CONTRIBUTION OF ENZYME PREPARATIONS TO THE LINALOOL CONTENT OF WINES MADE FROM THE NON-AROMATIC GRAPEVINE VARIETY ‘FURMINT’ (VITIS VINIFERA L.)

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Abstract

Aim: Terpene compounds were tentatively identified and quantified in musts and wines made from the non-aromatic grape variety ‘Furmint’ (Vitis vinifera L.) after individual treatments with seven different commercial enzyme preparations.

Methods and results: The identification and quantification of the terpene compounds were carried out with headspace solid phase microextraction (HS-SPME) coupled to gas chromatography-mass spectroscopy (GC-MS). The content of terpene compounds in the ‘Furmint’ grape pomace was below detectable level. After enzyme treatments [Lallzyme BETA (Lall), Rohavin VR-C (VRX), Rohapect VR-C (VRC), Rohavin MX (MX), Rohapect D5L (D5L), Endozym - Cultivar A (Cult. A) and β-glucosidase (Glucosidase)], only linalool was detected in semi-fermented musts, the must treated with β-glucosidase exhibiting the statistically highest linalool content (2.25 µg/L). ‘Furmint’ wine produced from must treated with Lall appeared to be the most aromatic (based on sensory analysis) as well as the one with the highest linalool content (2.14 µg/L).

Conclusion: Wines produced from non-aromatic ‘Furmint’ grape pomace treated with different pectolytic enzyme preparations did not generally contain statistically higher levels of terpene compounds.

Significance and impact of the study: This confirms the hypothesis that all enzyme preparations are not appropriate for every types of monovarietal grape pomace, especially regarding the increase of terpenic volatile compound contents.

Key words: wine, terpene, ‘Furmint’ grapes, glucosidase, pectolytic enzyme

Résumé

Objectif: Les composés terpéniques ont été identifiés et quantifiés dans des moûts et vins de la variété non-aromatique ‘Furmint’ (Vitis vinifera L.) après traitements individuels avec sept différentes préparations commerciales d’enzymes.

Méthodes et résultats: L’identification et la quantification de composés terpéniques ont été réalisées par couplage de microextraction en phase solide dans l’espace de tête (HS-SPME) et chromatographie en phase gazeuse-spectrométrie de masse (GC-MS). La concentration des composés terpéniques dans le marc de raisin ‘Furmint’ se situait en dessous du niveau de détection. Après traitements avec enzymes [Lallzyme BETA (Lall), Rohavin VR-C (VRX), Rohapect VR-C (VRC), Rohavin MX (MX), Rohapect D5L (D5L), Endozym - Cultivar A (Cult. A) et β-Glucosidase (Glucosidase)], uniquement le linalol a été détecté dans les moûts semi-fermentés, avec les moûts traités avec β-glucosidase contenant les plus grandes quantités statistiquement prouvées de linalol (2.25 µg/L). Le vin de ‘Furmint’ produit à partir de moûts traités avec Lall semble être le plus aromatique (d’après l’analyse sensorielle) et celui où les plus fortes concentrations de linalol (2.14 µg/L) ont été déterminées.

Conclusion: Les vins produits à partir de marc de raisin de la variété non-aromatique ‘Furmint’ traité avec différentes préparations d’enzymes pectolytiques ne contenaient généralement pas un niveau statistiquement plus élevé de composés terpéniques.

Signification et impact de l’étude: Cela confirme l’hypothèse selon laquelle toutes les préparations d’enzymes ne sont pas utiles pour toutes les monovariétés de marc de raisin, en particulier dans un contexte d’augmentation de la teneur en molécules volatiles issues de composés terpéniques.

Mots clés: vin, terpène, raisins ‘Furmint’, glucosidase, enzyme pectolytique
INTRODUCTION

Enzyme preparations have been widely used in the oenological industry to enhance important wine quality characteristics, which originate from the specific composition of grape skin and pulp, and which especially contribute to the colour and bouquet of the wine (Prosen et al., 2007). The main enzyme groups used in winemaking are pectinase, cellulase, hemicellulase, oxidoreductase, protease, and β-glucosidase, either alone or in different blends. In the European Union, the use of these enzyme preparations in wine production is regulated by the Commission Regulation (EC) No 606/2009.

The main groups of aromatic compounds are the aglycone moieties of glycosides including terpenes, straight-chain alcohols, benzene derivatives, C13 norisoprenoids and volatile phenols, in which the sugar moiety is represented by glucose or disaccharides (Günata et al., 1985; Prosen et al., 2007). Monoterpenes contribute to the characteristic odour of grapes and wines reminiscent of different flowers and are generally present only in low contents. Non-volatile glycosides (or glycosidically bound monoterpenes) have been shown to be tasteless at the levels present in grapes and wines, but they can significantly contribute to the aroma upon hydrolysis (Ribéreau-Gayon et al., 2006). Enzymatic hydrolysis of bound terpenes enhances the intensity of floral aromas in musts, where approximately 50 monoterpenes can be determined. Among them, limonol, geraniol, nerol, citronellol and terpenol are the most abundant. It has been proposed that pectolytic enzymes are suitable for achieving the same purpose (i.e., enhancing floral aroma), moreover, they may further increase the extraction of the main substances and importantly contributing to wine aroma.

