JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

Cite This: J. Agric. Food Chem. XXXX, XXX, XXX−XXX

pubs.acs.org/JAFC

Occurrence of Ehrlich-Derived and Varietal Polyfunctional Thiols in Belgian White Wines Made from Chardonnay and Solaris Grapes

Cécile Chenot, Laura Briffoz, Antonin Lomartire, and Sonia Collin*

Unité de Brasserie et des Industries Alimentaires, Earth and Life Institute (ELIM), Faculté des Bioingénieurs, Université catholique de Louvain. Croix du Sud 2, Box L7.05.07, B-1348 Louvain-la-Neuve, Belgium

ABSTRACT: Although almost disappeared at the end of the 20th century, Belgian wine production reached a million liters in 2017. The aim of the present work was to identify aroma markers for two white cultivars widespread in Belgium: Chardonnay (the most commercially grown cultivar worldwide) and Solaris (an interspecific fungus-resistant variety). Specific *p*-hydroxymercuribenzoic acid extraction followed by gas chromatography with a pulsed flame photometric detector was applied to single-varietal wine samples from 2015 to 2018 harvests. Among the Ehrlich-derived thiols, all found below their sensory thresholds, 3-sulfanylpropyl acetate usually outranged 3-sulfanylpropan-1-ol, while 2-sulfanylethan-1-ol concentrations always exceeded 2-sulfanylethyl acetate levels. The data confirm the occurrence, in both Chardonnay and Solaris wines, of 3-sulfanylhexan-1-ol and phenylmethanethiol (grapefruit and gunflint aroma, respectively), at concentrations far above their thresholds. This work also revealed, for the first time, the presence of 3-sulfanylheptan-1-ol, the branched alcohols 2-methyl-3-sulfanyl propan-1-ol, 2-methyl-3-sulfanylbutan-1-ol, and 3-sulfanyl-3-methylbutan-1-ol, and the carbonyls 3-sulfanylbutan-2-one (not in sparkling wines) and 3-sulfanylpentanal.

KEYWORDS: *Belgian white wine, polyfunctional thiols, Solaris, Chardonnay*

INTRODUCTION

Although Belgian wine production almost disappeared at the end of the 20th century, it approached a million liters in 2017 (including 361 000 L of still and 400 000 L of sparkling white wines), for a total of 350 ha.[1] The past decade has seen the creation of mostly small vineyards (<15 ha), some of which have gained a substantial reputation on international markets, especially vineyards producing sparkling Chardonnay wines.[1,2]

Thanks to its adaptability to various Huglin indices, Chardonnay, native to Burgundy, has become the most planted cultivar in the world.[2−4] Very famous for the typical flavors produced on Côte de Beaune and Chablis clay−limestone soils (gunflint, buttery, white flowers, citrus/tropical fruits, and hazelnut), Chardonnay grapes can give rise to various other interesting aroma profiles, highly dependent upon the climate and oenological practices.[2,5−7] Their sensitivity to late frosts, mildew, and oidium has recently led Belgian wine producers to test new, disease-tolerant cultivars, including Solaris (resistant to mildew and oidium but sensitive to *Botrytis cinerea* and coulure).[8,9] This German interspecific fungus-resistant variety (PIWI) results from multiple backcrosses of *Vitis amurensis* and *Vitis vinifera* cultivars in the 2000s. It exhales floral and fruity aromas, such as citrus, elderflower, peach/apricot, melon, banana, and strawberry.[10,11]

It is known that citrus, tropical fruit, gunflint, and hazelnut descriptors are often linked to the presence of polyfunctional thiols.[12,13] Most of these molecules have a 3-carbon distance between the SH group and the other chemical function (alcohol, ester, carbonyl, etc.). Of all of the plants analyzed to date for polyfunctional thiols, hop shows the highest diversity, with up to 41 different structures.[14,15] While 3-sulfanylhexan-1-ol (3SHol, grapefruit-like) and its derived ester, 3-sulfanylhexyl acetate (3SHA, passion fruit-like), appear relatively ubiquitous, the isomer 3-sulfanyl-4-methylpentan-1-ol (3S4MPol, grapefruit-like) has emerged as very specific to some hop varieties, including Nelson Sauvin, Hallertau Blanc, and Polaris. Similar ubiquity/specificity is observed for the corresponding cysteinylated and glutathionylated precursors.[15−17]

Although 3SHol, its acetate 3SHA, and 4-sulfanyl-4-methylpentan-2-one (4S4M2Pone, box-tree/catty flavor) were first identified as key varietal compounds in Sauvignon Blanc wines,[18,19] investigations of other wines have revealed high levels in Petit Manseng, Colombard, Muscat d'Alsace, Gewürztraminer, and Scheurebe (up to 5000 ng/L 3SHol, 150 ng/L 3SHA, and 400 ng/L 4S4M2Pone, with orthonasal thresholds in hydroalcoholic solution as low as 60, 4, and 0.8 ng/L, respectively).[19−21] Mateo-Vivaracho et al.[22] and Capone et al.[3] also found these compounds in Chardonnay wines but at lower concentrations (3SHol, 10−148 ng/L; 3SHA, 6−100 ng/L; and 4S4M2Pone, 7−23 ng/L). Except in Chardonnay, 3-sulfanyl-3-methylbutan-1-ol (3S3MBol, onion-like) has been found as an additional odorant thiol in the above-listed grape varieties but usually below its threshold of 1500 ng/L (orthonasal, value determined in hydroalcoholic solution).[19]

In botrytized wines, 3-sulfanylpentan-1-ol (3SPol, citrus/catty flavor), 3-sulfanylheptan-1-ol (3SHptol, citrus-like), and 2-methyl-3-sulfanylbutan-1-ol (2M3SBol, onion-like) are increased by the action of noble rot [up to 209 ng/L 3SPol, 51 ng/L 3SHptol, and 103 ng/L 2M3SBol on average, with

Special Issue: 12th Wartburg Symposium on Flavor Chemistry and Biology

Received: August 29, 2019
Revised: October 24, 2019
Accepted: October 29, 2019
Published: October 29, 2019

