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INFLUENCE OF GLYCOSIDASES ADDITION ON SELECTED MONOTERPENES CONTENTS IN MUSTS AND WHITE WINES FROM TWO GRAPE VARIETIES GROWN IN POLAND*

Mariusz Dziadas, Henryk Jeleń
Poznań University of Life Sciences

Background. Amount of monoterpene alcohol and their glycoside precursors in grapes is determined by the type of grapes, and the availability of specific hydrolytic enzymes during the manufacturing process. Addition of these enzymes, hydrolysing β-glycosidic bond, to the grape can be used to increase the potential of aromatic raw material, releasing odoriferous aglycons from non-volatile glycosides and in consequence to enhance the flavour of white wines. In this study complex enzyme preparations AR2000 and Rapidase X-Press were used as a supplement to the musts.
Material and methods. Two white grape varieties (Nachodka and Perła Zali) grown in Golesz vineyard in Jasło were used for winemaking. Aglycone analysis was conducted on the basis of fast acid hydrolysis and solid phase extraction (SPE-C18) with subsequent GC-MS analysis. Wines treated with enzymes and without enzyme treatment were prepared. Dominating terpenes (linalool, nerol, geraniol, β-citronelol, α-terpineol) were quantified in this study.
Results. Amount of free terpenes was comparable in musts of both varieties (42.7 μg/L for Perła Zali, 46.3 μg/L for Nachodka), however Nachodka had 228.9 μg/L of bound terpenes, compared to 88.8 μg/L in Perła Zali. Addition of glycosidases to musts resulted in an approximately 50 μg/L increase in investigated monoterpenes in Perła Zali and appr. 100 μg/L in Nachodka. Total amount of monoterpenes decreased after 6 months storage, however their levels in enzyme treated wines were significantly higher than in control samples. Enzyme treated wines were evaluated by sensory panel and perceived different from control samples with more pronounced selected notes mainly fruity and floral.
Conclusion. Addition of enzymes can be used to improve the flavour of white wines produced from the average quality of raw material, influencing the increase in aromatic

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Corresponding author – Adres do korespondencji: Prof. dr hab. Henryk H. Jeleń, Institute of Food Technology of Plant Origin of Poznań University of Life Sciences, Wojska Polskiego 31, 60-624 Poznań, Poland, e-mail: henrykj@up.poznan.pl

terpene alcohols. However, one must keep in mind that the changes in amount of released flavour compounds caused by the addition of enzymes may not always have a positive effect.

Key words: white wines, glycosides, monoterpenes, SPE, GC/MS

INTRODUCTION

Poland has not been regarded as a country with large scale wine production. Despite the tradition in certain regions, winemaking collapsed within last 50 years and now is gradually being rebuilt. As the climate in regions where vineyards exist is often harsh, varieties resistant to low temperatures are developed and grown. Application of enzymes in white wines production could be attractive for producers, as the varieties grown are often not very aromatic and wines are usually stored in bottles for maximum of 2-3 years. Flavour compounds that are released from their nonvolatile precursors by enzymes activity could improve flavour of such wines.

In winemaking use of exogenous enzyme preparations helps to overcome the problem of the insufficient activity of endogenous enzymes activity in grapes. Some biotechnological techniques have been of fundamental importance in oenology, among them enzymatic treatments by commercial preparation in free or immobilized form, selected yeasts, improvement of microbial starters and enzyme immobilization. But the use of the enzymes in the wine industry remains limited for two main reasons: traditionalism of winemakers and negative influence of physicochemical characteristics of musts and wines (pH, temperature, ethanol, sugars, polyphenols, etc.) on activity of enzymes [Colagrande et al. 1994].

The odouriferous aglycon moieties of glycosides occurring in plants and influencing their flavour include monoterpenes, C13-norisoprenoids, benzene derivatives, and aliphatic alcohols. The sugar part is represented by glucose or disaccharides: 6-*O*-α-L--arabinofuranosyl-ß-D-glucopyranosides, 6-*O*-α-L-arabino-pyranosyl-ß-D-glucopyranosides (vicianosides), 6-*O*-α-L-rhamnopyranosyl-ß-D-glucopyranosides (rutinosides), 6-*O*-ß-D-apiofuranosyl-ß-D-glucopyranosides, 6-*O*-ß-D-xylopyranosyl-ß-D-glucopyranosides (primeverosides), 6-*O*-ß-D-glucopyranosyl-ß-D-glucopyranosides (gentibiosides) [Stahl-Biskup et al. 1993, Vasserot et al. 1995, Di Stefano et al. 1997, Günata and Sarry 2004, Winterhalter and Skouroumounis 1997, Cabaroglu et al. 2003].