‘Furmint’ (Vitis vinifera L.) is a non-aromatic white grape variety from the Pontian Balkanica (Proles pontica) branch that is nowadays largely cultivated in Hungary, Slovenia, Croatia, Austria, and Slovakia. The aim of the present research was to investigate the potential contribution of several commercial enzyme preparations to the enhancement of monoterpenes in ‘Furmint’ musts and wines and evaluate their possible impacts on the sensorial profile of ‘Furmint’ wines.

MATERIALS AND METHODS

1. Grape and wine samples

‘Furmint’ grapes (syn. ‘Sipon’, ‘Moslavac’, ‘Mosler’) (Vitis vinifera L.) clone SI-14 grown in Podravje, the continental part of Slovenia, were used in this study. About 300 kg of grapes were harvested manually at full maturity, crushed and squeezed, and the obtained grape pomace was treated with sulphur (50 mg K₂S₂O₃ per L of must with skins). The grape pomace was subsequently treated with enzyme preparations Lallzyme BETA (Lall), Rohavin VR-C (VRX), Rohapect VR-C (VR-C), Rohavin MX (MX), Rohapect D5L (D5L), Endozym - Cultivar A (Cult. A) and β-glucosidase (Glucosidase), with four replicates per treatment, according to the manufacturers’ instructions (Table 1). The treated grape pomaces were left over night (8 h) at 17 °C and then mixed several times before inoculation with 0.25 g of yeast per litre of must (Saccharomyces cerevisiae, Uvaferm 43, Lallemand) to ensure the uniformity and to minimize the eventual impacts on the musts’ characteristics. Four samples were analysed for each treatment: during fermentation the semi-fermented musts were sampled on 16th Oct. and 20th Oct. 2009, and after fermentation the wines were sampled on 2nd Nov. (young wines) and 7th Dec. (after over 1 month of bottle aging). The samples (10 mL per replicate) were transferred into 20-mL glass vials, crimped and frozen (-20 °C) until analysis.

2. HS-SPME-GC-MS analysis

The extraction of aroma compounds from semi-fermented musts and wines followed the previously described procedure (Prosen et al., 2007). Briefly, each vial was placed for 15 min in a thermostatically controlled bath at 50 °C, then the volatile compounds were sampled for 35 min using headspace-solid phase microextraction (HS-SPME) with a 50/30 µm Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) fibre (Sigma Aldrich) inserted in the headspace. The fibre was subsequently inserted into the injector port of a gas chromatograph and desorbed for 10 min. The sampled volatile compounds were analysed by gas chromatography coupled to mass spectroscopy (GC-MS) using an Agilent 6890 Series GC System with Agilent 5973 Mass Selective Detector bound to an Rtx-20 column (60 m, 0.25 mm ID, 1.0 µm df, Restek, USA). The gas chromatograph was operated under the following conditions: injector, 250 °C; detector, 280 °C; column, initial temperature 50 °C (hold 2 min), then ramped at 10 °C min⁻¹ to 210 °C (hold 40 min). The mass spectrometer was operated in the electron impact (EI) ionization mode, whereas the chromatograms were recorded in the total ion current (TIC) mode. Compounds were tentatively identified on the basis of their retention times and spectra using the searchable EI-MS spectra library (NIST02).

3. Sensory evaluation

The aged wines from each fermentation quadruplicate of the seven enzyme preparations and a control (total of 32 wine samples) were compared among replicates and treatments by a panel of 10 trained wine tasters using the duo-trio difference testing. For assessment, 25 mL of each wine sample were served in covered ISO standard wine glasses at room temperature (22 °C). The sensory tasting
Table 1. Name and description of the studied enzyme preparations