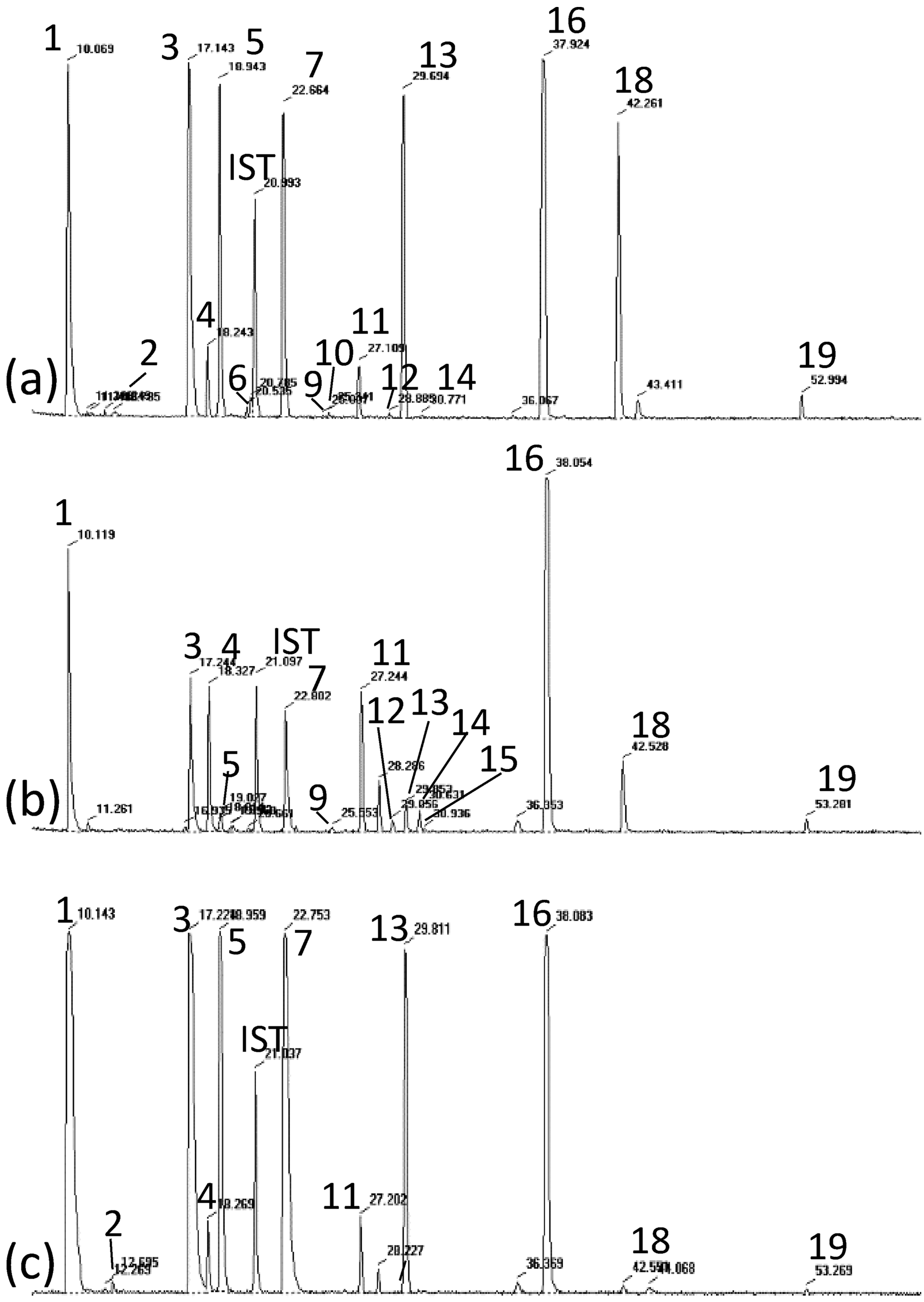

Figure 1. GC−PFPD chromatograms (CP-Sil5-CB column) of *p*HMB extracts obtained from the Belgian Chardonnay wines (a) Genoels-Elderen 2015 and (b) Chant d'Eole 2016 and the Solaris wine (c) Chenoy 2015 (variation coefficient of duplicates below 15%), with peak numbers referred in Tables 1 and 2.

DOI: 10.1021/acs.jafc.9b05478
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

Table 1. Concentrations (μg/L) of Polyfunctional Thiols in Belgian Chardonnay Wines, Determined by GC−PFPD[a]

	RI											
	CP-Sil5	FFAP	name and symbol	aroma descriptor(s)	Marnières	Genoels-Elderen	Bon Baron	Entre-deux-Monts	Bousval	Chant d'Ecole	Meerdael	Ruffus
1	722	1538	2-sulfanylethan-1-ol, 2SEol	grilled, gas	12.45 b,c	40.41 a	16.38 b,c	42.02 a	13.30 b,c	20.26 b	4.63 c	19.84 b
2	771	1295	3-sulfanylbutan-2-one, 3S2Bone	leek, cassis	nd	0.04 a	0.01 a	0.02 a	nd	nd	nd	nd
3	849	1620	3-sulfanylpropan-1-ol, 3SProl	potatoes, popcorn	0.27 c	5.07 a	0.99 b,c	0.61 c	0.22 c	1.64 b	0.30 c	0.74 c
4	868		unknown		0.86 b	0.68 b,c	0.35 c,d	0.24 d	0.29 c,d	2.01 a	0.53 b,c,d	0.51 b,c,d
5	880	1454	2-sulfanylethyl acetate, 2SEA	burnt, grill	2.27 b,c	4.24 b,c	2.98 b,c	15.91 a	8.78 b	0.19 c	0.23 c	2.82 b,c
6	897	1547	4-sulfanyl-4-methylpentan-2-one, 4S4M2Pone	citrus, catty	nd	0.10	nd	nd	nd	nd	nd	nd
7	922	1677	2-methyl-3-sulfanylpropan-1-ol, 2M3SProl	broth, leek	0.37 d	3.33 a	0.87 c	1.99 b	0.76 c	1.75 b	0.32 d	0.81 c
8	932	1470	2-ethyl-3-sulfanylpropanal, 2E3SPral	plastic	nd	nd	nd	nd	nd	nd	nd	0.04
9	938	1654	3-sulfanyl-3-methylbutan-1-ol, 3S3MBol	sulfur, soup	0.04 a	0.02 a	nd	nd	nd	0.05 a	nd	nd
10	942	1521	1-sulfanylpentan-3-one, 1S3Pone	sulfur, green	0.02 a,b	0.06 a	nd	0.01 b	nd	nd	nd	nd
11	966	1454	3-sulfanylpentanal, 3SPal	hoppy, flower	0.11 c,d	0.52 b	0.22 b,c,d	0.04 d	0.01 d	1.69 a	0.36 b,c	0.16 c,d
12	989	1738	2-methyl-3-sulfanylbutan-1-ol, 2M3SBol	leek	0.08 b	0.02 c	0.02 c	nd	nd	0.16 a	nd	0.06 b,c
13	1000	1579	3-sulfanylpropyl acetate, 3SPrA	grilled	1.50 c	4.46 a	0.91 d	2.30 b	0.80 d,e	0.33 e,f	0.21 f	1.51 c
14	1008	1760	3-sulfanylpentan-1-ol, 3SPol	catty, citrus	0.03 b	0.02 b	0.04 b	nd	nd	0.26 a	0.08 b	0.03 b
15	1011	1544	3-sulfanylhexanal, 3SHal	floral, citron	0.02 a	nd	nd	nd	nd	0.05 a	nd	nd
16	1070	1617	phenylmethanethiol, BM	gunflint	2.96 d	9.89 a	6.42 b,c	2.47 d	1.78 d	8.96 a,b	7.37 a,b,c	4.53 c,d
18	1094	1858	3-sulfanylhexan-1-ol, 3SHol	grapefruit	1.34 b	4.55 a	0.50 c	0.24 c	0.49 c	1.39 b	0.15 c	0.51 c
19	1176	1962	3-sulfanylheptan-1-ol, 3SHptol	citrus	0.12 c	0.34 a	0.06 c	nd	nd	0.29 a,b	0.15 b,c	0.16 b,c

