]. A detailed sensory characterization of Chilean País sparkling wines highlighted strong floral nuances, dependent on ethyl isobutyrate, isoamyl acetate, ethyl hexanoate, and β -phenylethanol, which are high-impact aromatic compounds [12]. Taken together, these studies reveal how the character of non-conventional grape cultivars may influence the innovative arena of sparkling wine production.

Distinctive characteristics of white grape cultivars can also be enhanced, for example, by applying skin pre-fermentative maceration, a technique that is usually performed in red and rosé sparkling wine production [39]. This strategy has been used to manufacture Spanish Pedro Ximenez sparkling wines, increasing the extraction of grape skin compounds that influence their ester profiles [33]. In the study by Ruiz Moreno et al. (2017), wines elaborated with pre-fermentative maceration displayed higher contents of ethyl esters of branched acids and cinnamates, while the absence of maceration rendered higher levels of ethyl esters of fatty acids and higher alcohol acetates [33]. Thus, classic methods used for red wine production may constitute alternative strategies for the elaboration of white sparkling wines with improved organoleptic characteristics.

Table 4. Chemical profiles of sparkling wines.

Grape Variety	Sparkling Wine	Aging Time (Months)	pH	Titrateable Acidity (g/L)	Volatile Acidity (g/L)	SO ₂ Total (mg/L)	Ethanol (% v/v)	Sugars (g/L)	Ref.
Albarin	White	9	2.76	7.2	0.39	35	12.2	ND	[25]
Viura	White	9	2.89	7.4	0.24	43	11.6	ND	[25]
Godello	White	9	2.82	7.2	0.32	43	12.2	ND	[25]
Malvasía	White	9	3.10	7.4	0.28	31	11.6	ND	[25]
Verdejo	White	9	2.94	7.4	0.30	33	11.6	ND	[25]
Torrontés Riojano	White	10	3.25 ± 0.04	4.85 ± 0.09	ND	ND	11.97 ± 0.09	2.07 ± 1.31	[6]
Pedro Ximénez	White	9	3.22	5.59	0.35	ND	12.6	2.4	[33]
Airén	White	4	3.39 ± 0.01	5.17 ± 0.06	0.36 ± 0.00	ND	10.75 ± 0.50	0.08 ± 0.01	[24]
Manzoni	White	18	ND	8.49 ± 0.13	0.36 ± 0.11	ND	10.8 ± 0.2	8.2 ± 0.2	[8]
Villenave	White	18	ND	4.36 ± 0.15	0.55 ± 0.10	ND	10.8 ± 0.3	1.0 ± 0.1	[8]
Moscato	White	18	ND	5.26 ± 0.25	0.63 ± 0.12	ND	12.3 ± 0.4	8.7 ± 0.2	[8]
Niágara	White	18	ND	4.88 ± 0.4	0.57 ± 0.12	ND	9.1 ± 0.2	1.0 ± 0.1	[8]
Goethe	White	18	ND	5.60 ± 0.17	0.75 ± 0.08	ND	11.4 ± 0.4	1.0 ± 0.1	[8]
Muscat Ottonel	White	15	3.1 ± 0.01	6.70 ± 0.02	0.30 ± 0.01	56 ± 0.47	11.6 ± 0.07	0.7 ± 0.02	[22]
Moscato Giallo	White	10	ND	6.69 ± 0.20	0.62 ± 0.05	ND	10.9 ± 0.1	1.10 ± 0.09	[10]
Chenin blanc	White	6	3.42 ± 0.02	9.68 ± 0.17	0.46 ± 0.03	ND	12.35 ± 0.37	2.60 ± 0.08	[11]
Syrah	White	6	3.58 ± 0.02	8.18 ± 0.07	0.48 ± 0.04	ND	13.20 ± 0.30	2.67 ± 0.15	[11]
Pinot gris and Pignoletto	White	12	3.11 ± 0.01	5.51 ± 0.01	0.28 ± 0.01	ND	11.30 ± 0.03	ND	[19]
Garnacha	Rosé	9	2.85	6.9	0.25	44	12.3	ND	[25]
Pietro Picudo	Rosé	9	3.02	7.1	0.29	21	12.5	ND	[25]
Tempranillo	Red	9	3.5 ± 0.1	4.7 ± 0.2	0.32 ± 0.04	ND	13.7 ± 0.2	ND	[34]
Tempranillo	Red	9	3.42 ± 0.1	5.6 ± 0.2	0.52 ± 0.05	ND	13.3 ± 0.2	ND	[36]
Tempranillo ¹	Red	9	3.49	5.2	0.32	ND	12.3	1.50	[35]
Tempranillo ²	Red	9	3.70	4.9	0.30	ND	14.0	1.70	[35]
Tempranillo	Red	4	3.38 ± 0.01	5.03 ± 0.06	0.45 ± 0.01	ND	10.76 ± 0.27	0.12 ± 0.10	[24]
S. blanc	White	18	ND	6.31 ± 0.12	0.65 ± 0.08	ND	12.9 ± 0.2	10.6 ± 0.3	[8]
Riesling Renano	White	18	ND	8.64 ± 0.25	0.40 ± 0.09	ND	11.2 ± 0.3	4.5 ± 0.3	[8]
Pinot grigio	White	18	ND	6.60 ± 0.13	0.71 ± 0.09	ND	10.5 ± 0.2	1.0 ± 0.1	[8]
Pinot noir	White	18	ND	5.33 ± 0.15	0.75 ± 0.10	ND	11.4 ± 0.2	1.0 ± 0.1	[8]
Chardonnay	White	18	ND	6.39 ± 0.12	0.77 ± 0.11	ND	11.8 ± 0.1	1.0 ± 0.1	[8]

