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Aroma Changes due to Second Fermentation and Glycosylated Precursors in Chardonnay and Riesling Sparkling Wines

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Supporting Information

ABSTRACT: Aroma changes in Chardonnay and Riesling base wines caused by the second fermentation were investigated by a targeted component analysis: A stable isotope dilution approach using headspace solid phase microextraction coupled online to gas chromatography mass spectrometry (HS-SPME-GC-MS) was applied to quantify 37 compounds relevant for sparkling wine aroma. In an enrichment experiment, glycosylated precursors isolated from one Chardonnay and one Riesling base wine were used to double the original amount in these base wines. Along with increased concentrations of precursor-derived volatiles after the second fermentation, descriptive sensory evaluation revealed an enhancement of fruity aroma impressions reminiscent of, for example, peach or cantaloupe. Except for benzyl alcohol, linalool, and 3-methylpentanol, no quantitative 2-fold increase of volatiles was found with a 2-fold increase in precursor concentration, as other metabolic pathways seem to interfere with aroma formation from glycosides.

KEYWORDS: aroma precursors, Chardonnay, glycosides, Riesling, sparkling wine, stable isotope dilution analysis

INTRODUCTION

Many of the substances that are produced during alcoholic fermentation of must or other sugar sources are volatile yeast metabolites. Whereas sugar itself is mainly converted into ethanol and carbon dioxide, important fermentation byproducts are higher alcohols, esters, carbonyls, and short-chain aliphatic acids, as well as sulfur- or nitrogen-containing compounds. Comprehensive reviews on these yeast metabolites have among others been published by Swiegers and co-workers.[1−3] However, most sparkling wines are products of two consecutive fermentation steps. Compared to the first fermentation, which converts must into wine, the second one is performed in a medium with elevated ethanol content and increasing carbon dioxide pressure, submitting yeast cells to a substantially altered environment. To reveal the effect of this modification on aroma development, we targeted a set of volatiles that we found to be relevant for the aroma of Chardonnay and Riesling sparkling wines.[4,5] The work published by Siebert and co-workers[6] describes a multicomponent HS-SPME-GC-MS method for the quantification of fermentation-derived volatiles, using stable isotopologue internal standards for each targeted analyte.

Apart from de novo syntheses, yeast is also responsible for liberating aroma compounds from nonvolatile precursors present in the wine matrix. Glycosylated aroma substances are quite common throughout the plant kingdom[7,8] and are of major importance in discussions of aroma development during both the making and aging processes of wine. Particularly, the presence of monoterpenyl glycosides in grapes[9,10] as well as their different accessibilities to yeast metabolism[11−13] has extensively been investigated. Apart from enzymatic cleavage,[14] aroma compounds can also be released from their precursors by hydrolysis under acidic conditions at wine pH.[15] Furthermore, subsequent rearrangement reactions and stereoselective reductions of liberated terpene alcohols and norisoprenoids contribute to aroma diversity and complexity.[16−18] It was shown that a combination of all of these effects contributes to bouquet formation during the first fermentation.[19] As aroma precursors are not entirely consumed during the first fermentation,[20] another scope of this work was to assess the importance of the remaining precursors for the second fermentation and evaluate their impact on sparkling wine sensory perception. It has therefore become necessary to extend the scope of compounds targeted by Siebert and co-workers[6] by monoterpene alcohols and linalool oxides. As the original fiber coating had been taken off the market, extraction conditions had to be modified, as well.

MATERIALS AND METHODS

Materials and Chemicals. [$^2H_3$]-Methyl iodide (99.5% d) was purchased from Acros Organics (Geel, Belgium). Gallic acid monohydrate (≥98%) and methyl 3-mercaptopropanoate were from Alfa Aesar (Karlsruhe, Germany). [$^2H_6$]-Ethanol (99.5% d), 2-([$^2H_5$]-phenyl)-ethanol (>99% d), and ([$^2H_{13}$]-hexan)-1-ol (>99% d)were from Dr. Ehrenstorfer (Augsburg, Germany). Geraniol, heptan-2-ol, linalool, linalool oxide (furanoid), and α-terpineol were from Fluka (Buchs, Switzerland). β-Damascenone was a gift from Haarmann & Reimer (now Symrise, Holzminden, Germany). Deuterium oxide (99.9% d) and Folin−Ciocalteu's phenol reagent were obtained from Merck

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(Darmstadt, Germany). Sterilization filter sheets size 6 (Pall-Seitz-Filter-Werke, Bad Kreuznach, Germany) were used for pressure filtration of sparkling wines. [$^2H_3$]-Acetyl chloride (>99% d), Amberlite XAD-2 polymeric adsorbent, [$^2H_7$]-2-bromopropane (98% d), ethyl 2-furoate, *cis*-hex-2-en-1-ol, *cis*-hex-3-en-1-ol, *trans*-hex-3-en-1-ol, 6-methyl-5-hepten-2-one, and 3-methylpentan-1-ol, as well as carbowax/divinylbenzene (70 μm CW/DVB) and divinylbenzene/carboxen/polydimethylsiloxane (50/30 μm DVB/CAR/PDMS) StableFlex SPME fibers for autosamplers, were purchased from Sigma-Aldrich (Steinheim, Germany).

Routine Chemical Wine Analysis. Prior to analyses, sparkling wine samples were passed through sterilization filter sheets with slightly increased pressure to remove discharging carbon dioxide. Routine chemical wine analysis was carried out using OIV compliant methods.[21,22] Extinction at λ = 420 nm was determined on a Cary 100 Conc spectrophotometer (Varian, Darmstadt, Germany) and was normalized to a 1 cm light path.

The total phenolic content was determined according to a photometric procedure (Folin-Ciocalteu) based on the method by Singleton and Rossi,[23] using a Konelab 20iXT instrument (ThermoFisher Scientific, Dreieich, Germany): A disposable multicell cuvette was filled with 20 μL of Folin−Ciocalteu's phenol reagent, 60 μL of purified water (Milli-Q, Waters, Eschborn, Germany), and 20 μL sample (diluted when necessary), mixed, and preincubated for 1 min at 37 °C. Saturated Na_2CO_3 solution (40 μL) and 60 μL of purified water are then added, and dead stop of the reaction was detected at 700 nm wavelength after 20 min of incubation at 37 °C. Results were calculated from triplicates as gallic acid equivalents by means of an equidistant five-point calibration, ranging from 30 to 300 mg/L gallic acid.

Isolation of Glycosylated Aroma Precursors. For this study, a dry Riesling and a dry Chardonnay wine, both from Germany, vintage 2006, monovarietal, were used as base wines for precursor isolation and sparkling wine production. Glycosylated aroma precursors were extracted from 9 L of Riesling base wine and 22.5 L of Chardonnay base wine by adsorption on Amberlite XAD-2 polymer.[24] The resin material was exhaustively extracted with methanol and filled into a glass column (80 cm × 6 cm). The polymeric adsorbent was washed and conditioned with water prior to sample application. To lower the alcohol content, the base wines were diluted 1:1 (v/v) with water and passed slowly through the column (approximately 2 drops per second). Sugars, acids, and other polar substances were rinsed off with water and discarded. Glycosides were eluted with methanol,[9] and the methanolic eluate was concentrated using a rotary evaporator. After removal of the free volatile compounds by extraction with diethyl ether, the glycosides were lyophilized at 50 °C and 22.5 mTorr (Thermo Savant SC 210 SpeedVac Plus centrifuge, Thermo Savant RVT 400 cryo trap, Thermo Savant VLP 80 vacuum pump). The Riesling base wine yielded 182 mg and the Chardonnay base wine, 161 mg, of lyophilisate per liter of base wine.