[a]Compounds were identified by coincidence with the GC−PFPD retention index of the pure compound on two capillary columns (CP-Sil5-CB and FFAP). Concentrations were determined in wine by GC−PFPD (μg/L, IST equivalents). nd = undetected. Variation coefficients were below 15%. Standard deviations have been considered in the Student−Newman−Keuls test. Values in the same row that do not share a common letter are significantly different ($p < 0.05$).

DOI: 10.1021/acs.jafc.9b05478
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

orthonasal detection thresholds of 950 and 35 ng/L (in hydroalcoholic solution) and 230 ng/L (in water), respectively].[23,24] Despite their low odor activity values, 3SPol and 3SHptol are also proposed to contribute to wine flavor through an additive effect, together with 3SHol.[23]

2-Sulfanylethyl acetate (2SEA) and 3-sulfanylpropyl acetate (3SPrA), associated with a grilled/roasted meat odor (both with an orthonasal threshold of 40 μg/L in beer), have been detected at up to 134 and 32 μg/L in Sauvignon and Semillon wines.[25,26] 2-Sulfanylethan-1-ol (2SEol, grilled/gas), ethyl 2-sulfanylpropanoate (2SPrE, fruity odor), and ethyl 3-sulfanylpropanoate (3SPrE, fruity/animal-like) have also been evidenced, especially in *Vitis labrusca* wines and Champagne [up to 2000 μg/L 2SEol, 800 ng/L 2SPrE, and 12 000 ng/L 3SPrE, with respective orthonasal thresholds of 2000 μg/L (in beer), 500 ng/L (in model wine), and 200 ng/L (in water)].[27−31]

2-Furfurylthiol (FFT, roasted coffee) and phenylmethanethiol (BM, gunflint) are other low-threshold thiols (retronasal values of 0.4 and 0.3 ng/L in hydroalcoholic solution, respectively) previously mentioned in white wines (up to 140 ng/L FFT in Champagne and up to 40 ng/L BM in Chardonnay).[32−34] Using a more oriented extraction method, Gros et al.[6] recently evidenced traces of two new thiols, 1-methyl- and 1-ethylpyrrole-2-methanethiol, as responsible for the typical hazelnut flavor of great Chardonnay wines (respective orthonasal thresholds of 0.7 and 1.4 ng/L in model wine).

The aim of this work was to identify the major polyfunctional thiols of Belgian white wines from Chardonnay and Solaris grapes. Thiol profiles of Solaris wines, even from other countries, have not been described to date. 4-Hydroxymercuribenzoic acid extraction, a method already extensively used on various wines from Bordeaux, was here followed by gas chromatography with a pulsed flame photometric detector (GC−PFPD) to investigate eight Chardonnay (five still and three sparkling) and three Solaris single-varietal wines from the harvest years 2015−2018.

■ MATERIALS AND METHODS

Chemicals. Absolute ethanol, dichloromethane, ethyl acetate, and 37% hydrochloric acid were purchased from VWR (Leuven, Belgium). Dowex resin 1 × 2 chloride form, anhydrous sodium acetate, 4-hydroxymercuribenzoic acid sodium salt (*p*HMB), >98% L-cysteine hydrochloride monohydrate, tris(hydroxymethyl)aminomethane (Tris), and 2-methyl-4-methoxy-butane-2-thiol were purchased from Sigma-Aldrich (Bornem, Belgium). Anhydrous sodium sulfate and sodium hydroxide were purchased from Acros Organics (Geel, Belgium). Milli-Q water was used (Millipore, Bedford, MA, U.S.A.). The reference standards (>97% purity) 2-sulfanylethan-1-ol, 3-sulfanylbutan-2-one, 3-sulfanylpropan-1-ol, 2-sulfanylethyl acetate, 3-sulfanyl-3-methylbutan-1-ol, 3-sulfanylpropyl acetate, 3-sulfanylpentan-1-ol, phenylmethanethiol, and 3-sulfanylhexan-1-ol were provided by Sigma-Aldrich (Bornem, Belgium). Commercially unavailable thiols were issued from combinatorial syntheses operated in our laboratory prior to this work.[35−37]

Wine Samples. A total of 11 Belgian white wines from 2 different grape varieties (Chardonnay and Solaris), 4 different harvest years (2015−2018), and 11 different Belgian vineyards were purchased in local supermarkets: Chardonnay Cuvée Sophie 2017 (Domaine des Marnières), Chardonnay Blauw 2015 (Château Genoels-Elderen), Chardonnay Bon Baron 2015 (Château Bon Baron), Chardonnay Entre-deux-Monts 2017 (Domaine Entre-deux-Monts), Chardonnay Bousval 2018 (Château de Bousval), Blanc de Blancs Brut 2016 (Domaine du Chant d'Eole), Chardonnay Meerdael Brut 2015 (Domaine de Meerdael), Ruffus Chardonnay Brut 2017 (Domaine des Agaises), Solaris Brin de Paille 2015 (Domaine du Ry d'Argent), Solaris La Foliette 2015 (Domaine du Chenoy), and Solaris Chapitre 2017 (Domaine du Chapitre).

***p*HMB Extraction of Polyfunctional Thiols from Wines.** Polyfunctional thiols were extracted from wines according to the procedure of Tominaga et al.[30] in the following steps: 300 mL of ethyl acetate liquid/liquid extraction of a 750 mL wine sample, extraction of the resulting organic phase with a *p*HMB solution, loading of the pooled aqueous phase onto a strong anion-exchanger resin, rinsing of impurities from the column with pH 6 acetate buffer, release of free thiols from *p*HMB by percolating a washed cysteine solution (4× 50 mL of dichloromethane for washing 640 mg of cysteine in 50 mL of water), final extraction with bidistilled dichloromethane, and concentration to 250 μL in a Kuderna Danish distillation apparatus and to 70 μL on a Dufton column. 2-Methyl-4-methoxy butane-2-thiol was added as an internal standard (IST, at 1.34 μg/L in the wine sample).

