Commercially Non-Saccharomyces Yeasts for Winemaking

Subjects: Food Science & Technology Contributor: Ricardo Vejarano

About 42 commercial products based on non-Saccharomyces yeasts are estimated as available on the market. being mostly pure cultures (79%), with a predominance of Torulaspora delbrueckii, Lachancea thermotolerans, and Metschnikowia pulcherrima. The others are multi-starter consortia that include non-

Saccharomyces/Saccharomyces mixtures or only non-Saccharomyces species. Several commercial yeasts have shown adequate biocompatibility with S. cerevisiae in mixed fermentations, allowing an increased contribution of metabolites of oenological interest, such as glycerol, esters, higher alcohols, acids, thiols, and terpenes, among others, in addition to a lower production of acetic acid, volatile phenols, biogenic amines, or urea. The studies conducted to date demonstrate the potential of these yeasts to improve the properties of wine as an alternative and complement to the traditional S. cerevisiae.

commercial non-Saccharomyces yeasts winemaking

biocompatibility

wine quality

1. Introduction

Traditionally, non-Saccharomyces yeasts have been considered as contaminants due to the production of undesirable metabolites by many of the species currently known. This aspect has been changing in recent years due to the growing interest in certain strains that contribute with metabolites that positively impact wine.

Most non-Saccharomyces yeasts are characterized by their low fermentative power and low ethanol tolerance, especially in the presence of SO₂ ^[1]; thus, to ensure a correct end of the fermentative process, its use necessarily requires the implementation of mixed fermentations (simultaneous or sequential) together with Saccharomyces cerevisiae ^[2].

In the last 20 years, this scenario has resulted in the search for and selection of new strains by the scientific community, as evidenced in the high number of publications related to non-Saccharomyces yeasts of oenological interest as well as in the development and market launch by commercial houses of products based on these selected non-Saccharomyces strains in various formats included in Resolution OIV-OENO 576B-2017 of the International Organization of Vine and Wine (OIV), which includes active dry yeast (ADY, dry matter > 92%), active frozen yeast (AFY, dry matter 40-85%), compressed yeast (COY, dry matter 30-35%), and cream yeast (CRY, dry matter 18-25%), in addition to encapsulated yeasts (pearls) or immobilized yeasts (ENY) with more than 86% dry matter. Additionally, several commercial products in the form of fresh liquid yeast (FLY) have been identified from an online review.

2. Non-Saccharomyces Yeasts Available on the Market

Various reviews have addressed aspects such as the metabolic characteristics and the most important contributions of non-*Saccharomyces* to wine ^{[3][4][5]}, improvement in wine properties such as acidity, and its influence on various oenological parameters ^[4], as well as statistical information regarding the providing companies, more commercialized species, quantity of commercial strains, regulations, and patents ^[5], among other aspects.

Based on an Internet search, as a part of this study, it is estimated that about 42 commercial products based on non-*Saccharomyces* yeasts are available for winemaking in different formats (**Figure 1**), of which 52% are represented by three species: *Torulaspora delbrueckii*, *Lachancea thermotolerans*, and *Metschnikowia pulcherrima*. In addition, 79% are marketed as pure cultures (monoculture) and the remaining available products are offered as multi-starters (blends of various yeast species). Four companies produce 52% of the supply.



Figure 1. (a) Percentages of non-*Saccharomyces* based products offered by main companies (adapted from Roudil et al. ^[5] with updated information). (b) Formats (%) in which non-*Saccharomyces* are offered. (c) Number of

commercial products based on non-Saccharomyces yeasts available in the market, by species.

According to the scientific literature, the use of non-*Saccharomyces* at the industrial level is still a pending issue since most applications have been conducted on the experimental scale. This indicates that the knowledge of these yeasts is still a field requiring development, considering the growing interest from the oenological sector in the production of wines with differentiated profiles, as reported by Roudil et al. ^[5], who described the evolution of the supply of these commercial yeasts in recent years. Several companies supply these yeasts for oenological applications (**Table 1**).

Table 1. Commercial non-*Saccharomyces* yeasts available on the market. Information obtained from the website of the companies that commercialize them (ADY: active dry yeast, CRY: cream yeast, AFY: active frozen yeast, ENY: encapsulated yeast, FLY: fresh liquid yeast).

Yeast Species	Commercial Brand	Providing Company (Country)	Format
Torulaspora delbrueckii	Biodiva TD291	Lallemand (Canada)	ADY
	Prelude	CHR Hansen (Denmark)	ADY
	Zymaflore Alpha	Laffort (France)	ADY
	Viniferm NSTD	Agrovin (Spain)	ADY
	EnartisFerm Qτ	Enartis (Italy)	ADY
	EnartisFerm Qτ Liquido	Enartis (Italy)	CRY
	Oenovin Torulaspora BIO	Oeno (Italy)	ADY
	Torulaspora delbrueckii	Probiotec (Italy)	FLY
	Torulaspora delbrueckii 12.2	Probiotec (Italy)	FLY
Lachancea thermotolerans	Laktia	Lallemand (Canada)	ADY
	Concerto	CHR Hansen (Denmark)	ADY
	Octave	CHR Hansen (Denmark)	ADY