Model Fermentations. Genuine base wines from Riesling and Chardonnay grapes were prepared as follows: Grapes were harvested in late October 2004 at 86 °Oe (20.7 °Bx), pressed, and treated with potassium bisulfite (5 g/hL). Chardonnay juice was clarified by flotation using nitrogen; Riesling juice was treated with 2 g/hL Panzym Clair Rapide G pectinase (Begerow, Langenlonsheim, Germany) and allowed to sediment. Aliquots of each juice (25 L) were fermented with 6.25 g of *Saccharomyces cerevisiae cerevisiae* cultured yeast (Fermicru VB 1, Max Keller, Mannheim, Germany), which was rehydrated and cultured with 7.5 g of Lalvin GoFerm yeast activator (Begerow, Langenlonsheim, Germany) prior to use. Musts were furthermore supplied with diammonium phosphate and thiamin (10 g of Vitamon Combi + 15 g of Vitamon B, both Erbslöh Geisenheim AG, Geisenheim, Germany). No malolactic fermentation was initiated. When the fermentation process was finished (Riesling, 2.4 g/L residual sugars after 10 days; Chardonnay, 1.9 g/L residual sugars after 15 days), wines were passed through a diatomaceous earth filter, sulfited to 40 g/L free SO_2, and stored topped up in carboys. These base wines were used for model sparkling wine productions.

From base wines of each variety, two sparkling wines were produced by méthode charmat on a small scale using different yeast strains, respectively *S. cerevisiae cerevisiae* (Fermicru VB 1) and *Saccharomyces cerevisiae bayanus* (IOC 18−2007 Champagne yeast, Wickert Kellereibedarf, Landau, Germany). Sucrose was added to aliquots of 25 L of base wine to raise residual sugars by 24 g/L. Dry cultured yeast (10 g) was rehydrated with 100 mL of water at 35 °C, fed stepwise with a total of 1.5 L of base wine and 375 mL of water, supplemented with diammonium phosphate and thiamin (1.25 g Vitamon Ultra, Erbslöh, Geisenheim AG), and cultured in an incubator at 22 °C for 18 h. The yeast suspension was poured into a DIN 6647-1 compliant 30 L stainless steel keg (PLUS KEG, Schäfer Werke GmbH, Neunkirchen, Germany) containing the remaining base wine. The stainless steel ascension pipe was screwed tightly into the bung hole, and a keg coupler equipped with a manometer was attached by means of a spring valve to the neck fitting. Kegs were stored at 19 ± 1 °C. The second fermentation was finished when the keg pressure remained at constant level, which was after 5−6 weeks in the case of VB 1 and after 8−9 weeks in the case of IOC 18-2007 yeast. Altogether, sparkling wines rested on the lees for 4 months and were then separated from the lees by isobarometric filtration using K 700 filter sheets and 1.2 μm membrane filters. Sparkling wines were sulfited prior to bottling to a level of 40 mg/L free SO_2.

In addition to a second fermentation in a closed vessel, base wines were also fermented in regular carboys equipped with airlocks, which allowed CO_2 to escape the vessel: Each 25 L base wine aliquot was inoculated with precultured VB 1 yeast and fermented in a 30 L carboy stored at 19 ± 1 °C, which was agitated daily. As soon as fermentation ended, fermented base wines were passed through a diatomaceous earth filter, sulfited to 40 g/L free SO_2, and bottled.

Base wines, sparkling wines, and fermented base wines underwent routine chemical analysis immediately before bottling. Results are listed in Table 1 for Chardonnay and Riesling.

Sparkling Wine Production from Enriched Base Wines. In each case, 6 L of Riesling and Chardonnay base wine was enriched with an amount of the respective glycosylated precursors equivalent to 6 L of base wine. Immediately before addition, lyophilisates were cleaned up by solid−liquid extraction with 30 mL of diethyl ether to avoid any contaminations by hydrolyzed aroma compounds. Along with 6 L of untreated base wines as controls, fermentation in crown capped bottles with *S. cerevisiae cerevisiae* yeast strain Fermicru VB 1 (Max Keller, Mannheim, Germany) was started. When fermentations were finished after 10 weeks, the sparkling wines were disgorged and underwent routine chemical analysis, GC-MS analyses, and sensory analyses. Results of fundamental chemical analysis are presented in Table 2 along with the data of the base wines after addition of tirage liqueur.

Sensory Evaluation. A three-alternative forced choice (3-AFC) test was conducted with the Chardonnay and Riesling sparkling wine samples from the latter experiment to determine whether any significant differences caused by base wine enrichment with glycosylated aroma precursors could be detected. For each grape variety, a tray with three opaque black wine testing glasses, one of them containing the control sparkling wine, was presented to 16 judges. The judges were asked to select the differing sample solely on the basis of orthonasal olfactory evaluation.

To characterize sensory differences, a descriptive sensory analysis was conducted. Sensory vocabulary was built up in two evaluation sessions, which were carried out by the five most experienced wine experts from our institute. After three training sessions, 14 judges (all of them experienced in wine and sparkling wine tasting) were asked to rate every sample regarding *color intensity*, *carbon dioxide perception*, *mouthfeel*, *sourness*, *bitterness*, and retronasal *fruitiness*, along with 11 orthonasal aroma sensations (*alcohol*, *icy drop candy*, *apple*, *peach*, *cantaloupe*, *grape must*, *elderflower*, *green bananas*, *grass*, *green bean*, *yeasty*). A scale

Table 1. Model Fermentations with the Fermicru VB 1/IOC 18-2007 Champagne Yeast Strain: Results from Routine Chemical Analysis of Chardonnay and Riesling Base Wines, Sparkling Wines, and Fermented Base Wines (n = 2)

		Chardonnay model fermentation				Riesling model fermentation			
	units	base wine	fermented base wine VB 1	sparkling wine VB 1	sparkling wine IOC	base wine	fermented base wine VB 1	sparkling wine VB 1	sparkling wine IOC
density $d^{20}/_{20}$		0.9938	0.9906	0.9949	0.9961	0.9956	0.9917	0.9920	0.9940
alcohol	% vol	11.9	13.5	12.9	12.8	10.5	11.9	12.1	11.8
total extract	g/L	25.6	21.4	30.2	33.1	25.4	19.7	20.5	24.8
residual sugars	g/L	5.6	1.4	10.6	13.8	5.9	0.3	2.0	5.8
glycerol	g/L	5.9	6.6	6.7	6.8	4.5	4.9	4.9	5.2
pH		3.2	3.2	3.2	3.2	3.3	3.2	3.2	3.2
total acidity[a]	g/L	6.8	6.7	6.8	6.8	6.9	6.6	6.7	6.9
volatile acidity[b]	g/L	0.45	0.49	0.59	0.62	0.26	0.28	0.31	0.39
tartaric acid	g/L	1.5	1.6	1.5	1.5	1.4	1.2	1.2	1.2
malic acid	g/L	3.7	3.4	3.3	3.4	4.7	4.5	4.6	4.6
lactic acid	g/L	0.1	0.2	0.2	0.2	0.1	0.1	0.1	0.1
total phenols[c]	mg/L	196	188	175	186	216	181	182	184
extinction at λ = 420 nm		0.0434	0.0480	0.0713	0.0667	0.0413	0.0366	0.0527	0.0527

[a] Calculated as tartaric acid. [b] Calculated as acetic acid. [c] Calculated as gallic acid equivalents.

