CHANGES IN WINE SECONDARY METABOLITE COMPOSITION BY THE TIMING OF INOCULATION WITH LACTIC ACID BACTERIA: IMPACT ON WINE AROMA [1]

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1. INTRODUCTION

Wine aroma is mainly impacted by compounds produced from the secondary metabolism of micro-organisms during the winemaking process. Malolactic fermentation (MLF) performed by lactic acid bacteria (LAB) plays a role in these aromatic changes.

In order to manage the MLF it is possible to introduce LAB starter cultures for direct inoculation in wine. Various studies have been carried out to determine the best moment for bacterial inoculation. However, the literature has very few data dealing with the impact of the timing of inoculation with LAB on the concentration levels of secondary metabolites and on the wine aroma. Only some compounds such as acetic acid, diacetyl and acetaldehyde have been studied specifically (Krieger, Arnink, 2003; Jussier *et al.,* 2006; Massera *et al*., 2009) and few sensory differences were found between sequential and simultaneous inoculation of musts with yeast and bacteria.

The present work reports an investigation of the impact of the time of inoculation with LAB on wine aromatic secondary metabolites. Six studies, conducted under winery conditions during 2 consecutive years on Merlot and Pinot noir wines, allowed the quantification of more than 60 molecules. Sensory profiles were performed in order to compare the aromatic notes of sequential and simultaneous inoculation of wines.

2. MATERIALS AND METHODS

2.1. Wines

Six mono-varietal wines were produced under winery conditions from grapes harvested from different regions and years. For each wine, two treatments were compared combining alcoholic fermentation (AF) by *S. cerevisiae* starter cultures with MLF by *O. oeni* starter cultures, where the lactic acid bacteria (LAB) were inoculated either during AF (simultaneous inoculation) or after completion of AF (sequential inoculation). Different yeast/LAB combinations were tested (tab. 1).

2.2. Chemical analyses

More than sixty aromatic compounds were quantified using different analytical methods developed in our laboratory (tab. 2).

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Tab. 1 - Experimental design.

Wine	Year	Origin	Yeast	LAB	Delay of bacteria adding	Sensorial analysis
Merlot A	2008	Switzerland	A	W	24h after yeast	Yes
Pinot noir	2008	Germany	B	X	24h after yeast	Yes
Merlot B	2009	France	C	W	2/3 AF	Yes
Merlot C	2009	France	D	Y	24h after yeast	No
Merlot D	2009	France	D	Y	24h after yeast	No
Merlot E	2009	France	D	Z	24h after yeast	No

Tab. 2 - Target compounds and techniques allowing their quantification.

Analytical techniques	Methods references	Quantified compounds
HS/SPME-GC/MS	(Antalick *et al.*, 2010)	**Apolar esters**: ethyl esters of straight-chain fatty acids, higher alcohol acetates, ethyl esters of branched aliphatic acids, cinnamates, methyl esters, isoamyl esters, minor esters
SBSE-GC/MS	(Antalick, 2010)	**C13-norisoprenoïds and lactones:** β-damascenone, β-damascone, β-ionone, α-ionone, γ-lactones (C8, C9, C10, C11, C12), δ-decalactone
Liq/liq extraction-GC/MS	(Antalick, 2010)	**Polar esters and branched fatty acids:** ethyl lactate, succinates, ethyl hydroxylated esters, isobutyric acid, 2-methylbutyric acid, isovaleric acid
Liq/liq extraction-GC/MS after derivatization	(de Revel *et al.*, 2000)	**Dicarbonyl compounds:** diacetyl, methylglyoxal, glyoxal, pentan-2,3-dione
Direct injection-GC/FID	(Falcao *et al.*, 2008)	Higher alcohols, methanol, ethyl acetate, acetaldehyde
Direct injection-GC/FID	(de Revel, 1992)	acetoin, butan-2,3-diols, acetol
HS-GC/FPD	(Falcao *et al.*, 2008)	Low boiling point sulfured compounds: DMS, H_2S

2.3. Sensory analysis

The sensory analyses were carried out by odor compared profile (orthonasal evaluation). Fifteen trained panelists described the wines evaluating the intensity on a scale from 1 to 7 on 4 aromatic attributes: fruity, milky, vegetable and smoky/toasty. The statistical treatment was performed by ANOVA.

