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TECHNIQUES FOR EXTRACTION AND ENHANCING FLAVOUR SUBSTANCES IN CHARDONNAY AND SAUVIGNON BLANC GRAPES BY ENZYME SUBSTRATE

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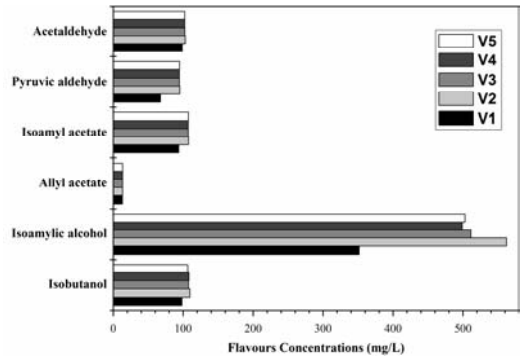
Identification and quantification of flavours through gas chromatography-mass spectrometry (GC-MS) from Chardonnay and Sauvignon blanc grapes is performed in the presence of proteolytic enzymes from pectinases class, which contains beta-glycosidase based activity grained pectinase. The used technique is skin maceration of grapes at 15°C, for 8 hours. Flavours are successively extracted with dichloromethane, then dried, concentrated and injected into GC-MS. The selected types of enzymes have led to the conclusion that independently of producers, the features of those enzymes boost the value of the flavours but also lessen the components that may damage the olfactory and sensorial qualities of the unfermented wine. Maceration enzymes improve the extraction degree of flavours.



INTRODUCTION

Chardonnay and Sauvignon blanc grape berries are semi-flavoured varieties which may accumulate volatile and highly volatile compounds under the influence of maceration enzymes, resulting fine quality rich-flavoured wines. Each variety possesses a certain feature according to the region of growth and climate, which has led to a more elaborate research in this field.[1] Many studies have shown that the pH and potassium concentration clearly influences the aroma of Chardonnay wines.[2]

In 1995, Ebadi, Coombe and May shown that the aromatic profile of the wines boosts by lowering the temperatures.[3] Also, the mould influences the aromatic potential of must and wines.[4] Flavours and aroma precursors have become a real challenge lately, a new series of chemical components being identified, including pyrazines, thiols, but also higher alcohols, acid esters and fatty acids.[5-7]

Recent studies on Sauvignon blanc grapes were elaborated in 2011, considering the rate of grapes ripening and all the factors that condition the sensory features of wines.[7]

On the assumption that the quality of raw materials influences the final products and that there are some factors that influence the aromatic features of wines, our study aims to assess the

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aromatic profile of the Chardonnay and Sauvignon blanc grapes semi-aromatic varieties, macerated in the presence of proteolytic enzymes from pectinases class, which contain beta-glycosidase based activity grained pectinase.

RESULTS

Chardonnay and Sauvignon blanc fully matured grapes harvested in 2013 from Recaş Vineyard were characterized by an average sugar content between 206 and 225 g/L, a pH between 3.29 and 3.45, and an acidity between 5.3 and 7.6.

Micro-vinification is a winemaking technique used often for experimental batches of wine fermented in small, specialized vats. Micro-vinification allows a viticulturist to express the most natural, unadultered characteristics of a single terroir, or vineyard block.[8,9]

Pectolytic enzymes are used for must clarification due to the fact that the method is simple and economic, with positive effects on must filterability and stabilization treatments, but also on the quality of the wine. Pectic substances are naturally found in the wine, their role being preventing wine clarification due to their colloidal character, but they can be enzymatic hydrolysed with pectolytic enzyme preparations.[10] The enzymes used in this study play an important role in collapsing the grape pulp and skin cell, and, in the same time they are able to split those chains and saccharide bonds between the chains, the time for reaction being 8 hours, at a temperature of 15°C.[11]

The GC-MS analysis emphasized volatile and highly volatile compounds from the selected grapes, indicating a high extraction for the variants resulted by enzymes fermentation, mostly for the V2 variant. The method enabled identifying and dosage of 22 compounds for Chardonnay must, from alcohol, organic acids, esters, acid anhydrides and aldehydes classes.