Characteristic flavour of white wines is determined by volatile flavour compounds that come from the grapes. Moreover, a significant part of many flavour compounds is accumulated in grape berries as odorless non-volatile glycosides. [Günata et al. 1985 a, 1988, Voirin et al. 1992, Mateo and Maicas 2005]. The volatile compounds from glycosides during winemaking process can be released by enzyme or acid hydrolyses (Fig. 1) [Williams et al. 1982, Ferreira et al. 2006, Tamborra et al. 2004, Günata and Sarry 2004, Mateo and Jimenez 2000, Mateo and Maicas 2005]. The acid hydrolysis occurs quite slowly in winemaking conditions. The rate of acid hydrolysis is closely dependent on the pH and temperature of the medium and on the structure of the aglycone moiety. Glycosides of tertiary alcohols such as linalool, linalool oxides and a-terpineol are more readily hydrolysed than those of primary alcohols such as geraniol and nerol as it was observed in wine [Günata et al. 1985 b]. After 2 years of storage at 10°C, more than half of the glycosides of geraniol were still present in a Muscat wine,

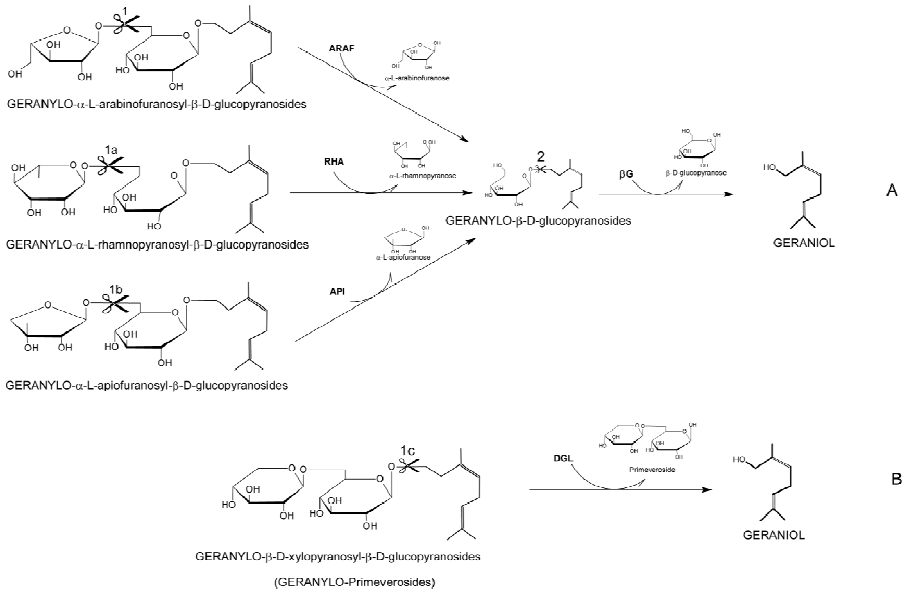

Fig. 1. Structures of glycosidic monoterpene precursors from grape berries and their hydrolysis by specifically enzymes (geraniol shown as example)

while glycosides of linalool were totally hydrolyzed [Günata and Sarry 2004]. A sequential reaction takes place in the enzymatic hydrolysis of diglycosides involving several glycosidases according to the sugars moiety of the substrates [Günata et al. 1988, Cabaroglu et al. 2003] (Fig. 1). Enzymatic release of flavour aglycones proceeds in sequential mode when for hydrolysis enzymes peculiar to chemicals structure of *O*-diglicosides were used. In first stage enzymes α-arabinofuranosidase (ARAF), α--rhamnopyranosidase (RHA), ß-xylopyranosidase (XYL) α-arabinopyranosidase (ARAP) or ß-apiofuranosidase (API), breaking of the linkage between inter-sugars liberating corresponding sugar and ß-D-glucopyranosides combined still with aglycon. In the second stage ß-D-glucosidase (ßG) catalyzes the hydrolysis of ß-D-glucopyranosides and liberates the corresponding aglycone and glucose (Fig. 1 A) [Günata et al. 1988, Mateo and Maicas 2005, Palmeri and Spagna 2007]. Some of enzymes have activity of diglicosidases such a primeverosidase, cutting bonds between disaccharides and aglycon compound (Fig. 1 B).