Conventional grape cultivars for sparkling wine production are shown in gray; ¹ Pre-mature and ² mature Tempranillo grapes; ND: not determined.

Table 5. Aromatic compounds in Muscat-related and Chardonnay, Pinot noir, and Riesling sparkling wines.

Compound ¹	Muscat Ottonel	Moscato Giallo	Moscato Embrapa	Torrontés Riojano	Chardonnay	Riesling	Pinot Noir	Descriptor	OPT ²
Ethyl hexanoate	ND	748.198 ± 10.000	841.6 ± 29.3	424.7 ± 146.0	744.0 ± 8.0	750.0 ± 40.0	154.1 ± 170.0	Apple, fruit	14 [48]
Ethyl octanoate	7998.72 ± 0.15	1229.184 ± 35.021	954.4 ± 32.1	322.0 ± 160.0	712.0 ± 7.0	670.0 ± 30.0	59.8 ± 6.9	Pineapple, pear, soapy	5 [49]
<i>Sum esters</i> ³	10,634.41	2614.719	2569.9	10,515 ± 195	75,921.6	28,771	267.4		
2-Phenylethanol	ND	8226.558 ± 12.664	11824.2 ± 162.7	29.9 ± 6.7	11.6 ± <0.1	9.1 ± 0.1	2990.5 ± 163.8	Rose, honey, woody	14,000 [8]
Nerol oxide	ND	26.303 ± 2.516	84.9 ± 5.3	88.6 ± 48.0	ND	ND	15.5 ± 2.5	Flower	400 [8]
Linalool	138.86 ± 0.06	1732.887 ± 7.311	169.0 ± 28.0	7.7 ± 4.0	<5	11.2 ± 0.1	42.2 ± 2.7	Flower, lavender	0.8 [50]
α-Terpineol	42.79 ± 0.40	1211.424 ± 11.521	97.1 ± 8.4	166.8 ± 23.0	3.4 ± <0.1	25.8 ± 0.3	9.5 ± 1.5	Citrus	250 [8]
<i>Sum terpenes</i> ³	181.65	3659.781	588.1	2890 ± 187	28	89.3	98.1		

¹ Ester and terpenic compounds (µg/L) identified in Muscat Ottonel (Ref. [22]), Muscat Giallo (Ref. [10]), Moscato Embrapa (Ref. [8]), Torrontes Riojano (Ref. [6]), Chardonnay (Ref. [42]), Riesling (Ref. [42]), and Pinot noir (Ref. [8]). ² OPT (Odor Perception Threshold; µg/L) and references are indicated. ³ Total esters and terpenes as reported in the references. Conventional grape cultivars for sparkling wine production are shown in gray. ND: not determined.