Yeast Species	Commercial Brand	Providing Company (Country)	Format
	EnartisFerm Qƙ	Enartis (Italy)	CRY
	Excellence X'Fresh	Lamothe-Abiet (France)	ADY
	LEVULIA Alcomeno	AEB Group (Italy)	ADY
	Kluyveromyces thermotolerans	Probiotec (Italy)	FLY
Metschnikowia pulcherrima	Flavia MP346	Lallemand (Canada)	ADY
	Oenoferm MProtect	Erbslöeh (Germany)	ADY
	AWRI Obsession	AB Biotek (United Kingdom)	ADY
	LEVULIA Pulcherrima	AEB Group (Italy)	ADY
	Primaflora VB BIO	AEB Group (Italy)	ADY
	Excellence B-Nature	Lamothe-Abiet (France)	ADY
Metschnikowia fructicola	Levia Nature	Oeno (Italy)	ADY
	Gaïa	Lallemand (Canada)	ADY
Schizosaccharomyces pombe	Atecrem 12H	BioEnologia (Italy)	CRY
	Promalic	Proenol (Portugal)	ENY
Wicheranomyces anomalus	Anti Brett 1	Probiotec (Italy)	FLY
Kluyveromyces wickerhamii	Anti Brett 2	Probiotec (Italy)	FLY
Starmerella bacillaris	Atecrem 11H	BioEnologia (Italy)	CRY
Zygosaccharomyces bailii	Fructoferm W3	Lallemand (Canada)	ADY
Zygosaccharomyces parabailii	Hardened Spaniard	Mainiacal Yeast (United States)	FLY

Yeast Species	Commercial Brand	Providing Company (Country)	Format
Pichia kluyveri	Frootzen	CHR Hansen (Denmark)	AFY
	Pichia kluveri MIP- 001	Propagate Lab (United States)	FLY
Pichia kluyveri + Kazachastania servazzii	Trillyeast	BioEnologia (Italy)	CRY
Torulaspora delbrueckii + Saccharomyces cerevisiae	Oenoferm Wild & Pure	Erbslöeh (Germany)	ADY
Torulaspora delbrueckii + Saccharomyces cerevisiae	New Nordic Ale Yeast	White Labs (United States)	FLY
Torulaspora delbrueckii + Metschnikowia pulcherrima	Zymaflore Égide	Laffort (France)	ADY
Metschnikowia pulcherrima + Saccharomyces cerevisiae	Primaflora VR BIO	AEB Group (Italy)	ADY
Lachancea thermotolerans + Saccharomyces cerevisiae	Symphony	CHR Hansen (Denmark)	ADY h
Lachance thermotolerans + Saccharomyces cerevisiae	Rhythm	CHR Hansen (Denmark)	ADY 1
Lachancea thermotolerans + Torulaspora delbrueckii + Saccharomyces cerevisiae	Harmony	CHR Hansen (Denmark)	ADY
Lachancea thermotolerans + Torulaspora delbrueckii + Saccharomyces cerevisiae	Melody	CHR Hansen (Denmark)	ADY r

with a high sugar content (between 30 and 42 °Brix), used to produce the Italian wines Amarone and Santo ^{[8][9]}, which makes it suitable for producing late-harvest wines. However, the commercial strain *T. delbrueckii* Zymaflore Alpha (Laffort, Bordeaux, France) does not show capacity to finish the fermentative process in Sauvignon Blanc musts, reaching only 6.2% *v/v* of ethanol and residual sugars > 100 g/L ^[10].

T. delbruekii shows the capacity to produce other metabolites of oenological interest, such as glycerol. Biodiva TD291 ^[11] and Oenoferm Wild & Pure (Erbslöh, Geisenheim, Germany) ^[12] show a high production of glycerol in sequential fermentations with *Saccharomyces*, indicating the potential of these yeasts to improve complexity and mouth-feel. Biodiva TD291 also shows less, or similar production, of acetic acid and volatile acidity compared to pure *S. cerevisiae* ^{[9][11][13]} as shown in **Table 2**.

2.2. Lachancea Thermotolerans

Lachancea thermotolerans was previously known as *Kluyveromyces thermotolerans*. The main advantage of this yeast is its capacity to produce lactic acid, improving the acidity of wine, along with its intensity and aromatic complexity [3][14], especially in sequential fermentations with *S. cerevisiae* [14]. In Riesling [15] and Tempranillo wines

^[16], *L. thermotolerans* Concerto (CHR Hansen) has shown capacity to increase the production of lactic acid compared to the traditional process that integrates alcoholic fermentation and malolactic fermentation (AF + MLF) (**Table 2**). The increase in lactic acid in Tempranillo contributed to improve the sensation of acidity and the sensory acceptability.

Another important contribution of *L. thermotolerans* Concerto is the highest production of pyruvic acid in sequential fermentations with *S. cerevisiae* and with *Schizosaccharomyces pombe* in Tempranillo wines ^[16] compared to the traditional AF + MLF process (**Table 2**). The increase in pyruvic acid also contributed to improve the color of the wines due to the synthesis of vitisin A, which is characterized by its increased stability ^[17].

In sequential fermentation with *S. cerevisiae*, *L. thermotolerans* Concerto showed a higher production of glycerol than *S. cerevisiae* ^[2]. In ternary fermentations (simultaneous fermentations), the multi-starter Melody (CHR Hansen), which contains *L. thermotolerans* ^[2], showed the capacity to reduce the alcohol content in Syrah wines (**Table 2**).

The improvement in acidity with *L. thermotolerans* can be a strategy that contributes to improving the composition and sensory profile in wines produced from grapes grown in warm regions where high temperatures may generate low acidity in the grape berries ^[18].

Table 2. Main changes produced by commercial non-*Saccharomyces* yeasts in the synthesis of metabolites, with their impact on the composition and sensory profile of the wine (increase \uparrow or decrease \downarrow in the content of each compound).