<table>
<thead>
<tr>
<th>Enzyme preparation</th>
<th>Abbreviation</th>
<th>Manufacturer</th>
<th>Manufacturer’s description of the enzyme preparation</th>
<th>Quantity added to grape pomace$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lallzyme-β</td>
<td>Lall</td>
<td>Lallemand</td>
<td>Granulated and formulated for use in white wine varieties high in 'bound' terpenols such as Gewürztraminer and Muscat. BETA is a blend of pectinases with β-glucosidase, rhamnosidase, apiosidase and arabinofuranosidase.</td>
<td>5 g / 100 L, in solution</td>
</tr>
<tr>
<td>Rohavin VR-C</td>
<td>VRX</td>
<td>AB Enzymes</td>
<td>Granulated; this pectinase combined with hemicellulase and protease is particularly suitable for making red wines. The effect of the combined enzymes improves the clarification and filtration of red wines. Pectolytic enzyme (from <em>Aspergillus</em>) with high pro teolytic and hemicellulolytic side activities.</td>
<td>5 g / 100 L, in solution</td>
</tr>
<tr>
<td>Rohapect VR-C</td>
<td>VRC</td>
<td>AB Enzymes</td>
<td>Granulated; pectolytic enzyme (from <em>Aspergillus</em>). This pectinase combined with hemicellulase and protease is particularly suitable for making red wines. The effect of the combined enzymes improves the colour extraction and stability as well as the clarification and filtration of red wines.</td>
<td>5 g / 100 L, in solution</td>
</tr>
<tr>
<td>Rohavin MX</td>
<td>MX</td>
<td>AB Enzymes</td>
<td>Liquid; highly purified pectolytic enzyme (from <em>Aspergillus</em>) for the treatment of crushed grape juice, supports the extraction of various bouquet compounds. Especially suitable for the skin maceration of white grapes or for making rosé wines. The highly specific pectinases primarily break down the soluble pectin and thus improve preliminary juice extraction.</td>
<td>5 mL / 100 L, in solution</td>
</tr>
<tr>
<td>Rohapect DSL</td>
<td>D5L</td>
<td>AB Enzymes</td>
<td>Liquid; pectolytic enzyme (from <em>Aspergillus</em>), universal application in juice processing.</td>
<td>5 mL / 100 L, in solution</td>
</tr>
<tr>
<td>Endozym - Variety A</td>
<td>Cult.A</td>
<td>Pascal Biotech</td>
<td>Microgranulated; pectolytic enzyme based on pectinase which ensures the extraction of varietal characteristic compounds during the grape treatments. The aromatic compounds present in molecules combined with sugars in the form of terpenic bi-glycosides are extracted in the volatile form in the end of the fermentation process.</td>
<td>5 g / 100 L, in solution</td>
</tr>
<tr>
<td>β-glucosidase</td>
<td>Glucosidase</td>
<td>Fluka</td>
<td>Microgranulated; from <em>Aspergillus niger</em>.</td>
<td>0.25 g / 100 L, directly</td>
</tr>
</tbody>
</table>

$^a$The use and dilution of the enzyme preparation was performed according to manufacturer’s instruction.
room was equipped in accordance with international wine competition standards (OIV, 1994). The tasters assessed and rated the aroma (by smelling) and flavour (by tasting) perceptions in triplicate, following a total of four training sessions. Prior to tasting, each taster was provided with information about the study and was instructed on how to complete the wine sensory evaluation sheet. At each session, each assessor evaluated the same four samples, with one example of each treatment in each session. Tasters were asked to rate each aroma and “in-mouth” character according to a descriptive 10-point intensity scale (0 - no perception, 2 - poor perception, 4 - average perception, 6 - above average perception, 8 - high perception, 10 - extremely high perception). Data acquisition was carried out using Statgraphics 4.0 software (Manugistics, Germany).

4. Statistical analysis

Data were analysed with Statgraphics 4.0 software. Statistical significance was performed using one-way ANOVA and Duncan test at $P \leq 0.05$ (*) and $P \leq 0.01$ (**).

RESULTS AND DISCUSSION

According to Ribéreau-Gayon et al. (2006) and Murányi and Kovács (2000), a wide range of monoterpenes was expected in ‘Furmint’ musts and wines. However, here the content of terpene compounds in ‘Furmint’ grape pomace was below detectable level (data not shown) and the only monoterpane compounds confirmed and quantified in enzyme-treated musts and wines was linalool. Irrespective of treatment, in semi-fermented musts (16th Oct.) the average linalool content was 2.00 µg/L, while the statistically highest average linalool content (2.25 µg/L) was determined in the same musts treated with $\beta$-glucosidase (Table 2). After 4 additional days of fermentation (20th Oct.), the average linalool contents generally decreased: the statistically highest linalool content was determined in musts from the control grape pomace (untreated; 2.11 µg/L), while the lowest was determined in musts from grape pomace treated with VRX and Lall (1.90 and 1.89 µg/L, respectively). After fermentation, the average linalool contents in young wines (2nd Nov.) decreased slightly, irrespective of the enzyme preparation, and reached an average value of 1.91 µg/L, with no statistically significant differences among treatments (Table 2). After one month of bottle aging (7th Dec.), the highest average linalool content was determined in wines deriving from grape pomace treated with Lall (2.14 µg/L), followed by those treated with Cult. A (2.01 µg/L). According to the manufacturer’s product description for Cult. A, the results of monoterpane enhancement in wines were expected. On the other hand, the considerable efficiency of Lall enzyme preparation could be explained by its specific composition of selected enzymes (pectinases with $\beta$-glucosidase, rhamnosidase, apiosidase and arabinofuranosidase), which are usually recommended for musts of aromatic varieties such as ‘Gewürztraminer’ or ‘Muscats’. The enzyme preparations VRC and VRX are recommended for red varieties, especially for colour extraction, stability and clarification, and therefore their effects on linalool contents in wines were also expected. Rohapext D5L enzyme preparation is recommended for universal use, but the results did not show statistically significant differences in linalool contents. The same result was observed when using pure $\beta$-glucosidase, which is contrary to the results reported by Sánchez-Palomo et al. (2005). The preliminary results of our study did not confirm the previously reported influence of enzyme preparations on a higher release of monoterpenes in wines, especially in those from less aromatic grape varieties.

