The Annals of the University Dunarea de Jos of Galati
Fascicle VI – Food Technology 36(1) 9-17

ORIGINAL RESEARCH PAPER

EFFECT OF β-GLUCOSIDASES IN THE MAKING OF CHARDONNAY WINES

CRISTIAN CODREŞI[1], GABRIELA RÂPEANU[2], PETRU ALEXE[2]
[1]*S.C. Sodinal SRL, 100 Timisoara str., Bucharest, Romania,*
[2]*Dunarea de Jos University of Galati, Faculty of Food Science and Engineering, 111 Domneasca Street, 800201Galati, Romania*
*Corresponding author: c.codresi@sodinal.com

Received on 13 February 2012
Revised on 26 March 2012

β-Glucosidase (βG, EC 3.2.1.21) is one of the most interesting glycosidases, especially for hydrolysis of glycoconjugated precursors, in musts and wines, and the release of active aromatic compounds.
The aim of this work was to study the efficiency β glucosidase in order to increase the aroma of Chardonnay wines.
An increase in concentrations of volatile terpenes and norisoprenoids was observed when β glucosidases were used after the finalization of alcoholic fermentation.

Keywords: wine flavor, terpenes, norisoprenoids, β glucosidases

Introduction

Wine aroma is due to a lot of volatile compounds with different chemical natures and origins, found at a wide range of concentrations. Today there is an increasing demand for young white wines with a fresh and fruity aroma, this being a major factor determining wine character and quality (Sanchez Palomo *et al.,* 2006).
Hydrolysis of flavor precursors during winemaking is possible under the action of enzymes from grapes, the enzyme related to yeast or by addition of exogenous enzyme preparations. These enzymes act in different process steps from grapes processing to the end of the winemaking process. Equally, there is a relation between odorant precursor's content of berries and olfactory intensity for each variety of wine with aromatic character (Canal-Llauberes, 2000).
Terpenes and C_{13} norisoprenoid compounds are important contributors to the aroma of Chardonnay wines. C_{13} norisoprenoid compounds are generated from carotenoids such as lutein and β carotene (Cox *et al*., 2005).
Enzyme preparations with β glucosidase activity are produced mainly from the *Aspergillus niger* mould. Selection of *Aspergillus niger* strain rich in β glucosidase activity is important to obtain balanced enzyme preparations used for the release of

aromatic compounds (odours) from glycosyl-glucose molecules (G-G molecules) (Cabaroglu *et al*., 2003).
The amount of odorant compounds related to sugars (bound fraction) of musts is higher than the quantity of free odorant compounds (free fraction). Treatment with exogenous enzymes allows hydrolysis of G-G molecules. Experiments have shown that the intensity of enzymatic hydrolysis depends on the variety and the efficacy of enzyme preparation used (Ribereau-Gayon *et al.,* 2006).
Enzymatic hydrolysis of glycoside extracts from Muscat, Riesling, Semillon, Chardonnay and Sirah varieties have induced the liberation not only of terpenes, but also of C_{13} norisoprenoids, such as 3-oxo-α-ionol and 3-hydroxy-β-damascenone.
These compounds are totally glycosilated in the grape and, unlike with terpenes, they are found in the same quantities in all grape varieties, aromatics or neutral, and they are capable of awarding certain typicity to the wine flavour because they have lower threshold values than terpenes and they contribute characteristic aromatic features (Mateo and Jimenez, 2000).
Overall, the proportion of different glucosides varies with the climatic conditions, the geographical location of the vineyard area and the ripeness of the grape.
The aim of this research which was carried out in microvinification conditions was to study the main effects of using enzymes with β glucosidase activity on the release of aroma substances in wines made from Chardonnay grapes.

Material and methods

Experiments were done on Chardonnay grapes from Murfatlar vineyard, Constanta, Romania in the climate conditions of the year 2009.
In Table 1 experimental variants and applied technology of Chardonnay wines are presented.