Commercial Yeast	Level	Fermentation	TYPE OF WINE	Changes with Respect to S. cerevisiae	Sensory Impact with Respect to S. cerevisiae	Reference
<i>Torulaspora delbrueckii</i> Biodiva TD291 (Lallemand, Montreal, QC, Canada)	Semi- industrial (Two wineries: 150 and 250 L)	Sequential and simultaneous + S. cerevisiae Lalvin EC1118 (Lallemand, Montreal, QC, Canada)	Amarone (Corvina, Rondinella, and Corvinone red grapes)	 2-phenylethanol; ethyl butyrate, ethyl lactate, isoamyl lactate; 4- carbethoxy-γ- butyrolactone, sherry lactones; α- terpineol, Ho- diendiol I, and endiol isoamyl acetate 	Higher aroma intensity, fruitiness, sweetness, ripe red fruit (cherry)	[<u>8]</u>
<i>Torulaspora delbrueckii</i> Biodiva TD291 (Lallemand, Montreal, QC, Canada)	Laboratory	Sequential + S. cerevisiae 734 *	Gewürztraminer	↑ linalool (OAV ≈ 1.0) ↓ citronellol and geraniol	Higher overall score (balance between terpenes)	<u>[19]</u>
Torulaspora delbrueckii Zymaflore Alpha	Semi- industrial	Sequential + <i>S. cerevisiae</i> Lalvin	Soave (Garganega	↑ 2-phenylethanol; diethyl succinate	Both wines: higher aroma intensity	[<u>9]</u>

Commercial Yeast	Level	Fermentation	TYPE OF WINE	Changes with Respect to S. cerevisiae	Sensory Impact with Respect to S cerevisiae	. Reference
(Laffort, Bordeaux, France) <i>Torulaspora delbrueckii</i> Biodiva TD291 (Lallemand, Montreal, QC, Canada)	(150 L)	EC1118 (Lallemand, Montreal, QC, Canada)	white grape) and Chardonnay	↓ 4-vinylguaiacol and 4-vinylphenol: with Alpha in both wines (4- vinylguaiacol OAV < 1.0) ↓ isoamyl acetate: Soave wine with Alpha; Chardonnay wine with Alpha and Biodiva)	and persistence, complexity, and body Better floral and tropical fruit attributes (especially in Soave wine)	
<i>Torulaspora delbrueckii</i> Biodiva TD291 (Lallemand, Montreal, QC, Canada)	Laboratory (500 mL)	Sequential + <i>S. cerevisiae</i> Lalvin EC1118 (Lallemand, Montreal, QC, Canada)	Santo (Sweet white wine from Nosiola grape)	 † 2-phenylethanol; ethyl lactate; sherry lactones ↓ 4-vinylphenol and ↓ vinylguaiacol ↓ isoamyl acetate † 3-methylthio-1- propanol 	Sensory analysis not performed	[<u>9]</u>
<i>Torulaspora delbrueckii</i> Zymaflore Alpha (Laffort, Bordeaux, France)	Laboratory (1.2 L)	Sequential and simultaneous + S. cerevisiae Zymaflore X5 (Laffort, Bordeaux, France)	Sauvignon Blanc	↑ isoamyl acetate (OAV > 1.0), isobutyl acetate, 2- phenylethyl acetate, ethyl isobutyrate, ethyl propanoate, ethyl dihydroxycinnamate	Sensory analysis not performed	[<u>10]</u>
<i>Torulaspora delbrueckii</i> Zymaflore Alpha (Laffort, Bordeaux, France)	Semi- industrial (150 L)	Sequential + S. cerevisiae Zymaflore FX10 (Laffort, Bordeaux, France)	Merlot	↑ isoamyl acetate (OAV > 1.0), ethyl isobutyrate (OAV > 1.0), isobutyl acetate, ethyl propanoate, ethyl dihydroxycinnamate	Higher complexity and fruity notes (interaction between esters)	[<u>10]</u>
Torulaspora delbrueckii Biodiva TD291 (Lallemand, Montreal, QC, Canada) Lachancea thermotolerans Concerto (CHR Hansen, Hørsholm, Denmark) Metschnikowia pulcherrima Flavia MP346	Laboratory (60 mL)	Monoculture Must/wine analyzed in the initial stages of the fermentation (2.0– 3.0% v/v ethanol)	Sauvignon Blanc and Syrah	Wines produced with <i>T.</i> <i>delbrueckii:</i> ↑ phenethyl propanoate (>50 times in both wines); linalool (both wines), β- damascenone (Sauvignon Blanc wine)	Sensory analysis not performed	(<u>20</u>)

Commercial Yeast	Level	Fermentation	TYPE OF WINE	Changes with Respect to S. cerevisiae	Sensory Impact with Respect to <i>S.</i> Reference <i>cerevisiae</i>
(Lallemand, Montreal, QC, Canada)				<pre>Wines produced with L. thermotolerans: ↑ in both wines: 2- phenylethanol; phenethyl propanoate, other esters; nerol, terpinen-4-ol ↑ in both wines: 3- methylthio-1- propanol Wines produced with M. pulcherrima: ↑ phenethyl propanoate, phenethyl butyrate, isoeugenil phenylacetate (Syrah wine); linalool (Syrah wine); β- damascenone (Sauvignon Blanc wine) ↑ 2-methoxy-4- vinylphenol (both wines), 3- methylthio-1- propanol (Syrah wine)</pre>	
Torulaspora delbrueckii Biodiva TD291 (Lallemand, Montreal, QC, Canada) Metschnikowia pulcherrima Flavia MP346 (Lallemand, Montreal, QC, Canada)	Semi- industrial (100 L)	Sequential + S. cerevisiae QA23 (Lallemand, Montreal, QC, Canada)	Base wine for Cava (Macabeo grape)	Wine produced with <i>T.</i> delbrueckii: ↑ glycerol ↑ foamability: Hm > 17%, foam persistence: Hs > 20% ↓ volatile acidity ↑ 4-ethylguaiacol, 4-ethylphenol, 4- vinylphenol Wine produced with <i>M.</i> pulcherrima: ↑ foam persistence:	Higher preference ^[11] for wine produced with <i>T. delbrueckii</i> (more similar to the control) Higher smoky and floral notes in wine produced with <i>M.</i> <i>pulcherrima</i>