Table 2. Enrichment Experiment: Results from Routine Chemical Analysis of Base Wines after Tirage, Sparkling Wines, and Sparkling Wines Fermented after Precursor Addition (n = 2)

		Chardonnay			Riesling		
			sparkling wine			sparkling wine	
	units	base wine after tirage	control	enriched	base wine after tirage	control	enriched
density $d^{20}/_{20}$		1.0053	0.9939	0.9939	1.0108	0.9950	0.9946
alcohol	% vol	10.8	12.2	12.2	11.6	13.6	13.6
total extract	g/L	50.9	25.6	25.6	67.7	32.6	31.6
residual sugars	g/L	26.5	<1.0	<1.0	46.4	9.8	7.9
glycerol	g/L	8.4	8.6	8.5	6.4	7.2	7.2
pH		3.4	3.4	3.5	3.2	3.2	3.3
total acidity[a]	g/L	7.4	7.1	7.5	7.2	7.0	7.0
volatile acidity[b]	g/L	0.48	0.44	0.46	0.35	0.34	0.34
tartaric acid	g/L	0.7	0.8	0.8	1.5	1.3	1.3
malic acid	g/L	4.8	4.5	4.5	3.5	3.2	3.2
lactic acid	g/L	0.2	0.2	0.2	0.6	0.5	0.5
total phenols[c]	mg/L	205 ± 11	187 ± 8	201 ± 11	316 ± 15	266 ± 21	278 ± 20
extinction at λ = 420 nm		0.0634	0.0904	0.1345	0.1031	0.1127	0.1386

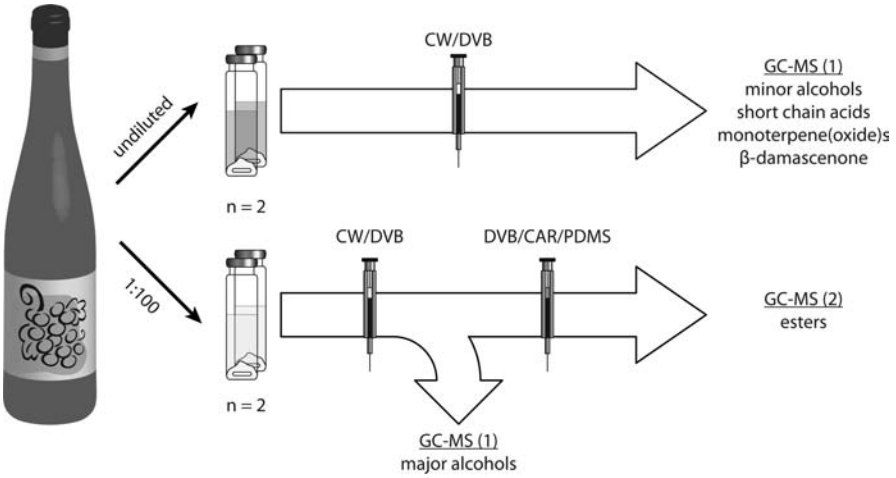
[a] Calculated as tartaric acid. [b] Calculated as acetic acid. [c] Average values and 95% confidence intervals from triplicates, calculated as gallic acid equivalents.

anchored at 0 (weak) and 10 (intense) was used for all attributes except *mouthfeel*, which was anchored at 0 (creamy) and 10 (coarse). Aliquots of about 30 mL of the sparkling wines were poured immediately before sensory evaluation, served at 8 °C, and presented in randomized order in coded DIN 10960 wine testing glasses (Schott, Mainz, Germany) covered with Petri dishes. While color and aroma attributes were evaluated for all samples comparatively, monadical scores for taste attributes and tactile descriptors were given. Aroma standards were prepared in neutral white wine cuvee according to Supporting Information Table S1 and presented along with the samples. Descriptive sensory data were collected using FIZZ for Windows software (version 2.00 D, Biosystemes, Couternon, France).

Syntheses of Deuterated Standards for SIDA. For all substances synthesized, spectroscopic data (MS, NMR) were in accordance with published data. Individual purity of ≥98% was confirmed by GC-MS analysis unless stated otherwise.

Deuterated Esters. Syntheses of 2-methylpropyl [$^2H_3$]-acetate, 3-methylbutyl [$^2H_3$]-acetate, and 2-phenylethyl [$^2H_3$]-acetate followed standard procedures adapted for small-scale preparation:[25] The corresponding alcohols (4 mmol) were esterified with [$^2H_3$]-acetyl chloride (5 mmol) in dry dichloromethane (10 mL). The products were purified and concentrated by microdistillation (Bemelmans apparatus[26]). Deuterated ethyl esters were prepared by small-scale reaction of [$^2H_6$]-ethanol (1 mL) with the corresponding acyl chlorides in 5 mL of dry dichloromethane.[25] After cleanup, careful concentration by microdistillation (Bemelmans apparatus) and evaporation of residual solvent under an argon stream, the deuterated standard compounds [$^2H_5$]-ethyl propanoate, [$^2H_5$]-ethyl butanoate, [$^2H_5$]-ethyl 3-methylbutanoate,

Scheme 1. Flow Diagram for Analysis of Volatiles: Sample Preparation of Wines and Degassed Sparkling Wine Samples



[$^2H_5$]-ethyl hexanoate, [$^2H_5$]-ethyl octanoate, and [$^2H_5$]-ethyl decanoate, as well as [$^2H_5$]-ethyl [$^2H_3$]-acetate, were obtained. [$^2H_5$]-Ethyl furoate was prepared by transesterification of 2.5 mmol of nondeuterated ethyl furoate with sodium dissolved in 1.5 mL of [$^2H_6$]-ethanol. After cleanup and purification, 220 mg of [$^2H_5$]-ethyl furoate was yielded.