Quantitation of Free Thiols in the Wine Samples by GC−PFPD. A total of 1 μL of *p*HMB-free thiol extract was analyzed with a ThermoFinnigan Trace GC 2000 gas chromatograph equipped with a splitless injector maintained at 250 °C. Compounds were analyzed with a wall-coated open tubular (WCOT) apolar CP-Sil5-CB (50 m × 0.32 mm inner diameter, 1.2 μm film thickness) capillary column [and on the polar FFAP, 25 m × 0.32 mm inner diameter, 0.3 μm film thickness, for retention index (RI) checking]. The carrier gas was helium, and the pressure was set at 50 kPa. The oven temperature was programmed to rise from 36 to 85 °C at 20 °C/min, then to 145 °C at 1 °C/min, finally to 220 °C at 3 °C/min, and held for 30 min. The column was connected to the OI Analytical PFPD (model 5380, combustor internal diameter of 2 mm). The following parameters were selected for the PFPD: temperature, 220 °C; voltage, 590 V; gate width, 18 ms; gate delay, 6 ms; trigger level, 400 mV; and pulse frequency, 3.33 Hz. PFPD chromatograms were recorded throughout elution. ChemStation software was used to process the resulting data. Identifications were performed as previously described by Gros et al.[14] The following equation was used for X quantitation: concentration of X (μg/L) = concentration of IST (μg/L) × (X peak area/IST peak area) × (X molecular weight/IST molecular weight) × (IST molar response coefficient/X molar response coefficient) × (IST recovery factor/X recovery factor). The IST relative recovery factor was set at 1 (experimental values from 0.8 to 1.2, previously determined by standard addition).[14] The good equimolarity of the PFPD enabled us to set the IST relative molar response coefficients at 1.

Statistical Analyses. All wine sample analyses were carried out in duplicate. Multiple comparisons of means were performed with Student−Newman−Keuls tests.

■ RESULTS AND DISCUSSION

Evidence of Ehrlich-Derived Polyfunctional Thiols in Still and Sparkling Belgian Chardonnay Wines. As previously evidenced in Sauternes wines before aging[26] and in beers,[27] four polyfunctional thiols are usually produced in fermented beverages by the Ehrlich yeast pathway: 2SEol (**1**) and 2SEA (**5**) issued from cysteine and 3-sulfanylpropan-1-ol (**3**, 3SProl, roasted/potatoes, orthonasal threshold of 400 μg/L in beer[24]) and 3SPrA (**13**) issued from homocysteine. As expected, all four were found in the eight Belgian Chardonnay wines investigated here (panels a and b of Figure 1 and Table 1).

2-Sulfanylethyl acetate and 3SPrA have been linked by Lavigne et al.[25] to the empyreumatic/grilled flavor of some Semillon and Sauvignon blanc wines. Neither has previously been listed as a chemical constituent of Chardonnay wines. Here, they were found below their sensory thresholds. The still wine Entre-deux-Monts showed the highest concentrations of 2SEA (15.91 μg/L) and 2SEol (42.02 μg/L, similar to the level in Genoels-Elderen). The highest levels of 3SPrA (4.46 μg/L) and 3SProl (5.07 μg/L) were found in another still wine, Genoels-Elderen. Among the sparkling wines, Ruffus showed the highest concentrations of 2SEA (2.82 μg/L) and 3SPrA

DOI: 10.1021/acs.jafc.9b05478
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

(1.51 μg/L), while Chant d'Eole was richest in 2SEol (20.26 μg/L, similar to the level in Ruffus) and 3SProl (1.64 μg/L).

The absence of correlation between the 2SEol and 3SProl concentrations (R^2 = 0.3871; n = 8) (Figure 2) probably

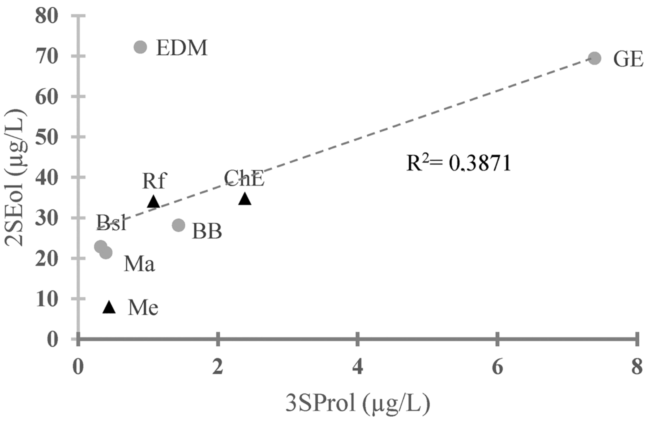

Figure 2. Correlation between the concentration values of 2-sulfanylethan-1-ol and 3-sulfanylpropan-1-ol in Chardonnay still (gray circles) and sparkling (black triangles) wines. Ma, Marnières 2017; GE, Genoels-Elderen 2015; BB, Bon Baron 2015; EDM, Entre-deux-Monts 2017; Bsl, Bousval 2018; ChE, Chant d'Ecole 2016; Me, Meerdael 2015; and Rf, Ruffus 2017.

indicates that yeast uses cysteine preferentially to homocysteine under some fermentation conditions, according to either the strain used or the must composition. Another big difference between 2SEol and 3SProl is that only the latter might also be issued from a glutathionylated precursor (derived from the addition of glutathione to propenal) or its derived cysteinyl adduct.[38]

As for other esters, the 2SEol/2SEA and 3SProl/3SPrA concentration ratios can be strongly influenced by fermentation parameters (yeast, oxygen, lipids, etc.).[2,39−41] Interestingly, however, 3SPrA outranged 3SProl in most samples (3SPrA/3SProl concentration ratio of 3.8 in Entre-Deux-Monts), while the 2SEol concentration always exceeded the 2SEA level (2SEol/2SEA concentration ratio up to 106 in Chant d'Eole) (Figure 3).

Evidence of Varietal Polyfunctional Thiols in Still and Sparkling Belgian Chardonnay Wines. As already reported for other Chardonnay wines,[3,22] 3SHol (**18**, 200−4500 ng/L) and BM (**16**, 1800−9900 ng/L) were found in all Belgian samples, well above their perception thresholds. Sulfanylakylketone 4S4M2Pone (**6**) was barely detectable in one still wine (Genoels-Elderen, 100 ng/L) and absent from all sparkling wines (Figure 3 and Table 1).

