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Food Chemistry 135 (2012) 80–87

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Release of wine monoterpenes from natural precursors by glycosidases from *Oenococcus oeni*

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A R T I C L E   I N F O

Article history:
Received 6 November 2011
Received in revised form 15 April 2012
Accepted 17 April 2012
Available online 27 April 2012

Keywords:
Oenococcus oeni
Wine
Aroma
Terpene
Glucosidase
Arabinosidase

A B S T R A C T

It is now well established that wine-related lactic acid bacteria (LAB), especially *Oenococcus oeni*, possess glycosidase activities that positively contribute to wine aroma through the hydrolysis of grape-derived aroma precursors. In our recent studies, we have identified and characterised several LAB glycosidases with potential in these terms. Here, we report that both a glucosidase and an arabinosidase from *O. oeni* can release high amounts of monoterpenes from natural substrates under optimal conditions, indicating that these intracellular enzymes might play a significant role in the hydrolysis of aroma precursors during malolactic fermentation. The enzymes from *O. oeni* exhibited broad substrate specificities (release of both primary/tertiary terpene alcohols) and were even active in grape juice. Further, a sensory panel clearly preferred enzyme-treated Riesling wines over the controls and affirmed that the glycosidases from *O. oeni* could improve the typical Riesling aroma.

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1. Introduction

Wines are highly complex beverages, various combinations of flavour components, such as acids, sugars, phenols and volatile aroma compounds generate a multitude of sensorial variations (Jackson, 2008). Although over 800 wine aroma compounds have been identified, only a limited number thereof makes a significant contribution to the wine aroma (Rapp & Mandery, 1986). Volatile constituents of the primary grape aroma, especially monoterpenes that are formed in the grapes during ripening, are the key components of the varietal wine bouquet. As demonstrated by Rapp (1992, 1998), GC fingerprint analysis of only a selected number of wine terpenes can be used to distinguish between grape varieties and even to determine the region of origin.

In addition, the sensory properties of wines are influenced by the microorganisms (yeasts, malolactic bacteria, molds) involved in the winemaking process and by the choice of vinification techniques (types of fermentors, aging style, blending, bottle closure, etc.) (Jackson, 2008). Market and consumer preferences exhibit a considerable influence on the style of wines produced as well, which not only affects the choice of grape varieties planted but also the applied viticultural and enological practices (Bruwer, Saliba, & Miller, 2011).

The recent developments in winemaking and marketing practices show that wine aroma composition has gained increasing importance in recent years (Bruwer et al., 2011). An important aspect in wine aroma tailoring is the fact that a significant fraction of the aroma compounds present in grapes and wine occurs as non-volatile odourless glycosides (Gunata, Bayonove, Baumes, & Cordonnier, 1985); these are mainly found in the grape juice rather than in the skin and pulp (Strauss, Wilson, Gooley, & Williams, 1986). The precursors of important monoterpenes (e.g., linalool, geraniol, nerol, β-citronellol and α-terpineol), C_{13}-norisoprenoids, benzene derivatives and phenols are synthesised during the early development of the grape berry. These precursors have been identified as monoglucosides and diglycosides; in the latter group, glucose can further be conjugated to apiose, arabinose, rhamnose or xylose (Gunata, Bitteur, Brillouet, Bayonove, & Cordonnier, 1988; Williams, 1993). With the aim of improving the characteristic varietal wine aroma, many authors have investigated the possibilities of sequential enzymatic hydrolysis of these aroma precursors by glycosidases (glucosidase, arabinosidase, rhamnosidase, apiosidase) (Maicas & Mateo, 2005; Palmeri & Spagna, 2007). It has been shown that fungal glycosidases that are often present as side activities in pectolytic enzyme preparations are suited for such a purpose (Maicas & Mateo, 2005). On the other hand, detailed studies have been committed to the impact of wine microorganisms, especially yeasts, on wine aroma (Antonelli, Castellari, Zambonelli, & Carnacini, 1999; Kotseridis & Baumes, 2000). Other authors have

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http://dx.doi.org/10.1016/j.foodchem.2012.04.099

focused on the aroma change caused by lactic acid bacteria (LAB) involved in malolactic fermentation (MLF) (Boido, Lloret, Medina, Carrau, & Dellacassa, 2002; D'Incecco et al., 2004; Ugliano, Genovese, & Moio, 2003). Grimaldi, Bartowsky, and Jiranek (2005a, 2005b) presented a comprehensive survey demonstrating that wine-related LAB (*Oenococcus oeni*, *Lactobacillus* spp. and *Pediococcus* spp.) possess the ability to hydrolyse various synthetic glycosides. Furthermore, it has been shown that high variations in glycosidase activities exist among isolates of *O. oeni* (Gagné et al., 2011; Ugliano & Moio, 2006). These studies indicated that wine LAB, in particular *O. oeni*, are indeed capable of releasing attractive aroma compounds during MLF and that LAB might be a promising source of novel glycosidases with oenological potential (Matthews et al., 2004). Therefore, it is of interest to identify the mechanisms that enable LAB, especially *O. oeni*, to release glycosylated aroma compounds. In our previous work, we were able to identify a glucosidase and an arabinosidase from *O. oeni* (Michlmayr, Schümann, Kulbe, & del Hierro, 2011; Michlmayr, Schümann, Barreira Braz Da Silva, Kulbe, & del Hierro, 2010). In the present study, we continued our research to determine if these glycosidases are capable of releasing monoterpenes from natural glycosidic precursors. Therefore, samples of Austrian wine and grape juice were prepared to perform assays with the aim of evaluating these enzymes' performance on different natural substrates under varying conditions (pH, sugar content) and in comparison to fungal glycosidases. Additionally, the results of applying both *O. oeni* glycosidases at an early stage (cold maceration) in the production of a typical Austrian white wine variety (Rheinriesling) are presented.

2. Materials and methods

2.1. Enzymes used in this study

A list of all enzyme preparations used in this study is provided in Table 1. The physicochemical and kinetic properties of the bacterial glycosidases involved have been reported before (references in Table 1). The fungal enzyme preparations are commercial products. The abbreviations (letter codes) as displayed in Table 1 are used throughout the paper, especially in the results section.

All bacterial glycosidases (GO, GL, AO, R) were heterologously expressed and purified as previously described (Michlmayr, Brandes et al., 2011; Michlmayr, Schümann et al., 2011; Michlmayr, Schümann, Wurbs et al., 2010). The resulting enzyme fractions were further purified by ion exchange chromatography (Source Q for GL, AA and Source S for GO, R; both from GE Healthcare, Uppsala, Sweden) following the suppliers' recommendations. The resulting enzyme fractions were dialysed over night against 20 mM citrate phosphate buffer, pH 7 (McIlvaine, 1921), at 4 °C and stored in this buffer. If required, the enzyme solutions were concentrated, using Amicon Ultra centrifugal filters (MWCO 10 kDa) (Millipore, Billerica, MA). All enzyme preparations were stored at 4 °C.

Glycosidase activities were determined with synthetic *p*-nitrophenyl (*p*NP) glycosides (all from Sigma–Aldrich, Vienna, Austria). The substrates used were *p*NP-β-D-glucopyranoside, *p*NP-β-D-galactopyranoside, *p*NP-β-D-xylopyranoside, *p*NP-α-L-arabinofuranoside, *p*NP-α-L-arabinopyranoside and *p*NP-α-L-rhamnopyranoside. The synthetic glycosides were dissolved in 10% (v/v) dimethyl sulfoxide. Unless mentioned otherwise, the conditions for all enzyme assays were: 10 mM substrate in 0.1 M McIlvaine buffer, pH 5.5, 37 °C, 10 min incubation time. The reactions were stopped with 0.5 M Na_2CO_3 (2-fold volumetric excess). The absorbance of *p*-nitrophenol was measured at 400 nm (ε_{400} = 18.300 M^{-1} cm^{-1} at pH 10.2) in a Beckman DU 800 spectrometer (Palo Alto, CA).