Deuterated Linalool and Linalool Oxides. 3-([$^2H_3$]-Methyl)-7-methyl-5,5-[$^2H_2$]-octa-1,6-dien-3-ol ([$^2H_5$]-linalool) was synthesized as described in detail by Luan[27] via Grignard reaction from vinylmagnesium bromide and 6-methyl-1,1,1,3,3-[$^2H_5$]-hept-5-en-2-one, which in turn was generated by H/^{2}H exchange from 6-methyl-hept-5-en-2-one according to ref 28. The resulting [$^2H_5$]-linalool was furthermore used for synthesis of linalool oxides based on oxidation with a peracid according to the method of Klein et al.[29] An aliquot of 550 mg of [$^2H_5$]-linalool was reacted in 15 mL of dry dichloromethane with 1.5 mL of a solution of formic acid and 30% hydrogen peroxide (1:1, v/v). The reaction was carried out at 0 °C and was stopped as soon as reaction control by GC-MS showed about 75% [$^2H_5$]-deuterated furanoid and pyranoid linalool oxides and about 20% residual [$^2H_5$]-linalool. Some minor byproducts were not considered further. The ratio of the linalool oxides produced was about 4:1 in favor of the furanoid form. After washing with potassium carbonate and brine and drying with magnesium sulfate, the final concentrations were determined by GC-MS using heptan-2-ol as internal standard. This mixture was used to prepare calibration standards without further purification.

Deuterated β-Damascenone. The preparation of deuterated β-damascenone was performed by H/^{2}H-exchange in a two-phase system of β-damascenone in dry tetrahydrofuran and deuterium oxide, with a catalytic amount of *n*-butyllithium in cyclohexane, according to a procedure described by Kotseridis et al.[30] The deuterated product showed an isotopologuos composition of primarily 46.6% 1-(2,6,6-trimethylcyclohexa-1,3-dienyl)-2,4,4,4-[$^2H_4$]-but-2-en-1-one ([$^2H_4$]-β-damascenone), 35.4% [$^2H_3$]-β-damascenone isomers, and 14.7% [$^2H_2$]-β-damascenone isomers. This was taken into account for the GC-MS method setup.

Deuterated 2-Methylpropanoic Acid. [$^2H_7$]-2-Methylpropanoic acid was synthesized via Grignard reaction from [$^2H_7$]-2-bromopropane and carbon dioxide as described earlier.[6]

Preparation of Internal Standard and Calibration Standard Mixtures. Mixtures of internal standards and calibration standards were prepared in ethanol separately for each of the five substance classes esters, major alcohols, short-chain aliphatic acids, β-damascenone, and minor alcohols + monoterpen(oxid)es. This was done to avoid chemical interactions between analytes during refrigerated storage. However, the deuterated ester standard solution suffers from long-term (>1 month) [$^2H_5$]-ethyl/ethyl exchange and should therefore be prepared in [$^2H_6$]-ethanol as solvent for subsequent analyses. Concentrations were chosen in a way that 25 μL of standard mixture added to 10 mL of calibration matrix yielded average natural concentrations in white wine (cf. Supporting Information Table S2).

Sample Preparation. Sparkling wine samples were passed through a paper filter with slightly increased pressure to remove discharging carbon dioxide. Scheme 1 gives an overview of the GC-MS analyses conducted. Major alcohols and esters were determined in an aqueous 1:100 dilution of a sample aliquot by successive HS-SPME using fibers with different polarities (CW/DVB and DVB/CAR/PDMS), whereas minor alcohols, short-chain aliphatic acids, monoterpene alcohols, linalool oxides, and β-damascenone were extracted in undiluted aliquots using the CW/DVB fiber. For HS-SPME-GC-MS, 10 mL of (diluted) sample, 3 g of NaCl, and 25 μL of each relevant internal standard solution were mixed in 20 mL headspace vials equipped with a 1 cm magnetic stir bar. All samples were analyzed in duplicates. In the same manner, two sets of seven calibration levels were prepared with 10 mL of purified water or 10 mL of model wine (12% vol, 3 g/L tartaric acid, pH 3.2) for diluted and undiluted samples, respectively, containing 2.5, 5, 12.5, 25, 50, 125, and 250 μL of each relevant calibration standard mixture. To compensate effects due to fiber wear, (sparkling) wine samples were bracketed by the calibration samples.

Automated HS-SPME-GC-MS. A Trace GC gas chromatograph (ThermoFisher Scientific, Dreieich, Germany) equipped with a programmed temperature vaporizing injector (PTV) coupled to a DSQ quadrupole mass spectrometer (ThermoFisher) was used. The system was furthermore equipped with a CombiPal autosampler (Firmware version 2.4.0, CTC Analytics, Zwingen, Switzerland) suitable for solid phase microextraction and a fiber conditioning station. Agitation and incubation was done with a single magnet mixer from Chromtech (Idstein, Germany). Samples were extracted at 35 °C for 20 min. Analytes were allowed to desorb in the injector at 230 °C for 5 min in splitless mode, and then the split ratio was set to 10:1. To minimize carry-over effects, the DVB/CAR/PDMS fiber was reconditioned at 270 °C and the CW/DVB fiber at 230 °C for 10 min between consecutive runs using a fiber conditioning station.

GC analysis was done with a 30 m × 0.25 mm i.d. fused silica capillary, coated with a polyethylene glycol stationary phase of 0.5 μm film thickness (ZB-Wax, Phenomenex, Aschaffenburg, Germany). Helium was used as carrier gas at a constant flow of 1.2 mL/min. The oven temperature was programmed to hold 35 °C for 5 min, increasing then at 4 °C/min to 240 °C, and held for 10 min. Analytes were detected by

quadrupole MS in selected ion mode (EI^+, 70 eV, source temperature 240 °C) according to Supporting Information Table S3 for quantification of esters and major alcohols (GC-MS (1) method) or according to Supporting Information Table S4 for quantification of the remaining substances (GC-MS (2) method). All ions were detected with a scan width of 1 amu. Instrument control and data acquisition were done with XCalibur software version 1.4 (ThermoFisher) and Cycle Composer software version 1.5.2 (CTC).

Nineteen compounds were quantified by stable isotope dilution analysis; for another 18 compounds, structurally related deuterated standards were used (for details see Supporting Information Tables S5, S6, and S7). Structural identity was confirmed by a positive match with the provided partial spectrum, for which only major fragments with mass abundances of >10% were considered as qualifier ions. Especially for small molecules, finding unique qualifier ions with fair abundances often was difficult. In these cases, integration of the highest or nearest peak in the respective target ion trace was a feasible alternative, when peak shapes were also considered; for example, 2/3-methylbutanoic acid gave a tailing signal with two apexes. As no baseline separation could be achieved for methylbutanoic acids, alcohols, and acetates, they were quantified as the sum of isomers. Calibration curves were obtained by applying a linear fit in the first instance, yet many curves gave better values for R^2, when a second order log−log fit was applied. Nonlinear calibration curves mainly result from the fact that many substances compete in adsorbing to the fiber surface:[31] this effect increases in high-level calibration standards, when fiber saturation can be an issue, as well. Also, at the highest calibration levels, which contain the 5- or 10-fold native amount of all calibrated substances, reproducibility tends to be rather imprecise, resulting in diverging calibration points for some compounds. In these cases, the highest calibrated amounts were excluded from the calibration curve, when appropriate (i.e., if at least one more calibration point lies between them and the highest amount to be quantified in a sample). Apart from highly volatile 2-methylpropyl acetate (R^2 = 0.823) and low-volatile ethyl decanoate (R^2 = 0.926), as well as octan-1-ol (R^2 = 0.939), *cis*-hex-2-enol (R^2 = 0.958), and α-terpineol (R^2 = 0.963), all calibration curves gave good fits with $R^2 \geq 0.97$.

