Abstract: Non-\textit{Saccharomyces} yeast species are currently a biotechnology trend in enology and broadly used to improve the sensory profile of wines because they affect aroma, color, and mouthfeel. They have become a powerful biotool to modulate the influence of global warming on grape varieties, helping to maintain the acidity, decrease the alcoholic degree, stabilize wine color, and increase freshness. In cool climates, some non-\textit{Saccharomyces} can promote demalication or color stability by the formation of stable derived pigments. Additionally, non-\textit{Saccharomyces} yeasts open new possibilities in biocontrol for removing spoilage yeast and bacteria or molds that can produce and release mycotoxins, and therefore, can help in reducing SO$_2$ levels. The promising species \textit{Hanseniaspora vineae} is analyzed in depth in this Special Issue in two articles, one concerning the glycolytic and fermentative metabolisms and its positive role and sensory impact by the production of aromatic esters and lysis products during fermentation are also assessed.

Keywords: non-\textit{Saccharomyces}; sensory improvement; dealcoholization; SO$_2$; grape variety; \textit{Hanseniaspora vineae}; \textit{Brettanomyces bruxellensis}; \textit{Pichia guilliermondii}; \textit{Metschnikowia pulcherrima}; \textit{Schizosaccharomyces pombe}; \textit{Lachancea thermotolerans}

Non-\textit{Saccharomyces} Species in Wine Biotechnology

Some non-\textit{Saccharomyces} yeast species have a powerful impact on wine aroma \cite{1-3} by the release of fermentative aromatic compounds (\textit{Torulaspora delbrueckii}, \textit{Candida stellate}, \textit{Starmerella bacillaris}, \textit{Wickerhamomyces anomalus}, \textit{Hanseniaspora vineae}, \textit{Schizosaccharomyces pombe}) \cite{4-11}, the production of varietal aromas such as thiols 3 MH and 3 MHA (\textit{Pichia kluivery}) \cite{3,12}, or the expression of exocellular enzymatic activities (\textit{Metschnikowia pulcherrima}) \cite{13}. The selection of the optimal strains of these species \cite{14} according to the specific production of the previously described aromatic compounds can even improve the effect on the sensory profile of wines during fermentation. Additionally, the weak implantation of non-\textit{Saccharomyces} species during must fermentation and the low competitiveness with \textit{Saccharomyces cerevisiae} make necessary the elimination of wild yeasts from grapes to ensure a suitable impact \cite{15}. To reach this goal, emerging non-thermal technologies open new possibilities in the effective implantation at an industrial scale of non-\textit{Saccharomyces} starters.

One of the key non-\textit{Saccharomyces} yeasts is currently \textit{Hanseniaspora vineae}, as it has high effectiveness in modulating the aromatic profile of neutral varieties by the production of acetate esters, especially 2-phenylethyl acetate \cite{3,10} and benzyl acetate \cite{3,16}, that are impact compounds of the floral aroma of rose petals and jasmine flowers \cite{3}. Additionally, \textit{H. vineae} releases during fermentation large amounts of cell wall polysaccharides that make it interesting in the fermentation and ageing of lees of white neutral varieties. Moreover, the better adaptation of \textit{H. vineae} to the fermentative process than other \textit{Hanseniaspora} fruit clade species has been highlighted, in terms of fermentative performance: growth, fermentation kinetics, and alcohol tolerance \cite{17}. The use of suitable levels of
SO₂ in grape must has been observed to have a positive effect in the selection of some *Hanseniaspora* spp. favoring the production of acetate esters, especially significant amounts of 2-phenylethyl acetate [18]. SO₂ management can be an interesting tool to modulate wild *non-Saccharomyces* populations for improving the aroma in uninoculated wines.

Another hot topic in the use of non-*Saccharomyces* yeasts is the adaptation to winemaking of specific grape varieties in global warming-affected climatic regions [19]. In warm areas, the winey and flat profile show even aromatic varieties can be improved by using non-*Saccharomyces* yeasts in mixed and sequential fermentations with *Saccharomyces cerevisiae*. In such conditions, some species behave as powerful biotools to improve wine freshness [3]. Among them, *Torulaspora delbrueckii*, *Lachancea thermotolerans*, and *Metschnikowia pulcherrima* [19,20], together with some apiculate yeasts [10,18,20], are key species. *Torulaspora delbrueckii* was the first species produced, and broadly used at an industrial level, because of the effect on wine aroma and mouthfeel [3,6]. *Lachancea thermotolerans* applications are increasing due its role in modulating wine acidity by the formation of lactic acid from sugars with a clear repercussion in pH control in warm areas [21,22]. The use of both species together in mixed fermentation helps to improve the sensory profile and freshness of wines from warm areas [19].

Another concomitant problem in warm areas is the excessive alcoholic degree, and several technologies are being developed to manage the high alcoholic degree. Among them, the use of non-*Saccharomyces* yeasts in which the metabolization of some sugars is derived to alternative products to ethanol is currently being studied [23,24]. The formation of glycerol, lactic acid, or yeast biomass [21,23,24] is a natural way to derive sugars used for the production of ethanol towards molecules or structures with repercussions in the sensory profile.