3. RESULTS AND DISCUSSION

3.1. Impact on secondary metabolites composition

This study revealed for the first time that the timing of inoculation with LAB could impact the levels of some wine aromatic compounds.

Amounts of varietal compounds coming from glycosides and other precursors (C13-norisoprenoïds, linalool, lactones) showed no significant differences as a result of changes in inoculation timing (data not shown). On the other hand, some fermentary compounds were much more influenced by the timing of inoculation with LAB. Levels of higher alcohols were minimally modified except for Merlot B. For this wine, the simultaneous inoculation performed at two thirds of AF leads to a significant 20 % decrease of higher alcohols concentration (data not shown).

Other metabolites produced by the yeasts during AF such as dimethyl sulfide, branched fatty acids and ethyl acetate were significantly changed by the timing of inoculation with LAB. For these compounds, the variations were not systematic (about the half of samples) and their trends and their levels depend on the micro-organisms combination (data not shown). No links can be established between the variations of these compounds and the method of inoculation.

Of all the studied compounds formed from yeast metabolism, the esters were by far the most impacted by the timing of inoculation with LAB. The variations of total apolar and polar esters levels were almost all significant (fig. 1). They can reach 930 µg L^{-1} for apolar esters and 170 mg L^{-1} for polar esters. These levels of variations are important because they can potentially lead to wine aroma modifications (Pineau *et al*. 2009). However, similar to the previous metabolites reported above, no trend is emphasized, the variations depending on the yeast/LAB combination.

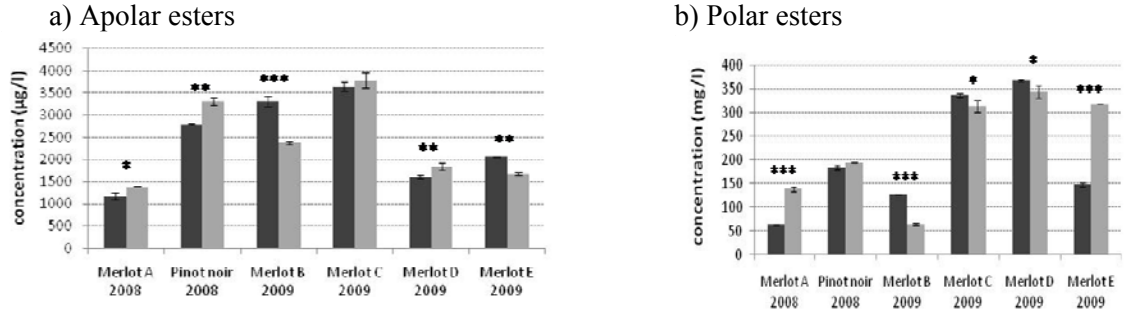

Fig. 1 - Mean of total esters levels of wines with different inoculation with LAB ways, with their corresponding standard deviation. Sequential inoculation (dark gray filled square) and simultaneous yeast/LAB inoculation (light gray filled square).

The modifications can be also different for a particular yeast/LAB combination according to the considered ester. For example, for Merlot C sample, no significant variation was observed for the total level of apolar esters whereas the concentrations of some esters families vary but not all in the same way (data not shown). In this case, the levels of ethyl esters of straight chain fatty acids and diethyl succinate increased with simultaneous inoculation whereas decreases occur for ethyl esters of branched aliphatic

acid, ethyl lactate and acetates, except for isoamyl acetate which was not impacted by the way of inoculation.

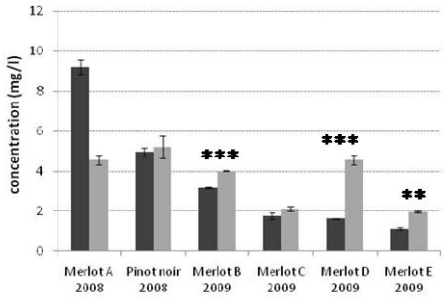

Fig. 2 - Mean of diacetyl levels of wines with different inoculation with LAB ways, with their corresponding standard deviation. Sequential inoculation (black filled square) and simultaneous yeast/LAB inoculation (gray filled square).