In warm and dry climates, like traditional Mediterranean winemaking regions, harvesting at optimal grape maturity may be associated with insufficient acidity for sparkling wine production [39,40]. Innovative base wines manufactured with Maresco and Grillo grape musts, however, show high total acidity even when fully ripened grapes are used [15,17]. Maresco grapes, a minor variety grown in the Apulia region of Italy, besides high acidity values, also showed floral, fruity, and fatty notes, as a result of the presence of linalool and phenyl acetate (floral descriptors), isoamyl acetate, ethyl hexanoate, ethyl octanoate, and ethyl decanoate (fruity descriptors), and octanoic acid (fatty descriptors) [17]. Sicilian Grillo grape musts, also from a dry Mediterranean region, have shown optimal concentrations of tartaric and malic acids, with the experimentally produced wines presenting remarkable values of total acidity and low pH values (Table 3) [15]. Similarly, in tropical semi-arid winemaking regions in Northeastern Brazil (i.e., São Francisco Valley), Syrah and Chenin Blanc are adapted varieties for the production of high quality sparkling wines (Table 4) [11]. These Syrah (*blanc de noir*) and Chenin Blanc (*blanc de blanc*) wines showed different varietal-related volatile profiles: Chenin Blanc was characterized by the presence of 2,3-butanediol, 3-ethoxypropan-1-ol, diethyl succinate, and ethyl decanoate, while Syrah by benzaldehyde, butyric acid, and some acetates [11]. Interestingly, due to high temperatures, solar radiation rates, and irrigation throughout the year, one vine can produce two harvests per year in the São Francisco Valley region [11].

In geographic regions where the agricultural conditions do not favor the proper development of most *V. vinifera* grapes, *V. non-vinifera* species and their hybrids are commonly cultivated [52]. In Brazil, non-*vinifera* grape varieties (e.g., Villenave -*V. labrusca* × *Riesling renano*-, Niagara, Goethe) and innovative *V. vinifera* varieties, such as Manzoni Bianco, mostly employed in the elaboration of grape juices and wines, express interesting characteristics of aroma and a good balance between acidity and sugar content, resulting in appealing alternatives for sparkling wines [7–9]. In this context, studies of changes in phenolic composition, browning index, and glutathione content during 18 months of biological aging *sur lies*, for sparkling wines produced with Villenave, Niagara, Manzoni, and Goethe cultivars, revealed highly suitable changes in the phenolic profiles during the aging period [7]. For example, (+)-catechin and (–)-epicatechin, flavanols with an important influence on the astringency and color of wines, show concentrations of 1.31–14.05 mg/L and 2.64–5.70 mg/L, respectively, in these non-conventional sparkling wines [7]. Catechin and epicatechin levels of 3.52–5.80 mg/L and 1.56–2.15 mg/L, respectively, were measured in sparkling wines made with Sauvignon blanc, Riesling Renano, Pinot Grigio, Pinot noir, and Chardonnay [7]. These studies show that non-*vinifera* grape varieties could enable sparkling wine production in latitudes where *V. vinifera* cultivars are more sensitive to different pests, environmental stresses and/or high annual rainfall [15].