One unit of glycosidase activity is expressed as 1 μmol of *p*-nitrophenol released per min at 37 °C.

2.2. Enzyme assays (terpene release) with commercial samples of wine and grape juice

Samples of wine and grape juice were prepared, to obtain controlled conditions for enzyme assays. White wine (Traminer 2010, Klöch, Austria) was evaporated (Heidolph Rotovapor; Heidolph, Schwabach, Germany) at 35 °C to remove ethanol and the background of volatile compounds; the pH was adjusted to 5.5 with $KHCO_3$. After readjustment to the original volume, the wine extract was sterilised by filtration (0.22 μm filter, Millipore). Two brands of commercial red grape juice ("St. Laurent", Stift Klosterneuburg, Austria and "Happy Day", Rauch, Rankweil, Austria) were sterilised by filtration as described above; if required, the pH was adjusted to 5.5 with $KHCO_3$ before filtration. The results of an analysis of the ingredients of wine extract and grape juices are shown in Table 2.

All enzyme assays (terpene release) were conducted using 10 mL of sample (triplicate determinations). The samples were treated with the enzyme preparations in excess (2 U/mL as determined with *p*NP-glycosides (Section 2.1) in different combinations. The arabinosidases (AO, AA) and a rhamnosidase (R) were each applied in combination with the glucosidase of *O. oeni* (GO). Naringinase (N) was applied alone or in combination with GO. All assays were performed under sterile conditions, the enzyme preparations were sterilised (0.22-μm filter) before application. The samples were incubated for 7 days at 15 °C. After the incubation period, the samples were frozen (−30 °C) until terpene analysis (Section 2.4) of the volatile fraction was performed.

2.3. Vinification experiments with glycosidases of O. oeni

Five hundred kilograms of Rheinriesling grapes, an aromatic white wine variety widely cultivated in Austria, were harvested

Table 1
Enzyme preparations included in this study.

Enzyme	GH[a]	Source organism	Code	Reference/supplier
β-D-glucosidase	3	*Oenococcus oeni* ATCC BAA-1163	GO	Michlmayr, Schümann, Wurbs et al. (2010)
β-D-glucosidase	3	*Lactobacillus brevis* SK3	GL	Michlmayr, Schümann et al. (2010), Michlmayr, Schümann, Wurbs et al. (2010)
β-D-glucosidase	3	*Aspergillus niger*	GA	Megazyme, Wicklow, Ireland
α-L-arabinofuranosidase	51	*Oenococcus oeni* ATCC BAA-1163	AO	Michlmayr, Schümann et al. (2011)
α-L-arabinofuranosidase	51	*Aspergillus niger*	AA	Megazyme, Wicklow, Ireland
α-L-rhamnopyranosidase	78	*Pediococcus acidilactici* DSM 20284	R	Michlmayr, Brandes et al. (2011)
Naringinase[b]	–[c]	*Penicillium decumbens*	N	Sigma–Aldrich, Vienna, Austria
Maceration C[d]	–	*Aspergillus niger*	MacC	Preziso (Lagerhaus), Austria
Trenolin Super DF[e]	–	(not specified)	–	Erbslöh, Geisenheim, Germany

[a] Glycoside hydrolase family (Cantarel et al., 2009; Henrissat & Davies, 1997).
[b] Commercial rhamnosidase preparation, side activities are shown in Fig. 1.
[c] Not specified by the supplier.
[d] Maceration preparation with side activities of β-D-glucosidase (3.5 U/g), β-D-xylosidase (1.3 U/g) and α-L-arabinofuranosidase (5.3 U/g).
[e] Pectinase preparation with side activities of β-D-xylosidase (0.2 U/mL) and α-L-arabinofuranosidase (3.9 U/mL).

Table 2
Composition of grape juice and wine extract used in enzyme assays. Analyses were performed using validated standard methods in an accredited test laboratory (ISO 17025).

	Grape juice "St Laurent"	Grape juice "Happy Day"	Wine extract (Traminer)
Fructose[a] (g/L)	85.2	75.3	1.7
Glucose[a] (g/L)	85.5	74.6	nd
Total sugar[b] (g/L)	189	162	–
Titratable acid[c] (g/L)	8.2	6.3	1.6
Tartrate[b] (g/L)	4.6	5.2	1.0
Malate[b] (g/L)	5.0	3.0	3.0
Lactate[b] (g/L)	nd	nd	nd
SO_2[d] (mg/L)	nd	nd	nd
K^+[e] (mg/L)	1286	1591	>2000
Na^+[e] (mg/L)	31	23	22
Mg^{2+}[e] (mg/L)	111	76	83
Ca^{2+}[e] (mg/L)	138	121	68
pH	3.3	3.0	5.5

nd, Not detectable.
[a] Enzymatic determination (test kits from Roche diagnostics).
[b] Fourier transform infrared spectroscopy (Foss WineScan FT 120).
[c] Expressed as tartrate.
[d] Determined per titration (NaOH).
[e] Atomic absorption spectroscopy.

(2010 vintage) at the vineyards of the College for Oenology and Viticulture in Klosterneuburg, Austria. After cleaning, destemming and sorting, the grapes were crushed (roller crusher QU75, Benczak GmbH & Co. KG, Siegendorf, Austria). During crushing, 125 mg/kg of dimethyl dicarbonate (DMDC) (Velcorin®, Lanxess GmbH, Leverkusen, Germany) were added to inhibit wild yeasts and lactic acid bacteria. The free run juice of the resulting mash had a pH of 2.9, a total acidity of 13.1 g/L and 163 g/L of reducing sugars. SO_2 (50 mg/kg as potassium metabisulfite; PMS) was added to the mash and the pH was adjusted to pH 4.0 using 480 g $CaCO_3$ and 275 g of $KHCO_3$. The mash was thoroughly mixed and kept at 8 °C for 24 h to give time for the DMDC to react. Subsequently, the mash was divided into pre-cleaned 45 L tanks and treated with enzyme preparations as follows: GO: 300, 200, 60 U/L; AO: 35 U/L; GO + AO: 150 + 25 U/L; Maceration C (Preziso, Austria) 3 g/hL; two tanks were kept without enzyme as controls. After thorough mixing, a further 20 mg of SO_2 (PMS) were added to each tank on the top of the mash. The tanks were tightly sealed and kept at 12 °C for 4 days. Before pressing, the mash of the recombinant enzyme treatments and one of the controls (C2) were supplemented with 8 mL/hL Pectinase (Trenolin Super DF, Erbslöh, Geisenheim) to facilitate must extraction (following the producers' recommendations 2 h before pressing). After pressing, the musts were chaptalised to a must weight of 18 °KMW ("Klosterneuburger Mostwaage", corresponding to 214 g/L total sugar or 12.8% (v/v) potential alcohol; Ribéreau-Gayon, Glories, Maujean, & Dubourdieu, 2006) with sucrose. Demijohn carboys (35 L each) were washed with 2% NaOH, 2% citric acid and rinsed with tap water. The musts were brought to 20 °C room temperature for the start of fermentation; 5 g of fermentation nutrient (Fermaid E, Lallemand, Vienna, Austria) were added to each demijohn. SO_2 was adjusted to 50 ppm free in each balloon to prevent wild yeast fermentation. The musts were fermented using Oenoferm Freddo yeast (Erbslöh Geisenheim, Germany) at the recommended rate for low temperature fermentation (15 g/hL). After the fermentation started and more than 1% (v/v) alcohol built up, the demijohns were cooled down to 12 °C. When the wines reached 8% (v/v) alcohol, a further 2 g of fermentation nutrient (Fermaid E) were added per demijohn and the fermentations were completed at 20 °C. The wines were then cooled to 4 °C and cross-flow filtered using a Lab4-102 (Romfil GmbH, Wolfsheim, Germany) filtration module of 0.2 μm at 1 bar. After filtration, 50 ppm SO_2 as PMS was added. All wines presented between 9.6 and 10.0 g/L total acidity and 6.8–7.0 g/L malic acid. Deacidification of the wines to 7 g/L total acidity was carried out by double salt deacidification, following the method proposed by Steidl (2001). After deacidification, all wines were adjusted to 45 mg/L free SO_2, microfiltered over a Cuno 3 M Zeta Plus H cartridge 80H05 (0.5 μm diameter pore cut-off), bottled in 375-mL bottles and stored at 10 °C.

