EFFECT OF MALOLACTIC FERMENTATION ON THE VOLATILE PROFILE OF WINES

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INTRODUCTION

LAB are mainly responsible for the conversion of L-malic acid into L-lactic acid and CO_2, resulting in less acidic wines. This process also enables wines to have a greater microbiological stability and better mouthfeel due to the loss of astringency and bitterness. LAB may influence wine aroma and flavour by various mechanisms, including the production of volatile compounds and the modification of grape constituents and yeast derived metabolites (1,2). The aromatic impact of bacterial activity can be variable depending on the wine type; indeed, the flavour attributes may also vary depending on the particular strain of malolactic organism employed (2-5).

AIMS

In order to understand the effect of MLF on the final composition of wines, in terms of desirable and undesirable compounds, an analytical characterisation was carried out before and after the occurrence of this microbiological step at industrial scale. The contribution of LAB to the production or modification of the content of heavy sulphur compounds, esters, alcohols and fatty acids of wines was studied.

MATERIALS AND METHODS

Fermentation conditions. Red grape must vinifications were performed at industrial scale (8500 l and 15000 l) from a single grape cultivar (Touriga Franca - TF) and a mixture of cultivars (MC) of the Douro Demarcated Region (Portugal). The grapes were destemmed and crushed. The must was treated with potassium metabisulfite and inoculated with *Saccharomyces cerevisiae* (commercial name VQ51, from Vinquiry). MLF was carried out through spontaneous fermentation. Two wine samples were collected before and after MLF. Wines presented 3 g/l of malic acid before MLF.

Analytical determinations. The determination of acetates of higher alcohols (isoamyl acetate, 2-phenylethyl acetate and hexyl acetate), ethyl esters of fatty acids (ethyl butyrate, ethyl hexanoate, ethyl octanoate and ethyl decanoate), 2-phenylethanol, ethyl lactate, volatile fatty acids (butyric, isobutyric and pentanoic acids) and free fatty acids (hexanoic, octanoic, decanoic and dodecanoic acids) was performed in a Hewlett–Packard (HP) 5890 gas chromatograph, equipped with a flame ionisation detector. Heavy sulphur compounds were determined by gas chromatography with flame photometric detection (GC/FPD), according to the method described by Moreira *et al*. (6).

Statistical analysis. An analysis of variance (ANOVA) was applied to the experimental data; results were considered significant if the associated P value was below 0.05. The significant differences were determined by Tukey tests. All statistical analyses were performed using the software SPSS® 17.0 for Windows® (SPSS Inc., Chicago, USA).

RESULTS

The highest concentrations of 2-phenylethyl acetate and hexyl acetate were observed in both wines after MLF. However, no significant differences were obtained in the isoamyl acetate content of both wines and in the total acetates of higher alcohols levels in MC wines (Fig. 1).

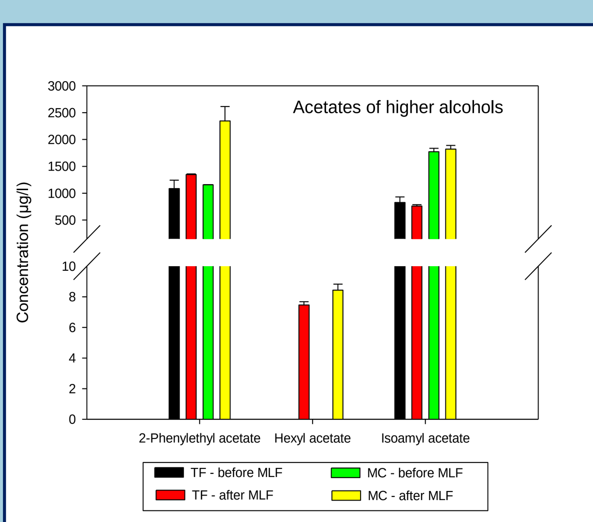

Fig. 1. Concentration of acetates of higher alcohols in Touriga Franca (TF) and Mixed cultures (MC) wines, before and after malolactic fermentation (MLF).

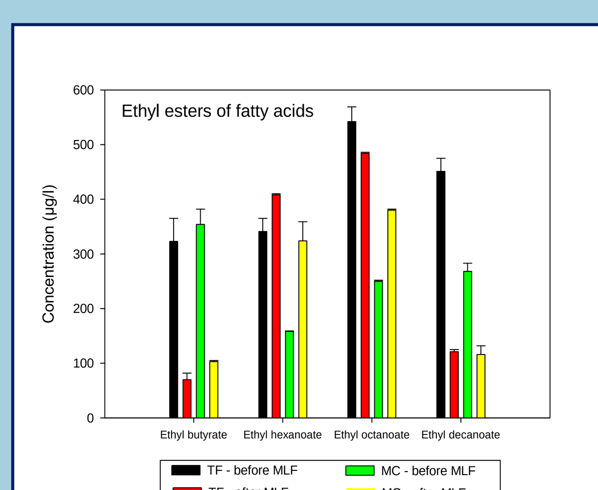

Fig. 2. Concentration of ethyl esters of fatty acids in Touriga Franca (TF) and Mixed cultures (MC) wines, before and after malolactic fermentation (MLF).

