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Analysis of volatile fungal metabolites in grape juice naturally contaminated with *Botrytis cinerea* and *Penicillium expansum*

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Abstract

The contamination of grapes by *Botrytis cinerea* is one of the most destructive diseases in vineyards. Besides the injuries caused to the grape and the undesirable oxidation of the botrytized musts, the grapes become more sensitive to the attack of other fungi thus facilitating the production of fungal volatile metabolites that can lead to the presence of off-odours in grape juice and wine. The presence of *Penicillium expansum* in botrytized grape juice is associated to the accumulation of geosmin (GEO) and 2-methylisoborneol (2MIB) in wine which are responsible of earthy odours.

The objective of this assay was to develop a method for the analysis of fungal volatile metabolites in naturally contaminated grape juices. The metabolites to be analyzed were GEO; 2-MIB; Fenchone; Fenchol; 1-octen-3-ol; 2,4,6-trichloroanisole; 2,3,4,6-tetrachloroanisole; 2,4,6-tribromoanisole and pentachloroanisole. For that purpose, a method for analysis of volatile compounds in wine (Boutou *et al.*, 2007) was adapted to grape juice.

Grape juice spiked with standards of the analytes was used during the whole process in order to take into account the matrix effect. The extraction of the compounds was carried out by Solid Phase Micro-extraction (SPME) and the analyses were performed by Gas Chromatography coupled with Mass Spectrometry (GC/MS). The extraction parameters a) time of extraction, b) content of ethanol, c) saturation of NaCl, d) pH and e) temperature were tested. Those conditions that maximized the extraction of GEO and 2MIB were chosen as optimal.

SPME extraction during 30 min at 50 °C optimized the extraction of GEO and MIB. Also, bigger peaks of both analytes were observed when the samples were saturated with NaCl and in the absence of ethanol. The pH of the sample did not seem to influence when saturated with NaCl and thus, the extractions were performed at grape juice pH. Good calibration curves, repeatability and recovery were obtained for GEO, MIB, fenchone, fenchol and 1-octen-3-ol. However, R^2 for calibration curves of anisoles was lower than 0,900.

This method permits to detect and quantify several volatile metabolites of fungal origin. The optimization of the extraction was performed in the basis of GEO and MIB because the olfactory threshold in wine of this compound is very low (50ng l⁻¹) and so the method should detect very low amounts of it. However, the anisoles can only be detected but not quantified.

Boutou, S.; Chatonnet, P. (2007). J. Chromat. A. 1141, 1-9