Table 1. Technological variants used for experiments

Variants	Used technology
Variant 1 (V1)	Free run must clarified by gravitational sedimentation and spontaneously fermented by epiphytic microbiota
Variant 2 (V2)	Free run must clarified by ZYMOCLAIRE High CG (1,5 g/100 kg grapes) and spontaneously fermented by epiphytic microbiota
Variant 3 (V3)	Free run must clarified by ZYMOCLAIRE High CG (1,5 g/100 kg grapes) and spontaneously fermented by epiphytic microbiota and finally treated with LALLZYME Beta (5g/hl wine)
Variant 4 (V4)	Free run must clarified by ZYMOCLAIRE High CG (1,5 g/100 kg grapes) and spontaneously fermented by epiphytic microbiota and finally treated with ZYMOVARIETAL Aroma G (3g/hl wine)

All variants have in common the addition of sulfur dioxide SO_2 by using a concentration of 50 mg/l directly applied after destemming and crushing. The clarification was done by gravitational sedimentation with or without enzymatic

preparation addition (ZYMOCLAIRE High CG). The difference between the variants was the mode of sedimentation (gravitational or enzymatic-gravitational). For each variant, the experiments were repeated three times. Each experiment was performed with homogeneous mash derived from grapes from a single cultivation area.

For enzymatic maceration, a pectolytic enzyme preparation was used. ZYMOCLAIRE High CG preparation produced by AEB Group, Spindal France presented good results in our previous tests.

Two types of enzymatic preparation with β glucosidase activity Lallzyme Beta (Lallemand Inc. Quebec, Canada) and Zymovarietal Aroma G (AEB Spindal, France) were used immediately after the end of the of alcoholic fermentation.

Terpenes and norisoprenoids separation and quantification

Terpenes separation and quantification was done by using a method described by Armada *et al.* (2010), with small modifications. A volume of 100 ml must/wine was applied to a preconditioned 500 mg RP C18 SPE column.

Preconditioning was performed by purging at 3 ml min^{-1} the column with 25 ml portions of methanol and water. After loading a sample onto the column it was washed with 150 ml of water. Non-polar fraction (NPF) was eluted using 25 ml of a mixture of pentane/dichloromethane (2/1, v/v). Subsequently, polar fraction (PF) was eluted using 25 ml of methanol and subjected to hydrolysis. Non-polar fraction was evaporated to approximately 500 µl firstly heating it at 30 °C water bath without mixing or stirring for 30 min, then in a delicate stream of nitrogen and 1 µl of it was introduced in a splitless mode into GC-MS system.

The terpenes and C_{13} norisoprenoids from wines were separated and measured using a Varian GC-MS with flame ionization detector (FID) and equipped with a STABILWAX fused silica capillary column (30 m × 0.32 mm i.d.; film thickness 0.5 µm).

A volume of 2 µl sample of the extract was injected in splitless mode (30 s). Temperature program: 1 min hold at 45°C, ramping at 3°C/minute to 230°C, and isotherm during 25min. Helium was used as the carrying gas (18°psi). Temperature of the injector and detector was 230°C.

Compounds identifications were performed by comparing linear retention index and electronic mass spectra with published data or authentic samples. All determinations were done in triplicate and the relative SDs were less than ±1%.

Statistical analyses

The statistical significance of the effect of the enzyme treatment on free and bound volatiles analyzed in triplicate was done by one way ANOVA using Statistica 8 (StatSoft, Inc.). Means between control and treated samples were compared at $P < 0.05$, $P < 0.01$, and $P < 0.001$ by Fisher's least significant difference test.

Sensorial analysis

Sensorial analysis of wine was conducted by a panel of 10 panelists (5 men and 5 women), all persons being certified as authorized wine tasters.

The following descriptors were chosen for Chardonnay wines sensory analysis: olfactory intensity, aroma purity, fruitiness, floral character, vegetable character, mineral character, bitterness, intensity bouquet, roundness, balance of taste, taste persistence. The maximum score of 5 points was awarded for excellent, 4 points for very good, 3 points for good, 2 points for less good and 1 point for poor.