The monoterpenes originating from grape is the most important aromatic fraction of many white wines. Some of the monoterpene odoriferous alcohols, especially linalool, α-terpineol, nerol, geraniol, ß-citronellol and hotrienol have floral aroma [Ribèreau--Gayon et al. 1975, Güth 1997, Ferreira et al. 2000, Mateo and Jimenez 2000]. The goal of described research was to evaluate the influence of enzymes addition on the release of main monoterpenes from their nonvolatile precursors from must and wine obtained from grape varieties grown in Poland, and their eventual influence on wine flavour.

MATERIAL AND METHODS

Chemical reagents

The following reagents were used: metanol, dichlorometane LiChrosolv (Merck, Darmstadt, Germany), pentane HPLC grade (Fluka, Schwitzerland), citric acid and Na_2HPO_4 (POCH, Poland), octan-2-ol (98%, Aldrich, Steinheim, Germany), linalol (98%), geraniol (97%), nerol (96%), β-citronelol (–97%), α-terpineol (95%). All terpene alcohols were purchased from Aldrich.

Winemaking and enzyme treatment

Musts from Perła Zali (*Traminer* × *Müller Thurgau*) and Nachodka (*Siewiernyj* × *Odeskij Ustojcziwyj*) cultivated in Golesz vineyard in Jasło (Podkarpacie region, Poland), were SO_2 (100 mg/L sodium metabisulfite $Na_2S_2O_5$) treated after putting into plastic tanks at the harvest day and transported to the laboratory immediately. The same day, they were inoculated with *Saccharomyces cerevisiae* CECT No. 10835 (Lallemand Corp.) at 10 mg/L and poured into 5 L glass vessels. For each must a control wine (without addition of enzymes) wine with addition of AR 2000, and wine with addition of Rapidase X-Press enzymes were prepared, each in duplicate. Enzymes used (AR2000 – activities in nkat/mg: BGLC – 5.7, API – 1.08, ARAB – 14.7, RHA – 0.3 [Günata and Sarry 2004] and Rapidase X-Press – activities not reported, both from DSM, Delft, The Netherlands) were added at a level of 5 mg/L. Fermentation was carried out at the room temperature (19-21°C), until complete metabolism of fermentable sugars (dry wine). Wine after fermentation was decanted from over sediment and stored at 10°C in the glass containers and analysed one day after fermentation termination and 6 months later.

Extraction and GC/MS analysis of free and glycosidic forms of monoterpenes from musts and wines samples

Musts or wines samples (100 ml sample + 150 µg 2-octanol in ethanol as internal standard), was analysed in twice replicate using combined methods of Mateo and Macais [2005], Di Stefano et al. [1997] and Pineiro et al. [2004]. Extraction was carried out on the Visiprep SPE station (Supelco Park, Bellefonte, PA) with the aid of large volume extractions kit (Supelco Park, Bellefonte, PA). For SPE 500 mg C-18 columns (Biotage, Uppsala, Sweden) were used, previously activated with 25 ml methanol and then 25 ml pure water. After extraction columns were rinsed with 150 ml pure water. Free forms of monoterpenes were eluted with 25 ml pentane:dichlorometane (2:1, v/v) and concentrated in a laboratory rotavapour (40°C) followed by concentration in a delicate stream of nitrogen to a volume of 500 µl. One microliter of this extract was injected in a splitless mode into a GC/MS. Bound fraction was eluted with 25 ml of methanol and evaporated to dryness in nitrogen stream. Then extract was dissolved in 5 ml of citric-phosphate buffer (0.2 M, pH = 2.5) and fast acid hydrolysed was applied [Ferreira et al. 2006]. Released aglycon was recovered by extractions on the previously activating 500 mg C-18 beds with 25 ml pentane:dichlorometane (2:1, v/v) and concentrated in laboratory rotavapour (40°C) to 500 µl and 1 µl this extract was analysed in splitless mode using GC/MS.