2.2. Rosé Sparkling Wines

For the production of rosé sparkling wines, red grape pressing conditions and/or maceration strategies render base wines with varying colors, depending on the concentration of color-determining compounds extracted from the grape skins [3]. The impact of various non-conventional red grape varieties on the quality of the resulting rosé sparkling wines has been evaluated. Pioneer studies using the red varieties Trepát and Monastrell, for the manufacture of rosé sparkling wine in Spain, largely contributed to the current use of these grapes for Cava production [31] (Tables 3 and 4). These original studies showed that rosé Trepát was more similar to the white Cava wines (using a conventional blend of Xarel.lo, Macabeo, and Parellada) than to the rosé wines manufactured with Monastrell [31]. Organoleptic characterization of these sparkling wines showed that Trepát and Monastrell had very good sensory attributes and even slightly better foam profiles than the white Cava [31]. Interestingly, it was also reported that the foamability and color intensity of the sparkling wines significantly increased when Trepát base wines were blended with white varieties (Macabeo, Xarel.lo, and Parellada) to manufacture Cava [32]. Thus, these qualities of Trepát grapes validated their use for elaborating either *blanc de noirs* sparkling wines or

novel versions of rosé Cava, and highlighted the value of autochthonous grape varieties for sparkling wine production.

Other Spanish cultivars such as Prieto Picudo, Tempranillo, and Garnacha, traditionally used to produce still wines, have also been characterized for their potential for manufacturing high quality rosé sparkling wines using the traditional method [25,27,28,53] (Tables 3 and 4). These wines are rich in typical ethyl esters and alcohol acetates that contribute to their fruity aroma [51] and preserve their varietal characteristics, even after a long aging time (i.e., thirty months) [27]. Furthermore, the sensory and analytical characteristics of five Garnacha Tinta rosé sparkling wines, where second fermentation was performed using five alternative yeast strains, showed that their organoleptic properties mostly depend on the grape variety itself rather than on the strain used for second fermentation [53]. Garnacha rosé has also shown high phenolic potential and hydroxycinnamic acid concentrations, while Prieto Picudo rosé sparkling wines had high color intensity and anthocyanin concentrations, with high olfactory intensity and freshness in sensory analyses [25]. Also, these sparkling wines, besides optimal chemical parameters (Table 4), presented good foaming characteristics, similar to high quality sparkling wines like Champagne and Cava, with low levels of biogenic amines, which are desirable attributes in sparkling wine production [25,27,51,53].

2.3. Red Sparkling Wines

Red sparkling or semi-sparkling wines represent a small fraction of the sparkling wine production worldwide, and are mostly characterized by a slight red color and a relatively poor complexity [34,35], because red grapes harvested at the proper time for sparkling wine production (i.e., <21 °Brix), have not yet achieved adequate phenolic maturity and, due to the relatively low final content of alcohol (i.e., 10.0–11.5% *v/v*; Table 3), the extraction of phenolic compounds and aroma precursors from the grape berries into the base wine is relatively low [35]. To face many of the challenges associated with red sparkling wine production, various agricultural and enological practices have been proposed, including alternative harvest dates, mixing of different grape varieties, pre-fermentative cold maceration, rack and return, carbonic maceration, and treatment of pre-mature grapes by applying pectolytic enzymes [23,34–36].

The impact of Tempranillo grape maturity on the alcohol concentrations, acidity, pH, and color intensity of red sparkling wine production and aging definitely depends on the ripening stage of grapes (Tables 3 and 4). Levels of soluble polysaccharides and oligosaccharides in Tempranillo base wines also increased with grape maturity, suggesting that these compounds are more easily extracted during maceration-fermentation [35]. Following these results, Pérez-Magariño et al. (2019) applied pre-fermentative cold maceration of pre-mature Tempranillo grapes, using dry ice to favor the extraction of skin compounds into the must [34]. The resulting wines showed a volatile composition similar to those produced from mature grapes, and also had good values in foam and sensory descriptors [34]. The study, however, also showed that the differences in overall volatile composition of the wines were impacted more by grape maturity than by the use of enological techniques [34]. In fact, sparkling wines made with mature Tempranillo grapes, despite their high alcohol content, showed better volatile composition and foam characteristics than those obtained from pre-mature Tempranillo grapes [34]. The use of pectinolytic enzymes and/or carbonic maceration on premature Tempranillo grapes, intended to contribute to the extraction of polyphenols and varietal aromas, did not improve the chemical and sensory qualities of the sparkling wines compared with those manufactured using mature Tempranillo grapes [36]. Moreover, Tempranillo red sparkling wines made with mature grapes have shown high contents of polyphenols, ethyl esters, alcohol acetates, and total volatile acids, as well as foam stability [34,36], while wines made using unripe grapes have vegetal aroma notes [34,36]. These studies suggest that mature grapes and traditional winemaking practices are options to be preferred for elaborating red sparkling wines using the traditional method (Tables 3 and 4).