2.4. Analysis of terpene compounds

Volatile compounds were analysed by gas chromatography–mass spectrometry (GC/MS). The analytical procedure is based on the method described by Skinkis, Bordelon, and Wood (2008). A 7890A GC system (Agilent technologies, Paolo Alto, CA) with a DB-5 capillary column (60 m × 0.25 mm, 0.25 μm; stationary phase 5% dimethyl polysiloxane, 95% phenyl polysiloxane), a CombiPal autosampler (CTC analytics, Zwingen, Switzerland) and a 5975C MS detector (Agilent) were used. The samples were prepared by solid-phase micro-extraction (SPME). Five millilitres of sample and 50 μL of the internal standard (4-chlorobutyl acetate) were added to a vial containing 2 g NaCl. SPME fibres (100 μm polydimethylsiloxane) from Supelco (Bellefonte, PA) were used as absorbant. Extraction was performed for 30 min at 50 °C, followed by desorption for 5 min at 250 °C. The samples were injected in splitless mode (3 min), the carrier gas was helium (99.999%; Air Liquide, Vienna, Austria) with a flow of 1.2 mL/min. The program for the oven temperature was as follows: initial temperature 50 °C for 3 min, temperature increase to 92 °C (1 °C/min), holding time 10 min; further increase to 127 °C (5 °C/min), then increase to 260 °C (40 °C/min), holding time 5 min. The transfer line temperature was 260 °C. Ionisation was performed at 70 eV. Ions were quantified by selected ion monitoring (SIM) relative to the internal standard 4-chlorobutyl acetate (m/z = 54). The following standards were applied (the m/z ratios used for quantification are shown in parentheses): *cis*/*trans*-linalool oxide (59), linalool (71), hotrienol (71), *cis*/*trans*-rose oxide (139), *cis*-limonene oxide (67), *trans*-limonene oxide (94), α-terpineol (93), β-terpineol (71) γ-terpineol (121), nerol (69), β-citronellol (69), geraniol (69), nerol oxide (68) and lavandulol (69). Rose oxide was obtained from Moellhausen (Vimercate, Italy), hotrienol from Treatt (Lakeland, Florida), nerol oxide from Roth (Karlsruhe, Germany). All other standards were obtained from Fluka (Sigma–Aldrich, Vienna, Austria).

The limit of quantification (LOQ) was determined as 0.3 μg/L, the relative standard deviation between repeated samples (repeatability) was below 6%.

2.5. Sensory analysis

The wines were evaluated in triplicate by a panel of seven trained tasters. The participants are officially approved tasters for the quality assessment of Austrian wines. The tasters are trained according to the Austrian wine law and their performance is evaluated annually. Assessment took place in a standard sensory analysis chamber (EN ISO 8589) equipped with separate booths under yellow light forcing the tasters to focus only on the aroma and taste of the wines. The tasters were presented with the wines in groups of five wine glasses each. The wines were presented in randomised order in coded standard tasting glasses (ISO 3591). In each group, the tasters were first asked to rank the wines on their aroma intensity using an unstructured scale ranging from 1 to 5 with 1 the highest and 5 the lowest aroma intensity. The wines where then sorted according to the perceived aroma intensity, following the method of Cartier et al. (2006).

Second, the tasters had to assess each wine based on their olfactory and taste sensations on an unstructured scale from 0 to 10,

with 10 reflecting the highest intensity of each attribute. The attributes involved were typical Riesling descriptors (stone fruit, citric, pomaceous fruit), attributes usually associated with white wines but not with Riesling (freshness, spice, tropical, candy). Further attributes were "floral" (corresponding to terpenoid aroma compounds) and "typicality" (Riesling).

2.6. Statistical analysis

The data from terpene analysis (Section 2.4) were statistically analysed with the software package SPSS 18. One-way analysis of variance (ANOVA) was applied to test for significant differences between the individual treatments. The results were analysed by Student's *t*-test (Student–Newman–Keuls) at a significance level of 95% ($\alpha = 0.05$).

The results from the sensory evaluation (Section 2.5) were analysed for significant differences ($\alpha = 0.05$) by ANOVA (Statgraphics).

3. Results and discussion

3.1. Effect of pH and sugar on terpene release

The first enzyme assays were performed under optimal enzyme conditions (pH 5.5, ethanol removed) using an extract from a white wine (Traminer, Austria). According to Mateo and Jiménez (2000), Traminer is classified as a non-Muscat, but aromatic variety, that depends on monoterpenes as major flavour components. The resulting concentrations of monoterpenes after enzyme treatment are shown in Table 3. Because β-citronellol and nerol could not sufficiently be separated by the analytical method applied, the corresponding results are displayed as sum of β-citronellol plus nerol throughout the paper. Regarding the used enzyme codes, the reader is again referred to Table 1.

As shown in Table 3, all β-glucosidase preparations (GL, GO, GA) were able to release monoterpenes, the highest concentrations were detected with GO. According to the scheme of sequential precursor hydrolysis as proposed by Gunata et al. (1988), arabinosidase and rhamnosidase preparations were always applied in combination with the β-glucosidase from *O. oeni* (GO). The use of the same glucosidase (GO) in all assays with enzyme combinations was intended to obtain comparable results. Fungal (GO/AA) and bacterial (GO/AO) arabinosidase could release equal amounts of total terpenes. Addition of the *Pediococcus acidilactici* rhamnosidase R to GO caused only a small further increase in terpene concentrations, compared to treatment with GO alone. The highest terpene concentrations were released when applying the combinations GO/AO/R and GO/N. At this point, it is important to note that N, which was applied as a fungal rhamnosidase preparation, is in fact a complex mixture containing additional activities of α-L-rhamnosidase, β-D-glucosidase, β-D-galactosidase, β-D-xylosidase, and α-L-arabinosidase (see activity profile in Fig. 1).