Biocontrol and bioprotection are research fields that are being strongly developed in wine biotechnology. Some non-*Saccharomyces* open interesting possibilities to exclude or eliminate undesired yeasts during fermentation because of their spoilage role in the production of defective compounds, such as ethylphenols by *Brettanomyces* or volatile acidity/ethyl acetate by some apiculate yeasts [25]. Some non-*Saccharomyces* with hydroxycinamatte decarboxylase activity can promote the formation of vinylphenolic pyranoanthocyanins during fermentation, therefore favoring the immobilization of ethylphenol precursors in stable pigments [26]. Additionally, the control and elimination of microorganisms that produce toxic molecules for human health, like biogenic amines or fungal toxins, have been studied [27,28].

Lastly, non-*Saccharomyces* species can be considered a new source of bioproducts or bioadditives with improved features that open new possibilities in wine biotechnology [28]. The use of non-*Saccharomyces* as a source of antimicrobial peptides can control toxin-producing or spoilage molds or undesired yeast or bacteria. Production in cocultures or the addition of molecules from non-*Saccharomyces* can promote the development of starters for alcoholic or malolactic fermentation. The application of non-*Saccharomyces* or their derivatives as oxygen consumers or reducers can control oxidation during fermentation and stabilization to reduce SO₂ levels. Many other alternative emerging uses of derived products from non-*Saccharomyces* species will be available for industrial applications soon.

**Conflicts of Interest:** The author declares no conflict of interest

**References**

1. Ciani, M.; Maccarelli, F. Oenological properties of non-*Saccharomyces* yeasts associated with wine-making. *World J. Microbiol. Biotechnol.* **1997**, *14*, 199–203. [CrossRef]
2. Jolly, N.P.; Augustyn, O.P.H.; Pretorius, I.S. The role and use of non-*Saccharomyces* yeasts in wine production. *S. Afr. J. Enol. Vitic.* **2006**, *27*, 15–39. [CrossRef]
3. Morata, A.; Escott, C.; Bañuelos, M.A.; Loira, I.; del Fresno, J.M.; González, C.; Suárez-Lepe, J.A. Contribution of Non-*Saccharomyces* Yeasts to Wine Freshness. A Review. *Biomolecules* **2020**, *10*, 34. [CrossRef] [PubMed]
4. Loira, I.; Vejarano, R.; Bañuelos, M.A.; Morata, A.; Tesfaye, W.; Uthurry, C.; Villa, A.; Cintora, I.; Suárez-Lepe, J.A. Influence of sequential fermentation with *Torulaspora delbrueckii* and *Saccharomyces cerevisiae* on wine quality. *LWT Food Sci. Technol.* 2014, 59, 915-922. [CrossRef]

5. Loira, I.; Morata, A.; Comuzzo, P.; Callejo, M.J.; González, C.; Calderón, F.; Suárez-Lepe, J.A. Use of *Schizosaccharomyces pombe* and *Torulaspora delbrueckii* strains in mixed and sequential fermentations to improve red wine sensory quality. *Food Res. Int.* 2015, 76, 325-333. [CrossRef]

6. Ramírez, M.; Velázquez, R. The Yeast *Torulaspora delbrueckii*: An Interesting but Difficult-To-Use Tool for Winemaking. *Fermentation* 2018, 4, 94. [CrossRef]

7. García, M.; Esteve-Zarzoso, B.; Cabellos, J.M.; Arroyo, T. Advances in the Study of *Candida stellata*. *Fermentation* 2018, 4, 74. [CrossRef]

8. Padilla, B.; Gil, J.V.; Manzanares, P. Challenges of the Non-Conventional Yeast *Wickerhamomyces anomalus* in Winemaking. *Fermentation* 2018, 4, 68. [CrossRef]

9. Martin, V.; Valera, M.J.; Medina, K.; Boido, E.; Carrau, F. Oenological Impact of the *Hansensiaspora/Kloeckera* Yeast Genus on Wines—A Review. *Fermentation* 2018, 4, 76. [CrossRef]

10. Del Fresno, J.M.; Escott, C.; Loira, I.; Herbert-Pucheta, J.E.; Schneider, R.; Carrau, E.; Cuerda, R.; Morata, A. Impact of *Hansensiaspora vineae* in Alcoholic Fermentation and Ageing on Lees of High-Quality White Wine. *Fermentation* 2020, 6, 66. [CrossRef]

11. Loira, I.; Morata, A.; Palomero, F.; González, C.; Suárez-Lepe, J.A. *Schizosaccharomyces pombe*: A Promising Biotechnology for Modulating Wine Composition. *Fermentation* 2018, 4, 70. [CrossRef]

12. Anfang, N.; Brajkovich, M.; Goddard, M.R. Co-fermentation with *Pichia kluyveri* increases varietal thiol concentrations in Sauvignon Blanc. *Aust. J. Grape Wine Res.* 2009, 15, 1–8. [CrossRef]