Table 1 shows that floral flavours given by decanoic, hexanoic, pentanoic, heptanoic acids and benzyl alcohol increase with enzyme maceration compared to the witness sample V1. The most effective enzyme was *Lafazyme CL* (V2). For the other enzymes, the values obtained are similar to the values obtain for V2, confirming the positive effect of the used enzymes in extraction and enhancing of grapes flavours.

Table 1

Flavours in untreated and enzyme-treated Chardonnay must from Recaş vineyard

No.	Compounds (mg/L)	Chardonnay must, Recaş, 2013				
		V1	V2	V3	V4	V5
1.	Isobutanol	98.331	109.425	107.552	108.127	106.583
2.	1-Pentanol	0.339	1.074	0.852	0.783	0.675
3.	Isoamylic alcohol	351.529	562.247	511.231	498.933	502.846
4.	1-Hexanol	3.012	3.988	3.224	3.176	3.431
5.	3-Metyl-3-pentanol	0.011	0.035	0.015	0.022	0.017
6.	Etylhexanol	0.093	0.194	0.125	0.141	0.153
7.	3-Metyl-2-heptanol	0.405	0.452	0.418	0.433	0.421
8.	Isooctanol	0.274	0.331	0.301	0.299	0.312
9.	1-Octanol	0.043	0.057	0.046	0.045	0.052
10.	Benzyl alcoohol	0.003	0.012	0.006	0.004	0.009
11.	Acetic acid	1.636	2.057	1.934	1.845	2.008
12.	Pentanoic acid	2.244	2.937	2.312	2.441	2.566
13.	Hexanoic acid	1.173	1.455	1.228	1.198	1.213
14.	Heptanoic acid	4.335	5.125	4.558	4.441	4.677
15.	Decanoic acid	5.622	6.199	5.727	5.991	5.887
16.	Isoamyl formate	7.115	7.542	7.488	7.533	7.549
17.	Ethyl acetate	3.383	3.452	3.391	3.385	3.888
18.	Allyl acetate	13.122	13.335	13.301	13.291	13.288
19.	Isoamyl acetate	93.557	107.591	106.995	107.125	107.258
20.	Pyruvic aldehyde	67.550	95.120	94.472	94.503	94.739
21.	3-Furaladehyde	1.833	2.150	2.088	2.065	2.091
22.	Acetaldehyde	98.649	103.050	102.235	102.177	102.202

The fruity character of Chardonnay must and the banana flavour increase due to the presence of 6% more of isoamyl formate and 15% more isoamyl acetate in V2, again the witness V1. The 3-furaldehyde that gives almond flavour increases as well with almost 17% and the vegetal flavour given by the 1-hexanol also increases with 32% in the V2 case.

The correlation is a statistical method used to determine the relationship between two or more variables.[12]

The correlation coefficient is a quantitative value that describes the relationship between two or more variables. It varies between -1 and +1, where extreme values assume a perfect relationship between variables while 0 means a complete lack of linear relationship. The most used coefficients are the Pearson correlation coefficient (r) for normal (uniform) distribution values and the Spearman correlation coefficient (rs) for unevenly distributed values.[12]

Pearson correlation coefficient (r) is independent of the measure unit. He assesses the degree of association between two variables. This refers to the intensity and variation direction of the values of one variable relative to the other, following a linear pattern. If the values of a variable follow, in direct sense, ascending or descending, the values of the other variable, then the two variables correlate with each other. The Pearson correlation coefficient (r) ranges between r = -1 (perfectly negative correlation, meaning that if scores of one variable increase, the scores for the other variable decrease) and r = +1 (perfectly positive correlation, which means that if a variable scores increase the scores of the other variable increase too). The absence of any link (correlation) between the variables translates as r = 0. In order to interpret the values of Pearson correlation coefficient, the following table (table 2) can be used: [12]

The Pearson correlation coefficient (Fig. 1, Fig. 2 and Table 3) of the V1-V2, V1-V3, V1-V4, V1-V5 variables computed with the help of the SPSS 16 soft program is 1, indicating a direct connection of maximum intensity between V1 witness variables and the variable values of the enzyme-treated variant. Sig (2-Tailed) value tell us if there is a statistically significant correlation between the two variables. In Table 3, the Sig. (2-tailed) value is 0.000 (less than 0.05), which means that there is a statistically strong correlation between the amount of enzyme added in the process and the flavour enhancement of the wine.