RESULTS

Results for the 30 volatile compounds quantified in Chardonnay and Riesling model samples are shown in Table 3. 2-Phenylethyl acetate could not be quantified, as its content was determined to be below the quantification limit of 55 μg/L (lowest calibrated concentration). Quantitative results of the enrichment experiment are shown in Table 4 for esters and in Table 5 for polar substances (30 compounds altogether). Ethyl furoate could not be quantified, as its content was below the quantification limit (5 μg/L in the lowest calibrated standard). All amounts are given as ranges from duplicate determinations.

Effect of Second Fermentation on Aroma Compound Composition. The general effect of a second fermentation on aroma-relevant compounds was comparable for model fermentations and enrichment experiments: The data show that a second fermentation leads to increasing amounts of short-chain aliphatic acids and decreasing amounts of medium-chain aliphatic acids. Ethyl esters are affected in the same manner throughout all variants (more short-chained and less medium-chained ethyl esters). Total ethyl ester concentrations were calculated from the sum of all molar concentrations of linear and branched ethyl alkanoates from propanoate to decanoate. In this respect it is noteworthy that the total amount of ethyl esters decreases or at least stays unchanged. Overall, more linear and branched alkanols were formed during the second fermentation. Only *cis*-hex-2-enol levels generally remained constant. All monoterpene alcohol and linalool oxide concentrations went up in Riesling and to a lesser degree in Chardonnay samples, the monoterpene alcohol levels of which were generally lower. Apart from α-terpineol, no such increase was observed in the Chardonnay sample used for model fermentation, having the least monoterpene alcohol concentrations. Contents of β-damascenone increased severely in sparkling wines of both varieties, whereas both fermented base wines showed no change at all.

Impact of Precursor Enrichment on Aroma Compound Composition. No substantial changes in ester concentrations were observed between sparkling wines made from base wines with and without added precursors. Although base wines were spiked up to the double precursor amount, a general 2-fold increase of volatiles could not be confirmed: only three volatiles investigated (benzyl alcohol and linalool in Riesling; 3-methylpentanol in both varieties) showed an almost quantitative 2-fold increase due to precursor addition. Whereas *cis*-hex-2-enol remained unaffected, *trans*- and *cis*-hex-3-enol as well as 3-methylpentanol increased considerably due to addition of precursor fractions to base wines. Likewise, addition of aroma precursors enhanced octan-1-ol, linalool, and β-damascenone contents in Riesling; if at all, only slightly more geraniol and α-terpineol were released from its precursor extract. On the other hand, concentrations of these two monoterpene alcohols rose substantially in Chardonnay sparkling wines. Benzyl alcohol experienced a major increase, as well. Whereas after enrichment even less 2-phenylethanol, *trans*-linalool oxide, and short-chain aliphatic acids were found in Chardonnay sparkling wines, these compounds remained unaffected in Riesling.

Impact of Precursor Enrichment on Sensory Perception. In the preceding 3-AFC test, significant differences between control sparkling wine and sparkling wine made from the enriched base wine were confirmed; 9 judges chose the differing Riesling sparkling wine correctly (α < 5%), and 14 judges chose the differing Chardonnay sparkling wine correctly (α < 0.1%). Aside from a slightly different degree of sugar fermentation in the Riesling samples, chemical properties of both corresponding sparkling wine variants were virtually identical (cf. Table 2). It was therefore deduced that the addition of glycosylated precursors had no influence on the general sparkling wine matrix and, hence, on overall aroma compound volatilization.

With organic acid profiles as well as alcohol and glycerol contents being almost identical for corresponding sparkling wines, descriptive sensory analysis could not provide evidence for a difference either: except for orthonasal attributes, all actually perceived differences were smaller than the least significant differences at p = 95%. Figure 1 gives an overview over all relevant sensory attributes in Chardonnay and Riesling sparkling wines that were affected by base wine enrichment with glycosylated precursors. As shown by mean sensory scores, both control sparkling wines were perceived as rather unobtrusive; whereas the Riesling sparkling wine was associated with ripe fruit, the Chardonnay sparkling wine had a rather yeasty and estery bouquet, combined with sensations of unripe fruit. Although scale normalization to 100% conceals these aspects, it was used anyway to visualize the different sensory effects of precursor enrichment on either of the two series: Cantaloupe was significantly enhanced in favor of yeasty impressions in the Chardonnay sparkling wine. The Riesling, however, was given higher peach and elderflower scores, and even green banana aroma sensations were intensified. Contrary to the Chardonnay, its entire bouquet

Table 3. Concentrations of Volatiles in Model Chardonnay and Riesling Samples Determined by HS-SPME-GC-MS[a]