Subsequently, two brands of red grape juice ("St. Laurent", "Happy Day"), both commercially available at Austrian markets, were used as substrates for enzyme assays. "St. Laurent" is a highly aromatic grape variety that is often cultivated in Eastern Austria (Lower Austria, Burgenland), while the latter is a commercial bulk product which is probably an undefined blend of several grape varieties. The aim of these assays was to take the effects of the juice matrix, especially of sugar inhibition at still optimal pH (adjusted to pH 5.5) into account (see Table 2 for juice composition). At first, the total amounts of released terpenes differed significantly between the two varieties (Table 4), most likely due to different concentrations of aroma precursors. The overall release of terpenes from "St. Laurent" was low, while higher concentrations were detected in "Happy Day" after enzyme treatment. Nevertheless, the results from both juices followed similar trends. Remarkably, both glucosidase (GA) and arabinosidase (GO/AA) from *Aspergillus niger* were almost inactive under these conditions (Table 4). These results are in agreement with the finding that the fungal glucosidase GA was strongly inhibited by glucose in tests with *p*NP-β-D-glucopyranoside (3.6% residual activity at 500 mM glucose, corresponding to 90 g/L). In contrast, GO still exhibited 36% residual activity at 500 mM glucose. However, the results for the fungal arabinosidase AA are rather surprising: AA was not inhibited by glucose in laboratory assays with *p*NP-α-L-arabinofuranoside (Michlmayr, Schümann et al., 2011). An explanation for its inactivity in the grape juice could be the effect of the complete juice matrix (Table 2). Although the combination GO/AA could release low amounts of α-terpineol, β-citronellol + nerol and geraniol (compared to GO

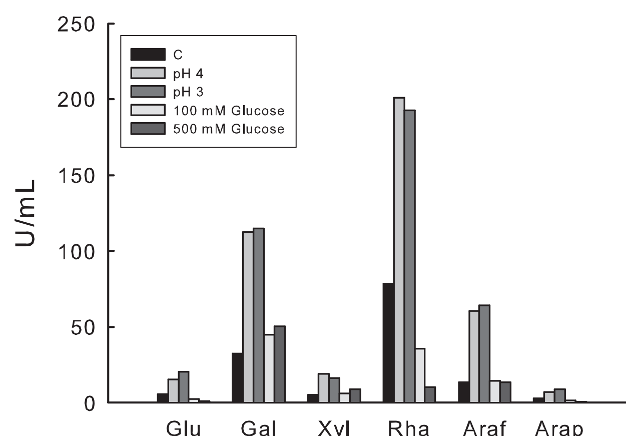

Fig. 1. Influence of pH and glucose concentration on the glycosidase activities of naringinase as determined with synthetic substrates. C, control (pH 5.5); Glu, *p*-nitrophenyl-β-D-glucopyranoside; Gal, *p*-nitrophenyl-β-D-galactopyranoside; Xyl, *p*-nitrophenyl-β-D-xylopyranoside; Rha, *p*-nitrophenyl-α-L-rhamnopyranoside; Araf, *p*-nitrophenyl-α-L-arabinofuranoside; Arap, *p*-nitrophenyl-α-L-arabinopyranoside. The data displayed represent the average of duplicate determinations.

Table 3
Terpene concentrations (μg/L) of the white wine extract (Traminer, Austria) after glycosidase treatment. C, control; the enzyme codes are the same as displayed in Table 1. All data represent the average of triplicate determination. The results were grouped by a *t*-test (Student–Newman–Keuls; $\alpha = 0.05$). Values labelled with the same letters (upper case) are not significantly different.

	C	GL	GO	GA	GO/AO	GO/AA	GO/R	GO/AO/R	GO/N
α-Terpineol	0.327^{A}	6.17^{B}	7.83^{B}	6.71^{B}	26.6^{D}	17.4^{C}	9.77^{B}	32.6^{E}	23.7^{D}
Linalool	<0.3	3.08^{A}	4.74^{B}	3.05^{A}	5.10^{B}	5.53^{B}	2.95^{A}	4.82^{B}	8.01^{C}
cis-Linalool oxide	4.10^{A}	31.8^{BC}	32.8^{BC}	18.1^{B}	70.2^{D}	46.9^{C}	30.5^{BC}	97.0^{E}	67.5^{D}
trans-Linalool oxide	2.45^{A}	34.9^{AB}	37.7^{AB}	14.6^{A}	154^{C}	41.8^{AB}	23.2^{AB}	213^{D}	58.6^{B}
β-Citronellol + nerol	2.55^{A}	78.8^{B}	130^{C}	62.4^{B}	218^{E}	247^{EF}	165^{D}	250^{EF}	269^{F}
Geraniol	11.7^{A}	249^{BC}	299^{C}	206^{B}	515^{E}	610^{F}	407^{D}	593^{F}	809^{G}
Hotrienol	<0.3	1.23^{B}	1.44^{BC}	1.31^{B}	1.79^{D}	1.27^{B}	0.74^{A}	2.23^{E}	1.67^{CD}
Sum of terpenes	21.7^{A}	406^{BC}	515^{CD}	314^{B}	993^{E}	971^{E}	640^{D}	1190^{F}	1240^{F}

Table 4
Total release of terpenes (μg/L) from two red grape juice varieties through glycosidase treatment. C, control; the enzyme codes are the same as displayed in Table 1. All data represent the average of triplicate determination. The results were grouped by a *t*-test (Student–Newman–Keuls; $\alpha = 0.05$). Values labelled with the same letters (upper case) are not significantly different.

	C	GO	GA	GO/AO	GO/AA	GO/R	GO/AO/R	N	GO/N
"St Laurent", pH 5.5									
α-Terpineol	2.08^{B}	2.15^{B}	1.91^{A}	2.80^{C}	2.20^{B}	2.17^{B}	3.19^{D}	3.42^{E}	3.91^{F}
Linalool	1.50^{B}	1.44^{B}	1.03^{A}	1.69^{B}	1.37^{B}	1.46^{B}	1.43^{B}	2.10^{C}	2.38^{D}
cis-Linalool oxide	6.04^{AB}	8.38^{C}	5.35^{A}	10.0^{D}	7.05^{BC}	7.93^{C}	11.6^{D}	11.0^{D}	14.4^{E}
trans-Linalool oxide	2.80^{A}	5.79^{C}	2.30^{A}	9.14^{E}	4.48^{B}	7.17^{D}	11.3^{F}	5.13^{BC}	13.9^{G}
β-Citronellol + nerol	<0.3	<0.3	<0.3	1.41^{B}	0.590^{A}	0.670^{A}	1.96^{C}	0.507^{A}	1.30^{B}
Geraniol	<0.3	0.397^{A}	<0.3	4.61^{D}	2.07^{C}	1.30^{B}	8.04^{E}	5.15^{D}	9.71^{F}
Hotrienol	1.05^{D}	1.00^{CD}	0.307^{A}	0.917^{C}	0.540^{B}	1.08^{D}	1.09^{D}	1.65^{E}	1.65^{E}
Sum of terpenes	14.4^{B}	20.0^{C}	11.5^{A}	31.5^{E}	18.8^{C}	22.9^{D}	39.5^{F}	29.9^{E}	48.6^{G}
"Happy Day", pH 5.5									
α-Terpineol	6.95^{A}	8.58^{B}	6.71^{A}	15.9^{E}	11.9^{C}	11.0^{C}	16.6^{E}	14.3^{D}	32.5^{F}
Linalool	1.56^{B}	1.62^{B}	1.11^{A}	1.66^{B}	1.44^{B}	1.57^{B}	1.56^{B}	2.88^{C}	3.35^{D}
cis-Linalool oxide	8.82^{A}	26.4^{C}	9.5^{A}	36.9^{E}	19.4^{B}	32.7^{D}	46.5^{F}	25.0^{C}	93.5^{G}
trans-Linalool oxide	5.57^{A}	15.0^{C}	5.54^{A}	25.4^{E}	11.3^{B}	17.7^{D}	32.5^{F}	15.6^{C}	55.8^{G}
β-Citronellol + nerol	<0.3	2.65^{B}	0.429^{A}	11.4^{F}	5.92^{C}	6.82^{D}	11.2^{F}	7.66^{E}	7.44^{E}
Geraniol	<0.3	5.05^{B}	1.30^{A}	18.3^{E}	10.8^{C}	12.0^{D}	18.6^{E}	23.0^{F}	26.5^{G}
Hotrienol	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	0.320
Sum of terpenes	23.8^{A}	59.9^{B}	24.9^{A}	110^{E}	61.2^{B}	82.5^{C}	127^{F}	99.0^{D}	221^{G}
"Happy Day", pH 3.0									
α-Terpineol	9.22^{B}	10.1^{C}	8.10^{A}	11.1^{D}	8.49^{A}	9.53^{B}	11.7^{E}	11.6^{E}	12.6^{F}
Linalool	2.13^{B}	2.49^{BC}	1.90^{AB}	2.86^{CD}	1.42^{A}	2.18^{B}	2.61^{BC}	3.74^{E}	3.36^{DE}
cis-Linalool oxide	13.7^{A}	15.0^{AB}	15.7^{ABC}	14.1^{A}	15.0^{AB}	17.1^{C}	16.8^{BC}	35.3^{D}	35.0^{D}
trans-Linalool oxide	7.67^{AB}	8.26^{B}	8.05^{AB}	8.26^{B}	7.34^{A}	8.13^{AB}	8.34^{B}	21.1^{C}	20.9^{C}
β-Citronellol + nerol	<0.3	1.02^{B}	1.16^{B}	1.84^{C}	<0.3	0.660^{A}	1.82^{C}	3.63^{D}	3.55^{D}
Geraniol	0.420^{A}	1.68^{C}	2.16^{D}	2.56^{E}	2.68^{E}	1.16^{B}	2.68^{E}	9.64^{F}	9.53^{F}
Hotrienol	0.710^{F}	0.693^{E}	0.533^{A}	0.657^{DE}	0.610^{BCD}	0.647^{CDE}	0.587^{B}	0.633^{BCD}	0.600^{BC}
Sum of terpenes	35.2^{A}	39.7^{BC}	38.0^{AB}	41.8^{C}	36.0^{A}	39.7^{BC}	45.0^{D}	86.2^{E}	86.1^{E}

