Development of a strategy for measuring fruity aroma potential in red wine

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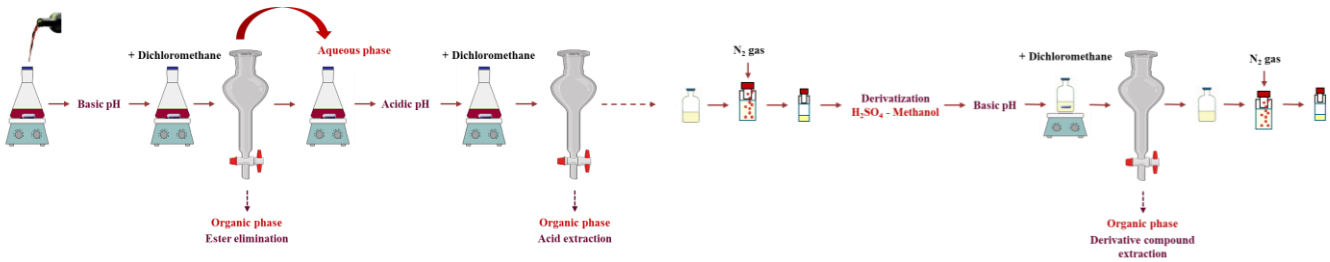
Introduction

Several studies demonstrated that, among red wine ethyl esters, those derived from short-chain substituted acids were strongly involved in the fruity aroma of red wines, playing the role of natural enhancers of fruity notes in red wine, even at subthreshold levels [1,2].
In contrast to the levels of most ethyl esters produced during alcoholic fermentation, substituted ester levels increase during aging [3].
Considering the importance of these substituted esters in the fruity aromas of red wines, the knowledge of their precursors remains indispensable [4].
The goal of this research was to develop a quantitation method for substituted acids including, where applicable, their various enantiomeric forms, preliminary to investigation into their formation pathways.

Development and Optimization of Hydroxycarboxylic Acid Quantitation and Enantiomeric Separation

- **ester elimination of wine sample : preliminary extraction was performed at basic pH to eliminate ethyl esters, thus avoiding their transesterification during derivatization**
- acid extraction, **derivatization**, extraction and analysis of derivative methyl esters by GC-MS



Quantitation of Substituted Ethyl Esters and Acids

Compound
Substituted ethyl esters
Ethyl 2-methylpropanoate
Ethyl 3-methylbutanoate
Ethyl (2*R*)-2-methylbutanoate
Ethyl (2*S*)-2-methylbutanoate
Ethyl (3*R*)-3-hydroxybutanoate
Ethyl (3*S*)-3-hydroxybutanoate
Ethyl (2*R*)-2-hydroxy-3-methylbutanoate
Ethyl (2*S*)-2-hydroxy-3-methylbutanoate
Ethyl (2*R*)-2-hydroxy-4-methylpentanoate
Ethyl (2*S*)-2-hydroxy-4-methylpentanoate
Ethyl 2-hydroxy-2-methylpropanoate (internal standard)
Alkyl-substituted acids
2-methylpropanoic acid
3-methylbutanoic acid
(2*R*)-2-methylbutanoic acid
(2*S*)-2-methylbutanoic acid
Hydroxycarboxylic acids
(3*S*)-3-hydroxybutanoic acid
(2*R*)-2-hydroxy-3-methylbutanoic acid
(2*S*)-2-hydroxy-3-methylbutanoic acid
(2*R*)-2-hydroxy-4-methylpentanoic acid
(2*S*)-2-hydroxy-4-methylpentanoic acid
2-hydroxy-2-methylpropanoic acid (internal standard)

- Substituted esters and acids were analyzed by GC-MS, using a **Chiraldex G-TA column**
- The quantitation and enantiomeric distribution of substituted esters and acids were established in **31 commercial Bordeaux red wines from 0 to 20 years old**

Variations in the Content of Substituted Esters over Time

Substituted ester levels were generally **higher in the oldest wines** than in younger ones

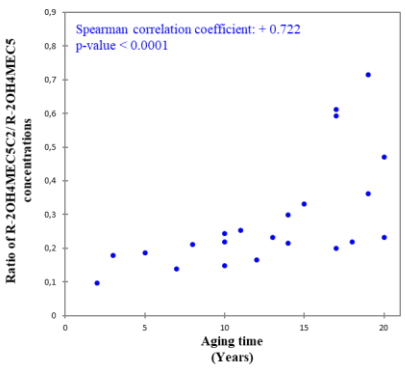
Only a few µg/L of these compounds are formed during alcoholic fermentation and wine aging is marked by **an increase in substituted ester concentrations over time**

Variations in the Content of Substituted Acids over Time

The **standard deviations** of average concentrations for each substituted acid during aging were **so large** that only few correlations between concentrations and age were observed

The **decrease** in (2R)-2-hydroxy-4-methylpentanoic acid concentration over time may be due to esterification, **leading to the corresponding ethyl ester**

The **increase** in (2S)-2-hydroxy-3-methylbutanoic acid and (2S)-2-hydroxy-4-methylpentanoic acid suggests that **another extra- alcoholic fermentation formation pathway may exist**



Ratio of substituted esters to the corresponding acids

- The **ratios** of ethyl 3-methylbutanoate, ethyl (2S)-2-methylbutanoate, ethyl (3R)-3-hydroxybutanoate, ethyl (2R)-2-hydroxy-3-methylbutanoate, ethyl (2S)-2-hydroxy-3-methylbutanoate, and ethyl (2R)-2-hydroxy-4-methylpentanoate to the corresponding substituted acids **increased slightly with the age of wine**
- These results support the hypothesis that **esterification of the corresponding substituted acids** resulted in the **accumulation of these esters**. In addition, this esterification may be related to the significant decrease in (2R)-2-hydroxy-4-ethylpentanoic acid observed over time

CONCLUSIONS

An assessment of the overall "aromatic potential" of these esters involved in red wine fruity aroma enhancement may be predicted, thanks to the development of the quantitation method of the corresponding acids

References
[1] Lytra, G.; Franc, C.; Cameleyre, M.; Barbe, J. C. J. Agric. Food Chem. 2017, 65, 5018-5025. [2] Lytra, G.; Tempere, S.; Le Floch, A.; de Revel, G.; Barbe, J.-C. J. Agric. Food Chem. 2013, 61, 8504−8513. [3] Díaz-Maroto, M. C.; Schneider, R.; Baumes, R. J. Agric. Food Chem. 2005, 53, 3503−3509. [4] Gracia-Moreno, E.; Lopez, R.; Ferreira, V. Anal. Bioanal. Chem. 2015, 407, 7925−7934.