After MLF, a decrease in the ethyl esters of fatty acids content was obtained in both wines, except for ethyl hexanoate, which suffered an increase (Fig. 2). As expected, the content of ethyl lactate increased with MLF (Table 1).

The concentration of 2-phenylethanol significantly increased in MC wine after MLF. No significant differences were observed in the content of this compound in TF wines.

Table 1. Concentration of volatile compounds in wines, before and after malolactic fermentation (MLF).

Concentration (µg/l)	Touriga Franca			Mixed Cultivars		
	Before MLF	After MLF	Sig.	Before MLF	After MLF	Sig.
Ethyl lactate	15763 (2179)	20383 (130)	**0.005**	16217 (47)	24790 (2476)	**0.000**
2-Phenylethanol	22331 (1024)	23406 (149)	0.073	29076 (190)	36807 (484)	**0.000**
Fatty acids						
Butyric acid	843 (17)	918 (1)	**0.000**	582 (42)	nd	**0.000**
Isobutyric acid	2329 (262)	2574 (27)	0.114	2331 (40)	2978 (303)	**0.005**
Pentanoic acid	1759 (143)	2100 (12)	**0.003**	1770 (38)	2435 (229)	**0.000**
Total volatile fatty acids	4724 (711)	5592 (14)	0.051	4407 (511)	5413 (499)	**0.018**
Hexanoic acid	1725 (60)	2122 (23)	0.108	956 (19)	1451 (116)	**0.000**
Octanoic acid	549 (3)	578 (0)	0.064	238 (2)	404 (3)	**0.000**
Decanoic acid	873 (98)	544 (14)	**0.001**	459 (31)	505 (73)	0.340
Dodecanoic acid	82.4 (9.2)	17.4 (0.2)	**0.000**	61.9 (11.9)	20.2 (2.6)	**0.001**
Total free fatty acids	3230 (152)	3261 (8)	0.688	1715 (25)	2380 (46)	**0.000**
Heavy sulphur compounds						
S-(Methylthio)acetate	4.96 (0.39)	nd	**0.000**	5.81 (0.87)	nd	**0.000**
Methionol	1594 (242)	2751 (260)	**0.001**	1407 (126)	2203 (311)	**0.003**
2-(Methylthio)ethanol	45.6 (0.3)	41.4 (2.2)	**0.009**	23.1 (4.3)	23.0 (0.5)	0.951
2MTHTP	58.3 (8.6)	70.2 (8.0)	**0.044**	88.3 (8.6)	78.1 (2.3)	0.060
3-Mercapto-1-propanol	5.11 (0.44)	nd	**0.000**	5.81 (0.4)	10.1 (0.7)	**0.000**
cis-2MTHTP [A]	15.5 (1.1)	25.9 (7.1)	**0.027**	40.3 (11.8)	50.8 (7.7)	0.188
trans-2MTHTP [A]	21.2 (5.9)	39.5 (14.2)	0.055	9.14 (2.56)	10.9 (4.7)	0.528
3-(Ethylthio)-1-propanol	36.9 (5.3)	33.3 (9.7)	0.545	44.0 (3.2)	46.7 (1.2)	0.177
4-(Methylthio)-1-butanol	14.5 (3.3)	10.8 (1.2)	0.075	12.2 (1.8)	11.1 (0.4)	0.288
Dimethyl sulphone	70.1 (11.4)	146 (39)	**0.010**	73.0 (21.5)	69.2 (27.3)	0.832

Values in parenthesis are standard deviations from four determinations. Sig.: significance; bold values represent a *p*-value lower than 0.05. nd: not detected.
2MTHTP: 2-Methyltetrahydrothiophen-3-one, *cis*-2MTHTP: *cis*-2-Methyltetrahydrothiophene-3-ol, *trans*-2MTHTP: *trans*-2-Methyltetrahydrothiophene-3-ol.
[A]Peak area x 10^3 / peak area of internal standard.

No significant differences were found in the total volatile and free fatty acids in TF wines before and after MLF, except for butyric and pentanoic acids whose concentrations increased and for decanoic and dodecanoic acid whose levels decreased.