Table 2. Average linalool content (µg/L) in ‘Furmint’ semi-fermented musts (16th Oct. and 20th Oct.) and wines (young wine, 2nd Nov.; aging wine, 7th Dec.) after treatment with enzyme preparation (data are means of 4 replicates ± SD; n = 32)

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>16th Oct.</th>
<th>20th Oct.</th>
<th>2nd Nov.</th>
<th>7th Dec.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lall</td>
<td>2.03 ± 1.51 ab</td>
<td>1.89 ± 1.11 ab</td>
<td>2.00 ± 0.38</td>
<td>2.14 ± 0.12 c</td>
</tr>
<tr>
<td>VRX</td>
<td>1.89 ± 0.38 a</td>
<td>1.90 ± 1.32 a</td>
<td>1.89 ± 0.37</td>
<td>1.91 ± 0.13 b</td>
</tr>
<tr>
<td>D5L</td>
<td>1.92 ± 1.48 a</td>
<td>2.01 ± 1.43 abc</td>
<td>1.88 ± 1.26</td>
<td>n.d.</td>
</tr>
<tr>
<td>MX</td>
<td>2.03 ± 0.51 ab</td>
<td>2.02 ± 1.23 abc</td>
<td>1.90 ± 0.31</td>
<td>1.81 ± 0.11 ab</td>
</tr>
<tr>
<td>VRC</td>
<td>2.08 ± 1.14 bc</td>
<td>2.01 ± 0.44 abc</td>
<td>1.91 ± 0.53</td>
<td>1.89 ± 0.08 ab</td>
</tr>
<tr>
<td>Cult. A</td>
<td>1.93 ± 1.29 a</td>
<td>2.01 ± 1.48 abc</td>
<td>1.87 ± 0.68</td>
<td>2.01 ± 0.08 c</td>
</tr>
<tr>
<td>Glucosidase</td>
<td>2.25 ± 0.10 c</td>
<td>2.02 ± 1.28 dc</td>
<td>1.90 ± 0.78</td>
<td>1.78 ± 0.18 a</td>
</tr>
<tr>
<td>Control</td>
<td>1.92 ± 1.73 a</td>
<td>2.11 ± 0.51 c</td>
<td>1.91 ± 0.79</td>
<td>1.91 ± 0.05 b</td>
</tr>
</tbody>
</table>

Identical letters within column denote no significant difference between data at $P < 0.05$ (Duncan test); n.d. – not defined
The descriptive sensory evaluation of ‘Furmint’ wines is given in Figure 1. Based on the chemical analysis results, the wines produced from grape pomace treated with Lall were expected to score higher in sensory tasting. This was confirmed since these wines showed statistically significant odours reminiscent of fresh cut grass, paprika, spices ($P \leq 0.05$) and other green aromas ($P \leq 0.01$). These wines were also the most pleasant but scored lower for harmony, which can partly be explained by the excessive cumulative odour. Unexpectedly, the control wine obtained the second highest scores for aroma reminiscent of grass, but medium scores for other sensory attributes. Wines made from grape pomace treated with VRX also exhibited high intensities for citrus, paprika, green beans and other green aromas, however, these were not significantly different from control. These wines also scored high for impression and pleasure (Figure 1). The most unpleasant wines were those produced from grape pomace treated with β-glucosidase, Cult. A and DSL.

CONCLUSION

It can be generally concluded that the treatments with the studied enzyme preparations did not significantly contribute to the enhancement of monoterpenes and, consequently, the sensory evaluation of wines from the non-aromatic grape variety ‘Furmint’. It should be pointed out, however, that some of the other evaluated odours are caused by aromatic compounds such as esters, aldehydes, ketones, C13 norisoprenoids, etc. which were not the object of our research. The significant impact of Lall enzyme preparation on the wine bouquet can be explained by its composition, that is, the unique blend of pectinases with β-glucosidase, rhamnosidase, apiosidase and arabinofuranosidase.

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