Validation of a high-throughput method for the quantification of volatile carbonyl compounds (VCCs) in wine and its application for the determination of their formation during accelerated ageing.

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INTRODUCTION & AIM

VCCs are widely present in foods and beverages, either as aldehydes or ketones. Their formation is due to chemical reactions and biological processes where oxygen plays a key role [1]. However, many of these are side transformations that can modify the oxidative status with a consequent loss in varietal aroma and an increase in off flavors and defects [2]. At the same time, several carbonyls are related to pleasant scents so their presence is desirable and the winemaking of many oxidized wines like Madeira, Port, Vin Santo is tailored to emphasize their productions.

The aim of this research is the optimization and validation of a high-throughput method for the measure of the concentration of carbonyls that could be added as a new quality control tool for the evaluation of a complete fermentation, correct winemaking style, and proper bottling and storage [3].

MATERALS & METHODS

Various local white wines (cv. Gewurztraminer) and red wines (cv. Teroldego) were submitted to accelerated-ageing process. All bottles were opened under inert atmosphere, sampled in duplicate and submitted to the accelerated-ageing procedure at 50°C, according to Oliveira and Ferreira [4] (Figure 1). Oxygen concentration was measured daily using a NomaSense oxygen analyzer.

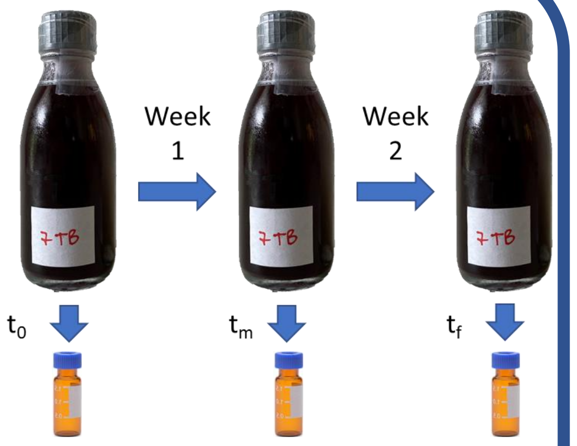


Figure 1. Accelerated ageing procedure.

The extraction procedure was based on the protocol purposed by Moreira et. al. [5], upgraded with a fully automated sample preparation performed by a CTC-PAL3 autosampler (Figure 2). GC-MS analysis was carried out using a TSQ Quantum XLS Ultra Triple Quadrupole GC-MS/MS equipped with a 30 m x 0.25 mm ID x 0.25 µm Restek Rx Sil MS w/Integra-Guard® column. The MS signal was obtained by EI at 70 eV using MRM acquisition. Calibration curves were acquired in matrix using a commercial white wine treated with Geosorb activated carbon. Acetone d6, 4-methyl-4-penten-2-one d10, Octanal d16 and 4-fluorobenzaldehyde were the internal standards. As many as 56 compounds, selected among linear aldehydes, Strecker aldehydes, ketones and many other carbonyls were the analytes under investigation. Metaboanalyst was the software used for statistical analysis.

Samples were analyzed as they are (t_0), after 1 week (t_m), and after 2 weeks (t_f) in randomized session.