If wine metabolites coming from yeast metabolism can be impacted by the timing of inoculation with LAB, it is also the case for compounds mainly derived from LAB activity such as diacetyl. Indeed, previous studies (Krieger, Arnink, 2003) have shown that MLF carried out in the presence of yeast active lees can lead to a decrease of diacetyl levels by the strongly reductive character of the lees. In the present study, this phenomenon was observed for Merlot A sample for which simultaneous inoculation displays a lower diacetyl level than the sequential way (fig. 2). However, for the first time, significant increases of diacetyl levels have also been observed for simultaneous inoculation with LAB for Merlot B, D and E (fig. 2). It should be pointed out that no correlation was found between diacetyl variations and the levels of compounds derived from its reduction (acetoin, butan-2,3-diol) (data not shown).

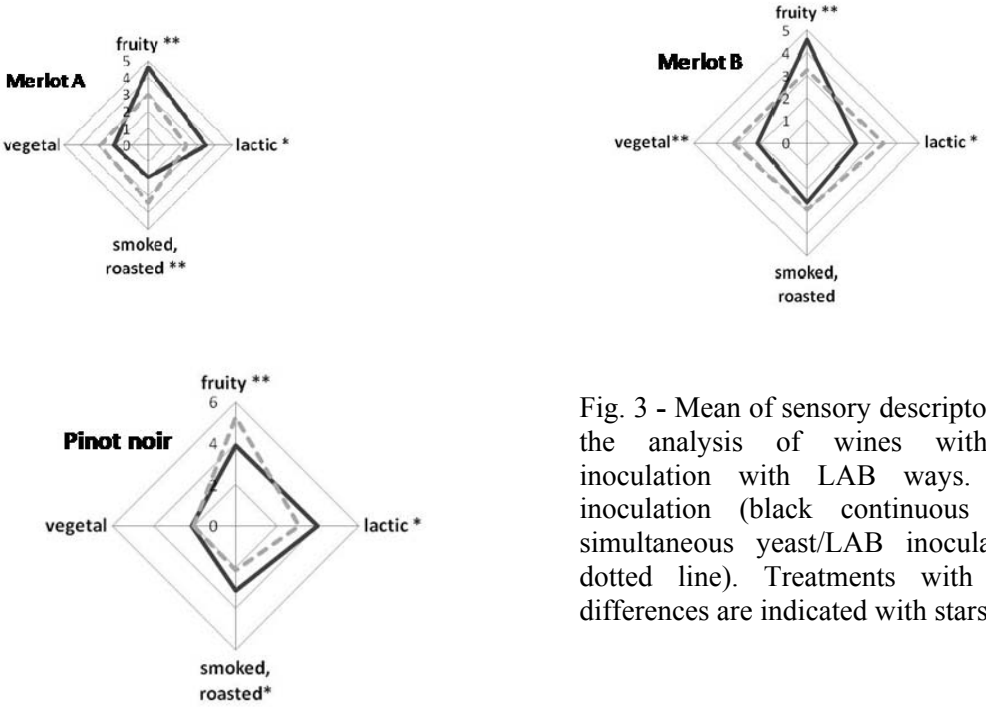

Fig. 3 - Mean of sensory descriptors values in the analysis of wines with different inoculation with LAB ways. Sequential inoculation (black continuous line) and simultaneous yeast/LAB inoculation (gray dotted line). Treatments with significant differences are indicated with stars.

Thus, the variations of diacetyl levels generated by different times of inoculation are not only due to red-ox effects; microbiological interactions should be considered too.

3.2. Impact on wine aroma

The 3 sensory profiles compared in this study display significant aromatic differences on 4 chosen descriptors with regard to the times of inoculation with bacteria (fig. 3). As for secondary metabolites composition, no trend was emphasized for the two inoculation techniques and the results were dependent on the yeast/LAB combination.

Abstract
For the first time, it was established that the timing of inoculation with LAB could significantly impact the concentration of many secondary metabolites leading to significant aromatic changes. From studied compounds, the most influenced were esters and diacetyl. No trend was emphasized according to the methods of inoculation with LAB. The sensory analyses performed in this study displayed significant differences in the aromatic profiles according the way of inoculation with LAB.

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