Results and discussions

In Table 2 are presented data obtained for quantification of free terpenes content from free run wine obtained without treatment with enzyme preparations (variant V1), and treated with maceration enzyme (variant V2) and wine made from must treated with maceration enzymes and β glucosidase enzyme (variant V3 and variant V4).

Table 2. Free terpenes from enzymatically treated and untreated variants

Terpenes, µg/l	Variant V1 [a]	Variant V2 [a]	Significance[b]	Variant V3 [a]	Significance[b]	Variant V4 [a]	Significance[b]
α Terpineol	4.4	5.9	**	6.2	**	9.2	ns
Geraniol	10.2	13.4	ns	13.6	*	15.2	**
Linalool	2.0	2.8	*	3.1	**	4.7	**
Total	16.6	22.1		22.9		29.1	

[a] average of triplicate analyses
[b] significance at which means differ between control and treated samples as shown by ANOVA
(*, **, ***) denote significances at $P < 0.05$, $P < 0.01$, respectively $P < 0.001$; ns: not significant.

As it can be seen in Table 2, the total of terpenes compounds was 16.6 µg/l for variant V1, in the case of V2 variant was 22.1 µg/l. By using the enzymatic preparation with β glucosidase activity the total terpenes increased to 22.9 µg/l and 29.1 µg/l for V3 and V4 variant.

The results are in line with the ones obtained by Castro Vazquez *et al.*, 2002 who observed an increase in linalool content by 64.2% in the case of Chardonnay wine made from grapes after a treatment with AR-2000 commercial enzyme preparation. The same author reported an increase in α terpineol by 25% and in geraniol by 50% when AR-2000 commercial enzyme preparation was added.

As shown in Figure 1, the level of 3-oxo-α ionol varied depending on the enzymatic treatment in must and wine.

Thus, it can be observed that the addition of enzymes at the end of alcoholic fermentation has an important role in content norisoprenoids content.

In the case of the 3-oxo-α ionol, the content increased by 19.5% when LALLZYME Beta preparation (5 g/hl wine) was used and by 35.9% when ZYMOVARIETAL Aroma G preparation (3 g/hl of wine) was used compared to variant V1 (Figure 1).

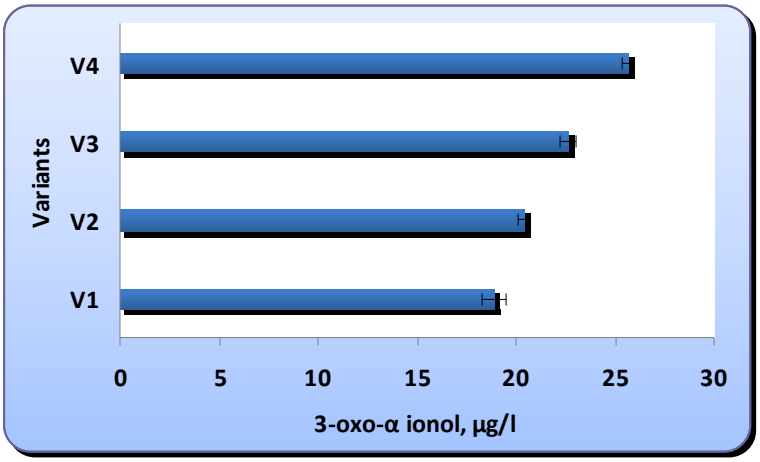

Figure 1. The level of 3-oxo-α ionol for the studied variants

The increasing of the 3-oxo-α ionol value was lower by 10.7% when Beta LALLZYME preparation (5 g/hl wine) was added and by 25.9% when ZYMOVARIETAL Aroma G preparation (3 g/hl of wine) was used compared to variant V2. In the case of 3-hydroxy-7,8-dehydro-β ionol level, an increase by 24.3% was observed when Beta LALLZYME preparation (5 g/hl of wine) was used compared to variant V1.
The increase was higher by 46.0% when Aroma ZYMOVARIETAL G preparation (3 g/hl of wine) was used compared to variant V1 (Figure 2).