		Chardonnay				Riesling			
	units	base wine	fermented base wine (VB 1)	sparkling wine (VB 1)	sparkling wine (IOC)	base wine	fermented base wine (VB 1)	sparkling wine (VB 1)	sparkling wine (IOC)
ethyl acetate	mg/L	64 ± <1	**70 ± 2**	**74 ± < 1**	**86 ± 3**	23.1 ± 0.1	**24.3 ± 0.4**	**27.3 ± 0.5**	**38.4 ± 0.6**
ethyl propanoate	μg/L	95 ± 4	**115 ± 4**	**115 ± 3**	**118 ± 7**	90 ± 4	**117 ± 2**	**109 ± 7**	**101 ± 1**
ethyl butanoate	μg/L	197 ± 2	**210 ± 5**	200 ± 3	220 ± 20	135 ± 7	133 ± 1	**122 ± 3**	128 ± 5
ethyl 2-methylbutanoate	μg/L	8.4 ± 0.2	**14,8 ± 0.3**	**14.4 ± 0.7**	**16.7 ± 1.5**	nd	nd	nd	nd
ethyl 3-methylbutanoate	μg/L	11.0 ± 0.2	**18,7 ± 0.6**	**16.2 ± < 0.1**	**21.8 ± 2.0**	nd	nd	nd	nd
ethyl hexanoate	μg/L	713 ± <1	**764 ± 2**	**744 ± 8**	**790 ± 70**	790 ± 20	**759 ± 2**	750 ± 40	**635 ± 6**
ethyl octanoate	μg/L	800 ± 6	810 ± 10	**712 ± 7**	820 ± 40	900 ± 60	**720 ± 20**	**670 ± 30**	**540 ± 20**
ethyl decanoate	μg/L	200 ± 50	**126 ± 2**	**<120**	**<120**	<120	<120	<120	<120
total ethyl esters	μmol/L	13.4 ± 0.4	13.8 ± 0.2	<13.0 ± 0.2	<14.2 ± 1.0	12.8 ± 0.6	**11.8 ± 0.2**	**11.2 ± 0.5**	**9.6 ± 0.2**
ethyl 2-furoate	μg/L	<5	<5	<5	<5	7 ± 1	8 ± 1	8 ± 2	**<5**
2-methylpropyl acetate	μg/L	26.5 ± 1.1	26.7 ± 0.8	**33.2 ± 0.2**	**32.4 ± 0.7**	<12	<12	<12	<12
2/3-methylbutyl acetate (sum)	μg/L	169 ± 5	**211 ± 7**	**192 ± 7**	170 ± 20	nd	nd	nd	nd
2-methylpropanol	mg/L	50 ± 2	**56 ± 1**	51 ± 1	48 ± 4	9.0 ± <0.1	**14.2 ± 0.4**	**14.7 ± 0.2**	**11.9 ± 0.3**
2/3-methylbutanol (sum)	mg/L	140 ± 10	**160 ± 6**	136 ± 1	136 ± 7	81 ± 7	**124 ± 2**	**108 ± 1**	**103 ± 8**
hexan-1-ol	mg/L	2.84 ± 0.17	2.70 ± 0.06	**2.61 ± 0.02**	2.65 ± 0.10	1.92 ± 0.03	**2.06 ± 0.01**	**2.08 ± 0.04**	1.97 ± 0.14
trans-hex-3-enol	μg/L	21.0 ± 0.6	**26.3 ± 0.4**	**23.5 ± <0.1**	**28.6 ± 3.5**	51.8 ± 2.3	**57.4 ± 0.6**	**54.7 ± <0.1**	49.4 ± 0.8
cis-hex-3-enol	μg/L	95 ± 1	**116 ± 2**	**111 ± 1**	**130 ± 20**	67 ± 3	**82 ± 1**	**95 ± 1**	**86 ± 2**
cis-hex-2-enol	μg/L	12.7 ± 1.0	13.6 ± 0.7	14.0 ± 0.4	**16.0 ± 0.9**	21.1 ± 1.2	21.5 ± 0.1	23.0 ± 0.7	21.9 ± 0.3
3-methylpentanol	μg/L	22.3 ± 0.1	**29.5 ± 0.5**	**26.5 ± 0.3**	**32.0 ± 5.7**	30.6 ± 1.6	**38.8 ± 0.7**	**38.3 ± 0.3**	**35.7 ± 0.8**
octan-1-ol	μg/L	4.3 ± 0.1	**5.8 ± 0.2**	**4.9 ± 0.1**	**5.5 ± 0.1**	8.2 ± 0.2	**13.9 ± 0.3**	**11.4 ± <0.1**	**9.5 ± 0.2**
benzyl alcohol	μg/L	55 ± 5	**88 ± 4**	48 ± 2	48 ± 5	90 ± 10	**118 ± 6**	94 ± 2	**65 ± 7**
2-phenylethanol	mg/L	10.7 ± 0.2	**12.3 ± <0.1**	**11.6 ± <0.1**	11.5 ± 0.6	5.8 ± 0.7	**7.8 ± 1.1**	**9.1 ± 0.1**	**8.0 ± 0.4**
2-methylpropanoic acid	mg/L	1.12 ± 0.02	**1.42 ± <0.01**	**1.64 ± 0.01**	**1.93 ± 0.09**	0.70 ± <0.01	**0.87 ± 0.03**	**0.88 ± 0.01**	**1.19 ± 0.01**
2/3-methylbutanoic acid (sum)	mg/L	0.50 ± 0.01	**0.72 ± 0.01**	**0.73 ± 0.01**	**0.81 ± 0.04**	<0.1	<0.1	<0.1	<0.1
hexanoic acid	mg/L	8.3 ± 0.4	7.8 ± 0.2	7.8 ± 0.2	8.0 ± 0.4	12.5 ± 1.4	**10.0 ± <0.1**	11.4 ± 0.5	**9.2 ± 0.1**
linalool	μg/L	<5	<5	<5	<5	5.5 ± 0.1	**6.0 ± 0.1**	**11.2 ± 0.1**	**8.4 ± 0.1**
trans-linalool oxide (furanoid)	μg/L	12.6 ± 1.4	13.7 ± 0.8	11.6 ± < 0.1	**9.1 ± < 0.1**	19.4 ± 0.1	**25.5 ± 0.4**	**24.9 ± 0.2**	**22.2 ± 0.9**
cis-linalool oxide (furanoid)	μg/L	7.8 ± 0.6	7.9 ± 0.1	8.0 ± 1.5	8.2 ± 0.7	19.8 ± 0.1	**24.1 ± 0.2**	**24.7 ± 0.3**	**22.6 ± 0.2**
α-terpineol	μg/L	2.2 ± 0.1	**2.8 ± 0.1**	**3.4 ± < 0.1**	**3.9 ± 0.3**	17.6 ± 0.7	**22.8 ± 0.3**	**25.8 ± 0.3**	**22.0 ± 0.4**
geraniol	μg/L	<1	<1	<1	<1	1.5 ± 0.1	1.,4 ± < 0.1	**2.7 ± 0.1**	**2.0 ± 0.1**
β-damascenone	μg/L	1.01 ± 0.03	0.99 ± 0.11	**2.87 ± 0.09**	**3.04 ± 0.21**	0.20 ± 0.01	0.21 ± < 0.01	**2.25 ± 0.03**	**1.96 ± 0.01**

[a] Values given as ranges from duplicates. Substantial changes of base wine contents due to the second fermentation using the Fermicru VB 1/IOC 18-2007 Champagne yeast strain are printed in bold type.

was characterized by a higher aroma complexity instead of being dominated by single odors.

DISCUSSION

One major effect due to the second fermentation of base wines regards changes in the fatty acid spectrum and thus the corresponding ethyl esters: Up to a certain chain length, concentrations will rise but begin to decrease subsequently. However, the position of that split point, that is, the carbon number of the acid moiety showing no quantitative changes, seems to vary depending on individual undetermined fermentation factors. Considering that the second fermentation causes total ethyl esters to either stagnate or decrease, no net synthesis of ethyl esters seems to take place. Ester formation is closely related to the fatty acid metabolism, requiring acyl-CoA and therefore energy.[32,33] Hence, a metabolic shift toward shorter chain lengths reflects net energy consumption by yeast cells during sparkling wine production, which is reasonable in a closed system.

On the other hand, acetate esters did not show consistent behavior. Investigations of Ramey and Ough have shown that acetate esters are more easily affected by hydrolyzation processes than ethyl esters.[34] The above-mentioned experiments differed in base wine pH, ethanol content, and degree of fortification, which might be the explanation for the nonuniform changes observed.

In both varieties, more short-chain branched alcohols were generated in the model fermentation experiment, originating either from

Table 4. Ester Concentrations in Chardonnay and Riesling Samples Determined by HS-SPME-GC-MS[a]