alone, Table 4), regarding the sum of terpenes, no further significant increase of free terpenes could be observed by adding AA to GO. The relatively high activity of N in grape juice compared to the enzyme preparations from *A. niger* might be caused by the comparably low effect of glucose on the glycosidase activities of N. As shown in Fig. 1, the rhamnosidase activity of N was clearly inhibited by glucose (13% residual activity at 500 mM glucose), but other glycosidase side activities of N were affected less or even increased in the presence of high glucose concentrations.

At natural juice pH (Table 4, assays only performed with "Happy Day") the bacterial enzymes could still release statistically significant amounts of terpenes, although at a low magnitude. Only the fungal preparation N could release higher amounts of terpenes at pH 3.0, which is consistent with the results obtained with synthetic glycosides shown in Fig. 1, suggesting a high increase of glycosidase activities toward lower pH. The addition of GO to N caused no further increase of terpene concentrations.

3.2. Terpene profiles

In addition to the total amount of terpenes released under given conditions, it is important to consider the characteristic profile of free terpenes generated by an enzyme preparation in more detail. The corresponding observations are discussed in the present section. For this purpose, the results shown in Tables 3 and 4 are additionally presented in graphical form as Supplementary online content (Figs. S1 and S2).

The resulting terpene profiles in the Traminer wine extract (Table 3, Supplementary Fig. S1) suggest rather similar substrate specificities for the β-glucosidases GL, GO and GA. Although all these enzymes are classified into the same glycoside hydrolase family (GH 3, see also Table 1), both bacterial glucosidases possess additional side activities of xylosidase and arabinosidase (Michlmayr, Schümann, Barreira Braz Da Silva, Kulbe, del Hierro, 2010; Michlmayr, Schümann, Wurbs et al., 2010), while such side activities could not be detected in GA. Although it might be expected that these side activities of GL and GO would contribute to a distinct aroma profile compared to GA, such an effect was not observed.

A rather interesting observation was that (in combination with GO) the arabinosidase from *O. oeni* (AO) significantly produced higher amounts of the tertiary terpene alcohols α-terpineol, *cis*/*trans*-linalool oxide and hotrienol than the arabinosidase from *A. niger* (GO/AA; Table 3, Fig. S1). In contrast, AA released higher amounts of the primary terpenols geraniol and β-citronellol + nerol than AO. A similar effect was observed comparing the combinations GO/AO/R and GO/N. While the overall terpene concentrations produced were equal, GO/AO/R released higher amounts of α-terpineol, linalool oxides and hotrienol than GO/N. In the case of linalool, which is a tertiary terpene alcohol as well, no significant increase could be detected after addition of AO and R to GO. In contrast, the highest concentrations of linalool were released by the combination GO/N.

Regarding the complex composition of N (Fig. 1), it is interesting to observe that although the addition of N to GO could further increase the total concentrations of free terpenes, the resulting terpene profiles of GO and GO/N were rather similar in the wine extract (Supplementary Fig. S1). The same effect was observed in "Happy Day" grape juice at pH 5.5 (Supplementary Fig. S2). Fig. S2 also shows that the profiles generated by N and GO/N are clearly distinct, as the addition of GO to N caused a further significant increase of the tertiary terpenols α-terpineol and *cis*/*trans*-linalool oxides, implying synergistic effects between these preparations. Further, comparing the terpene profiles generated by N at pH 3.0 and pH 5.5, it is obvious that the resulting profiles were remarkably different (Fig. S2). This may indicate that the enzymes that contribute to aroma release by N respond differently to pH.

Fig. S2 also demonstrates that in the grape juice ("Happy Day", pH 5.5), addition of AO and/or R to GO could further increase the

concentrations of free α-terpineol, *cis*/*trans*-linalool oxide, β-citronellol + nerol, and geraniol, compared to samples treated with GO only.

The results presented above indicate that the glycosidases from *O. oeni* are capable of releasing terpenes from natural glycosylated precursors, suggesting that these intracellular enzymes might contribute to the release of glycosylated aroma compounds during malolactic fermentation. Further, the bacterial glycosidases demonstrated interesting characteristics in comparison to the fungal enzymes. Besides the lower inhibition of the *O. oeni* glycosidases in juice conditions, a general observation made here is that the bacterial enzymes, especially the arabinosidase from *O. oeni*, possess capacities to release both primary and tertiary terpene alcohols (terpenols), while the fungal enzymes preferentially released primary terpenols. These findings seem to contradict the results of Ugliano et al. (2003), and Ugliano and Moio (2006), who reported that *O. oeni* mainly released primary terpenols during MLF. However, it remains to be investigated to what extent such glycosidase genes are distributed in *O. oeni* genomes and further, whether such enzymes are actually expressed during MLF. Due the reported genetic heterogeneity of *O. oeni* (Bartowsky & Borneman, 2011; Borneman, Bartowsky, McCarthy, & Chambers, 2010), it can be expected that variations with regard to the presence of glycosidase genes and their regulation exist between individual *O. oeni* isolates. This is also indicated by the fact that glycosidase activity profiles have been shown to be highly strain-dependent (Gagné et al., 2011; Grimaldi et al., 2005b). Furthermore, *O. oeni* possesses several GH 1 phospho-β-glucosidase genes related to the cellobiose/β-glucoside specific phosphotransferase system (Capaldo, Walker, Ford, & Jiranek, 2011a, 2011b). It is not yet established, whether this enzyme class can be made responsible for the release of glycosylated aroma compounds during MLF.