GC-MS analysis

Analyses of free and liberated after hydrolysis monoterpenes were performed using 7890A gas chromatograph coupled to 5975C TAD quadrupole mass spectrometer (both from Agilent Technologies, Santa Clara, CA). A fused capillary column DB-5 MS, (25 m × 0.25 mm × 0.25 µm, J&W, Folsom, CA) was used for compounds separation (He flow 0.8 ml/min). Temperatures of heated zones were following: transfer line – 280°C, injector – 240°C, oven was programmed at 40°C (0.5 min), then 5°C/min to 220°C (5 min), ion source – 230°C. Spectra (70eV) were acquired in a range of 33--333 Da. For the identification of compounds MSD Chemstation ver. E.02.00 search engine, AMDIS ver. 2.65, NIST 05 library and the AMDIS-created library based on retention index were used.

Method validation

Selected method performance parameters such as limits of detection (LOD), limit of quantification (LOQ) were evaluated. LOD was calculated from the signal to noise (S/N) ratio in Chemstation software obtained in the analysis of the mixture of monoterpenes dissolved in ethyl acetate. LOQ was calculated from calibrations curves for LOD mean data. For the determination of recoveries of the free forms of monoterpenes, a 100 ml (n = 9) of white wine was spiked with authentic linalool, α-terpineol, ß-citronelol, nerol and geraniol standards at a concentration of approximately 12 µg/100 ml each.

RESULTS AND DISCUSSION

Limits of detection and quantification were determined to evaluate method and confirm its usefulness in quantification of low levels of monoterpenes in musts and wines. Table 1 shows limits of detection estimated for the instrument (detector) which ranged from 0.34 to 0.49 ng/µl (injection) depending on a compound. Estimated limit of quantification for the whole method ranged from 0.57 µg/L for β-citronellol to 0.82 µg/L for linalool. Recoveries of analysed compounds varied from 89.7% (linalool) to 101.8% (nerol). These values allowed precise determination of monoterpenes in free form and also these released from glycosides during enzymatic hydrolysis in winemaking process.

When both musts were compared in terms of total monoterpenes (free and liberated in the hydrolysis) contents Nachodka contained twice as much compared to Perła Zali (Fig. 2). The amount of terpenes in free forms was approximately the same in both musts, the most significant difference was observed in the contents of terpenes released after hydrolysis. Their contents in Nachodka was over 2.5 times higher than Perła Zali, indicating that the former variety can yield more odouriferous monoterpenes during wine production using enzymes.

The use of exogenous glycosidases (AR2000 and Rapidase X-Press) increased the final total contents of monoterpenes in investigated wines to which were added, compared to the control wines without enzymes addition. An increase of analysed compounds in Perła Zali in control wine sample from 43 µg/L in must to 94 µg/L after fermentation was observed. In young wine samples to which AR2000 and Rapidase

Table 1. Selected parameters of method used in determination of monoterpenes in musts and wines

	RF (TIC/2octanol)	REC %	LOD[a] ng/µl	LOQ[a] ng/µl	LOD[b] µg/L	LOQ[b] µg/L	OT µg/L	References
Linalool	1.18	89.7	0.049	0.165	0.25	0.82	25	Ferreira et al. [2000]
α-terpineol	1.35	100.9	0.035	0.116	0.17	0.58	250	Ferreira et al. [2000]
ß-citronellol	1.08	91.6	0.034	0.114	0.17	0.57	18	Ribèreau et al. [1975]
Nerol	1.22	101.8	0.047	0.157	0.24	0.78	15	Güth [1997]
Geraniol	1.05	90.3	0.048	0.161	0.24	0.81	30	Etiévant [1991]

RF – response factors used for quantification (based on total ion current – TIC) with 2-octanol as an internal standard.

REC – recovery based on the concentration of approximately 12 µg/L of each monoterpene spiked into wine, n = 9.

LOD[a], LOQ[a] – limit of detection and limit of quantitation respectively for the MS detector based on the injection of pure standards dissolved in ethyl acetate.

LOD[b], LOQ[b] – limit of detection and limit of quantitation respectively calculated for the SPE method.

OT – odour threshold for analysed compound in wine originating from cited reference works (References).