Commercial Yeast	Level	Fermentation	TYPE OF WINE	Changes with Respect to S. cerevisiae	Sensory Impact with Respect to S. F cerevisiae	Reference
				Hs > 35% ↓ esters ↑ 4-ethylguaiacol, 4-vinylphenol, 2- methoxyphenol, 2,6- dimethoxyphenol (2,6- dimethoxyphenol: OAV > 1.0, smoky aroma)		
Lachancea thermotolerans Concerto (CHR Hansen, Hørsholm, Denmark) <i>Metschnikowia</i> <i>pulcherrima</i> Flavia MP346 (Lallemand, Montreal, QC, Canada) <i>Pichia kluyveri</i> FrootZen (CHR Hansen, Hørsholm, Denmark)	Laboratory (5 L)	Sequential + S. cerevisiae Lalvin EC1118 (Lallemand, Montreal, QC, Canada)	Riesling	Wine produced with <i>L</i> . thermotolerans: 1 lactic acid; ethyl esters; terpenes 4 2-phenylethyl acetate; acetaldehyde Wine produced with <i>M.</i> pulcherrima: 4 2-phenylethanol, other higher alcohols; acetate esters; acetaldehyde Wine produced with <i>P. kluyveri</i> : 1 2-phenylethyl acetate 4 isoamyl acetate; acetaldehyde	All wines: higher preference and Riesling typicity; lower oxidation, acetaldehyde, and ethyl acetate perception Higher perception peach/apricot (<i>L.</i> <i>thermotolerans</i> and <i>P. kluyveri</i>), citrus/grapefruit (<i>M.</i> <i>pulcherrima</i>)	[15]
Hanseniaspora vineae T02/5AF (from Uruguayan vineyards)	Semi- industrial (100 L)	Monoculture Control: S. cerevisiae QA23 (Lallemand, Montreal, QC, Canada)	Macabeo	 t 2-phenylethyl acetate (50 times higher than S. cerevisiae), isobutyl acetate, ethyl lactate; α-terpineol 4 acetoin (73% lower than S. cerevisiae) 4 higher alcohols Synthesis of N- acetiltiramine and 1H-indole-3-ethanol acetate (not 	Higher preference, fruity, and floral scores	[21]

Commercial Yeast	Level	Fermentation	TYPE OF WINE	Changes with Respect to S. cerevisiae	Sensory Impact with Respect to S. cerevisiae	Reference
				synthesized by S. cerevisiae)		
<i>Torulaspora delbrueckii</i> Zymaflore Alpha (Laffort, Bordeaux, France)	Laboratory (1.2 L)	Sequential and simultaneous + S. cerevisiae Zymaflore X5 (Laffort, Bordeaux, France)	Sauvignon Blanc	↑ aromatic thiols: 3SH and 3SHA	Sensory analysis not performed	[<u>22</u>]
<i>Lachancea thermotolerans</i> Viniflora Concerto (CHR Hansen, Hørsholm, Denmark)	Laboratory (5 L)	Sequential + Schizosaccharomyces pombe V2 * or Sequential + S. cerevisiae 88 *	Tempranillo	↑ lactic acid and pyruvic acid (>2.0 and >3.7, respectively, respect to AF + MLF) ↑ vitisin A and vitisin B (>1.5 and >2.6, respectively, respect to AF + MLF) ↑ total anthocyanins (>1.6 respect to AF + MLF) S. pombe: residual urea (97% lower than AF + MLF)	<i>L.</i> <i>thermotolerans/S.</i> <i>pombe</i> Higher acidity Higher aroma intensity and quality, sensory acceptability	[<u>16]</u>
<i>Metschnikowia pulcherrima</i> AWRI Obsession (AB Biotek, London, United Kingdom)	Semi- industrial (50 kg of grape)	Simultaneous + <i>S. cerevisiae</i> AWRI838	Merlot	I alcohol degree (< 1.0% v/v) ↑ total esters; High score: ref higher alcohols ↑ sulfur flavor and fruit compounds: H ₂ S general (>22 times), Low score: vege dimethyl sulfide meat, and (>2.1 times), ethanethiol, methanethiol		[<u>23</u>]
Torulaspora delbrueckii Zymaflore Alpha (Laffort, Bordeaux, France) Torulaspora delbrueckii Biodiva TD291 (Lallemand, Montreal, QC, Canada) Torulaspora delbrueckii Prelude (CHR Hansen, Hørsholm,	Laboratory (20 L)	Sequential + S. cerevisiae PDM (Maurivin, Australia) and Multi-starter Melody	Shiraz (Two different ripeness level: 24 and 29 °Brix)	Wine produced from must of 24 °Brix: ↓ alcohol degree: <0.6% v/v (multi- starter Melody) ↑ glycerol: >0.85 g/L (Concerto), >1.84 g/L (Flavia) ↑ isoamyl acetate	Wine produced from must of 24 °Brix: Better aroma intensity, floral attribute, perception of red fruit (Melody, Biodiva, Alpha, Flavia)	2

2.3. Metschnikowia Pulcherrima

Metschnikowia pulcherrima is known for its capacity to reduce alcohol content ^{[23][24]}, which has piqued commercial interest, especially for wines produced from grapes grown in warm regions that are characterized by a high sugar