The MLF significantly influenced the content of total volatile and free fatty acids in MC wines; despite of no variation in decanoic acid level and a reduction of butyric and dodecanoic acids concentrations were observed with MLF, an increase in free and volatile fatty acids contents was detected.

The highest levels in methionol concentration were found in wines after the occurrence of MLF.

The effect of MLF on other heavy sulphur compounds was more evident in TF wines than in MC wines. Besides methionol, an increase of 2-methyltetrahydrothiophen-3-one (metallic, natural gas, butane-like odour), dimethyl sulphone (odourless), *cis*- (odourless) and *trans*-2-methyltetrahydrothiophen-3-ol (onion, chive-garlic aroma) levels were observed in TF wines; however, a reduction of *S*-(methylthio)acetate (rotten/cooked vegetables, sulphurous odour), 2-(methylthio)ethanol (french bean, cauliflower) and 3-mercapto-1-propanol (roasted, potato, sweat odour) concentrations were found. Some unidentified sulphur compounds were detected in lower amounts after MLF. Further research was performed in order to identify those compounds. As presented in Fig. 3, the chromatogram of TF wine after MLF shows less chromatographic peaks than before MLF.

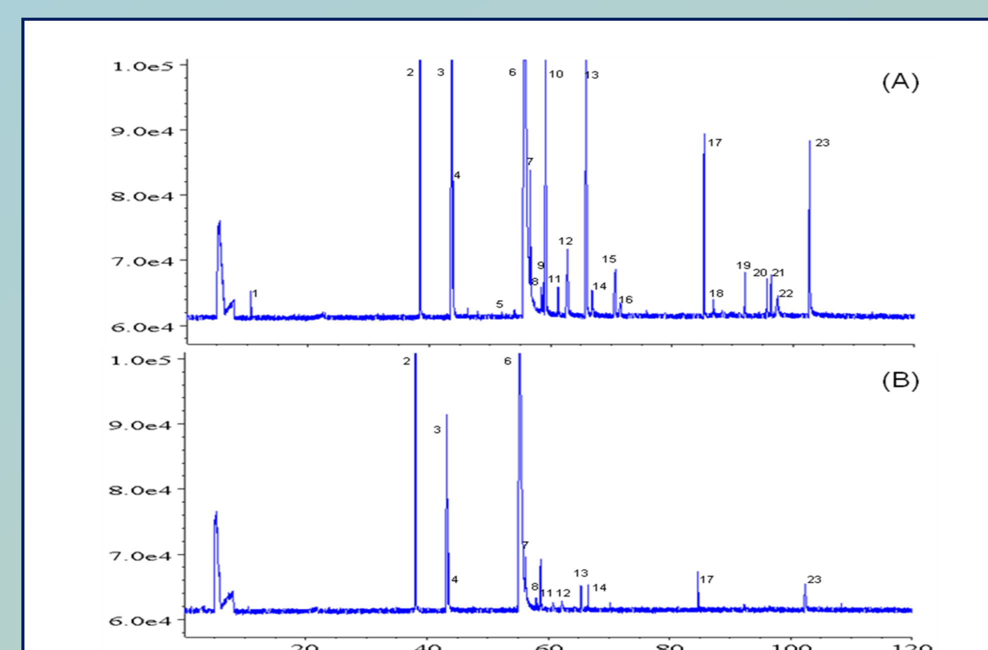

Fig. 3. Chromatogram of sulphur compounds in Touriga Franca wines (A) before and (B) after malolactic fermentation: (1) *S*-(methylthio)acetate, (2) IS, (3) 2-(methylthio)ethanol, (4) 2-methyltetrahydrothiophen-3-one, (5) 3-mercapto-1-propanol, (6) 3-(methylthio)-1-propanol, (7) peak 1[A], (8) *cis*-2-methyltetrahydrothiophen-3-ol, (9) peak 2[A], (10) 3-(ethylthio)-1-propanol, (11) peak 3[A], (12) *trans*-2-methyltetrahydrothiophen-3-ol, (13) 4-(methylthio)-1-butanol, (14) dimethyl sulphone, (15) peak 4[A], (16) peak 5[A], (17) peak 6[A], (18) peak 7[A], (19) peak 8[A], (20) peak 9[A], (21) peak 10[A], (22) peak 11[A], (23) peak 13[A]. [A]Unidentified substance. IS-internal standard, ethyl (methylthio)acetate.

CONCLUSIONS

This work highlights the effect of MLF on the volatile composition of wines. MLF can increase or decrease the concentration of several compounds known to play an important role on wine aroma such as esters, fatty acids, sulphur compounds and 2-phenylethanol. The impact of MLF in some compounds was found to be wine dependent. However, the influence of the MLF on heavy sulphur compounds is still scarce and requires further investigation.

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