3. Indigenous Yeast Starters for First and Second Fermentation of Sparkling Wines Using Non-Conventional Grape Varieties and the Traditional Method

Yeast starters used in both grape must fermentations and secondary fermentations of base wines are exposed to highly hostile physicochemical environments. Grape juices and musts destined for base wine production have high osmotic pressure (i.e., 16–21 °Brix), high titratable acidity (i.e., >8 g/L tartaric acid), low pH (i.e., 2.9–3.2), growing ethanol and glycerol amounts, presence of sulfites, and progressive consumption of nutrients [2]. On the other hand, yeast starters used for second fermentation must meet additional technological requisites to those needed for first fermentation [2]. Second fermentation of base wines in bottles requires yeast strains capable of growing under high ethanol contents (10–12% *v/v*), low pH (2.9–3.2), low temperature (10–15 °C), high CO₂ pressure (up to 6 atmospheres), relatively high levels of SO₂ (50–80 mg/L), and high total acidity (7–10 g/L; measured as tartaric acid) (Table 3) [2]. In addition, these second-fermentation yeast strains should be able to properly flocculate and autolysate during aging [1,3,54,55], allowing the wines to remain in contact with the lees (i.e., mostly yeast cell debris and clarifying agents), shaping their sensory complexity [4,56,57]. The aging process also contributes to other sparkling wine properties such as foam (i.e., maximum height and stability) and bubble (i.e., size and persistence) profiles [25,27,39,58]. Thus, because sparkling wine organoleptic properties largely correlate with the physiological and metabolic characteristics of the yeasts used for fermentation, a proper selection of starters is essential [14,54,59].

Although a wide variety of *Saccharomyces cerevisiae* strains are commercialized for first and second fermentation of grape musts and base wines, respectively, there is increased interest in indigenous *S. cerevisiae* and non-*Saccharomyces* yeasts to improve the sensory attributes of the resulting wines [15,54,59]. Producing both base and sparkling wines with native yeast starters can give typicality to the wines and may offer valuable technological alternatives to the application of commercial starters that can lead to wine flavor standardization [54,59]. Interesting technological screening methods have been proposed to identify, characterize, and select novel and indigenous yeast strains with valuable potential in the sparkling wine industry [2,6,14,54,57,59–61]. Enological and technological traits (i.e., fermenting power and vigor, SO₂ tolerance, alcohol tolerance, flocculence, production of acetic acid, glycerol, H₂S, and volatile compounds, autolytic capacity, and foaming properties), sensory analyses, and genotypic screenings are currently being used as selection strategies for these yeast strains [6,14,54,57,59,60] (Figure 2). As a result, various non-commercial *S. cerevisiae* strains for first and second fermentation have been proposed to provide regional character to sparkling wines as a driver of innovation/segmentation in this market [14,54,57,59,60].