[<u>18][26]</u> Commercial Yeast	Level	Fermentation	TYPE OF WINE	Changes with Respect to S. cerevisiae	Sensory Impact with Respect to S. Reference cerevisiae	
Denmark)				(Prelude, Melody),	Wine produced	specially
Viniflora Concerto (CHR Hansen, Hørsholm, 2 ^[1]				acetate and ethyl isobutyrate (Alpha,	°Brix: Sweetness (Alpha,	$\left[\frac{12}{2}\right]$. This
Denmark) Metschnikowia	via Biodiva, Prelude), Biodiva, Prelude) via					uction of
pt <mark>yg</mark> jerrima Flavia MP346				(Prelude, Concerto, Melody)		stages of
(Lallemand, Montreal, QC, Canada) Melody (Torulaspora delbrueckii/Lachancea thermotolerans/Saccharomyces	[<u>20]</u>		[<u>2][12]</u>	↑ terpenes: Alpha, Biodiva, Prelude ↓ tannins: Alpha, Biodiva, Prelude, Concerto, Flavia	[<u>13][22][25]</u>	glycerol,
cerevisiae) (CHR Hansen, Hørsholm,				Wine produced from must of 29		with S.
Denmark)				° Brix: 2-phenyletnanol		reported
				(mainly Alpha, Biodiva, Prelude;		% v/v of
	[<u>24</u>]			and to a lesser		erol was
				Flavia, Melody)		n with S
	[<u>2</u>]			↑ terpenes: Alpha, Biodiva, Prelude Residual sugars: >5 g/L (Alpha, Biodiva, Prelude)		n with 0.
[<u>12</u>]						e of Sila
Metschnikowia pulcherrima	_aboratory	Sequential + <i>S. cerevisiae</i> Viniferm Diana (Agrovin, Alcázar de San Juan, Spain)		M. purcherrimals. cerevisiae Diana: ↓ alcohol degree: <0.62% v/v ↑ 4MSP (≈28 ng/L vs. ≈4 ng/L in S. cerevisiae control) ↑ glycerol (>0.72 g/L) ↓ higher alcohols	Both wines: highest scores in	erevisiae addition,
NS-EM-34 (Reported as pre-commercial	(5 L)	or Sequential	Verdejo	M. pulcherrimalS. cerevisiae	fruity, intensity, and	by CHR
strain by authors)		+ S. cerevisiae Viniferm Revelacion		Revelacion: 1 alcohol degree:	aromatic quality	Enologia.
		(Agrovin, Alcázar de		<0.63% V/V		ma. WA
		σαιι συαπ, σμαπ)		vs. 0 ng/L in <i>S.</i> cerevisiae control) ↑ glycerol (>0.52 g/L) ↓ higher alcohols	[27]	t (ADY),

Viniflora FrootZen is commercialized and stored in freezing conditions (-45 °C) and inoculated directly into the grape must without prior rehydration. The multi-starter Trillyeast is commercialized in the form of cream yeast (CRY) (Table 1).

Regarding the contributions of this yeast, the existing evidence reflects the need for more studies to optimize the fermentative conditions to take advantage of the benefits mentioned in the technical datasheets of these commercial strains, in order to improve certain aspects such as the high production of metabolites that negatively affect wine.

2.5. Schizosaccharomyces Pombe

Commercial Yeast	Level	Fermentation	TYPE OF WINE	Changes with Respect to S. cerevisiae	Sensory Impact with Respect to S. Re cerevisiae	eference	-iologica
Hanseniaspora vineae (Currently under evaluation by Oenobrands, Montpellier, France)	Semi- industrial (120 L)	Monoculture Control: S. cerevisiae Fermivin 3C (Oenobrands, Montpellier, France)	[<u>4]</u> Albillo	↑ esters, especially 2-phenylethyl acetate (OAV = 31.84)	[28] Sensory analysis not performed	[25]	ay finis up to 2 ind MA
Torulaspora delbrueckii Oenoferm Wild & Pure (Erbslöh, Geisenheim, Germany) <i>Metschnikowia pulcherrima</i> Flavia MP346 (Lallemand, Montreal, QC, Canada)	Laboratory (10 L)	Sequential + S. cerevisiae Oenoferm Bouquet (Erbslöh, Geisenheim, Germany) + S. bayanus LittoLevure CHA (Erbslöh, Geisenheim, Germany)	[<u>28]</u> Sila [29	Decrease in alcohol degree M. pulcherrima/S. bayanus/S. cerevisiae: <0.91% V/v M. pulcherrima/S. bayanus: <0.62% v/v Glycerol production (S. cerevisiae control: 5.7 g/L) T. delbrueckii/S. bayanus: 7.0 g/L M. pulcherrima/S. bayanus/S. cerevisiae: 6.7 g/L	Higher score of aroma and overall flavor: <i>M.</i> <i>pulcherrima/S.</i> <i>bayanus</i> and <i>T.</i> <i>delbrueckii/S.</i> <i>bayanus</i> Higher score: citrus flavor (<i>M.</i> <i>pulcherrima/S.</i> <i>bayanus</i>), melon and banana flavor (<i>M. pulcherrima/S.</i> <i>cerevisiae</i>)	[12]	nmercia uction o selectio nentativ ween <i>l</i> d conter
Torulaspora delbrueckii Biodiva TD291 (Lallemand, Montreal, QC, Canada) Metschnikowia pulcherrima Flavia MP346 (Lallemand, Montreal, QC, Canada)	Semi- industrial (50 L)	Monoculture Control: 3 commercial strains of S. <i>cerevisiae</i>	[<u>30]</u> Base wine for Cava (Chardonnay and Xarel.lo)	Base wines with M. pulcherrima High content of proteins. High foamability (Hm) and foars persistence (HS) Cava wines with T. delbrueckii Highest concentrations of esters, especially ethyl isovalerate (120–126 µg/L) in both wines	Cava wines Better fruity and fresh aromatic profiles, especially with T. <i>delbrueckii</i>	[7]	d wines quality o levels o ential fo
Torulaspora delbrueckii Biodiva TD291 (Lallemand, Montreal, QC, Canada) Metschnikowia pulcherrima Flavia MP346 (Lallemand, Montreal, QC, Canada)	Laboratory (1 L)	Simultaneous at the beginning of fermentation L. thermotolerans + T. delbrueckii; L. thermotolerans + M. pulcherrima; L. thermotolerans + H.	Airén	L. thermotolerans/M. pulcherrima + S. cerevisiae ↑ lactic acid: up to 3.27 g/L ↓ pH: reduction to 3.42 (grape must	L. thermotoleransIM. pulcherrima + S. cerevisiae Higher overall score Higher acidity	[<u>26</u>]	nocultur no majo s slight