As far as possible, the fungal enzymes (*A. niger*) used in this study were chosen due to their assignment to the same GH families as the bacterial glycosidases involved (glucosidases GH 3, arabinosidases GH 51, Table 1). However, it should be noted that the above discussed differences in substrate specificities are most likely not directly related to the bacterial or fungal origin of the involved glycosidases. It would be worthwhile to investigate whether the capability to release primary and/or tertiary terpenols is related to the empirical distinction between aryl/alkyl glycosidases on one hand and glycosidases specific for short chain oligosaccharides on the other hand, which is especially well documented in the case of β-glucosidases (Bhatia, Mishra, & Bisaria, 2002). Our previous results suggest that both glucosidase and arabinosidase from *O. oeni* can be classified as true aryl/alkyl glycosidases, while both *A. niger* glycosidases showed a high preference in hydrolysing disaccharides (Michlmayr, Schümann et al., 2011; Michlmayr, Schümann, Wurbs et al., 2010). Further, our recent work (Michlmayr, Brandes et al., 2011) on two bacterial rhamnosidases, both assigned to GH 78, revealed that Ram ("R" in the present study) can be classified as an aryl-glycosidase, while Ram2 (not involved in the present study) displayed its highest catalytic efficiency with the disaccharide rutinose. Interestingly, Ram (R) could release both primary and tertiary terpenols in a Muscat wine extract, while Ram2 could only release primary terpenols under the same conditions.

3.3. Vinification experiments with glucosidase and arabinosidase from *O. oeni*

Small-scale vinification experiments were conducted to perform an initial evaluation on whether the glycosidases from *O. oeni* are in principle suited for application in winemaking. Therefore, both glucosidase and arabinosidase from *O. oeni* were applied during the cold maceration stage of a Riesling wine. The total terpene contents of the musts extracted from the Riesling mash after enzyme treatment and that of the resulting wines are shown in Table 5. Additionally, graphical representations of these data can be found in the supplementary online content of this paper (Supplementary Figs. S3 and S4). Interpreting these data, it is not clear whether the bacterial enzymes could hydrolyse aroma precursors during the cold maceration period. The highest concentrations of terpenes were detected in samples treated with the commercial preparation Maceration C (MacC), followed by the two controls (In C1, no pectinase was added before pressing). The increase of free terpenes in both controls compared to the mash (determined in the free run juice of the mash) before the maceration period might be explained by the extraction of terpenes from the skin during maceration or the action of a grape glycosidase (Maicas & Mateo, 2005). The total terpene concentrations in the musts treated with GO were even lower than that of the controls, an explanation for this could be the absorption of volatile compounds on hydrophobic regions of the protein. In contrast to the experiments described in Section 3.1, only low enzyme concentrations were used in these vinification experiments, causing an expectedly low

Table 5
Terpene concentrations (μg/L) in the Riesling musts after enzyme treatment during cold maceration and of the corresponding final wines after alcoholic fermentation. M, mash before cold maceration; C1, control without pectinase treatment before pressing; MacC, Maceration C (Preziso, Austria), 3 g/hL; GO, with 60, 200, 300 U/L. AO, 35 U/L; GO/AO: 150 U/L GO + 25 U/L AO. All data represent the average of triplicate determination. The results were grouped by a *t*-test (Student–Newman–Keuls; $\alpha = 0.05$). Values labelled with the same letters (upper case) are not significantly different.

	M	C1	C2	MacC	GO60	GO200	GO300	AO	GO/AO
Musts after enzyme treatment									
α-Terpineol	0.543^{A}	14.6^{D}	15.2^{D}	20.4^{E}	8.08^{B}	11.6^{C}	13.0^{C}	16.3^{D}	15.5^{D}
Linalool	0.963^{A}	18.4^{DE}	18.3^{DE}	20.5^{E}	10.2^{B}	15.4^{C}	16.5^{CD}	19.4^{E}	18.6^{DE}
cis-Linalool oxide	1.76^{A}	7.07^{C}	8.35^{D}	12.2^{F}	5.84^{B}	6.61^{BC}	7.06^{C}	9.52^{E}	9.10^{DE}
trans-Linalool oxide	0.753^{A}	2.57^{B}	2.71^{B}	4.13^{D}	2.28^{B}	2.32^{B}	2.68^{B}	3.39^{C}	3.26^{C}
β-Citronellol + Nerol	<0.3	5.07^{E}	4.48^{D}	2.30^{A}	3.12^{B}	4.30^{CD}	3.89^{BCD}	3.57^{BC}	3.72^{BCD}
Geraniol	0.353^{A}	17.4^{F}	15.1^{E}	6.90^{B}	10.8^{C}	15.2^{E}	13.8^{DE}	12.8^{CD}	13.2^{DE}
Hotrienol	2.92^{A}	28.9^{D}	31.8^{D}	39.7^{E}	16.1^{B}	22.6^{C}	24.1^{C}	30.2^{D}	30.5^{D}
Sum of terpenes	7.48^{A}	94.9^{D}	96.8^{D}	107^{E}	56.8^{B}	78.6^{C}	81.7^{C}	95.8^{D}	94.6^{D}
Riesling wines									
α-Terpineol		7.25^{A}	7.87^{AB}	10.1^{D}	7.25^{A}	7.60^{AB}	8.11^{B}	9.19^{C}	9.02^{C}
Linalool		26.4^{ABC}	27.7^{BC}	30.1^{D}	26.1^{B}	25.7^{A}	27.8^{BC}	30.1^{D}	28.3^{C}
cis-Linalool oxide		2.91^{A}	3.26^{BC}	3.76^{D}	3.12^{AB}	3.26^{BC}	3.52^{CD}	3.68^{D}	3.58^{CD}
trans-Linalool oxide		1.06^{A}	1.11^{A}	1.35^{B}	1.07^{A}	1.09^{A}	1.16^{AB}	1.22^{AB}	1.23^{AB}
β-Citronellol + Nerol		3.57^{A}	3.73^{AB}	3.50^{A}	3.92^{B}	3.67^{AB}	3.45^{A}	3.73^{AB}	3.88^{B}
Geraniol		5.36^{A}	5.23^{A}	5.76^{B}	5.72^{B}	5.48^{AB}	5.73^{B}	6.32^{C}	6.75^{D}
Hotrienol		13.7^{A}	17.9^{CD}	22.2^{E}	15.7^{B}	16.4^{B}	17.6^{C}	19.2^{D}	18.6^{CD}
Sum of terpenes		66.3^{A}	73.3^{B}	83.3^{D}	69.3^{AB}	69.0^{AB}	72.6^{B}	80.0^{CD}	77.9^{C}

release of terpenes. Nevertheless, with increasing dose of GO the total terpene concentrations increased as well, suggesting that GO was not completely inactive, although the detected changes were at a low level (see Table 5 and Fig. S3). Interestingly, both samples treated with GO/AO and AO displayed terpene concentrations equal to those of the controls. In tests with the Traminer extract, it was shown that AO could release only low levels of terpenes compared to the control without the presence of the glucosidase (21 ppm total compared to the controls). Considering the fact that it was reported that grape glucosidases mainly hydrolyse primary terpenols (Maicas & Mateo, 2005), and further regarding the differences in the release of primary/tertiary terpenols by different enzyme preparations as discussed in Section 3.2, it could be expected that different enzyme treatments would result in recognisably distinct terpene profiles in the musts. However, such an observation cannot be confirmed.

The analysis of terpenes after the alcoholic fermentation (Table 5, see also Supplementary Figs. S3 and S4) shows that the differences in terpene concentrations between the treatments are less distinct than before the alcoholic fermentation. Further, the total terpene concentrations in the wines were lower than in the musts. However, it is interesting to observe that the overall trends observed in the musts are still recognisable after alcoholic fermentation, which is evident when comparing the results in the Supplementary Figs. S3 and S4. This indicates that dependent on the glycosidase activity profile of the yeast involved, enzyme treatment at an early stage of winemaking, as presented here, may indeed affect the sensory properties of the final product.