Producing sparkling wine with the use of alternative indigenous yeast strains renders wines with varying organoleptic profiles [14,15,60]. What is more, native *S. cerevisiae* strains have revealed similar [53,59] and even improved [54,62] physicochemical parameters when compared with commercial starters for second fermentation [53,54,59,62] (Figure 2). Studies by Di Gianvito et al. (2018) compared the second fermentation performance of the commercial strains FI (i.e., a mixture of strains IOC-18-2007 and FRC) and EC1118 with six indigenous Italian *S. cerevisiae* wine strains (i.e., F6789, F6030, F10599, F10477, F10471, and F7101), selected on the basis of their degree of flocculation and autolytic capacity [54]. Their results showed that the indigenous yeast strains had an enological performance comparable, in terms of fermentation rate and the maximum pressure reached, to the commercial strains [54]. This study also highlighted the phenotypic differences of some yeasts, in terms of their autolysis profiles, as well as the different levels of aromatic compounds released after six months of aging [54]. Sparkling wines obtained with strains F10471 and F10477 presented the highest amount of esters (e.g., 3-methylbut-1-yl ethanoate, ethyl ethanoate, ethyl octanoate, ethyl decanoate and ethyl dodecanoate), which can be attributed to the strong autolytic ability of these strains [54]. In similar studies, Martí-Raga et al. (2016) showed that Cava wines fermented with the indigenous yeast strain P29, isolated from the Penedes grape-growing region in Spain, reached a remarkable foam

height and sensory profiles compared to sparkling wines elaborated using the commercial *S. cerevisiae* strains PDM and ARM [62].