3. Improvement in Fermentative Aromatic Profile Regarding Saccharomyces

3.1. Torulaspora Delbrueckii

The strains Biodiva TD291 (Lallemand, Montreal, QC, Canada), Zymaflore Alpha (Laffort, Bordeaux, France), and Prelude (CHR Hansen, Hørsholm, Denmark) show the capacity to increase the production of 2-phenylethanol in sequential fermentations with *S. cerevisiae* ^{[2][9]} and in ternary (simultaneous) fermentations with *L. thermotolerans* and *S. cerevisiae* (multi-starter Melody, CHR Hansen) ^[2]. Biodiva TD291 demonstrates this property in musts with a high sugar content (42 °Brix) ^[8] (**Table 2**). At levels above its threshold of perception (14 mg/L ^[31]), 2-phenylethanol confers rose aromas and is one of the volatile compounds of oenological interest.

[<u>32</u>] Commercial Yeast	Level Fermentation	TYPE OF WINE	Changes with Respect to S.	Sensory Impact with Respect to S. Reference	ude and
			cerevisiae	cerevisiae	im-chain
Hanseniaspora vineae	vineae		3.84)		
(Currently under evaluation by	+		↓ alcohol degree:		
Oenobrands, Montpellier,	Addition on day 8: S.		<0.66% v/v		
France)	cerevisiae 7VA *		(residual sugars =		
Lachancea thermotolerans L31			0)		
*			↑ higher alcohols;		a nigner
			esters		rain was

the only one to produce isoamyl acetate, amyl acetate, ethyl hexanoate, and ethyl octanoate. However, in the Nenriched medium, the three strains of *T. delbrueckii* produced higher amounts of H₂S, especially Zymaflore Alpha, which may be related to the presence of sulfur amino acids in the enriched medium.

Regarding the improvement in the synthesis of esters, sequential and simultaneous fermentations in Sauvignon Blanc and sequential fermentations in Merlot, with *T. delbrueckii* Zymaflore Alpha and *S. cerevisiae*, produced an increase in isoamyl acetate (banana), isobutyl acetate (banana), 2-phenylethyl acetate (rose), ethyl isobutyrate (strawberry, red fruit), ethyl propanoate (strawberry), and ethyl dihydroxycinnamate (pineapple, almond) ^[10]. The authors considered that ethyl propanoate, ethyl isobutanoate, and ethyl dihydroxycinnamate may be considered aromatic markers for *T. delbrueckii* Zymaflore Alpha, since they are usually not synthesized at important levels by *S. cerevisiae*. All these esters contributed to improving the complexity and fruity note in Merlot wines, produced at a semi-industrial level (**Table 2**).

The high production of isoamyl acetate and isobutyl acetate in Merlot wines indicates a positive interaction between both yeasts, also observed in sequential fermentations with *T. delbrueckii* Prelude and *S. cerevisiae* in Shiraz wines (**Table 2**), as well as 2-phenylethyl acetate and ethyl isobutyrate in sequential fermentations involving Zymaflore Alpha, Biodiva TD291, and Prelude, with a consequent improvement in the aromatic quality, especially the fruity notes ^[2].

However, other authors reported a decrease in the content of esters in sequential fermentations with Zymaflore Alpha and Biodiva TD291, especially isoamyl acetate in Chardonnay, Soave, Amarone, and Santo wines ^{[8][9]}, although, in all cases, it was produced above its threshold (30 μ g/L ^[31]), showing that the capacity to produce esters, in addition to the grape variety and the type of winemaking, depends on the intra-species variability of each yeast, based on its enzymatic esterase and acetyltransferase activities ^[10].

The production of aromatic compounds in the early stages of fermentation (alcohol degree about 2–3% v/v) with pure *T. delbrueckii* Biodiva TD291 was also evaluated ^[20]. The authors obtained an increase in phenethyl propanoate (rose aroma) (**Table 2**), which is not commonly synthesized by *S. cerevisiae*. The authors indicated the need to evaluate whether this ester persists until the end of fermentative process, for example, in sequential fermentations with *S. cerevisiae*.

An increase in the content of lactones was obtained with Biodiva TD291 in Amarone wines, especially 4carbethoxy- γ -butyrolactone (sweet coconut aroma), at concentrations higher than its threshold of 400 μ g/L, in agreement with the sensory analysis in which wines were described with greater aromatic intensity and sweetness ^[8]. However, the authors emphasized that these results should be considered with caution since Amarone wines can be commercialized after two years of ageing.

Regarding the production of metabolites with negative connotations, sequential fermentations with the Zymaflore Alpha strain showed the capacity to reduce the content of 4-vinylphenol and 4-vinylguaiacol in Soave and Chardonnay wines ^[9], and in the case of 4-vinylguaiacol, to levels below its threshold (40 μ g/L ^[33]). Biodiva TD291 showed the same effect in sequential fermentations in Santo sweet wine ^[9].

In contrast, sequential fermentations of Biodiva TD291 with *S. cerevisiae* in Sauvignon Blanc wines increased the production of 3-methylthio-1-propanol (sulfur, onion, raw potato), 3-(2-hydroxyethyl)thio-1-propanol (sulfur, onion), and ethyl ester of 3-methylthio-propanoic acid (metallic, pineapple, fruity, ripe pulpy tomato) ^[13]. The origin of these thiols may be the metabolism of amino acids such as methionine, or fermentations with grape musts poor in amino acids ^[34], such that the results indicate a grape must with low levels of amino acids, that *T. delbrueckii* catabolized methionine more easily, or that it generated an impoverishment in amino acids in the medium facilitating the subsequent synthesis of these thiols by *S. cerevisiae* ^[13].