Three new, additional varietal thiols were detected, often above their respective sensory thresholds: 2-methyl-3-sulfanylpropan-1-ol (**7**, 2M3SProl, broth/leek/sweat, 300−3330 ng/L, with a retronasal threshold of 20−130 μg/L in model wine),[42] 3SHptol (**19**, 60−340 ng/L), and 3-sulfanylbutan-2-one (**2**, 3S2Bone, broth/blackcurrant, 10−40 ng/L). 2-Methyl-3-sulfanylpropan-1-ol was initially identified in Merlot and Cabernet Sauvignon red wines.[43] Surprisingly 3SHptol, evidenced here, is also proposed to be linked to the action of noble rot in Sauvignon and Semillon grapes.[44] The sulfanylalkylketone 3S2Bone was absent from all tested sparkling wines [as was 1-sulfanylpentan-3-one (**10**, 1S3Pone, green/mineral), another ketone detected only at trace levels in some still wines].

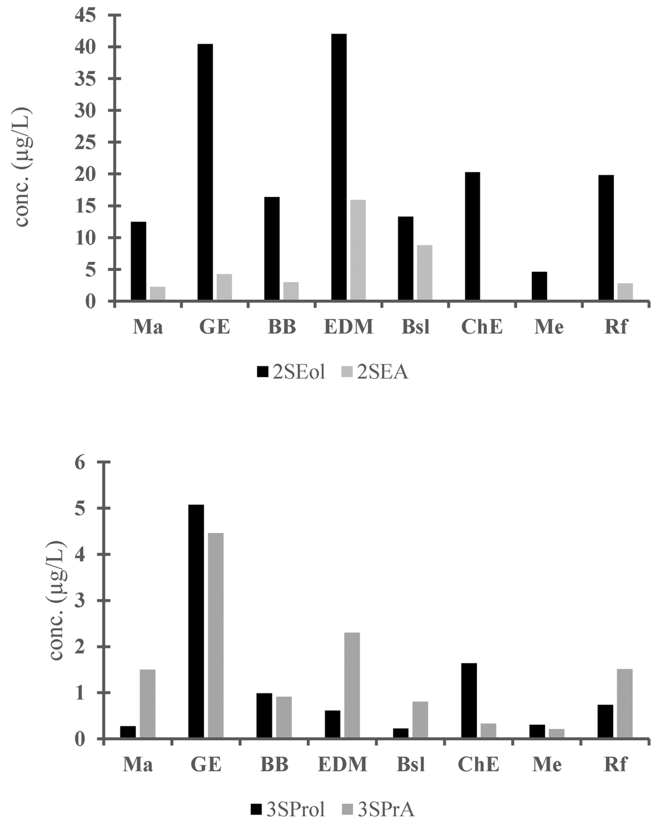

Figure 3. Concentrations (μg/L) of 2-sulfanylethan-1-ol, 3-sulfanylpropan-1-ol, and their derived esters in Belgian Chardonnay wines. Ma, Marnières 2017; GE, Genoels-Elderen 2015; BB, Bon Baron 2015; EDM, Entre-deux-Monts 2017; Bsl, Bousval 2018; ChE, Chant d'Ecole 2016; Me, Meerdael 2015; and Rf, Ruffus 2017.

Additional interesting polyfunctional thiols liable to influence the flavor of Belgian Chardonnay wines include the unknown at RI = 868 (**4**, 0.2−2.0 μg/L) and 3-sulfanylpentanal (**11**, 3SPal, flowery/hoppy, 0.01−1.7 μg/L), never investigated in wine. While 3SHol (0.2−4.5 μg/L) was always present at a higher concentration than 3-sulfanylhexanal (**15**, 3SHal, <0.02 μg/L, flowery), 3SPal was found at a higher concentration than 3SPol (**14**, <0.04 μg/L). Among the eight Belgian Chardonnay wines investigated, the still wine Genoels-Elderen emerged as the richest in most varietal thiols (3S2Bone, 4S4M2Pone, 2M3SProl, 3SHol, and 3SHptol; Figure 4). The sparkling wine Chant d'Eole, however, proved much richer in 3SPal and BM. The latter was probably issued from another aldehyde (addition of hydrogen sulfide to benzaldehyde) (Figure 4).[12,30]

Finally, this work has evidenced 3S3MBol (**9**, 20−50 ng/L) and 2M3SBol (**12**, 20−160 ng/L) for the first time in Chardonnay wines. On the other hand, 1-methyl- and 1-ethylpyrrole-2-methanethiol, 3SHA, and FFT were not detected in the *p*HMB extracts (detection limit of around 10 ng/L). Their impact on Belgian Chardonnay wine aroma needs to be investigated with even more specific methods.

Comparison to Polyfunctional Thiols Found in Belgian Solaris Wines. As in the tested Chardonnay still wines, the four Ehrlich-derived thiols and the varietal thiols 3SHol (0.1−0.4 μg/L), BM (3.1−4.8 μg/L), 4S4M2Pone (0.01−0.1 μg/L), 2M3SProl (0.1−9.9 μg/L), 3SHptol (0.04−0.07 μg/L), 3S2Bone (0.07 μg/L), 3SPal (0.1−0.4 μg/L), and RI = 868 (0.3−1.5 μg/L) were found in the Belgian Solaris wines (Figure 1c and Table 2). Our method also failed to detect any FFT,

DOI: 10.1021/acs.jafc.9b05478
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