In the case of Sauvignon blanc must, the GC-MS method allows the identification and dosage of 24 flavours (Table 4), from the alcohols, organic acids, esters, acid anhydrides and aldehydes classes. Table 4 shows a boost of aromatic features given by enzymes extraction and skin maceration, the variant V2 indicating the highest potential, although the variants V3, V4 and V5 show also great results.

The fruity character of Sauvignon blanc must is evidenced due to the increasing concentration of isoamyl formate, the banana flavour is given by isoamyl acetate and the almond flavour by the 3-furaldehyde increasing. The vegetal aroma is due to the presence of 1-hexanol and the peach flavour is given by 1-penthanol.

Enzyme-treated Sauvignon blanc must contains ethyl octanoate with 10-18% more than the V1 witness, the citrus fruit flavour lasting longer than the witness sample's.

Also, in the case of Sauvignon blanc, it was used the Chardonnay must previous model, the Pearson correlation coefficient (Fig. 3, Fig. 4 and Table 5) for the variants V1-V2, V1-V3, V1-V4, V1-V5, computed with the help of SPSS 16 soft program, being 1, indicating a direct connection of maximum intensity b between V1 witness variables and the variable values of the enzyme-treated variant. In table 5, the Sig. (2-tailed) value is 0.000 (less than 0.05), which means that there is a statistically strong correlation between the amount of enzyme added in the process and the flavour enhancement of the wine.

Table 2

Pearson correlation coefficient interpretation

Pearson correlation coefficient (r) range	Interpretation
[0; 0.2]	Very low intensity correlation
[0.2; 0.4]	Low intensity correlation
[0.4; 0.6]	Medium intensity correlation
[0.6; 0.8]	High intensity correlation
[0.8; 1]	Highest intensity correlation

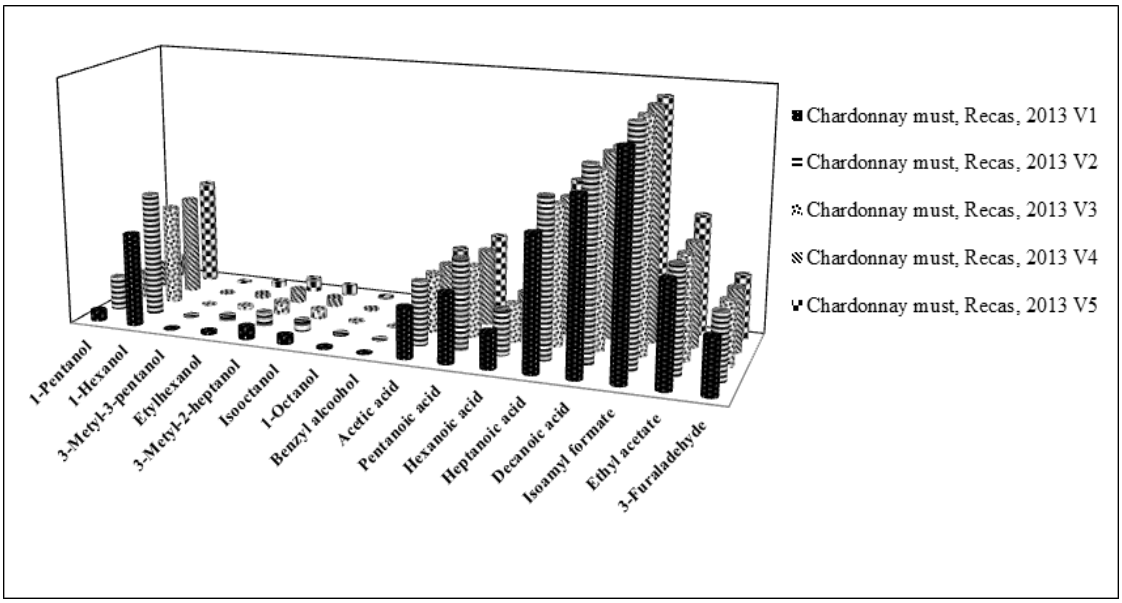

Fig. 1 – Low concentration flavours corresponding to untreated and enzyme-treated Chardonnay must from Recaș vineyard.