However, the concentrations of these thiols were not high enough to be detected in sensory analysis ^[13], which indicates the need for further studies on the catabolism of amino acids by *T. delbrueckii* to elucidate the mechanism through which thiols are synthesized and to establish strategies to decrease their production.

3.2. Lachancea Thermotolerans

L. thermotolerans, in addition to acidity, can improve the aromatic profile of wine. The use of the Concerto strain (CHR Hansen, Hørsholm, Denmark) in monoculture was reported to result in a high production of 2-phenylethanol, phenethyl propionate (rose), and other esters in the initial stages of fermentation of Sauvignon Blanc and Syrah musts and wines (alcohol degree between 2.0% and 3.0% v/v) ^[20]. Phenethyl propionate is commonly not synthesized by *S. cerevisiae*; thus, its synthesis constitutes a strategy to improve the aromatic profile in wine through mixed fermentations with *S. cerevisiae*.

The Concerto strain, in sequential fermentations with *S. cerevisiae*, also showed a higher production of 2-phenylethanol in high-alcohol Syrah wines ^[2] and ethyl esters in Riesling wines ^[15]. In Sauvignon Blanc wines, it produced high amounts of isoamyl acetate and citronellol acetate ^[13] and isobutyl acetate in Syrah wines ^[2] (**Table 2**).

In Syrah wines, a higher content of isoamyl acetate and isobutyl acetate was obtained with the Melody multi-starter (CHR Hansen, Hørsholm, Denmark), which contains a strain of *L. thermotolerans* as well as a higher production of 2-phenylethanol in Syrah wine produced from over-ripe grapes (29 °Brix) ^[2].

Another metabolite of interest is acetaldehyde, whose production was decreased in sequential fermentations of *L*. *thermotolerans* Concerto with *S. cerevisiae* in Riesling wines ^[15], contributing to higher preference and Riesling

typicity, higher fruit perception (peach and apricot) and aromatic quality, and lower oxidation, acetaldehyde, and ethyl acetate perception.

However, in Beckner Whitener et al. ^[20], *L. thermotolerans* Concerto produced higher amounts than the control wine of 3-methylthio-1-propanol (**Table 2**), which negatively impacts wine (sulfur aroma, onion). The persistence of this compound can be evaluated in sequential fermentations with *S. cerevisiae*, as its presence was detected in the early stages of fermentation (alcohol degree between 2.0% and 3.0% v/v).

3.3. Metschnikowia Pulcherrima

In the early stages of fermentation (between 2.0% and 3.0% v/v ethanol) in Syrah musts ^[20], *M. pulcherrima* Flavia MP346 (monoculture) showed the capacity to synthesize isoeugenil phenylacetate and phenethyl propionate (rose aroma), which are not usually produced by *S. cerevisiae* (**Table 2**). However, it is necessary to assess whether these esters persist until the end of fermentative process, for example, in sequential fermentations.

In sequential fermentations with *S. cerevisiae* ^[13], Flavia MP346 also showed the capacity to produce methyl-butyl, methyl-propyl, and phenylethyl esters in Sauvignon Blanc wines (**Table 2**). On the contrary, in Riesling wines, a decrease in acetate esters was obtained ^[15]. In ternary fermentations *L. thermotolerans/M. pulcherrima* Flavia MP346 + *S. cerevisiae*, Vaquero et al. ^[26] obtained a higher production of esters in Airén wines.

M. pulcherrima AWRI Obsession (AB Biotek, London, United Kingdom) showed increased production of total esters in simultaneous fermentations with *S. cerevisiae* in Merlot wines ^[23], obtaining high scores in aroma and fruity flavor, and a sensory profile similar to the wine produced with *S. cerevisiae*.

Regarding higher alcohol content, in sequential fermentations of *M. pulcherrima* Flavia MP346 with *S. cerevisiae*, variable results have been obtained, with an increase in 2-phenylethanol in high-alcoholic Syrah wines ^[2] or a decrease in the content of 2-phenylethanol and higher alcohols in general in Riesling wines ^[15]. *M. pulcherrima* NS-EM-34 (reported as precommercial) in sequential fermentation with *S. cerevisiae* demonstrated a lower production of higher alcohols in Verdejo wines ^[24].

M. pulcherrima AWRI Obsession showed a higher production of higher alcohol in simultaneous fermentations with *S. cerevisiae* in Merlot wines ^[23]. In ternary fermentations of *L. thermotolerans/M. pulcherrima* Flavia MP346 + *S. cerevisiae*, Vaquero et al. ^[26] reported a higher production of higher alcohol in Airén wines.

The increased synthesis of compounds with negative, such as 2-methoxy-4-vinylphenol in Sauvignon Blanc and Syrah musts and wines ^[20], was also reported with *M. pulcherrima* Flavia MP346 in monoculture (**Table 2**), which indicates the presence of hydroxycinnamate decarboxylase activity, in addition to 3-methylthio-1-propanol in Syrah wines (sulfur, onion aroma). It will be necessary to evaluate their evolution in mixed fermentations with *S. cerevisiae*, as well as to study the evolution of these compounds on a larger scale to optimize fermentation conditions that help reduce their production and impact on the wine.