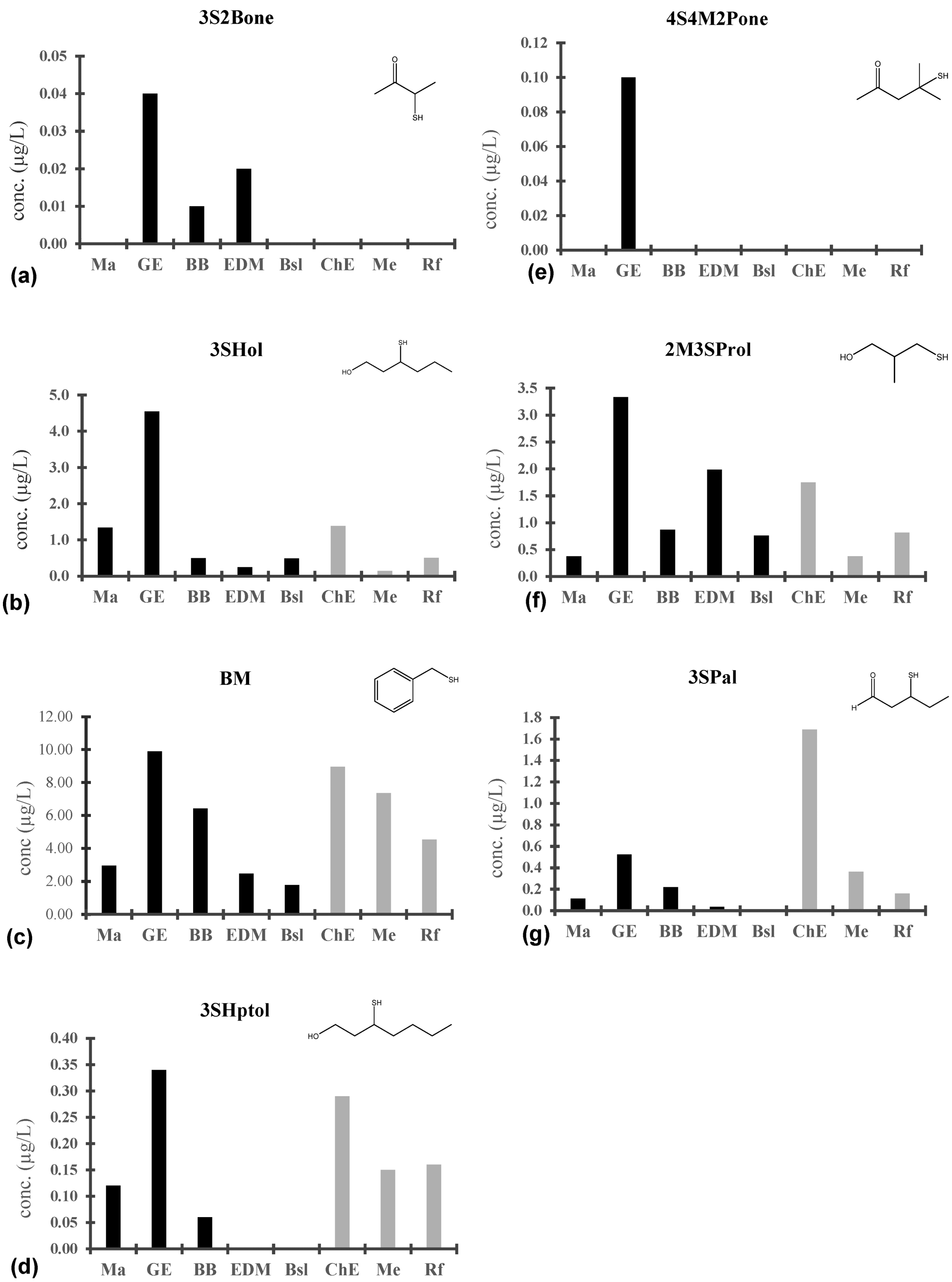

Figure 4. Concentrations (μg/L) of (a) 3-sulfanylbutan-2-one, (b) 3-sulfanylhexan-1-ol, (c) phenylmethanethiol, (d) 3-sulfanylheptan-1-ol, (e) 4-sulfanyl-4-methylpentan-2-one, (f) 2-methyl-3-sulfanylpropan-1-ol, and (g) 3-sulfanylpentanal in Belgian (black bars) still and (gray bars) sparkling Chardonnay wines. Ma, Marnières 2017; GE, Genoels-Elderen 2015; BB, Bon Baron 2015; EDM, Entre-deux-Monts 2017; Bsl, Bousval 2018; ChE, Chant d'Ecole 2016; Me, Meerdael 2015; and Rf, Ruffus 2017.

3SHA, 1-methylpyrrole-2-methanethiol, or 1-ethylpyrrole-2-methanethiol in them.

A few minor thiols distinguished the Chardonnay samples: the alcohols 3S3MBol, 2M3SBol, and 3SPol and the carbonyls 2-ethyl-3-sulfanylpropanal (**8**, 2E3SPral), 1S3Pone, and 3SHal. On the other hand, 2-methyl-3-sulfanylpentan-1-ol (**17**, 2M3SPol, saucy odor, 0.03 μg/L) was found in only one wine made from Solaris grapes (Ry d'Argent).

In conclusion, the present work confirms the occurrence of several polyfunctional thiols, at concentrations above their thresholds, in Belgian white wines from both Solaris and Chardonnay grapes. Complementary research should focus on

DOI: 10.1021/acs.jafc.9b05478
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

Table 2. Concentrations (μg/L) of Polyfunctional Thiols in Belgian Solaris Wines, Determined by GC−PFPD[a]

	RI						
	CP-Sil5	FFAP	name and symbol	aroma descriptor(s)	Ry d'Argent	Chenoy	Chapitre
1	722	1538	2-sulfanylethan-1-ol, 2SEol	grilled, gas	11.67 c	94.53 a	24.55 b
2	771	1295	3-sulfanylbutan-2-one, 3S2Bone	leek, cassis	nd	0.07	nd
3	849	1620	3-sulfanylpropan-1-ol, 3SProl	potatoes, popcorn	0.33 b	8.43 a	0.55 b
4	868		unknown		0.31 b	0.40 b	1.55 a
5	880	1454	2-sulfanylethyl acetate, 2SEA	burnt, grill	0.32 b	7.49 a	0.62 b
6	897	1547	4-sulfanyl-4-methylpentan-2-one, 4S4M2Pone	citrus, catty	0.01 b	nd	0.11 a
7	922	1677	2-methyl-3-sulfanylpropan-1-ol, 2M3SProl	broth, leek	0.09 b	9.86 a	0.11 b
11	966	1454	3-sulfanylpentanal, 3SPal	hoppy, flower	0.09 b	0.44 a	0.11 b
13	1000	1579	3-sulfanylpropyl acetate, 3SPrA	grilled	0.37 b	3.06 a	0.37 b
16	1070	1617	phenylmethanethiol, BM	gunflint	3.11 b	4.83 a	3.41 b
17	1071	1822	2-methyl-3-sulfanylpentan-1-ol, 2M3SPol	saucy	0.03	nd	nd
18	1094	1858	3-sulfanylhexan-1-ol, 3SHol	grapefruit	0.40 a	0.13 b	0.43 a
19	1176	1962	3-sulfanylheptan-1-ol, 3SHptol	citrus	0.07 a	0.06 a	0.04 a

[a]Compounds were identified by coincidence with the GC−PFPD retention index of the pure compound on two capillary columns (CP-Sil5-CB and FFAP). Concentrations in wine (μg/L, IST equivalents) were determined by GC−PFPD. nd = undetected. Variation coefficients were below 15%. Standard deviations were considered in the Student−Newman−Keuls test. Values in the same row that do not share a common letter are significantly different ($p < 0.05$).

the impact of soil, harvest year, and fermentation conditions on concentrations of these compounds in Belgian white wines.