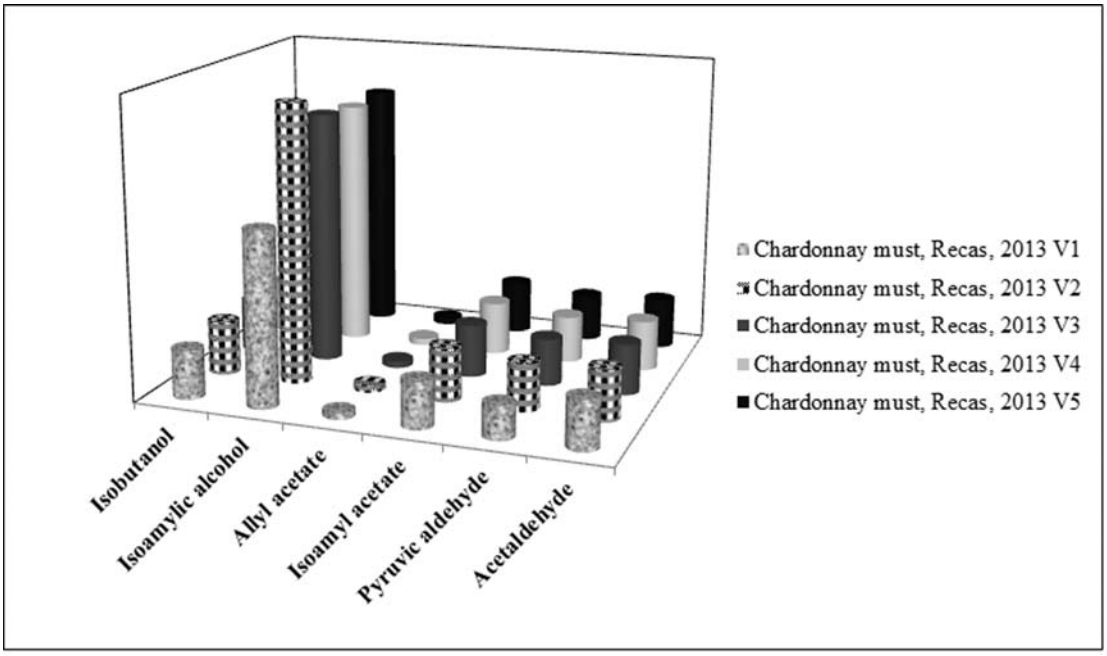

Fig. 2 – High concentration flavours corresponding to untreated and enzyme-treated Chardonnay must from Recaș vineyard.

Table 3

Correlation is significant at the 0.01 level (2-tailed)

		V1	V2,V3,V4,V5
V1	Pearson Correlation	1.000	1.000
	Sig. (2-tailed)		.000
	N	22.000	22
V2	Pearson Correlation	1.000	1.000
	Sig. (2-tailed)	.000	
	N	22	22.000

Table 4

Flavours in untreated and enzyme-treated Sauvignon blanc must from Recaş vineyard

No.	Compounds (mg/L)	Must Sauvignon blanc, Recaş, 2013				
		V1	V2	V3	V4	V5
1.	Isobutanol	68.450	75.225	69.023	70.055	69.667
2.	1-Pentanol	0.102	0.211	0.122	0.150	0.148
3.	Isoamylic alcohol	440.125	502.033	460.350	451.022	467.375
4.	1-Hexanol	4.302	5.175	4.908	4.756	5.088
5.	3-Metyl-3-pentanol	0.022	0.065	0.034	0.042	0.045
6.	Etylhexanol	0.075	0.108	0.064	0.092	0.091
7.	3-Metyl-2-heptanol	0.078	0.156	0.124	0.128	0.275
8.	Isooctanol	0.333	0.370	0.362	0.345	0.351
9.	1-Octanol	0.067	0.083	0.071	0.069	0.075
10.	Benzyl alcohol	0.007	0.015	0.009	0.010	0.008
11.	Phenyl-ethyl alcohol	6.378	8.605	7.422	7.211	7.140
12.	Acetic acid	2.026	2.308	2.056	2.103	2.066
13.	Diethyl-acetic acid	7.761	7.805	7.775	7.801	7.925
14.	Pentanoic acid	3.755	4.045	3.862	3.744	3.788
15.	Hexanoic acid	1.173	1.274	1.203	1.198	1.907
16.	Heptanoic acid	3.583	4.007	3.750	3.795	3.802
17.	Decanoic acid	6.607	6.866	6.623	6.708	6.699
18.	Isoamyl formate	5.311	5.330	5.321	5.314	5.315
19.	Allyl acetate	7.022	7.629	7.204	7.245	7.305
20.	Isoamyl acetate	65.133	68.988	66.365	65.277	65.905
21.	Isopropyl acetate	92.155	95.507	92.988	93.456	93.307
22.	Ethyl octanoate	7.955	9.456	8.122	8.304	8.655
23.	3-Furaldehyde	2.002	2.033	2.025	2.009	2.005
24.	Acetaldehyde	78.808	79.055	78.811	78.809	78.903