Regarding other sulfur compounds, in simultaneous fermentations of *M. pulcherrima* AWRI Obsession and *S. cerevisiae* in Merlot wines, an increase in H_2S , dimethyl sulfide, ethanethiol, and methanethiol was obtained (**Table 2**) with respect to the control wine ^[23], being the first study to report the production of these compounds by *M. pulcherrima*. However, in the sensory analysis, the presence of these compounds was not detected, highlighting, on the contrary, fruity aromas. The perception of fruity aromas may be related to the lower alcohol content and higher levels of esters and higher alcohol. Previously, it was reported that lower levels of ethanol can contribute to a better expression of fruit aromas ^[35].

3.4. Pichia Kluyveri

In sequential fermentations with *S. cerevisiae, P. kluyveri* Viniflora FrootZen (CHR Hansen, Hørsholm, Denmark) showed the capacity to increase the 2-phenylethyl acetate content and release high amounts of amino acids in Riesling wines ^[15] while reducing the contents of acetaldehyde and isoamyl acetate. However, these properties do not seem to affect the acceptability of wines, showing increased preference by the sensory panel, a lower perception of oxidation, acetaldehyde, and ethyl acetate, and greater value for the peach and apricot attribute.

In a subsequent study with Viniflora FrootZen, in sequential fermentations with *S. cerevisiae* in Sauvignon Blanc musts ^[13], high production of 3-methyl-butanoic acid (isovaleric acid) was obtained (sour, sweaty, cheese-like aroma) (**Table 2**), which derives from the catabolism of L-leucine. However, the concentration of this acid was not high enough to be detected in the sensory analysis. It was suggested that the synthesis of isovaleric acid can be considered a criterion for the selection of *P. kluyveri* ^[27] and that its esterification can produce the ethyl ester of 3-methyl-butanoic acid, which has a pleasant fruity aroma.

Based on those results, together with the absence of positive sensory attributes, Beckner Whitener et al. ^[13] suggested that Viniflora FrootZen strain would not be a good candidate to produce Sauvignon Blanc wines, in addition to the high levels of phenylethylamine detected in these wines.

Since few commercial products are based on *P. kluyveri*, the available field of study to select new strains with commercial potential is wide, as well as to develop fermentative strategies that, by using the strains currently available, take advantage of the benefits reported for this yeast in its technical datasheet (<u>Table S1</u>).

The capacity of this yeast to form films on the surface of wine may also be used ^[27], for example, through its industrial application as a "flower-film yeast", as an alternative to the traditional *Saccharomyces* used for the production of Sherry wines.

3.5. Hanseniaspora Vineae

The strain *H. vineae* T02/5AF (of Uruguayan origin) was used in monoculture to produce Macabeo wine with increased contents of 2-phenylethyl acetate, isobutyl acetate, and ethyl lactate with respect to *S. cerevisiae* ^[36], in addition to a lower acetoin content (**Table 2**). In this study, a lower synthesis of higher alcohols was obtained, in addition to the synthesis of N-acetiltiramine and 1H-indole-3-ethanol acetate (not synthesized by *S. cerevisiae*). In

addition, the wine produced with *H. vineae* T02/5AF received a higher preference score and a higher score for the fruity and floral attribute in the sensory analysis, which indicated the positive contribution of the esters.

More recently, an increase in the production of 2-phenylethyl acetate was obtained in Albillo wines with a pure precommercial strain of *H. vineae* ^[25] as shown in **Table 2**.

3.6. Commercial Non-Saccharomyces Yeasts in Sparkling Wines

The current literature reports few studies with commercial non-*Saccharomyces* yeasts in sparkling wines, both at the base wine level (first fermentation) and during the stage of second fermentation and bottle ageing.

One of the pioneering studies was conducted by González-Royo et al. ^[11] with *T. delbrueckii* Biodiva TD291 (Lallemand, Montreal, QC, Canada), in sequential fermentation with *S. cerevisiae* to produce base wine from Macabeo grapes, resulting in an increase in glycerol, a decrease in volatile acidity, and better foam properties (**Table 2**). The sensory acceptability of this wine was also higher. However, a higher production of volatile phenols with *T. delbrueckii* Biodiva TD291 and *M. pulcherrima* Flavia MP346 was obtained, although, in all cases, within the desired sensory limits. In addition, 2,6-dimethoxyphenol was produced by Flavia MP346 at levels higher than its threshold (OAV > 1.0), producing a marked smoky aroma in the sensory analysis.

Despite the improvements over the base wines, authors ^[11] highlighted the need to assess the long-term impact on the corresponding sparkling wine to determine whether the properties are maintained, or whether they are modified by the action of second fermentation and bottle ageing.

More recently, the same yeasts (Biodiva TD291 and Flavia MP346), in addition to three strains of *S. cerevisiae*, were used in monoculture to produce base wine from Chardonnay and Xarel.lo grape musts ^[Z]. All wines showed residual sugar levels below 0.4 g/L. An increased amount of proteins was also obtained, especially in wines fermented with Flavia MP346, conferring better foam properties (**Table 2**) as reported by González-Royo et al. ^[11]. All the wines were subsequently fermented and bottle aged for 18 months with a commercial strain of *Saccharomyces bayanus*. *T. delbrueckii* Biodiva TD291 wines showed a higher content of esters, especially ethyl isovalerate (aroma of pineapple, apple, pear, anise, and flowers) in both Cava wines (Chardonnay and Xarel.lo), in addition to isoamyl acetate and hexyl acetate. This indicates the contribution of Biodiva TD291 to the fruity character, in agreement with the sensory analysis (**Table 2**). Furthermore, unlike the study of González-Royo et al. ^[11], undesirable compounds such as volatile phenols were not detected.

Based on the results of *T. delbrueckii* Biodiva TD291 and *M. pulcherrima* Flavia MP346 in both studies, its biocompatibility in ternary fermentations could be evaluated, in addition to designing and implementing fermentative strategies that take advantage of the positive effects of both yeasts on the quality of foam and sensory profile in sparkling wines, as both studies were conducted at a semi-industrial level.

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