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Book of Abstracts

Evaluation of antioxidant supplementation in must on the development and potential reduction of different compounds involved in atypical ageing of wine using HPLC-HQOMS

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Summary: *High performance liquid chromatography coupled with Q-Exactive hybrid quadrupole-orbitrap mass spectrometer (HPLC-HQOMS) was used to evaluate the AAP precursor content in wines after different adjuvant treatments and subsequently the AAP content after an accelerated ageing. Different precursors were quantified and qualified through both a targeted approach and a suspect screening approach.*

Keywords: *Atypical ageing, 2-Aminoacetophenone, HPLC-HRMS*

1 Introduction

The development in young white wines of 2-aminoacetophenone (AAP), commonly known as atypical ageing defect (UTA), leads to unpleasant notes such as mothball, wet mop, and sweaty with the loss of the typical vine-bouquet and consequent depreciation of the product. Formation of AAP occurs mainly during wine aging and, to a lesser extent, during alcoholic fermentation [1] deriving from non-volatile precursors, among which free indole-3-acetic acid (IAA), the main auxin of plants, is accepted to be the most important. The concentration of this hormone increases in stressed plant, resulted for example, of an excessive rains or high temperatures. The formation of AAP is caused by the oxidative degradation of the IAA, triggered by sulfuration after fermentation [2].

This study aims to evaluate the effectiveness of different oenological adjuvants (ascorbic acid, Asc; glutathione, GSH; galla tannin, GaT; ellagic tannin, ET; grape tannins, GrT) added to musts before fermentation for preventing the possible development of ATA.

High performance liquid chromatography coupled with hybrid quadrupole-orbitrap mass spectrometry (HPLC-HQOMS) was used to evaluate the AAP precursor content in wines after each of the different adjuvant treatments and subsequently the AAP content after an accelerated ageing. Moreover, different precursors were quantified and qualified through both a targeted approach and a suspect screening approach. The formation and consumption kinetics of the reaction metabolites from oxidative degradation of the most prominent IAA were evaluated for the first time. ANCOVA modelling was used to predict the possible AAP production considering grape varieties, treatments, and IAA content in young wine as known variables.

2 Experimental

Chromatographic separation was carried on using Thermo Ultimate R3000 ultra-high performance liquid chromatography (UHPLC; Thermo Scientific) coupled with a Q-Exactive hybrid quadrupole-orbitrap mass spectrometer (HQOMS, Thermo Scientific) equipped with a heated electrospray ionization (HESI-II) interface working in positive and negative ionization. A column Raptor Biphenyl 3 × 150 mm (2.7 µm particle size, Restek®, PA, USA) with a ternary mobile phase at 0.3 mL min^{-1} was used. Mass spectra were acquired with a full MS–dd MS/MS experiment. Mass resolution was set at 70,000 full width at half-maximum (FWHM, calculated for m/z 200, 1.5 Hz) for full MS spectra and at 17,500 FWHM (12 Hz) for dd-MS2. The precursors of 14 pure standards, corresponding to the protonated molecule $[M+H]^+$, were detected in the extracted ion chromatograms (EICs) and used for the quantification. Matching of m/z values with a mass tolerance <5 ppm, RTs and dd-MS/MS spectra were used for the qualification.

For the qualitative method, a suspect-screening approach was performed using the *in-house* prepared standard. The EICs corresponding to the exact mass of the deprotonated molecule $[M+H]^-$ (IAA-SO_3H) and protonated molecule $[M+H]^+$ (radical cation, FAP and Ox-IAA) were used to evaluate the correct RT of the compounds and the relative dd-MS/MS spectra for fragmentation study. The IAA-hexoside RT was studied with a Full MS/AIF/NL dd-MS2 experiment in positive ion mode.