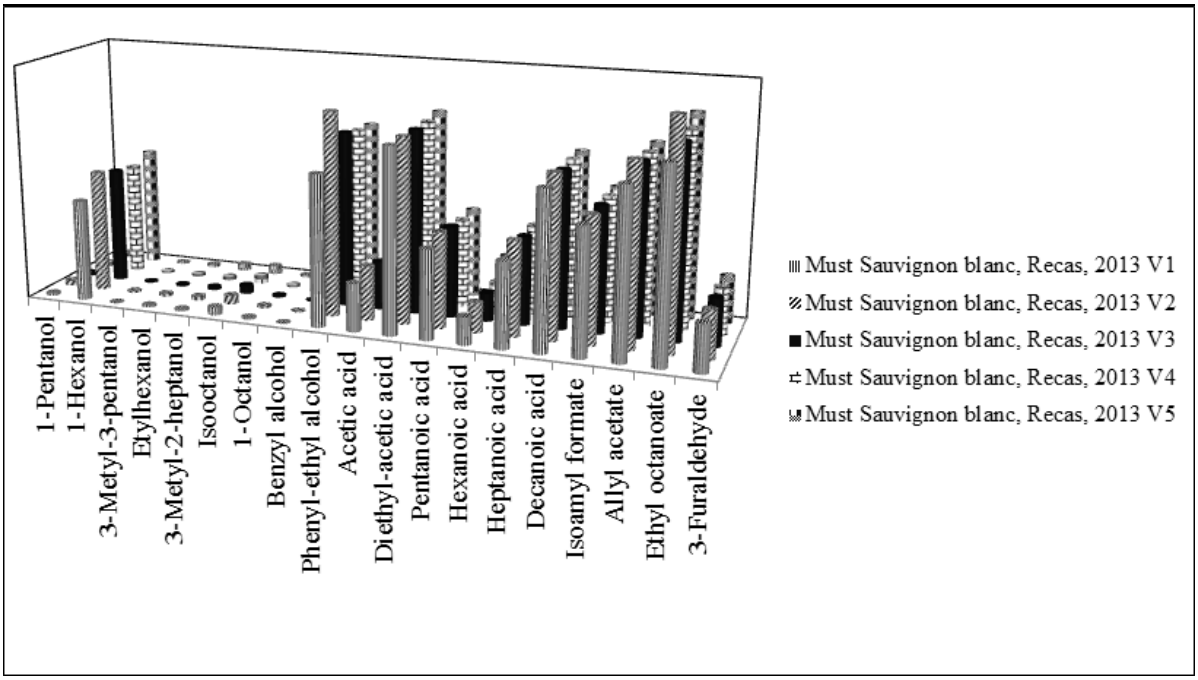

Fig. 3 – Low concentration flavours corresponding to untreated and enzyme-treated Sauvignon blanc must from Recaş vineyard.