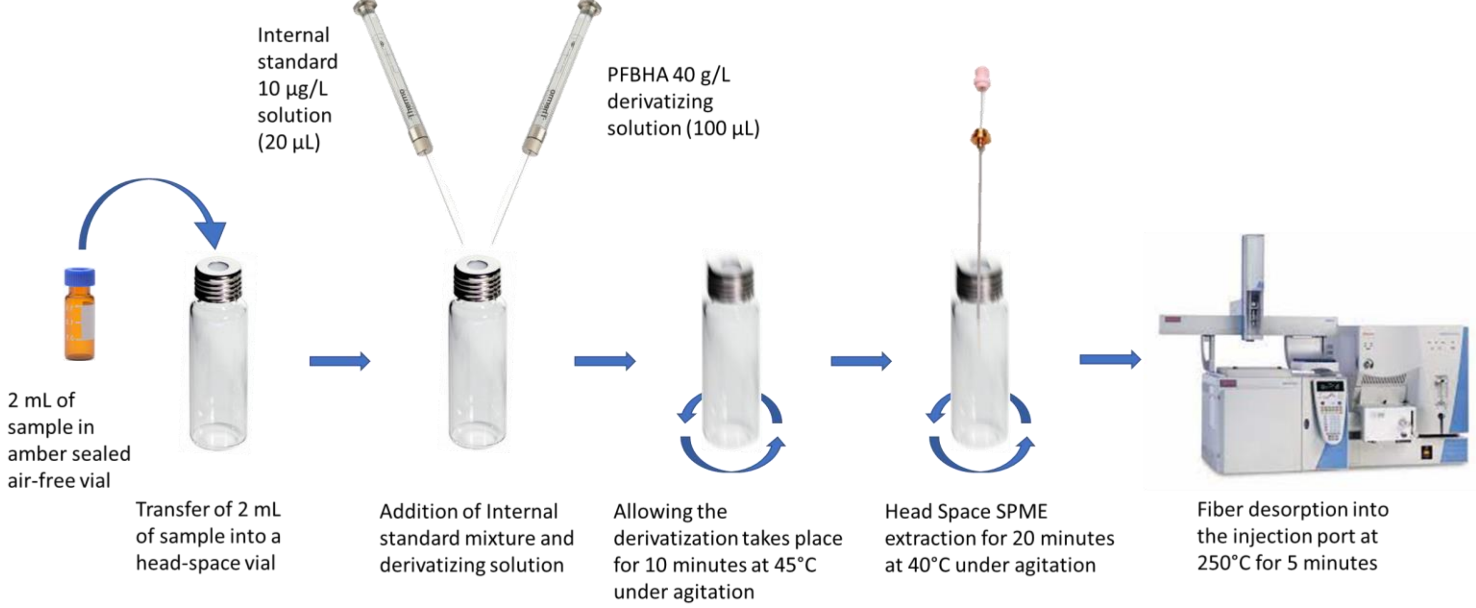


Figure 2. Work-flow of the automated procedure sample preparation.

RESULTS & DISCUSSION

After the instrumental optimization the method was validated. All compounds showed a good linearity spanning from approximately 0.1 to 50 µg/L ($R^2 > 0.99$). Intra-day and 5 days repeatability showed an RSD lower than 30% at 5 and 50 µg/L. IS recoveries have RSD comprised between 4.86% and 17.58%. Matrix effect was determined comparing a commercial white wine with the same sample spiked at 5 µg/L; recoveries were satisfactory with all compounds comprised in a ±50% and most of them <±30%.

With regard the wine samples submitted to the accelerated-ageing process, the oxygen content was daily measured; regardless the initial amount the concentration decrease under 10 µg/L after 3 days showing the same behavior for both types of wine (Figure 3).

Teroldego and Gewürztraminer show a very similar behavior during the accelerated-ageing process since the production of many oxidation products was detected in all samples. Key results are shown in Figure 4 (Gewürztraminer) and Figure 5 (Teroldego).

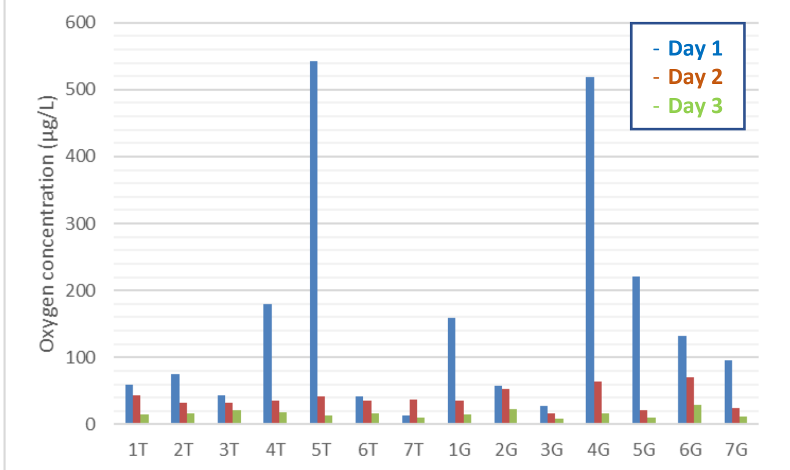
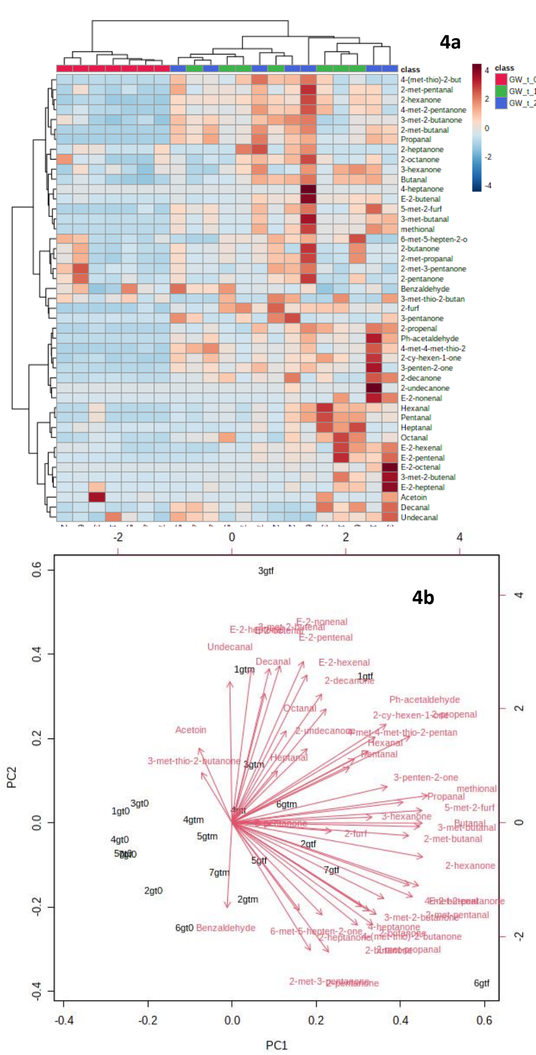


Figure 3. Oxygen concentration during the accelerated-ageing process.