		Chardonnay			Riesling		
	units	base wine	sparkling wine (control)	sparkling wine (enriched)	base wine	sparkling wine (control)	sparkling wine (enriched)
ethyl acetate	mg/L	47 ± 1	**52 ± 1**	52 ± <1	51 ± 1	55 ± 3	54 ± 1
ethyl propanoate	μg/L	74 ± 1	**106 ± 5**	118 ± 8	48 ± <1	**73 ± 6**	72 ± 2
ethyl butanoate	μg/L	104 ± 2	**122 ± 6**	131 ± 8	200 ± 10	200 ± 20	191 ± 3
ethyl 2-methylbutanoate	μg/L	1.5 ± 0.2	**2.7 ± 0.2**	3.3 ± 1.1	0.9 ± 0.1	**2.5 ± 0.3**	2.2 ± 0.2
ethyl 3-methylbutanoate	μg/L	3.7 ± 0.5	**6.8 ± 0.6**	7 ± 0.1	4 ± 0.3	**7.1 ± 1.0**	6.1 ± 0.4
ethyl hexanoate	μg/L	710 ± 40	750 ± 10	**690 ± 10**	850 ± 10	860 ± 50	810 ± 50
ethyl octanoate	μg/L	798 ± 4	820 ± 70	820 ± 20	1200 ± 100	1000 ± 100	890 ± 20
ethyl decanoate	μg/L	480 ± 60	**250 ± 20**	280 ± 40	700 ± 20	**315 ± <1**	310 ± 20
total ethyl esters	μmol/L	13.6 ± 0.4	13.4 ± 0.7	13.3 ± 0.5	18.6 ± 0.8	**15.9 ± 1.2**	14.8 ± 0.6
2-methylpropyl acetate	μg/L	88 ± 1	**74 ± 5**	82 ± 6	39 ± 3	**45 ± 2**	45 ± 1
2/3-methylbutyl acetate (sum)	mg/L	3.6 ± 0.1	**2.8 ± 0.2**	2.92 ± 0.02	0.94 ± 0.06	**0.73 ± 0.08**	0.73 ± 0.01
2-phenylethyl acetate	μg/L	255 ± 4	**212 ± 7**	217 ± 7	140 ± 20	**90 ± 10**	90 ± 10

[a] Values given as ranges from duplicates. Substantial changes due to the second fermentation (base wine vs control, central columns) or to precursor addition (control vs enriched, right columns) are printed in bold type.

Table 5. Concentrations of Hydroxyl Compounds and β-Damascenone in Chardonnay and Riesling Samples Determined by HS-SPME-GC-MS[a]

		Chardonnay			Riesling		
	units	base wine	sparkling wine (control)	sparkling wine (enriched)	base wine	sparkling wine (control)	sparkling wine (enriched)
2-methylpropanol	mg/L	20 ± 3	23 ± 5	27 ± 1	41 ± 4	44 ± 3	41 ± 1
2/3-methylbutanol (sum)	mg/L	120 ± 20	120 ± 30	130 ± 10	88 ± 4	96 ± 7	91 ± 3
hexan-1-ol	mg/L	1.4 ± 0.2	1.3 ± 0.3	1.4 ± 0.1	2.5 ± 0.1	2.4 ± 0.2	2.14 ± 0.01
trans-hex-3-enol	μg/L	31 ± 3	**35 ± 1**	**39 ± 1**	84 ± 2	**102 ± 8**	**115 ± 3**
cis-hex-3-enol	μg/L	150 ± 10	**180 ± 10**	**207 ± 4**	87 ± 2	**112 ± 9**	**126 ± 2**
cis-hex-2-enol	μg/L	13 ± 1	13 ± 1	13 ± 2	18.1 ± 0.4	19.1 ± 0.9	18.8 ± 0.8
3-methylpentanol	μg/L	80 ± 10	**90 ± 10**	**109 ± 2**	36 ± 1	**49 ± 5**	**60 ± 5**
octan-1-ol	μg/L	6 ± 0.7	**11.2 ± 0.5**	11.5 ± 0.2	10.3 ± 0.4	**11.9 ± 1.2**	**13.4 ± 0.1**
benzyl alcohol	μg/L	60 ± 10	**110 ± 10**	**170 ± 20**	42 ± 3	**90 ± 20**	120 ± 20
2-phenylethanol	mg/L	8.0 ± 0.8	**9.6 ± 0.3**	**8.2 ± 0.5**	8 ± 1	**10 ± 1**	11 ± 1
2-methylpropanoic acid	mg/L	1.80 ± 0.07	**1.94 ± 0.06**	**1.74 ± 0.02**	1.41 ± 0.01	**1.55 ± 0.02**	1.58 ± 0.04
2/3-methylbutanoic acid (sum)	μg/L	1620 ± 40	**1840 ± 40**	**1630 ± 10**	659 ± 7	**925 ± 1**	**953 ± 8**
hexanoic acid	mg/L	12.8 ± <0.1	**11.2 ± 0.1**	**10.4 ± 0.5**	10.3 ± 0.3	**9.7 ± 0.2**	10 ± 0.3
linalool	μg/L	18 ± 1	**26 ± 2**	28 ± <1	110 ± 3	**125 ± 8**	**138 ± 3**
trans-linalool oxide (fur.)	μg/L	7.6 ± 0.3	**9.1 ± 0.4**	**7.3 ± <0.1**	35.0 ± 0.5	**41.8 ± 0.8**	38.8 ± 3.3
cis-linalool oxide (furanoid)	μg/L	2.4 ± 0.1	**3.4 ± 0.3**	3.1 ± 0.2	10.8 ± 0.4	**13.1 ± 0.6**	11.3 ± 1.3
α-terpineol	μg/L	7.7 ± 0.4	**13.5 ± 0.2**	**14.6 ± 0.2**	43 ± 1	**63 ± 5**	70 ± 3
geraniol	μg/L	8.7 ± 0.8	9.6 ± 0.8	**11.5 ± 0.8**	20 ± 1	**24 ± 2**	27 ± 2
β-damascenone	μg/L	3.4 ± 0.1	**4 ± 0.3**	4 ± 0.1	4.4 ± 0.1	**5.5 ± 0.1**	**6.9 ± 0.4**

[a] Values given as ranges from duplicates. Substantial changes due to the second fermentation (base wine vs control, central columns) or to precursor addition (control vs enriched, right columns) are printed in bold type.

decarboxylation and subsequent NAD^+ mediated reduction of valine, leucine, and isoleucine (Ehrlich pathway) or from glucose degradation via α-ketoacids.[35] Increasing amounts of 2-phenylethanol were also detected, originating from phenylalanine via the Ehrlich pathway.[36]

Model fermentation of Riesling showed that a second fermentation leads to increasing amounts of some monoterpene alcohols and linalool oxides. Using synthetic must, Carrau et al. described the de novo synthesis in *S. cerevisiae* to be on the magnitude of

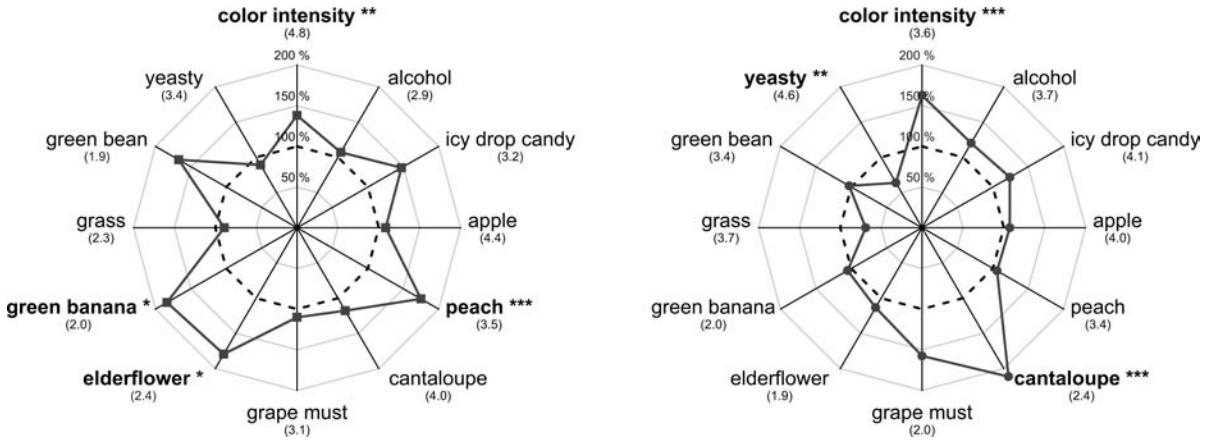


Figure 1. Sensory changes of Chardonnay (●) and Riesling sparkling wines (■) due to base wine enrichment with glycosylated precursors rated by 14 judges. Sparkling wines made of native base wines (controls) were normalized to 100% (dashed line). Scores of the control are shown in parentheses; significant changes are indicated by asterisks and bold type.