13. Morata, A.; Loira, I.; Escott, C.; del Fresno, J.M.; Bañuelos, M.A.; Suárez-Lepe, J.A. Applications of *Metschnikowia pulcherrima* in Wine Biotechnology. *Fermentation* 2019, 5, 63. [CrossRef]

14. Suárez-Lepe, J.A.; Morata, A. New trends in yeast selection for winemaking. *Trends Food Sci. Technol.* 2012, 23, 39–50. [CrossRef]

15. Morata, A.; Loira, I.; Vejarano, R.; González, C.; Callejo, M.J.; Suárez-Lepe, J.A. Emerging preservation technologies in grapes for winemaking. *Trends Food Sci. Technol.* 2017, 67, 36–43. [CrossRef]

16. Martin, V.; Giorello, F.; Fariña, L.; Minteguiaga, M.; Salzman, V.; Boido, E.; Aguilar, P.S.; Gaggero, C.; Dellacassa, E.; Mas, A.; et al. De novo synthesis of benzenoid compounds by the yeast *Hansensiaspora vineae* increases the flavor diversity of wines. *J. Agric. Food Chem.* 2016, 64, 4574–4583. [CrossRef] [PubMed]

17. Valera, M.J.; Boido, E.; Dellacassa, E.; Carrau, F. Comparison of the Glycolytic and Alcoholic Fermentation Pathways of *Hansensiaspora vineae* with *Saccharomyces cerevisiae* Wine Yeasts. *Fermentation* 2020, 6, 78. [CrossRef]

18. Cuijvers, K.; Van Den Heuvel, S.; Varela, C.; Rullo, M.; Solomon, M.; Schmidt, S.; Borneman, A. Alterations in Yeast Species Composition of Uninoculated Wine Ferments by the Addition of Sulphur Dioxide. *Fermentation* 2020, 6, 62. [CrossRef]

19. Escribano-Viana, R.; Garijo, P.; López-Alfaro, I.; López, R.; Santamaría, P.; Gutiérrez, A.R.; González-Arenzana, L. Do Non-*Saccharomyces* Yeasts Work Equally with Three Different Red Grape Varieties? *Fermentation* 2020, 6, 3. [CrossRef]

20. Romani, C.; Lencioni, L.; Biondi Bartolini, A.; Ciani, M.; Mannazzu, I.; Domizio, P. Pilot Scale Fermentations of Sangiovese: An Overview on the Impact of *Saccharomyces* and Non-*Saccharomyces* Wine Yeasts. *Fermentation* 2020, 6, 63. [CrossRef]

21. Morata, A.; Loira, I.; Tesfaye, W.; Bañuelos, M.A.; González, C.; Suárez Lepe, J.A. *Lachancea thermotolerans* Applications in Wine Technology. *Fermentation* 2018, 4, 53. [CrossRef]

22. Vaquero, C.; Loira, I.; Bañuelos, M.A.; Heras, J.M.; Cuerda, R.; Morata, A. Industrial Performance of Several *Lachancea thermotolerans* Strains for pH Control in White Wines from Warm Areas. *Microorganisms* 2020, 8, 830. [CrossRef] [PubMed]

23. García, M.; Esteve-Zarzoso, B.; Cabellos, J.M.; Arroyo, T. Sequential Non-*Saccharomyces* and *Saccharomyces cerevisiae* Fermentations to Reduce the Alcohol Content in Wine. *Fermentation* 2020, 6, 60. [CrossRef]

24. Ivit, N.N.; Longo, R.; Kemp, B. The Effect of Non-*Saccharomyces* and *Saccharomyces* Non-*Cerevisiae* Yeasts on Ethanol and Glycerol Levels in Wine. *Fermentation* 2020, 6, 77. [CrossRef]
25. Peña, R.; Vilches, J.; Poblete, C.; Ganga, M.A. Effect of Candida intermedia LAMAP1790 Antimicrobial Peptides against Wine-Spoilage Yeasts Brettanomyces bruxellensis and Pichia guilliermondii. *Fermentation* 2020, 6, 65. [CrossRef]

26. Morata, A.; Escott, C.; Loira, I.; Del Fresno, J.M.; González, C.; Suárez-Lepe, J.A. Influence of Saccharomyces and non-Saccharomyces Yeasts in the Formation of Pyranoanthocyanins and Polymeric Pigments during Red Wine Making. *Molecules* 2019, 24, 4490. [CrossRef]

27. Vilela, A. Non-Saccharomyces Yeasts and Organic Wines Fermentation: Implications on Human Health. *Fermentation* 2020, 6, 54. [CrossRef]

28. Vejarano, R. Non-Saccharomyces in Winemaking: Source of Mannoproteins, Nitrogen, Enzymes, and Antimicrobial Compounds. *Fermentation* 2020, 6, 76. [CrossRef]

**Publisher’s Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© 2020 by the author. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).