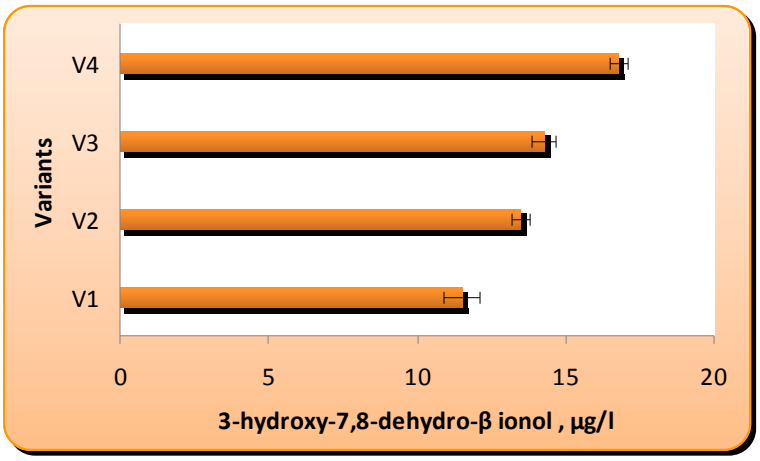

Figure 2. The content of 3-hydroxy-7,8-dehydro-β ionol for the studied variants

If the results are compared to variant V2, the level of 3-hydroxy-7,8-dehydro-β ionol increased only by 5.9% for variant V3 and by 24.4% for variant V4.

This compound 3-hydroxy-7,8-dehydro-β ionol is not odorous but undergoes slowly acidic rearrangement during wine storage and generates 3-hydroxy-β damascenone which is an odorant component (Cabaroglu *et al*., 2003).
In Figure 3 is presented the variation of 3-hydroxy-β damascenone content according to the variants studied. By comparing the content of 3-hydroxy-β damascenone for variants V3 and V4 it was observed that for the variant V3 the quantity was higher by 1.4 µg/l compared to the control variant V1. The content of 3-hydroxy-β damascenone for variant V4 is higher by 3 µg/l compared to the control variant V1.
3-hydroxy-β damascenone presents a flowery, quince like odor and its odor threshold has reported very low, 2 ng/l in water (Buttery *et al*., 1988) and 50 ng/l in 10% alcoholic solution (Guth, 1997).
The total amount of norisoprenoidic compounds was 36.5 µg/l and 41.0 mg/l in the case of V1 and V2 variants.

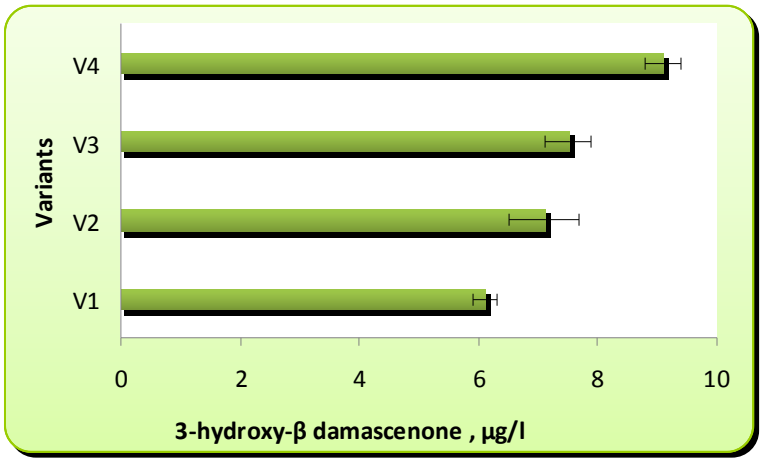

Figure 3. The content of 3-hydroxy-β damascenone for the studied variants

By using the exogenous β-glucosidase, the total norisoprenoids increased to 44.4 µg/l in case of variant V3 and to 51.6 µg/l for variant V4 (Figure 4).
The addition of enzyme preparation with high concentrations in glucosidases acting in the first stage mechanism and in the enzyme β-glucosidase acting on the second stage of the enzymatic mechanism causes high increasing of terpenes and norisoprenoidic compounds concentrations in the case of Chardonnay grapes.