1 μg/L geraniol, 4 μg/L linalool, and 4 μg/L α-terpineol,[37] which is similar to our findings. The same publication reported no detectable production of linalool oxides by yeast, whereas we determined about 5 μg/L additional linalool oxides after the second fermentation. Altogether, we assume that these substances originate from the wine matrix itself rather than being formed using yeast material as in Chardonnay only α-terpineol was increased, although the same yeast strain was used. It is known that generally lower levels of monoterpene alcohols are found in Chardonnays than in Riesling wines.[38] Further support for the liberation of linalool oxides from such precursors was found with the isolation and identification of 6-*O*-glucopyranosides of furanoid and pyranoid linalool oxides by Witte.[24]

The presence of glycosylated aroma precursors in *Vitis vinifera* grapes and their potential for aroma contribution to wines has already been described in the literature.[9,10,15] It has also been shown that glycosylated precursors are not fully consumed by a first fermentation.[20] In this respect, the contribution of the remaining glycosylated precursors was investigated in a second experiment by precursor addition to base wines.

In both cultivars, *cis*- and *trans*-hex-3-enol, hexanol, and octanol were formed during the second fermentation. The question arises as to whether these substances are lipid oxidation metabolites (reduction of corresponding aldehydes, as proposed by Herraiz et al. [39]) or if they directly originate from glycosylated precursors. Hexanol and *cis*-hex-3-enol have already been found in the juice of Muscat of Alexandria grapes after enzymatic hydrolysis,[40] and our results of the precursor enrichment assay suggest that hex-3-enyl glycosides are still present in Riesling and Chardonnay base wines. On the other hand, Ferreira et al. found lower *cis*-hex-3-enol contents after the first fermentation when Macabeo grape juice had been supplemented with aroma precursors.[19] Although our results suggest glycosylated precursors for octanol in Riesling, hexanol contents remained unaffected by elevated amounts of glycosylated precursors in the base wine. Altogether, a universal answer cannot be given for the higher alcohols here, as both pathways seem to take place to different degrees, depending on individual fermentation conditions.

Despite the negligible increase of benzyl alcohol during model fermentations, spiking the Chardonnay base wine with precursor extract doubled the additional benzyl alcohol content. Sugar moieties of potential glycosylated precursors were described to be either disaccharides or glucose,[10] of which the benzyl monoglycoside has been described as ubiquitous by Skouroumounis and Winterhalter.[41] Similar precursors for 2-phenylethanol are known; however, its content could not be consistently related to precursor addition. It was already stated that 2-phenylethanol originates rather from yeast metabolism than from glycosylated precursors.[10,11] Accordingly, the main source for other major alcohols seem to be amino acids already present in the base wine[36] instead of glycosylated precursors, as the contents of 2-methylpropanol and 2/3-methylbutanol remained unaffected by precursor addition.

Apart from nerol, our study targets the same monoterpene alcohols as Zoecklein et al. had quantified in White Riesling wines, which were subjected to conventional aging and thermal storage;[13] their results suggest that all of these compounds are liberated during the first fermentation. Indeed, additional linalool was formed from the Riesling precursor extract during second fermentation, whereas the Chardonnay sparkling wine yielded higher geraniol and α-terpineol concentrations. Even though volatiles were determined directly after disgorging, acid-catalyzed rearrangement reactions could have taken place already,[20] so that the amount of these monoterpene alcohols released from precursors does not approximate the extent of the first fermentation. Zoecklein et al. could not relate additionally liberated monoterpene contents to the results obtained from their glycosyl-glucose assay, as well.

Intensification of sensory properties in sparkling wines due to precursor addition is generally in line with increasing amounts of volatiles. Although the weaker perception of yeasty and grassy aroma impressions in Chardonnay might seem to be inconsistent with increasing amounts of hex-3-enols, this is more likely to be a side effect to an intensified perception of cantaloupe. However, it could not be elucidated which substances were responsible for this. The volatile fraction of cantaloupe is rich in sulfurous compounds originating, for example, from methionine degradation;[42] sulfur-containing compounds should therefore be incorporated as analytes for future investigations. Due to the low odor threshold of β-damascenone (50 ng/L in 10% aqueous ethanol[43]) and its ability to enhance overall fruitiness of red wines,[44] increasing amounts of β-damascenone are probably responsible for the overall boost of many aroma nuances and enhanced complexity in Riesling sparkling wines made from enriched base wines. With regard to its formation, different

pathways from intermediates such as grasshopper ketone[45] are discussed.[46−48] Moreover, the 3-*O*-glucopyranoside of grasshopper ketone has been identified in a similar precursor extract by Witte.[24]

Although our method for determining changes in the composition of volatiles by HS-SPME in wine and sparkling wine samples proved to be convenient, it remains a demanding task to chemically analyze aroma as a whole within a reasonable amount of time. Alternative approaches, such as nontargeted profiling analysis, for example, based on comprehensive two-dimensional gas chromatographic analysis (GC×GC), are promising options in this respect.[49] As mentioned earlier, the analytical method described in this work is being continuously improved. Major modifications have become necessary, as the supply of the carbowax/divinylbenzene SPME fiber was discontinued. The two consecutive SPME extraction steps have meanwhile been replaced by one solid phase extraction step. Additional aroma relevant compounds have also been incorporated.

Altogether, this work has shown that aroma generation during a second fermentation in principle is not substantially different from processes known from the first fermentation. Many desirable aroma-relevant volatiles and sensory impressions were enhanced by addition of aroma precursors to base wines. The additional increase of volatiles caused by spiking the base wine to double-precursor content was considerable for many substances with hydroxyl groups, yet this effect seemed to be less than the effect of the second fermentation alone. Obviously, alternate metabolic pathways seem to interfere with aroma formation from glycosides. Because our conclusions are based upon measured concentrations of liberated compounds, future investigations should include quantification of precursors per se before and after fermentation.

■ ASSOCIATED CONTENT

Supporting Information. Additional tables. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

3-AFC, three-alternative forced choice; CAL, calibration standard; CAR, carboxen; CW, carbowax; DVB, divinylbenzene; HS, headspace; ISTD, internal standard; OIV, International Organisation of Vine and Wine; PDMS, polydimethylsiloxane; SIDA, stable isotope dilution analysis; SPME, solid phase microextraction

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