Fig. 2. Contents of free and bound (aglycons) monoterpenes in Perła Zali and Nachodka musts

X-Press enzymes were added the concentration of analysed monoterpenes increased to 141 µg/L and 139 µg/L respectively (Table 2). After 6 months storage a decrease in the contents of free monoterpenes was observed, which can be attributed probably either to their oxidation or transformation to compounds not quantified. However, when free monoterpenes were compared in wine after 6 months storage a significant increase was observed for both enzyme treatments (Table 2). The increase of free monoterpenes in wines was associated with a decrease in the contents of bound ones, determined using acid hydrolysis. The initial reservoir of monoterpenes available to enzymes activity in must (89 µg/L) decreased in control wine, but to higher extend in enzyme treated wines. After 6 months storage only minute amounts (approximately 2 µg/L) of bound

Table 2. Changes of investigated monoterpene contents in Perła Zali must and wines treated with enzymes. Bound monoterpenes determined as liberated aglycons using acid hydrolysis of must, control wines and wines treated with enzymes

	Must	Wine AF control	Wine AF RXP	Wine AF AR2000	Wine 6M control	Wine 6M RXP	Wine 6M AR2000	Must	Wine AF control	Wine AF RXP	Wine AF AR2000	Wine 6M control	Wine 6M RXP	Wine 6M AR2000
	free monoterpenes							bound monoterpenes						
Linalool														
µg/L	11.91	21.07	33.73	33.29	25.92	30.78	30.54	34.8	3.71	3.17	2.81	2.65	0.71	0.46
SD	1.09	1.65	0.67	4.15	1.65	0.67	4.15	0.64	0.2	0.23	0.17	0.61	0.05	0.03
α-Terpineol														
µg/L	9.38	19.6	22.56	21.1	21.12	24.72	32.81	16.96	4.7	3.85	3.15	3.26	1.13	1.3
SD	0.01	1.55	8.05	7.06	0.55	8.05	7.06	1.59	0.54	0.28	0.21	0.47	0.09	0.09
Nerol and β-Citronellol														
µg/L	11.05	34.26	56.26	60.61	28.73	37.8	36.91	21.35	1.38	0	0	1.12	0	0
SD	2.31	3.43	6.13	4.48	5.43	6.13	4.48	5.32	0.01	0	0	0.17	0	0
Geraniol														
µg/L	10.4	19.21	26.51	25.81	18.83	20.31	23.66	15.65	2.31	0	0	2.01	0	0
SD	0.65	3.95	2.68	4.9	3.95	2.68	4.9	0.04	0.03	0	0	0.32	0	0
Total, µg/L	42.74	94.14	139.06	140.81	94.6	113.61	123.92	88.76	12.1	7.02	5.96	9.04	1.84	1.76

Wine AF, Wine 6M – wine after fermentation and wine after 6 months respectively.
Control, RXP, AR2000 – wine untreated, treated with Rapidase X-Press and with AR2000 respectively.

monoterpenes were left. All investigated monoterpene alcohols were present in Perła Zali must in comparable levels of approximately 10 µg/L in their free forms, whereas linalool prevailed in bound monoterpenes fraction (Table 2). After fermentation nerol and β-citronellol increased most significantly compared to the remaining monoterpenes. Even after 6 months storage of wine nerol and β-citronellol prevailed.

Nachodka presented a higher potential for monoterpenes release than Perła Zali. Despite the comparable initial concentrations of free terpenes (Fig. 2) the amount of bound monoterpenes was much higher in Nachodka (229 µg/L) than in Perła Zali (89 µg/L). This resulted in much higher amounts of free monoterpenes measured in wine after fermentation and storage. The amount of free monoterpenes in wine after fermentation reached 332 µg/L (for Rapidase X-Press treated wine). However, the decrease in free monoterpenes concentrations was higher for Nachodka than for Perła Zali. Despite this fact due to high initial concentration of monoterpenes their levels in stored Nachodka wines were twice as high as for Perła Zali. As a result of enzymatic release of monoterpenes in production of Nachodka wine bound terpenes decreased to approximately 12 µg/L (Table 3). Contrary to Perła Zali in Nachodka must linalool prevailed in free terpenes fraction, but the most abundant monoterpene in must was α-terpineol. In the fermentation process α-terpineol was a dominating terpene present in free form, whereas after storage the contents of α-terpineol was comparable with nerol and citronellol.

Differences between concentrations of total monoterpenes in control samples and samples with enzymes addition were significant at the end of fermentation, as well as after 6 month storage. The significance of differences in total contents of selected freed monterpenes between enzymes treated and nontreated wine samples was estimated using Tukey test for determination of NIR ($\alpha = 0.05$). According to it, the significant difference was 4.74 ppb, which makes all the samples treated with enzymes significantly different in terms of monoterpenes contents than control samples.