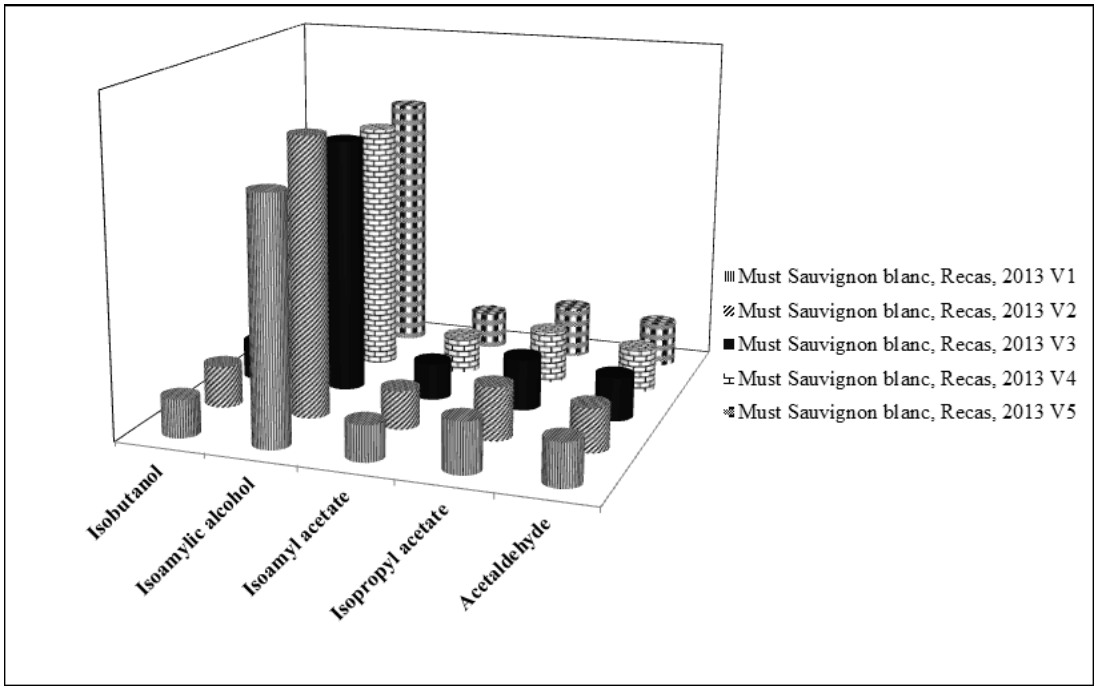

Fig. 4 – High concentration flavours corresponding to untreated and enzyme-treated Sauvignon blanc must from Recaş vineyard.

Table 5

Correlation is significant at the 0.01 level (2-tailed)

		V1	V2,V3,V4,V5
V1	Pearson Correlation	1.000	1.000
	Sig. (2-tailed)		.000
	N	24.000	24
V2	Pearson Correlation	1.000	1.000
	Sig. (2-tailed)	.000	
	N	24	24.000

EXPERIMENTAL

Raw materials and extraction procedures

Chardonnay and Sauvignon blanc fully matured grapes harvested in 2013 from Recaş (Romania) vineyards have been selected for the study.

There were used proteolytic enzymes such as: Lafazyme CL (Laffort SA - Bordeaux Cedex, France), Sihazym Extro (Eaton Begerow Product Line – Langenlonsheim, Germany), Siha Panzym Extract G (Eaton Begerow Product Line – Langenlonsheim, Germany), Enovin Varietal (Focşani – Vrancea, Roumania). Dichloromethane, anhydrous potassium sulphate and absolute ethanol were purchased from Merck (Darmstadt, Germany).

Enzyme substrate contained grained pectinase on beta-glucosidase activity and led to a superior extraction of aromatic substances from grape berries.

5g/100kg of enzyme substrate has been laid directly on grapes. Technological versions were focused on the use of various extraction enzymes (Lafazyme CL, Sihazym Extro, Siha Panzym Extract G, Enovin Varietal) for maceration, at 15°C for 8 hours.

Maceration versions for Chardonnay and Sauvignon blanc types

Version I (V1) blank

Chardonnay and Sauvignon blanc grapes were enzyme-free macerated, for 8 hours at a temperature of 15°C.

Version II (V2/Lafazyme CL enzyme)

Chardonnay and Sauvignon blanc were skin macerated with Lafazyme CL, for 8 hours, at a temperature of 15°C.

Version III (V3/Sihazym Extro enzyme)

Chardonnay and Sauvignon blanc grapes were skin macerated with Sihazym Extro enzymes, for 8 hours, at a temperature of 15°C.

Version IV (V4/Siha Panzym Extract G enzyme)

Chardonnay and Sauvignon blanc grapes were skin macerated with Siha Panzym Extract G enzyme, for 8 hours, at a temperature of 15°C.