3 Results

The most relevant AAP precursor IAA was found to be present with a concentration ranging from 20 to 120 µg/L. Asc and GrT treated samples had a significantly lower IAA content (Friedman's test, p value < 0.05), while ET and GaT treated wines resulted in significantly higher concentrations. The effect of the different antioxidant treatments was evaluated by quantifying the AAP content in the artificially aged wines (40 °C; 3 days, T3; 6 days, T6). Fig. 1 shows the box plots of the AAP content distribution during the accelerated aging treatment. IAA and its conjugates, radical cation, FAP and Ox-IAA were evaluated with a suspect screening approach during 25 days of accelerated ageing of wines with an Asc treatment and without treatment. The initial (day 0) IAA contents for both treatments were different, 90 µg/L and 33 µg/L for the untreated wines and Asc-treated wines, respectively, which shows the difference in IAA accumulation resulting from the treatment. After accelerated ageing, the IAA content decreased from 90 to 13 µg/L and from 33 to 3 µg/L, for untreated and Asc-treated wines respectively. IAA-SO3H showed an increase throughout the accelerated ageing process. This accumulation occurred more rapidly in the untreated wine. Also for the IAA-hexoside an initial formation was observed, reaching the maximum more rapidly in the Asc-treated wine compared to the untreated wine and was followed by degradation in both cases. The addition of Asc limited both the production of the precursor IAA and the final product AAP which directly involves the defect of ATA. Nevertheless, a reserve of IAA and FAP remained present in the wines after 25 days of accelerated ageing which is considered a possible reservoir of AAP release over time.

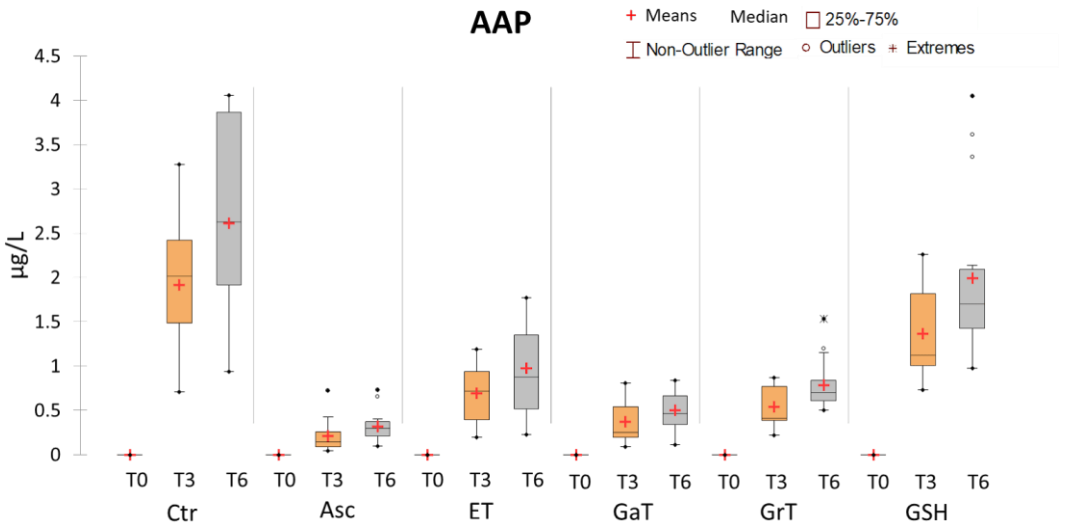

Fig. 1. Box plots of distribution of 2-aminoacetophenone in wines with different treatments (Asc = ascorbic acid, GSH = glutathione, ET = ellagic tannin, GrT = grape tannin, GaT = galla tannin) during the accelerated aging (40 °C; T0 = young wine, T3 = after 3 days heating, T6 = after 6 days heating); --- Median trend line (forced through T0).

4 Conclusions

The most effectiveness adjuvants against the possible development of ATA were Asc, GrT and GaT. Asc and GrT addition induced a reduced production of the IAA precursors, whereas GaT provided protection particularly during the storing period by preventing the AAP formation despite the IAA content. The ANCOVA linear modelling showed a promising capability to predict the possible development of the AAP in wine during fining processing.

References

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