Simultaneous determination of eight organic acids in base wines and cavas by HPLC-UV/vis: Method validation and application of Principal Components Analysis (PCA) for data treatment.

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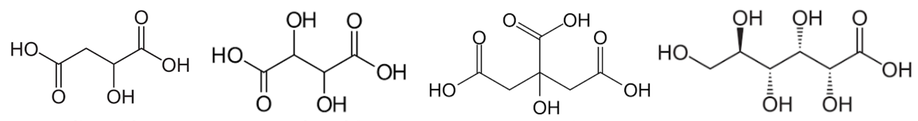
Introduction

Cava is a highly popular sparkling wine of high quality with Protected Designation of Origin (PDO) produced under the Champenoise method based on second fermentation and aging in its own bottle. Nowadays, cava is the most exported Spanish wine. Among other natural constituents of wines, low molecular weight organic acids are relevant species influencing on the taste and sensorial equilibrium. The most abundant acids of wines, (e.g., tartaric, gluconic, malic and citric acids) are originally present in the grape while others (e.g., succinic, fumaric, lactic and acetic acid) appear during subsequent fermentations until obtaining cava. The determination of organic acids in wines is important in quality control as well as in the monitoring of the evolution of winemaking processes.

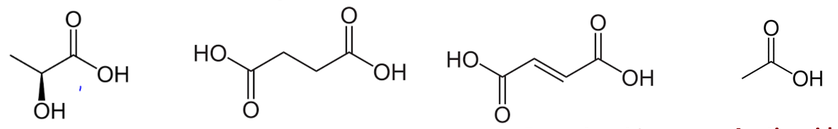
In this poster, a new method based on HPLC with UV detection to determine the main organic acids is presented. A preliminary study is carried out to establish the most suitable separation mode, including reversed-phase, HILIC and anion exchange. Finally, an alkyl-based column especially designed for polar compounds is chosen to separate the analytes under isocratic elution with water/acetonitrile solution (95:5 v/v, pH 2). More than 50 base wines and 160 cava wines produced from different grape varieties and blends are analyzed. Data consisting of compositional profiles of organic acids in the sets of samples is used for characterization and classification purposes. Box plots, radial diagrams and principal components analysis are used to try to find descriptors of varietal classes and winemaking factors. Results indicate that organic acids are good descriptors of base wine features while show a limited descriptive performance in the case of cavas.

Organic acids

Organic acids originally present in the grapes



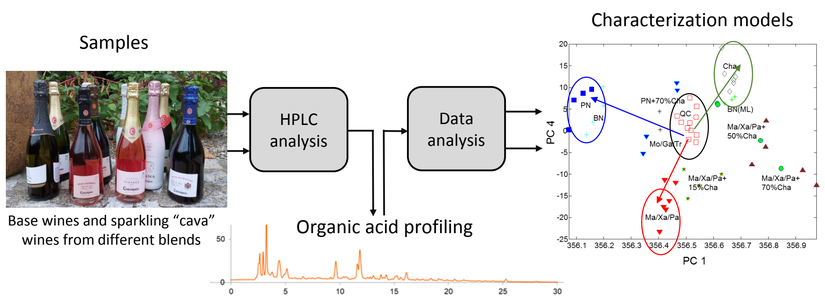
Organic acids from fermentative processes



Grape varieties

Experimental section

Working flowchart



Scheme of the overall working strategy:
from samples to data analysis

Samples

Samples	Composition
Coupage C	100% Macabeu, Xarel·lo, Parellada (MXP) with MLF
Coupage W	100% Blanc de Noirs with malolactic fermentation (MLF)
Coupage P	100% Pinot Noir (PN) with MLF
Coupage I	100% Blanc de Noirs without MLF
Coupage G	100% Chardonnay (CHA) with MLF
Coupage A	70% Macabeu, Xarel·lo, Parellada / 30% Chardonnay with MLF
Coupage E	90% Macabeu, Xarel·lo, Parellada / 10% Chardonnay with MLF
Coupage K (only in cava samples)	70% Macabeu, Xarel·lo, Parellada / 30% Chardonnay with MLF (15-30 month in contact with lees) with MLF
Coupage S	50% Macabeu, Xarel·lo / 50% Chardonnay with MLF
Coupage T	70% Pinot Noir / 30% Chardonnay with MLF
Coupage V	Pinot Noir / Garnatxa negra / Trepat with MLF