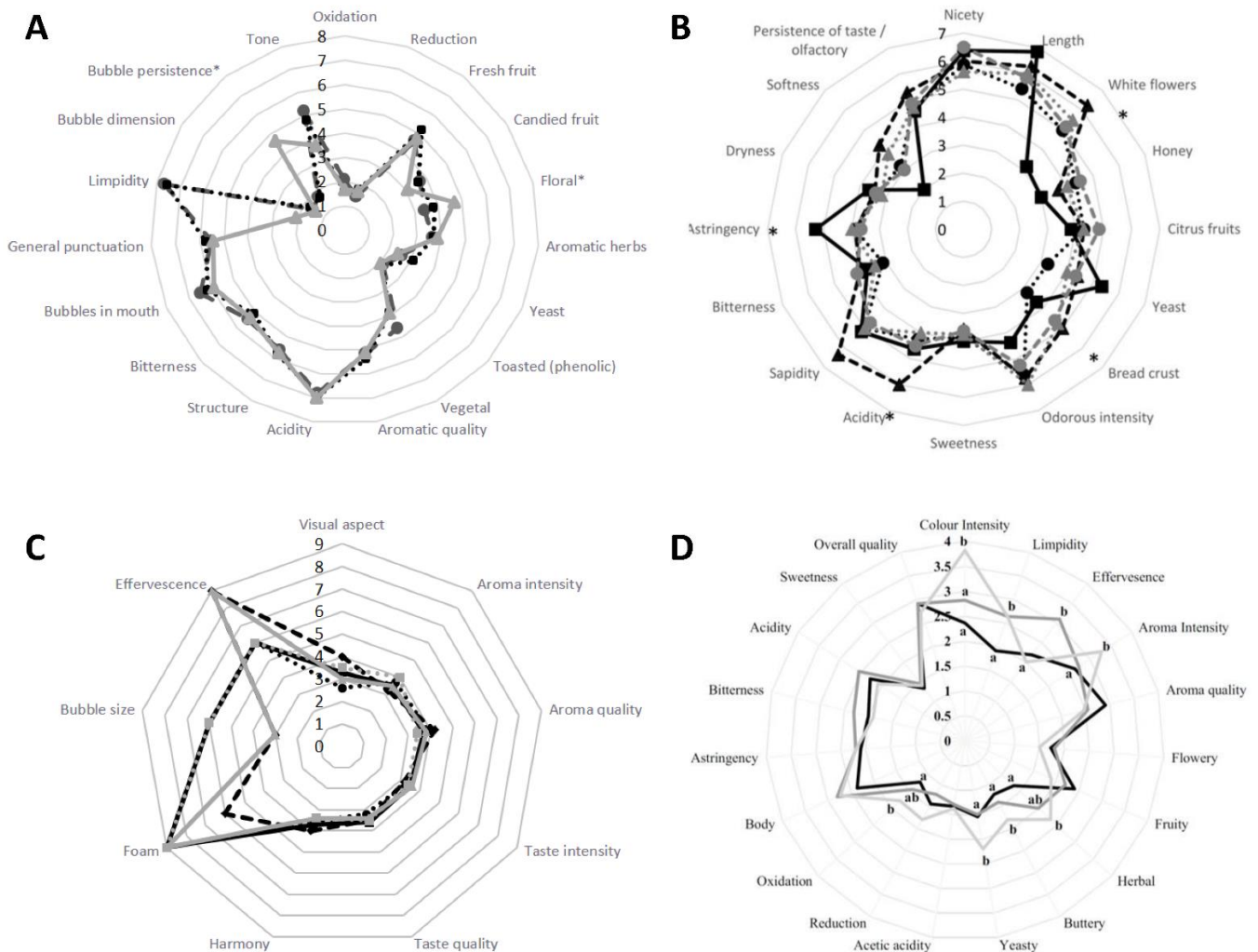


Figure 2. Influence of yeast starters, used in secondary fermentation of base wines, on the sensory qualities of white, rosé, and red sparkling wines. (A) Mean ratings of the attributes of Torrontés Riojano white sparkling wines after 10 months of aging on lees, obtained using commercial yeasts EC1118 (gray dashes), bayanus C12 (black dots), and mutant *S. cerevisiae* strain IFI473I (gray line). (A) was prepared based on data from reference [6]. (*) Attributes with significant differences between treatments ($p < 0.05$). (B) Mean ratings of the attributes of Verdicchio white sparkling wines obtained using the following single or combined starters: *S. cerevisiae* DiSVA 527 (black line), *T. delbrueckii* DiSVA130 (black dashes), DiSVA 313 (black dots), *S. cerevisiae* DiSVA 527 plus *T. delbrueckii* DiSVA 130 (gray dashes), and *S. cerevisiae* DiSVA 527 plus *T. delbrueckii* DiSVA 313 (gray dots). The figure was licensed from reference [18]. (*) Attributes with significant differences between treatments ($p < 0.05$). (C) Mean ratings of the attributes of Garnacha tinta rosé sparkling wines obtained with the following yeast starters: *S. cerevisiae* IMIA-2010 (black line), IMIA-2011 (black dashes), IMIA-2012 (black dots), IMIA-2013 (gray dashes), and EC1118 (gray line). The figure was prepared based on data from reference [53]. (D) Mean ratings of the attributes of Tempranillo red sparkling wines obtained using the following yeast starters: *S. cerevisiae* 7VA (black line), *Saccharomyces ludwigii* 979 (dark gray line), and *Schizosaccharomyces pombe* 938 (light gray line). Values in the same line with the same letter are not significantly different ($p < 0.05$). The figure was licensed from reference [24].

In a detailed study of first and second fermentation of Apulian grape varieties (i.e., Nero di Troia and Bombino bianco) using native yeast strains for the production of white and rosé sparkling wines, Garofalo et al. (2018) reported a yeast strain-dependent profile of the release of volatile compounds [14]. Interestingly, they also showed that alternative native yeast strains influenced the final CO₂ pressure values of white Bambino bianco

sparkling wines, confirming the suitability of native *S. cerevisiae* strains for improving the quality of these wines [14]. Indigenous Italian yeast strains have also been tested for the production of Grillo base wines in dry Italian Mediterranean climates [15]. These native strains (i.e., CS182, GR1, MSE13 and MSE41) showed their ability to start fermentation at low pH and in the presence of high amounts of organic acids, contributing to some herbaceous and vegetal, floral, and exotic fruit descriptors that clearly distinguished each of the Grillo base wines produced [15]. Taken together, these results support the idea that indigenous *S. cerevisiae* yeasts could be exploited as starter cultures, differentiating between sparkling wines, and linking them with their region of production.