The results of the sensory evaluation are shown in Fig. 2. "Riesling HBLA" was included as a further control for the sensory tests. This wine was produced from the same harvest of grapes without cold maceration but otherwise the same fermentation conditions. Interestingly, in the aroma intensity ranking (Fig. 2a), the highest intensity was recognised in the wines treated with AO only, while wines treated with GO/AO received the lowest rating (except "Riesling HBLA"). This is remarkable as treatment with AO and GO/AO did not result in analytically distinct terpene concentrations compared to the controls. Further, wines treated with β-glucosidase (GO) alone were marked as significantly different to the controls. The analysis of variance of the aroma intensity ratings (Fig. 2a) showed that in the glucosidase-treated wines, aroma intensity significantly (significance level = 0.01) correlated with increasing enzyme dose. Additionally, the perceived intensity of the glucosidase-treated wines highly correlates to the stone fruit (0.01 level), citrus (0.05 level) descriptors; the intensity perceived for the arabinosidase (AO) and arabinosidase with glucosidase (GO/AO) treated wines highly correlates with pomaceous fruits (0.001 level), citrus (0.05 level), stone fruit (0.05) and freshness (0.01 level) (Fig. 2b). Therefore, it seems that wines with the treatment of AO and GO/AO were described with the typical Riesling descriptors (stone fruit, citrus, pomaceous). However, the tasters did not see an increase in floral, candied, tropical aromas. Interestingly, in the typicality rating, the external control wine "Riesling HBLA" was not recognised as a typical Riesling wine by the tasters (rating 37%); the controls (MacC, C1 and C2) received ratings between 57% and 64%. The wines treated with the bacterial enzymes were most often marked as typical (GO/AO and GO200 treatments 78%, GO60 81%, AO 90% and GO300 93%).

The major drawback in the results presented above is that a clear correlation between analytical and sensory evaluation cannot not be made. It is conceivable that due to the low perception thresholds of volatile compounds (Mateo & Jiménez, 2000), significant differences in aroma composition may already be recognised on a subjective level where the corresponding chemical changes are not even detectable/distinguishable by analytical methods. Synergistic/additive effects between aroma compounds resulting

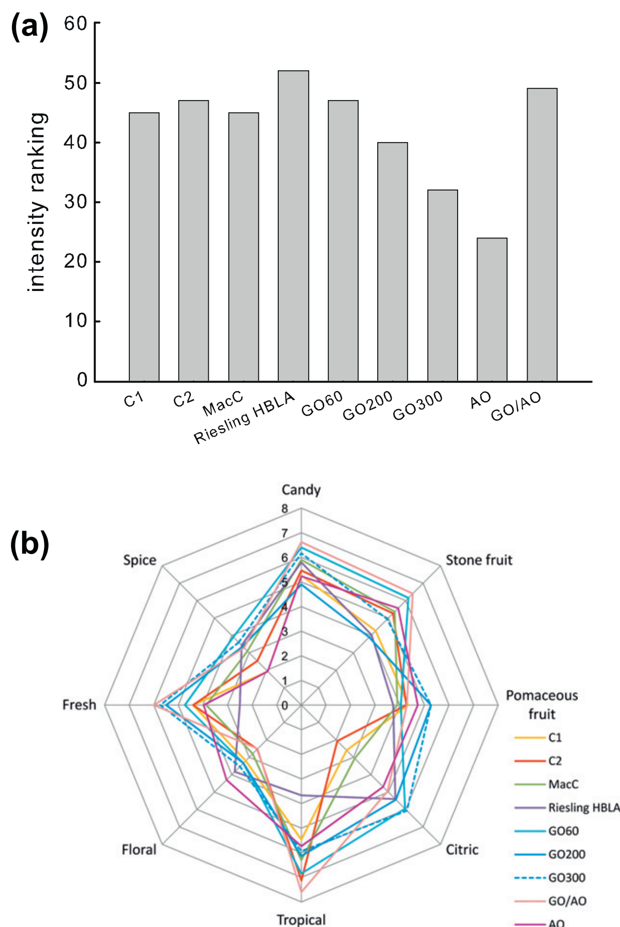

Fig. 2. Analysis of the sensory evaluation (professional panel, $n = 7$) of the enzyme treated wines. (a) Intensity ranking, increasing aroma intensity correlates with less points. (b) Spider plot of the intensity of the attributes included. C1, control without pectinase treatment; MacC, Maceration C (Preziso, Austria), 3 g/hL; GO, with 60, 200, 300 U/L. AO, 35 U/L; GO/AO: 150 U/L GO + 25 U/L AO. "Riesling HBLA" is a wine produced with the same grapes, but without cold maceration.

in lowered perception thresholds have been described as well (Rapp & Mandery, 1986). Therefore, the question whether a given enzyme is a valuable tool for winemaking may be a matter of sensory and personal preferences rather than an analytical one. Accordingly, apart from a biochemical characterisation, it is most important to understand how an enzyme preparation influences the characteristic varietal aroma bouquet in sensory terms.

4. Conclusion

To the best of our knowledge, this is the first study reporting the properties of cell-free glycosidases from *O. oeni* to release aroma compounds from natural substrates like wine and fruit juice. From a biological point of view, this is an essential step towards understanding how *O. oeni* is capable of releasing grape-derived aroma compounds from wine. It will further be necessary to determine how such glycosidase genes are regulated during the MLF. Further, due to the intracellular nature of both glucosidase and arabinosidase of *O. oeni*, studies on the mechanisms involved in substrate import will be required as well to gain a complete understanding of the mechanisms that govern the aroma release by wine lactic acid bacteria.

The results of the present study further indicate that the enzymes of *O. oeni* could be interesting tools for the early stages of

winemaking, especially since the wines produced from them were preferred by the tasting panel, and enzyme treatment could evidently contribute positively to the "typical" Riesling aroma. However, further detailed experiments using different wine varieties and fermentation conditions (e.g., yeasts) will be required in order to confirm this conclusion. Regarding a possible application of such glycosidases, it is necessary to mention that a direct application of bacterial enzymes in winemaking is at present not realistic. A major obstacle is the necessity of recombinant enzyme production. The use of recombinant techniques in the food industry has a rather negative image due to consumer and market preferences. An attractive alternative (although recombinant as well) could be the use of LAB as GRAS/food grade expressions systems, which is a developing field of intensive research (Peterbauer, Maischberger, & Haltrich, 2011).

Acknowledgements

This work was supported by a Grant (FWF Project 20246-B11) given to K.D.K. by the Austrian Science Fund. We thank Yiqun Wu for assistance in sample preparation.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2012.04.099.

References

Antonelli, A., Castellari, L., Zambonelli, C., & Carnacini, A. (1999). Yeast influence on volatile composition of wines. *Journal of Agricultural and Food Chemistry, 47*, 1139–1144.

Bartowsky, E. J., & Borneman, A. R. (2011). Genomic variations of *Oenococcus oeni* strains and the potential to impact on malolactic fermentation and aroma compounds in wine. *Applied Microbiology and Biotechnology, 92*, 441–447.

Bhatia, Y., Mishra, S., & Bisaria, V. S. (2002). Microbial β-glucosidases: Cloning, properties, and applications. *Critical Reviews in Biotechnology, 22*, 375–407.

Boido, E., Lloret, A., Medina, K., Carrau, F., & Dellacassa, E. (2002). Effect of β-glycosidase activity of *Oenococcus oeni* on the glycosylated flavor precursors of Tannat wine during malolactic fermentation. *Journal of Agricultural and Food Chemistry, 50*, 2344–2349.

Borneman, A. R., Bartowsky, E. J., McCarthy, J., & Chambers, P. J. (2010). Genotypic diversity in *Oenococcus oeni* by high-density microarray comparative genome hybridization and whole genome sequencing. *Applied Microbiology and Biotechnology, 86*, 681–691.

Bruwer, J., Saliba, A., & Miller, B. (2011). Consumer behaviour and sensory preference differences: Implications for wine product marketing. *Journal of Consumer Marketing, 28*, 5–18.