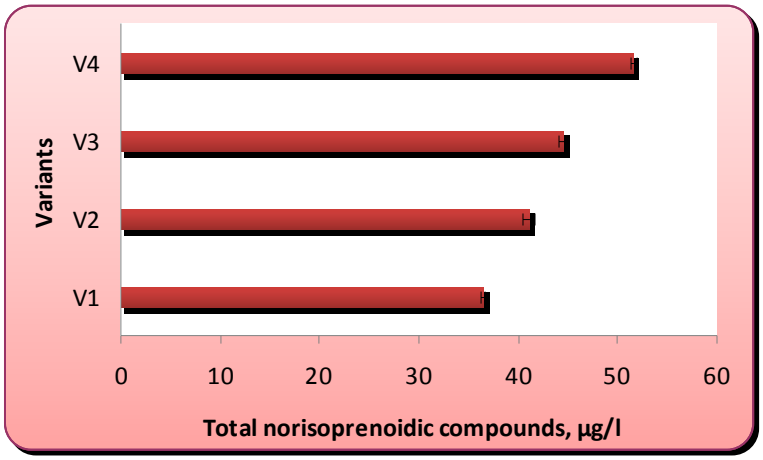

Figure 4. *Total C_{13} norisoprenoidic compounds for the studied variants*

Aromatic profile of wines made by variant V1, variant V2, variant V3 and variant V4 is represented in the radar diagram (Figure 5).

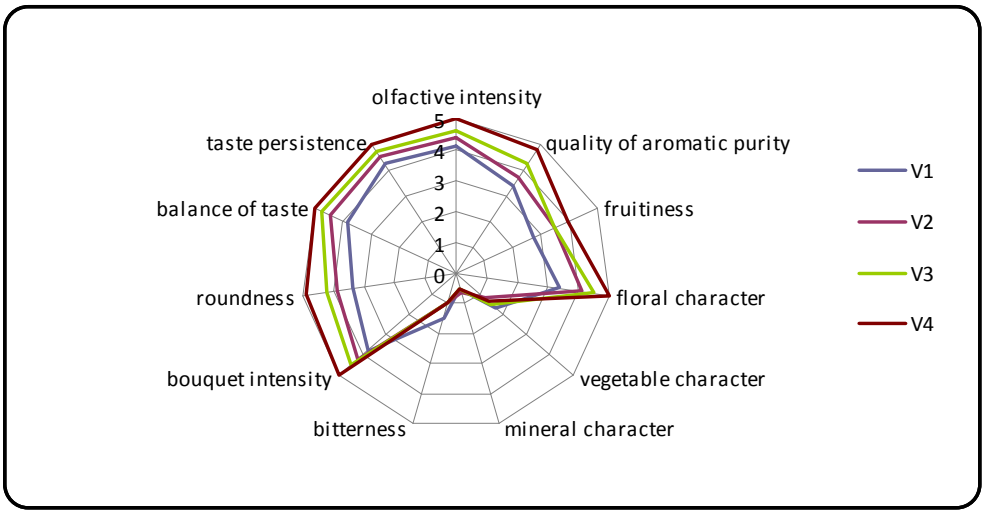

Figure 5. *Aromatic profile of wines*

Wine made from grapes treated with enzyme preparation ZYMOVARIETAL Aroma G (variant V4) present higher sensory flavor characteristics compared to variant V1 and variant V3 treated with enzyme preparation Lallzyme Beta.
Variant V4 treated with ZYMOVARIETAL Aroma G ensures superior sensorial quality wines, with roundness, fruitiness and typical varietal aromas of Chardonnay grape variety.
This benefit is possible to highlight more pronounced of the richness and purity of the varietal flavors typical of the Chardonnay wine variety and area of origin. The

characteristics of fruitiness and stability of odors are maintained during normal maturation and aging of Chardonnay wines.
To the wines made with ZYMOVARIETAL Aroma G enzymatic preparation after alcoholic fermentation, the presence of lower amounts of vegetal volatile compounds that affect the quality of Chardonnay wines was observed.