AUTHOR INFORMATION

Corresponding Author

*Fax: +32-10-47-21-78. E-mail: sonia.collin@uclouvain.be.

ORCID

Cécile Chenot: 0000-0003-4071-2523

Sonia Collin: 0000-0001-6574-8237

Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

IST, internal standard; *p*HMB, 4-hydroxymercuribenzoic acid sodium salt; PFPD, pulsed flame photometric detector

REFERENCES

(1) SPF Economie, P.M.E., Classes Moyennes et Energie. *La Viticulture Belge en Chiffres: Récolte et Agréments en 2017*; SPF Economie, P.M.E., Classes Moyennes et Energie: Brussels, Belgium, 2018; pp 34.

(2) Gambetta, J. M.; Bastian, S. E. P.; Cozzolino, D.; Jeffery, D. W. Factors Influencing the Aroma Composition of Chardonnay Wines. *J. Agric. Food Chem.* **2014**, *62*, 6512−6534.

(3) Capone, D. L.; Barker, A.; Williamson, P. O.; Francis, I. L. The Role of Potent Thiols in Chardonnay Wine Aroma. *Aust. J. Grape Wine Res.* **2018**, *24* (1), 38−50.

(4) Anderson, K.; Aryal, N. R. *Which Winegrape Varieties are Grown Where?: A Global Empirical Picture*. University of Adelaide Press: Adelaide, Australia, 2013; pp 690.

(5) Simpson, R. F.; Miller, G. C. Aroma Composition of Chardonnay Wine. *Vitis* **1984**, *23* (2), 143−158.

(6) Gros, J.; Lavigne, V.; Thibaud, F.; Gammacurta, M.; Moine, V.; Dubourdieu, D.; Darriet, P.; Marchal, A. Toward a Molecular Understanding of the Typicality of Chardonnay Wines: Identification of Powerful Aromatic Compounds Reminiscent of Hazelnut. *J. Agric. Food Chem.* **2017**, *65*, 1058−1069.

(7) Saliba, A. J.; Heymann, H.; Blackman, J. W.; MacDonald, J. B. Consumer-Sensory Evaluation of Australian Chardonnay. *Wine Vitic. J.* **2013**, *28* (3), 64.

(8) Ehrhardt, C.; Arapitsas, P.; Stefanini, M.; Flick, G.; Mattivi, F. Analysis of the Phenolic Composition of Fungus Resistant Grape varieties Cultivated in Italy and Germany Using UHPLC-MS/MS. *J. Mass Spectrom.* **2014**, *49*, 860−869.

(9) Zhang, S.; Petersen, M. A.; Liu, J.; Toldam-Andersen, T. B. Influence of Pre-Fermentation Treatments on Wine Volatile and Sensory Profile of the New Disease Tolerant Cultivar Solaris. *Molecules* **2015**, *20*, 21609−21625.

(10) Boso, S.; Kassemeyer, H. H. Different Susceptibility of European Grapevine Cultivars for Downy Mildew. *Vitis* **2008**, *47* (1), 39−49.

(11) Liu, J.; Toldam-Andersen, T. B.; Petersen, M. A.; Zhang, S.; Arneborg, N.; Bredie, W. L. Instrumental and Sensory Characterisation of Solaris White Wines in Denmark. *Food Chem.* **2015**, *166*, 133−142.

(12) Roland, A.; Schneider, R.; Razungles, A.; Cavelier, F. Varietal Thiols in Wine: Discovery, Analysis and Applications. *Chem. Rev.* **2011**, *111*, 7355−7376.

(13) Tominaga, T.; Furrer, A.; Henry, R.; Dubourdieu, D. Identification of New Volatile Thiols in the Aroma of *Vitis vinifera* L. var. Sauvignon Blanc Wines. *Flavour Fragrance J.* **1998**, *13*, 159−162.

(14) Gros, J.; Nizet, S.; Collin, S. Occurrence of Odorant Polyfunctional Thiols in the Super Alpha Tomahawk Hop Cultivar. Comparison with the Thiol-rich Nelson Sauvin Bitter Variety. *J. Agric. Food Chem.* **2011**, *59*, 8853−8865.

(15) Gros, J.; Peeters, F.; Collin, S. Occurrence of Odorant Polyfunctional Thiols in Beers Hopped with Different Cultivars. First Evidence of an *S*-Cysteine Conjugate in Hop (*Humulus lupulus* L.). *J. Agric. Food Chem.* **2012**, *60*, 7805−7816.

(16) Kankolongo Cibaka, M. L.; Decourrière, L.; Lorenzo-Alonso, C. J.; Bodart, E.; Robiette, R.; Collin, S. 3-Sulfanyl-4-methylpentan-1-ol in Dry-Hopped Beers: First Evidence of Glutathione S-Conjugates in Hop (*Humulus lupulus* L.). *J. Agric. Food Chem.* **2016**, *64* (45), 8572−8582.

(17) Chenot, C.; Robiette, R.; Collin, S. First Evidence of the Cysteine and Glutathione Conjugates of 3-Sulfanylpentan-1-Ol in Hop (*Humulus Lupulus L.*). *J. Agric. Food Chem.* **2019**, *67* (14), 4002−4010.

(18) Darriet, P.; Tominaga, T.; Lavigne, V.; Boidron, J.-N.; Dubourdieu, J. Identification of a Powerful Aromatic Component of *Vitis vinifera* L. var. Sauvignon Wines: 4-Mercapto-4-methylpentan-2-one. *Flavour Fragrance J.* **1995**, *10*, 385−392.

(19) Tominaga, T.; Murat, M.-L.; Dubourdieu, D. Development of a Method for Analyzing the Volatile Thiols Involved in the Characteristic Aroma of Wines Made from *Vitis vinifera* L. Cv. Sauvignon Blanc. *J. Agric. Food Chem.* **1998**, *46*, 1044−1048.

(20) Tominaga, T.; Baltenweck-Guyot, R.; Peyrot des Gachons, C.; Dubourdieu, D. Contribution of Volatile Thiols to the Aromas of White Wines Made From Several Vitis vinifera Grape Varietes. *Am. J. Enol. Vitic.* **2000**, *51*, 178−181.

DOI: 10.1021/acs.jafc.9b05478
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

(21) Rodríguez-Bencomo, J. J.; Schneider, R.; Lepoutre, J. P.; Rigou, P. Improved Method to Quantitatively Determine Powerful Odorant Volatile Thiols in Wine by Headspace Solid-Phase Microextraction after Derivatization. *J. Chromatogr. A* **2009**, *1216*, 5640−5646.