HPLC-UV method

- Instrument: HPLC Agilent Series 1100 HPLC chromatograph with a diode array detector
- Separation column: Agilent Zorbax SB-Aq (4.6 mm ID x 150 mm, 5µm)
- Mobile phase: 20 mmol L^{-1} H_3PO_4/acetonitrile (95:5, v:v)
- Elution mode: isocratic
- Flow rate: 1 mL min^{-1}
- Injection volume: 5 µL
- Detection wavelength: 210 nm

Results and discussion

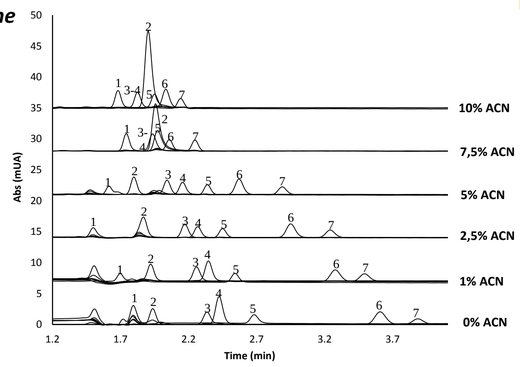
Optimization studies

Experimental conditions considered

Column	Mobile phase	ACN range (%)	pH range	Flow rate (mL min^{-1})	Temperature (°C)	Elution mode
Kinetex C_{18} polar	$HCl_{(aq)}$, $H_3PO_{4(aq)}$, $H_2SO_{4(aq)}$	0	1.0-7.0	0.5	Ambient	Isocratic
Spherisorb S10 NH_2	$H_3PO_{4(aq)}$	0-1	1.5-7.0	0.5-1.5	23-60	Isocratic
Xterra ® C_{18}	$HCl_{(aq)}$, $H_3PO_{4(aq)}$	0	1.0-7.0	0.5	Ambient	Isocratic
Rezex ROA	$H_2SO_{4(aq)}$	0	1.4-6.0	0.5	23-40	Isocratic
Syncronis™ HILIC	$H_3PO_{4(aq)}$	0	2.0-6.5	0.5	Ambient	Isocratic
Zorbax SB-Aq	$H_3PO_{4(aq)}$	0-10	2.0-3.0	0.7-1.0	Ambient	Isocratic / gradient

Influence of the ACN% in the separation using a Zorbax SB-Aq column

Peak assignment:
1 = Gluconic acid
2 = Tartaric acid
3 = Malic acid
4 = Lactic acid
5 = Acetic acid
6 = Citric acid
7 = Succinic acid