Conclusions

Addition of β-glucosidases at the end of alcoholic fermentation plays an important role for free terpenes and norisoprenoids content in final wine.
The total content of terpenes was 16.6 μg/l for variant V1 and 22.1 μg/l for the variant V2. Due to the use of β glucosidases, the total terpenes content increased to 22.9 μg/l for variant V3 and to 29.1 μg/l for variant V4.
The total amount of norisoprenoidic compounds was 36.5 μg/l for variant V1 and 41.0 μg/l for variant V2. Due to the use of β glucosidases, the total C_{13} norisoprenoidic compounds increased to 44.4 μg/l (variant V3) and to 51.6 μg/l (variant V4).
The most efficient treatment for flavor compounds release involved the addition of ZYMOVARIETAL Aroma G preparation. It is possible due to the increase in the compounds responsible for aromatic typicity of wines made from Chardonnay grapes, which is reflected by an increase in the real value of quality wines obtained.

Acknowledgements

The authors acknowledge the funding support from S.C. Sodinal SRL, Bucharest, Romania and S.C. Vinex Murfatlar S.R.L. Cernavoda, Romania.

References

Armada, L., Fernández, E., and Falqué, E. 2010. Influence of several enzymatic treatments on aromatic composition of white wines. *LWT - Food Science and Technology*, **43**, 1517-1525.

Buttery, R.G., Terashini, R.,and Ling, L.C. 1988. Identification of damascenone on tomato volatiles, *Chem. Ind*., **7**, 238-241.

Cabaroglu, T., Selli, S., Canbas, A., Lepoutre, J.P., and Günata., Z. 2003. Wine flavor enhancement through the use of exogenous fungal glycosidases. *Enzyme Microbial Technology*, **33**, 581–587.

Canal-Llauberes, R.M. 2000. Les enzymes et leur application en œnologie. Ed. Ferret, Bordeaux dans *Produits de traitement et auxiliaires d'élaboration des moûts et des vins*, 43-60.

Castro Vázquez L., Pérez-Coello M.S., and Cabezudo, M.D. 2002. Effects of enzyme treatment and skin extraction on varietal volatiles in Spanish wines made from Chardonnay, Muscat, Airén, and Macabeo grapes, *Analytica Chimica Acta*, **458**, 39-44.

Cox, A., Capaone, D.L., Elsey, G.M., Perkins, M.V., and Sefton, M.A. 2005. Quantitative analysis, occurrence, and stability of (E)-1-(2, 3, 6-trimethylphenyl)buta-1, 3-diene in wine. *Journal of Agricultural and Food Chemistry*, **53**, 3584-3591.

Guth, H. 1997. Identification of character impact odorants of different white wine varieties, *Journal of Agricultural and Food Chemistry,* **45**, 3027-3032.

Mateo J.J. and Jimenez M., 2000. Monoterpenes in grape juice and wines, *Journal of Chromatography A*, **881**, 557–567.

Ribereau-Gayon P., Glories Y., Maujean A. and Dubourdieu, D. 2006. *Handbook of Enology*, Wiley, Chichester, UK.

Sanchez Palomo E., Perez-Coello M.S., Diaz-Maroto M.C., Gonzalez Vinas M.A., and Cabezudo, M.D. 2006. Contribution of free and glycosidically-bound volatile compounds to the aroma of muscat ‘‘a petit grains’’ wines and effect of skin contact, *Food Chemistry*, **95**, 279–289.