Cantarel, B. I., Coutinho, P. M., Rancurel, C., Bernard, T., Lombard, V., & Henrissat, B. (2009). The Carbohydrate-Active EnZymes database (CAZy): An expert resource for glycogenomics. *Nucleic Acids Research, 37*, D233–D238.

Capaldo, A., Walker, M. E., Ford, C. M., & Jiranek, V. (2011a). β-Glucoside metabolism in *Oenococcus oeni*: Cloning and characterisation of the phospho-β-glucosidase bglD. *Food Chemistry, 125*, 476–482.

Capaldo, A., Walker, M. E., Ford, C. M., & Jiranek, V. (2011b). β-Glucoside metabolism in *Oenococcus oeni*: Cloning and characterization of the phospho-β-glucosidase CelD. *Journal of Molecular Catalysis B: Enzymatic, 69*, 27–34.

Cartier, R., Rytz, A., Lecomte, A., Poblete, F., Krystlik, J., Belin, E., et al. (2006). Sorting procedure as an alternative to quantitative descriptive analysis to obtain a product sensory map. *Food Quality and Preference, 17*, 562–571.

D'Incecco, N., Bartowsky, E., Kassara, S., Lante, A., Spettoli, P., & Henschke, P. (2004). Release of glycosidically bound flavour compounds of Chardonnay by *Oenococcus oeni* during malolactic fermentation. *Food Microbiology, 21*, 257–265.

Gagné, S., Lucas, P. M., Perello, M. C., Claisse, O., Lonvaud-Funel, A., & de Revel, G. (2011). Variety and variability of glycosidase activities in an *Oenococcus oeni* strain collection tested with synthetic and natural substrates. *Journal of Applied Microbiology, 110*, 218–228.

Grimaldi, A., Bartowsky, E., & Jiranek, V. (2005a). Screening of *Lactobacillus* spp. and *Pediococcus* spp. for glycosidase activities that are important in oenology. *Journal of Applied Microbiology, 99*, 1061–1069.

Grimaldi, A., Bartowsky, E., & Jiranek, V. (2005b). A survey of glycosidase activities of commercial wine strains of *Oenococcus oeni*. *International Journal of Food Microbiology, 105*, 233–244.

Gunata, Y. Z., Bayonove, C. L., Baumes, R. L., & Cordonnier, R. E. (1985). The aroma of grapes I. Extraction and determination of free and glycosidically bound fractions of some grape aroma components. *Journal of Chromatography A, 331*, 83–90.

Gunata, Z., Bitteur, S., Brillouet, J. M., Bayonove, C., & Cordonnier, R. (1988). Sequential enzymic hydrolysis of potentially aromatic glycosides from grape. *Carbohydrate Research, 184*, 139–149.

Henrissat, B., & Davies, G. (1997). Structural and sequence-based classification of glycoside hydrolases. *Current Opinion in Structural Biology, 7*, 637–644.

Jackson, R. S. (2008). *Wine Science. Principles and applications* (3rd ed.). Burlington: Academic Press.

Kotseridis, Y., & Baumes, R. (2000). Identification of impact odorants in Bordeaux red grape juice, in the commercial yeast used for its fermentation, and in the produced wine. *Journal of Agricultural and Food Chemistry, 48*, 400–406.

Maicas, S., & Mateo, J. J. (2005). Hydrolysis of terpenyl glycosides in grape juice and other fruit juices: A review. *Applied Microbiology and Biotechnology, 67*, 322–335.

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

Matthews, A., Grimaldi, A., Walker, M., Bartowsky, E., Grbin, P., & Jiranek, V. (2004). Lactic acid bacteria as a potential source of enzymes for use in vinification. *Applied and Environmental Microbiology, 70*, 5715–5731.

McIlvaine, T. C. (1921). A buffer solution for colorimetric comparison. *The Journal of Biological Chemistry, 49*, 183–186.

Michlmayr, H., Brandes, W., Eder, R., Schümann, C., del Hierro, A., & Kulbe, K. D. (2011). Characterization of two distinct GH Family 78 α-L-rhamnosidases from *Pediococcus acidilactici*. *Applied and Environmental Microbiology, 77*, 6524–6530.

Michlmayr, H., Schümann, C., Barreira Braz Da Silva, N. M., Kulbe, K. D., & del Hierro, A. M. (2010). Isolation and basic characterization of a β-glucosidase from a strain of *Lactobacillus brevis* isolated from a malolactic starter culture. *Journal of Applied Microbiology, 108*, 550–559.

Michlmayr, H., Schümann, C., Kulbe, K. D., & del Hierro, A. M. (2011). Heterologously expressed family 51 α-L-arabinofuranosidases from *Oenococcus oeni* and *Lactobacillus brevis*. *Applied and Environmental Microbiology, 77*, 1528–1531.

Michlmayr, H., Schümann, C., Wurbs, P., da Silva, N. M. B. B., Rogl, V., Kulbe, K. D., et al. (2010). A β-glucosidase from *Oenococcus oeni* ATCC BAA-1163 with potential for aroma release in wine: Cloning and expression in *E. coli*. *World Journal of Microbiology and Biotechnology, 26*, 1281–1289.

Palmeri, R., & Spagna, G. (2007). β-Glucosidase in cellular and acellular form for winemaking application. *Enzyme and Microbial Technology, 40*, 382–389.

Peterbauer, C., Maischberger, T., & Haltrich, D. (2011). Food-grade gene expression in lactic acid bacteria. *Biotechnology Journal, 6*, 1147–1161.

Rapp, A. (1992). Aromastoffe des Weines. *Chemie in unserer Zeit, 26*, 273–284.

Rapp, A. (1998). Volatile flavour of wine: Correlation between instrumental analysis and sensory perception. *Nahrung – Food, 42*, 351–363.

Rapp, A., & Mandery, H. (1986). Wine aroma. *Experientia, 42*, 873–884.

Ribéreau-Gayon, P., Glories, Y., Maujean, A., & Dubourdieu, D. (2006). *Handbook of enology volume 2: The chemistry of wine: Stabilization and treatments* (2nd ed.). Chichester, England: John Wiley & Sons Ltd..

Skinkis, P. A., Bordelon, B. P., & Wood, K. V. (2008). Comparison of monoterpene constituents in Traminette, Gewürztraminer, and Riesling winegrapes. *American Journal of Enology and Viticulture, 59*, 440–445.

Steidl, R. (2001). *Kellerwirtschaft* (7th ed.). Leopoldsdorf, Austria: Österreichischer Agrarverlag.

Strauss, C. R., Wilson, B., Gooley, P. R., & Williams, P. J. (1986). Role of monoterpenes in grape and wine flavor. In T. H. Parliment & R. Croteau (Eds.). *Biogeneration of Aromas, ACS Symposium series* (Vol. 317, pp. 222–242). USA: American Chemical Society.

Ugliano, M., Genovese, A., & Moio, L. (2003). Hydrolysis of wine aroma precursors during malolactic fermentation with four commercial starter cultures of *Oenococcus oeni*. *Journal of Agricultural and Food Chemistry, 51*, 5073–5078.

Ugliano, M., & Moio, L. (2006). The influence of malolactic fermentation and *Oenococcus oeni* strain on glycosidic aroma precursors and related volatile compounds of red wine. *Journal of the Science of Food and Agriculture, 86*, 2468–2476.

Williams, P. J. (1993). Hydrolytic flavor release in fruit and wines through hydrolysis of nonvolatile precursors. In T. E. Acree & R. Teranishi (Eds.), *Flavor science – sensible principles and techniques* (pp. 287–308). Washington, DC: American Chemical Society.