Table 3. Changes of investigated monoterpenes contents in Nachodka must and wines treated with enzymes. Bound monoterpenes determined as liberated aglycons using acid hydrolysis of must, control wines and wines treated with enzymes

	Must	Wine AF control	Wine AF RXP	Wine AF AR2000	Wine 6M control	Wine 6M RXP	Wine 6M AR2000	Must	Wine AF control	Wine AF RXP	Wine AF AR2000	Wine 6M control	Wine 6M RXP	Wine 6M AR2000
	free monoterpenes							bound monoterpenes						
Linalool														
µg/L	16.91	48.07	56.67	60.52	34.12	31.18	37.11	41.8	7.11	5.18	3.71	5.48	1.02	1.19
SD	5.09	1.65	6.07	5.28	6.92	8.71	4.15	4.04	0.15	0.32	0.11	0.1	0.03	0.01
α-Terpineol														
µg/L	13.38	109.6	181.17	151.12	83.23	109.18	91.01	127.03	14.36	14	13.06	12.48	11.52	10.42
SD	2.41	1.55	21.15	12.87	10.96	21.3	11.59	6.12	0.21	1.8	0.18	1.26	1.23	1.23
Nerol and β-Citronellol														
µg/L	7.81	44.26	62.67	61.6	93.12	103.81	93.47	36.87	13.04	0.44	0.64	11.97	0	0
SD	3.78	3.43	8.14	9.18	20.12	13.21	22.11	5.13	0.23	0.02	0.02	2.21	0	0
Geraniol														
µg/L	8.24	18.21	31.98	35.22	26.83	35.31	23.66	23.23	12.81	0.3	0.87	10.12	0	0
SD	3.02	3.95	3.17	5.12	4.12	5.32	4.9	3.41	0.30	0.02	0.03	1.64	0	0
Total, µg/L	46.34	220.14	332.49	308.46	237.3	279.48	245.25	228.93	47.32	19.92	18.28	40.05	12.54	11.61

Wine AF, Wine 6M – wine after fermentation and wine after 6 months respectively.
Control, RXP, AR2000 – wine untreated, treated with Rapidase X-Press and with AR2000 respectively.

Sensory profile analysis

Wines were also assessed using sensory profile analysis. At the first stage main flavour descriptors were estimated by 10 people panel, who has been performing sensory analysis regularly at the Faculty of Food Science and Nutrition. Fruity, spicy, caramel, floral, earthy, wood, oxidative, microbial notes were assessed for both enzyme treated wines (Fig. 3). The sensory panel members distinguished the differences in flavour between wines with and without addition of enzymes. The aroma of enzyme treated wines

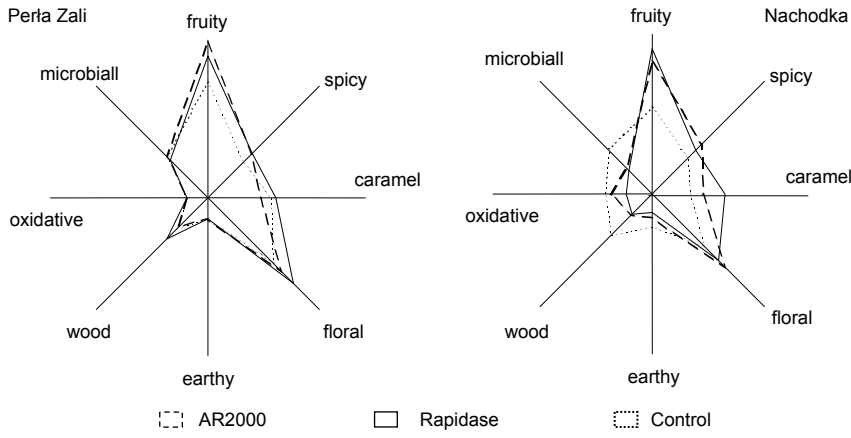

Fig. 3. Sensory profile comparison for wines produced from Perła Zali and Nachodka grape varieties with Rapidase X-Press and AR2000 enzymes

were different than the control wine. For Perła Zali the differences were the most pronounced in fruity notes, in Nachodka enzymes treated samples the differences were even more evident, with fruity notes dominating in enzyme-treated samples. Also floral, caramel, spicy notes were more intensive. A decrease was observed in wood, earthy and microbial notes.