Most significant compounds were propanal, 2-furfural, 5-methyl-2-furfural, 3-penten-2-one, 2-methylpropanal, 2-methylbutanal, 2-methylpentanal, and E-2-butenal.

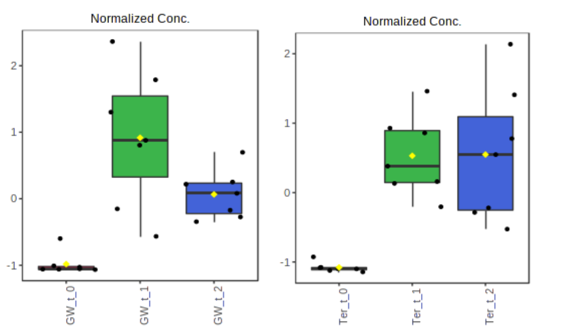
All this molecules (except 3-penten-2-one) are oxidation markers who are excepted to be produced when Fenton oxidation of alcohols, oxidation of fatty acids and Strecker degradation of amino acids take place.

The increase of 3-penten-2-one is correlated to the production of most Strecker aldehydes and takes place in both wines; this results are in accordance with Pinto et.al. who described the same behavior in Chardonnay.

2-furfural behaved differently since its concentration increased during the first week and then decreased during the second in both type of wine (Figure 6).

Methional, 2-cyclohexen-1-one and benzaldehyde increased more significantly in Gewürztraminer rather than Teroldego.

Differently, 2-butanone, 4-methylthio-2-butanone and butanal production was more significant in Teroldego.



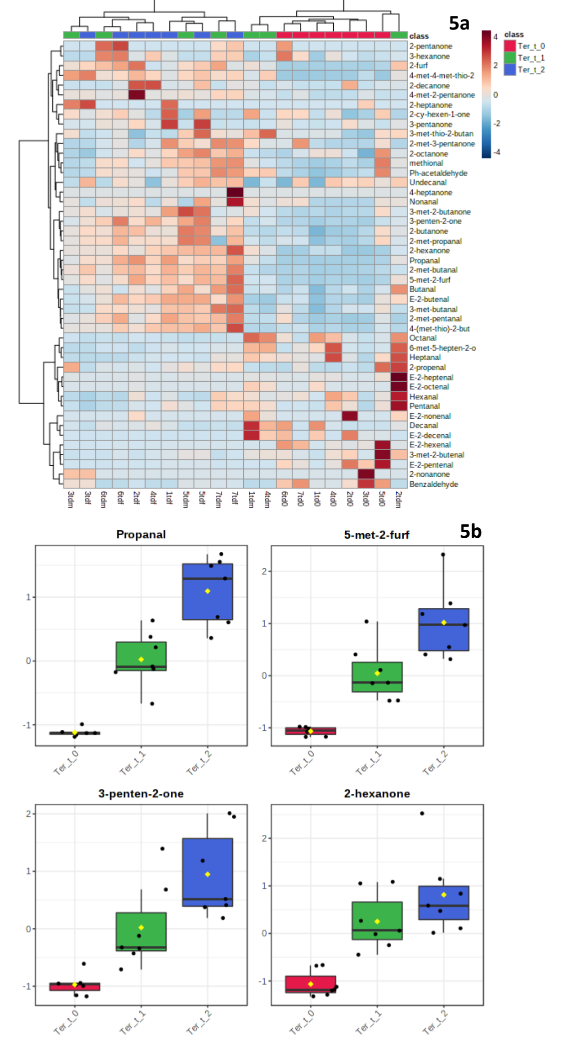


Figure 4. Heatmap (a) and PCA-biplot (b) in Gewürztraminer. *Figure 6. 2-furfural evolution in Gewürztraminer (a) and Teroldego (b).* *Figure 5. Heatmap (a) and key-compound increase (b) in Teroldego.*

CONCLUSIONS

A fully robotized method for the determination of volatile carbonyls is presented; thanks to its high automation ratio an improved robustness and high-troughtput was achieved. Good performance and repeatability, suitable for the use in the quality control, were demonstrated.

Despite a very different amount of oxygen in the bottled samples, it was consumed in the first days of accelerated-ageing. However, the production of oxidized molecules continued also in the days after so different source of oxygen were involved.

The production of many oxidation markers was detected according with previous experiments reported in literature.

3-penten-2-one increased significantly its concentration in all samples so it can be included in the oxidation markers group of molecules according to Pinto et.al.

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