(22) Mateo-Vivaracho, L.; Zapata, J.; Cacho, J.; Ferreira, V. Analysis, Occurrence, and Potential Sensory Significance of Five Polyfunctional Mercaptans in White Wines. *J. Agric. Food Chem.* **2010**, *58*, 10184−10194.

(23) Sarrazin, E.; Shinkaruk, S.; Tominaga, T.; Bennetau, B.; Frérot, E.; Dubourdieu, D. Odorous Impact of Volatile Thiols on the Aroma of Young Botrytized Sweet Wines: Identification and Quantification of New Sulfanyl Alcohols. *J. Agric. Food Chem.* **2007**, *55*, 1437−1444.

(24) Widder, S.; Dittner, T.; Sabater-Lüntzel, C.; Pickenhagen, W.; Vollhardt, J. 3-Mercapto-2-alkyl-alkan-1-ols and Their Use as Perfuming and Flavouring Agents. European Patent EP0924198, June 23, 1999.

(25) Lavigne, V.; Henry, R.; Dubourdieu, D. Identification et Dosage de Composés Soufrés Intervenant Dans l'Arôme Grillé des Vins. *Sci. Alim.* **1998**, *18*, 175−191.

(26) Bailly, S.; Jerkovic, V.; Meurée, A.; Timmermans, A.; Collin, S. Fate of Key Odorants in Sauternes Wines through Aging. *J. Agric. Food Chem.* **2009**, *57* (18), 8557−8563.

(27) Tran, T. T. H.; Gros, J.; Bailly, S.; Nizet, S.; Collin, S. Fate of 2-Sulfanylethyl Acetate and 3-Sulfanylpropyl Acetate Through Beer Aging. *J. Inst. Brew.* **2012**, *118* (2), 198−204.

(28) Anocibar Beloqui, A.; Bertrand, A. Study of Sulfur Compounds in Wine. Preliminary Results. *J. Food Sci.* **1995**, *3*, 279.

(29) Blanchard, L. Recherche sur la Contribution de Certains Thiols Volatils à l'Arôme des Vins Rouges. Etude de leur Genèse et de leur Stabilité. Ph.D. Thesis, Université Bordeaux 2, Bordeaux, France, 2000.

(30) Tominaga, T.; Guimbertau, G.; Dubourdieu, D. Role of Certain Volatile Thiols in the Bouquet of Aged Champagne Wines. *J. Agric. Food Chem.* **2003**, *51* (4), 1016−1020.

(31) Kolor, M. G. Identification of an Important New Flavor Compound in Concord Grape: Ethyl-3-mercaptopropionate. *J. Agric. Food Chem.* **1983**, *31* (5), 1125−1127.

(32) Tominaga, T.; Guimbertau, G.; Dubourdieu, D. Contribution of Benzenemethanethiol to Smoky Aroma of Certain *Vitis vinifera* L. Wines. *J. Agric. Food Chem.* **2003**, *51*, 1373−1376.

(33) Tominaga, T.; Blanchard, L.; Darriet, P.; Dubourdieu, D. A Powerful Aromatic Volatile Thiol, 2-Furanmethanethiol, Exhibiting Roast Coffee Aroma in Wines Made from Several *Vitis vinifera* Grape Varieties. *J. Agric. Food Chem.* **2000**, *48*, 1799−1802.

(34) Tominaga, T.; Dubourdieu, D. A Novel Method for Quantification of 2-Methyl-3-furanthiol and 2-Furanmethanethiol in Wines Made from *Vitis vinifera* Grape Varieties. *J. Agric. Food Chem.* **2006**, *54*, 29−33.

(35) Vermeulen, C.; Pellaud, J.; Gijs, L.; Collin, S. Combinatorial Synthesis and Sensorial Properties of Polyfunctional Thiols. *J. Agric. Food Chem.* **2001**, *49* (11), 5445−5449.

(36) Vermeulen, C.; Collin, S. Synthesis and Sensorial Properties of Mercaptoaldehydes. *J. Agric. Food Chem.* **2002**, *50* (20), 5654−5659.

(37) Vermeulen, C.; Guyot-Declerck, C.; Collin, S. Combinatorial Synthesis and Sensorial Properties of Mercapto Primary Alcohols and Analogues. *J. Agric. Food Chem.* **2003**, *51* (12), 3623−3628.

(38) Gros, J.; Tran, T. T. H.; Collin, S. Enzymatic Release of Odourant Polyfunctional Thiols from Cysteine Conjugates in Hop. *J. Inst. Brew.* **2013**, *119* (4), 221−227.

(39) Masneuf-Pomarède, I.; Mansour, C.; Murat, M.-L.; Tominaga, T.; Dubourdieu, D. Influence of Fermentation Temperature on Volatile Thiols Concentrations in Sauvignon Blanc Wines. *Int. J. Food Microbiol.* **2006**, *108* (3), 385−390.

(40) Deed, R. C.; Fedrizzi, B.; Gardner, R. C. Influence of Fermentation Temperature, Yeast Strain, and Grape Juice on the Aroma Chemistry and Sensory Profile of Sauvignon Blanc Wines. *J. Agric. Food Chem.* **2017**, *65* (40), 8902−8912.

(41) Saerens, S. M. G.; Delvaux, F.; Verstrepen, K. J.; Van Dijck, P.; Thevelein, J. M.; Delvaux, F. R. Parameters Affecting Ethyl Ester Production by *Saccharomyces cerevisiae* during Fermentation. *Appl. Environ. Microbiol.* **2008**, *74* (2), 454.

(42) Bouchilloux, P.; Darriet, P.; Dubourdieu, D.; Henry, R.; Reichert, S.; Mosandl, A. Stereodifferentiation of 3-Mercapto-2-Methylpropanol in Wine. *Eur. Food Res. Technol.* **2000**, *210* (5), 349−352.

(43) Bouchilloux, P.; Darriet, P.; Henry, R.; Lavigne-Cruège, V.; Dubourdieu, D. Identification of Volatile and Powerful Odorous Thiols in Bordeaux Red Wine Varieties. *J. Agric. Food Chem.* **1998**, *46*, 3095−3099.

(44) Thibon, C.; Shinkaruk, S.; Jourdes, M.; Bennetau, B.; Dubourdieu, D.; Tominaga, T. Aromatic Potential of Botrytized White Wine Grapes: Identification and Quantification of new Cysteine-S-Conjugate Flavor Precursors. *Anal. Chim. Acta* **2010**, *660* (1−2), 190−196.

DOI: 10.1021/acs.jafc.9b05478
J. Agric. Food Chem. XXXX, XXX, XXX−XXX