CONCLUSIONS

Terpenes present in wine are compounds that for some wines (such as Muscat) are their key odorants. As grape derived compounds their levels are not directly influenced by fermentation, however production process may change their contents mainly due to their release from their bound forms. The monoterpenes fraction due to the instability of some of the compounds is in a delicate equilibrium in a wine making process. The changes in concentration of monoterpenes in the production of wine can be attributed to several reasons: release of aglycons from bound forms induced by acid hydrolysis in wine (pH = 2.9-3.0) during maturation or due to enzymes activity in enzyme treated wines. It has to be remembered that enzyme activity will be different from optimal at wine storage temperature (10°C). Chemical transformation of one monoterpenes into another will also take place. Odour thresholds of investigated monoterpenes provided in Table 1 indicate, that even their minor change (in terms of concentration) in wine can influence its flavour.

Liberation of monoterpene aglycones by using exogenous glycosidases can enhance the floral and fruity aroma of wines, but these enzymes can also generate other flavour compounds of different character. Therefore the enzyme addition can be a double sided sword for the wine flavour, on one side improving its flavour by a release of positively associated with flavour compounds, on the other hand can disturb the balance in wine aroma and bouquet, or even release compounds that can cause an off flavour in such enzyme treated wine.

REFERENCES

Cabaroglu T., Selli S., Canbas A., Leputre J.P., Günata Z., 2003. Wine flavour enhancement through the use of exogenous fungal glycosidases. Enzyme Microbial Technol. 33, 581-587.

Colagrande O., Silva A., Fumi M.D., 1994. Recent application of biotechnology wine production – review. Biotechnol. Prog. 10, 2-18.

Di Stefano R., Mateo J.J., Jimenez M., Gentilini N., Huerta T., 1997. Fractionation of glycoside precusrsors of aroma in grapes and wine. J. Chromatogr. A, 778, 219-224.

Etiévant P.X., 1991. Wine. In: Volatile compounds in foods and beverages. Ed. H. Marse. Marcel Dekker New York, 483-533.

Ferreira V., Lopez R., Cacho J.F., 2000. Quantitative determination of the odorants of young red wines from different grape varieties. J. Sci. Food Agric. 80, 1659-1668.

Ferreira V., Jesus-Ibarz M., Hernandez-Orte P., Loscos N., Cacho J., 2006. Optimization and evaluation of a procedure for the gas chromatographic-mass spectrometric analysis of the aromas generated by fast acid hydrolysis of flavour precursors extracted from grape. J. Chromatogr. A, 1116, 217-229.

Günata Z., Bayonove C.L., Baumes R.L., Cordonnier R.E., 1985 a. The aroma of grapes. I. Extraction and determination of free and glycosidically bound fraction of some grape aroma components. J. Chromatogr. A, 331, 83-90.
Günata Z., Bayonove C., Baumes R., Cordonnier R., 1985 b. Stability of free and bound fractions of some aroma components of grape cv. Muscat during the wine processing. Am. J. Enol. Viticult. 37, 112-114.
Günata Z., Bitteur S., Brillouet J.M., Bayonove C.L., Cordonnier R.E., 1988. Sequential enzymic hydrolysis of potentially aromatic glycosides from grape. Carbohydr. Res., 184, 139-149.
Günata Z., Sarry J.E., 2004. Plant and microbial glycoside hydrolases: Volatile release from glycosidic aroma precursors. Food Chem. 87, 509-521.
Güth H., 1997. Quantitation and sensory studies of character impact odorants of different white wine varieties. J. Agric. Food Chem. 45, 3027-3035.
Mateo J.J., Jimenez M., 2000. Monoterpenes in grape juice and wines. J. Chromatogr. A, 881, 557-567.
Mateo J.J., Maicas S.J., 2005. Hydrolysis of terpenyl glycosides in grape juice and other fruit juices: a review. Appl. Microb. Biotech. 67, 322-335.
Palmeri R., Spagna G., 2007. Glucosidase in cellular and acellular form for winemaking application. Enzym. Microbiol. Techn. 40, 382-389.
Pineiro Z., Palma M., Barroso C.G., 2004. Determination of terpenoids in wines by solid phase extraction and gas chromatography. Anal. Chim. Acta 513, 209-214.
Ribèreau-Gayon P., Boidron J.N., Terrier A.J., 1975. Aroma of Muscat grape varieties. Agric. Food Chem. 23, 1042-1048.
Tamborra P., Martino N., Esti M., 2004. Laboratory tests on glycosidase preparations in wine. Anal. Chim. Acta 513, 299-303.
Stahl-Biskup E., Intert F., Holthuijzen J., Stengele M., Schulz G., 1993. Glycosidically bound volatiles – A review 1986-1991. Flavour Fragr. J. 8, 61-80.
Vasserot V., Arnaud A., Galzy P., 1995 Monoterpenol glycosides in plants and their biotechnological transformation. Acta Biotechn. 15, 77-95.
Voirin S.G., Baumes R.L., Sapis J.C., Bayonove C.L., 1992. Analytical methods for monoterpenes glycosides in grape and wine. II. Qualitative and quantitative determinations of monoterpene glycosides in grape. J. Chromatogr. 595, 269-281.
Williams P.J., Strauss C.R., Wilson B., Massy-Westropp R.A., 1982. Studies on the hydrolysis of vitis viniferamonoterpene precursor compounds and model monoterpene beta-D-glucosides rationalizing the monoterpene composition of grapes. J. Agric. Food Chem. 30, 1219-1223.
Winterhalter P., Skouroumounis G.K., 1997. Glycoconjugated aroma compounds: occurrence, role and biotechnological transformation. In: Advances in biochemical engineering/biotechnology. Ed. T. Scheper. Springer Heidelberg-Berlin, 74-105.