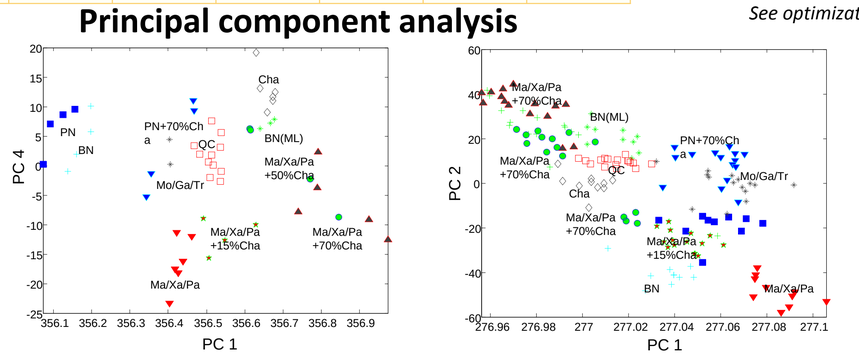


Figures of merit

Validation of the HPLC-UV method at 210 nm

Compound	RT (min)	Linear range* (g L^{-1})	Sensitivity (mAU min L g^{-1})	R^2	Repeatability peak area (sd)	LOD (mg L^{-1})
Gluconic acid	1.61±0.005	0.02-1.0	0.0072	0.9948	0.021	0.22
Tartaric acid	1.80±0.004	0.005-8.0	0.0164	0.9974	0.267	0.80
Malic acid	2.04±0.004	0.01-3.5	0.0129	1.0000	0.018	0.16
Lactic acid	2.16±0.005	0.01-3.5	0.0276	0.9999	0.023	1.4
Acetic acid	2.34±0.005	0.005-1.0	0.0089	0.9930	0.018	0.1
Citric acid	2.57±0.000	0.01-2.0	0.0139	0.9999	0.012	0.26
Succinic acid	2.88±0.005	0.02-0.8	0.0077	0.9982	0.023	0.13
Fumaric acid	3.08±0.004	0.001-0.1	0.8403	0.9973	0.023	0.02

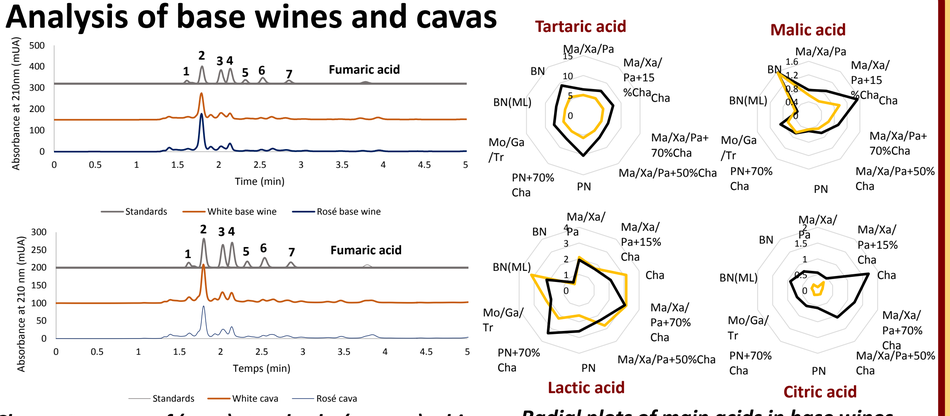
Principal component analysis



Plots of scores from principal component analysis from the study of chromatograms of base wines (a) and cava classes (b)

Wine assignation:
Ma = Macabeu
Xa = Xarel·lo
Pa = Parellada
BN =Blanc de Noirs
PN = Pinot Noir
Tr = Trepat
Ga = Garnatxa
Mo = Monastrell
Cha = Chardonnay

Analysis of base wines and cavas



Chromatograms of (grey) standards, (orange) white base wine and cava (c) rosé base wine and cava.
See optimization for peak assignment

Radial plots of main acids in base wines (black) and cavas (orange) as a function of coupages

Conclusions

✓The HPLC-UV method developed here was applied successfully to the determination of organics acids in base wine and cava samples. Among the diverse separation possibilities, the reversed-phase mode especially adapted to polar compounds provided the best results.

✓Analytes were chromatographically resolved without interferences from other endogenous wine species.

✓Organic acids may result in useful descriptors of varieties and blends at the stage of base wine but they offer limited possibilities for cava classes because the corrective oenological processes applied during the winemaking processes.

References

[1] http://www.institutdelcava.com/en/ (accessed 19th November, 2019).

[2] Mato, I., Suarez-Luque, S., Huidobro, J.F. (2005). A review of the analytical methods to determine organic acids in grape juices and wines. Food Research International, 38, 1175-1188.

Acknowledgements

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