Besides the selection of native *S. cerevisiae* strains, *Saccharomyces non-cerevisiae* and non-*Saccharomyces* yeasts have also been proposed as alternatives for improving the enological features and flavor complexity of second fermentations of base wines [37,38,63,64]. *Saccharomyces bayanus* and *Saccharomyces oviformis* have been used both in free forms and immobilized within coated alginate beads as inoculums for the second fermentation of Turkish Emir and Drimit sparkling wines [37,38]. During the second fermentation of Emir and Drimit base wines, no significant differences were found in free amino acids and amino acids in peptides between the use of immobilized and free yeasts [37,38]. However, significant differences in these compounds were found due to the aging time and the yeast strains used [37,38]. Free amino acids were higher in the Emir sparkling wine made with *S. oviformis* than with *S. bayanus* [38], and in the Drimit sparkling wine fermented with *S. cerevisiae* than with *S. bayanus* [37].

Among non-*Saccharomyces* species, *Metschnikowia pulcherrima* (Flavia® strain) and *Torulaspora delbrueckii* (Biodiva™ strain) have been tested, in sequential inoculations with *S. cerevisiae*, for their impact on the composition and quality of Macabeo base wine for sparkling wine production [64]. This study showed that base wines fermented with the addition of *M. pulcherrima* resulted in an increase in foam persistence and changes in the aromatic profile, characterized by smoky and flowery notes [64]. Sequential addition of *T. delbrueckii* increased glycerol concentration, reduced volatile acidity, and exerted a positive effect on foaming properties when compared with control base wines fermented with *S. cerevisiae* [64]. Further studies showed that the foaming properties of the sparkling wines obtained by sequential inoculation with these *T. delbrueckii* and *S. cerevisiae* strains resulted in sparkling wines with significantly higher maximum foam heights than conventional *S. cerevisiae* inoculation, probably due to the autolysis of *T. delbrueckii* cells in the base wine [65]. Another report used *T. delbrueckii* strains (i.e., Td130/313) to conduct second fermentations of Verdicchio base wine, both in mixed fermentations with *S. cerevisiae* and as pure inocula [18] (Figure 2B). The *T. delbrueckii* strains tested were able to complete the secondary fermentation and confirmed the previously reported behavior of *T. delbrueckii* in still wines, leading to a reduction in acetaldehyde and some higher alcohols, and increasing the production of ester compounds [18]. Thus, *T. delbrueckii* sparkling wines showed overall different aromatic compositions and sensory profiles to those of pure *S. cerevisiae* starters, with higher scores for positive aromatic descriptors [18] (Figure 2B).

Finally, physicochemical properties and sensory evaluations of sparkling wines made by second fermentation in bottle with *Saccharomyces ludwigii* and *Schizosaccharomyces pombe* have been studied by Ivit et al. (2018) [24] (Figure 2D). These non-*Saccharomyces* yeasts properly completed the second fermentation of Airén white base wine and of Tempranillo red base wine, showing significant differences in acidity parameters, non-volatile compound levels, and sensory evaluations compared to control sparkling wines produced with *S. cerevisiae* [24] (Figure 2D). These studies demonstrated the potential of non-*Saccharomyces* yeasts to develop flavor complexity in sparkling wine production. The wide diversity of non-*Saccharomyces* species and strains constitute a great resource in the arena of sparkling wine innovation. Further studies are needed to define the potential of these non-*Saccharomyces* yeast starters [2].

4. Conclusions

Innovation and diversification in the manufacturing of high-quality sparkling wines is largely associated with the use of non-conventional grape varieties and yeast starters. A worldwide repertoire of prestigious grape cultivars, traditionally used in the production of high quality still wines, has demonstrated suitable enological properties for elaborating white, rosé, and red sparkling wines. These non-conventional grape varieties can positively contribute to the sensory diversification of sparkling wines, while showing comparable physicochemical profiles to Champagne and Cava. The diversity of these non-conventional grape varieties opens a window of opportunities for winemakers to innovate and satisfy consumer expectations. Novel and/or indigenous yeast starters for first and/or second fermentation can make interesting contributions. Native *S. cerevisiae* strains and/or *S. non-cerevisiae* and/or non-*Saccharomyces* yeasts offer new tools for innovation. The creative use of alternative technological operations in winemaking would also contribute to diversifying the sensory profiles of the growing number of non-conventional sparkling wines.

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