WPŁYW DODATKU GLIKOZYDAZ NA ZAWARTOŚĆ WYBRANYCH MONOTERPENÓW W MOSZCZACH I BIAŁYCH WINACH Z DWÓCH ODMIAN WINOROŚLI ROSNĄCEJ W POLSCE

Wstęp. Zawartość alkoholi monoterpenowych i ich prekursorów glikozydowych w winogronach jest kształtowana przez odmianę i dostępność specyficznych enzymów hydrolitycznych w trakcie procesu produkcji. Dodatek tych enzymów hydrolizujących wiązanie β-glikozydowe do moszczu może być wykorzystany do oceny potencjału aromatycznego surowca, który jest unieruchomiony w nieaktywnych zapachowo o-glikozydach oraz do poprawy aromatu win gronowych białych. W pracy zastosowano kompleksowe preparaty enzymatyczne AR2000 i Rapidase X-Press jako dodatek do moszczu w celu uwol-

nienia aglikonów terpenowych z cząsteczek glikozydowych. Analizowano wolne aglikony po szybkiej hydrolizie kwasowej za pomocą ekstrakcji do fazy stałej (C18 RP), a następnie techniką chromatografii gazowej i spektrometrii mas (GC/MS).
Materiały i metody. Przebadano dwie odmiany białej winorośli pochodzące z winnicy Golesz w Jaśle (Nachodka i Perła Zali) cechujące się średnią zawartością związków terpenowych (odmiany niearomatyczne). Porównano zawartości aglikonów uwolnionych z glikozydów obecnych w moszczach oraz winach z dodatkiem i bez dodatku enzymów. Alkohole (linalool, nerol, geraniol, β-citronelol, α-terpineol) i ich glikozydowe prekursory ekstrahowano na kolumnach SPE (C-18) i analizowano techniką GC/MS.
Wyniki. Zawartość wolnych alkoholi terpenowych w moszczach wynosiła 42,7 μg/L dla Perła Zali, 46,3 μg/L dla Nachodki, lecz zawartość aglikonów terpenowych z glikozydów wynosiła odpowiednio 228,9 μg/L dla Nachodki i 88,8 μg/L dla Perły Zali. Dodatek glikozydaz do moszczy wpłynął na zwiększenie stężenia badanych monoterpenów o około 50 μg/L u Perły Zali i ok. 100 μg/L u Nachodki. Całkowita ilość monoterpenów obniżyła się podczas 6-miesięcznego leżakowania wina, jednakże w winach z dodatkiem enzymów była istotnie wyższa w porównaniu z próbą kontrolną. Wina z dodatkiem enzymów zostały poddane ocenie sensorycznej i zaobserwowano wyraźne różnice w stosunku do próby kontrolnej w nutach kwiatowych i owocowych.
Wnioski. Dodatek enzymów może być stosowany do poprawy aromatu win gronowych białych produkowanych ze średniej jakości surowca, wpływając na zwiększenie aromatycznych alkoholi terpenowych. Jednakże trzeba mieć na uwadze, iż zmiany wywołane dodatkiem enzymów nie zawsze mogą mieć charakter pozytywny.

Słowa kluczowe: białe wino, glikozydy, monoterpeny, SPE, GC/MS

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