Version V (V5/Enovin Varietal enzyme)

Chardonnay and Sauvignon blanc grapes were skin macerated with Enovin Varietal enzyme, for 8 hours, at a temperature of 15°C.

GC-MS analysis

The musts obtained by previously described maceration methods are injected into GC-MS system, using Headspace Gas Chromatography method. The system included the Varian 450GC gas-chromatograph coupled with Varian 240MS mass spectrometer model (Varian Inc – California, USA), fitted with a Thermo Scientific TG-WAXMS capillary column (Waltham, MA USA) (60m x 0.32mm x 0.25 μm). The sample volume was 1 mL, injected by a 1 mL syringe into the 1:10 splittered injection system.

The injector temperature for volatile compounds was 150°C; the column temperature is rising from the initial temperature of 30°C with 3°C/minute until it reaches 120°C and then it grows to 220°C. The carrier gas was He at a flow rate of 1.2 mL/min.

The identification of the aroma compounds is performed by comparing the mass spectrum obtained by the available spectrometer soft database (NIST Mass Spectral Search Program version 2.0/2006) or by comparing to the mass spectrum of the standard compounds and retention times.

Statistical analysis

The Pearson correlation coefficient of the variables V1-V2, V1-V3, V1-V4 and V1-V5 was computed with the help of SPSS Inc. Released 2007. SPSS for Windows, Version 16.0 (SPSS Inc., Chicago, USA).

CONCLUSIONS

This study presented an overview of the usage of enzymes in the maceration pre-fermentation phase and their influence upon the flavours enhancement in the resulted must. The high concentration of volatile compounds due to enzymes treatment may lead to their recommendation for wine production in technological processes. The variants selected for this study proved that the usage of these enzymes can increase aromas concentrations. Also, it was evidenced the positive effect of maceration enzymes upon flavours extraction, the V2 variant for Chardonnay and Sauvignon blanc having the most effective results, concluding that *Lafazyme CL* enzyme had major influence on flavours enhancement.

REFERENCES

1. O. Makhotkina, B. Pineau and P.A. Kilmartin, *Aust. J. Grape Wine Res.,* **2012,** *18*, 91-99.
2. R.R. Walker and D.H. Blackmore, *Aust. J. Grape Wine Res*, **2012**, *18*, 183-193.
3. A. Ebadi, B.G. Coombe and P. May, *Aust. J. Grape Wine Res.*, **1995**, *1*, 3-10.
4. B. Stummer, F. Leigh, A. Markides, and E. Scott, *Aust. J. Grape Wine Res.*, **2003**, *9*, 28-39.
5. K.S. Howell, J.H. Swiegers, G.M. Elsey, T.E. Siebert, E.J. Bartowsky, G.H. Fleet, I.S. Prtorius and M.A. De Barros Lopes, *FEMS Microbiol Lett.,* **2004,** *240*, 125-129.
6. A. Bellincontro, A. Fardelli, D. De Santis, R. Botondi and F. Mencarelli, *Aust. J. Grape Wine Res.,* **2006**, *12*, 141-149.
7. B. Pineau, M.C.T. Trought, K. Stronge, M.K. Beresford, M.W. Wohlers and S.R. Jaeger, *Aust. J. Grape Wine Res.,* **2011**, *17*, 358-367.
8. D. Dubourdie, T. Tominaga, I. Masneuf, C. Peyrot des Gachons and M.I. Murat, *Am J. Enol. Vitic*, **2006**, *57*, 81-88.
9. E.S. King, I.L. Francis, J.H. Swiegers and C. Curtin, *Am J. Enol. Vitic*, **2011**, *62*, 366-370.
10. R. M. Canal-Llauberes, "Les enzymes et leur application en oenologie", Ed. Ferret, "Bordeaux dans Produits de traitement et auxiliaires d'élaboration des moûts et des vins", 2000, p. 43-60.
11. P. Ribereau-Gayon, Y. Glories, A. Maujean and D. Dubourdieu, "Handbook of Enology", Wiley, Chichester, 2006, p. 285-333.
12. E. Jaba, A. Grama, "Analiza statistica cu SPSS sub Windows", Polirom Press, Iaşi, 2004.