Supporting Information for
https://doi.org/10.1021/acs.jafc.2c00243

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Qualitative Screening of Volatile Thiols in Wine by Selective Silver Ion Solid-Phase Extraction with Heart-Cutting Multidimensional Gas Chromatography Mass Spectrometry/Olfactometry

Liang Chen* and Philippe Darriet

Université de Bordeaux, INRAE, Bordeaux INP, UMR1366 Œnologie, ISVV, F-33140 Villenave d'Ornon, France

Table of Content:

Section I. LLE and pHPLC for isolating thiol-related odors in a red wine

Sensory profiling of W1. One red wine from Bordeaux region (W1) was freshly open and an aliquot of 30 mL was decanted to a standard wine glass; only for orthonasal evaluation. Treated wine was derived from adding Cu^{2+} (final concentration 50 mg/L, as the form of $CuSO_4$) to the original wine; 30 mL presented in standard wine glass; only for orthonasal evaluation.

***Sample preparation*. Liquid-liquid extraction (LLE)** An aliquot of original wine sample W1 (300 mL) was extracted with DCM (15 mL, 15 mL, 15 min × 3) for three time. Organic phases were separated, pooled, dried over anhydrous Na_2SO_4, and concentrated under N_2 to 300 µL.

pHPLC fractionation 250 µL of concentrated LLE extract was fractionated by pHPLC. A total of 50 fractions were collected as 1 mL solution in 2 mL HPLC vails. All fractions were subjected to a sensory panel and the results are shown in Table S1.

Discussion. Before any attempt to develop a new method for thiol extraction was made, the analytical performances of some existing extraction methods were assessed. Odor screening for the prepared extracts was conducted by GC–O and a sensory panel. One red wine (W1) from Bordeaux region was selected for its intense bouquet of thiol-related aromas (such aroma disappeared after the supplementation of 50 mg/L $CuSO_4$ to wine matrix, evaluated by a sensory panel of 5 participants). One bottle (750 mL) of W1 was extracted by nonselective LLE and analyzed by GC–O. The GC–O results (two judges, duplicate sessions, on both a DB-Wax and a DB-5 column) showed the usual and persistent odor regions (e.g., volatile acetates, esters, and higher alcohols etc.), but no distinctive thiol odors were noticed (data not shown). The masking of the odors of interest by other major co-eluting wine volatile compounds during GC–O was well recognized when only LLE was used for sample preparation.[1] The LLE extract was fractionated by pHPLC to aqueous alcoholic subfractions (n=50). The subfractions were assessed by a sensory panel (n=4). This sensory-guided approach has been successful for locating the odors of interest in wine matrix.[2–4] In the current study, however, no typical thiol-like aromas were clearly found in the subfractions (**Table S1**). The sensory results for some subfractions (e.g., fraction 40: "banana, fruity") were very similar to previously reported data for other wine varieties using the same approach[3] indicating good reliability of pHPLC fractionation technique. The incapability of pHPLC fractionation to reveal odor of interests could be the results of low concentration of potential thiols in the wine matrices, the loss of such analytes during pHPLC run, or the masking effects in the subfractions. Since neither traditional LLE nor pHPLC approach was successful for screening thiol related odors, the necessity for developing an alternative sample preparation method became evidently clear.

Section II. Testing a previously reported Ag^+ SPE procedure

A previously reported Ag^+ SPE procedure[5] was tested for its suitability for qualitative screening experiment. **Figure S1** illustrates the SPE steps.

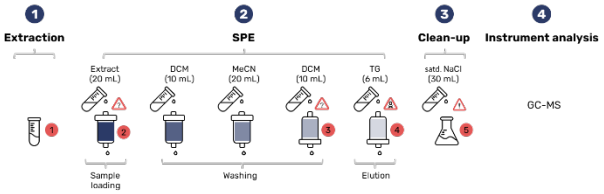

FIGURE S1. Overview of the extraction steps used for experiment in this section.

***Sample preparation* LLE** An aliquot of 25 mL bag-in-box red wine (W0) was extracted with 5 mL, 2.5 mL, 2.5 mL of DCM (15 min × 3). The combined organic extracts were dried with anhydrous Na_2SO_4 and concentrated under N_2 to 500 µL. This extract was analyzed by GC–MS and the total ion chromatogram (TIC) is shown in **Figure S2**.

Table S1. Odor descriptors of pHPLC subfractions of an LLE extract from a red wine (W1)

subfraction	Participant 1	Participant 2	Participant 3	Participant 4
1	spirit (bourbon, cognac), vanilla	/	/	/
2	spirit (bourbon, cognac), vanilla	fermentative, fruity	fruity	/
3	/	/	moisty	/
4	/	fermentative, fruity	/	/
5	acetate	/	/	/
6	/	/	moisty	/
7	/	/	/	/
8	/	/	/	/
9	/	fruity	fruity, butter	/
10	glue	acetate	acetic acid, vinegar	/
11	vinegar	/	cooked butter	/
12	yogurt	buttery	butter	/
13	lactic acid	buttery	butter	fatty
14	lactic acid	buttery	butter	fatty
15	lactic acid	buttery	yogurt	cream
16	lactic acid, buttery	spicy	caramel, strawberry	/
17	buttery	/	vinegar	/
18	truffle	/	potatoes	spicy, smoke
19	truffle	/	cheese, yogurt, rancid	/
20	truffle	/	cheese, yogurt, rancid	/
21	cheese	/	cheese, rancid	/
22	blue cheese	fermentative	fruity, yogurt	/
23	solvent	fermentative	apple	/
24	solvent	fermentative	apple	flowers, cream
25	bakery, biscuits	/	chocolate, coffee	/
26	cooked sugar	sugar	chocolate, cake	smoke
27	cooked sugar	/	coffee, fruity	/
28	olive	leather, animal	savory, salty	floral
29	olive, woody, amber	/	rancid, floral, moisty	garlic
30	blue cheese, herbal notes	alcohol	salty, floral	butter
31	fresh herbs	rose	white flowers	rose
32	solvent	fermentative	floral, leather	/
33	musky, animal	solvent	orange blossom	/
34	fruity	solvent	garlic	/
35	fruity	fruity, spicy	plastic	/
36	fruity	/	fruity, sea water	citrus
37	woody	whiskey, coconut	coconut, horse	spicy
38	orange	fruity, oaky	fruity, fatty	candy
39	fruity	lactones	peach, lactones	/
40	honeysuckle	lactones	smokey	candy
41	fruity, candy	fermentative acetates	banana	strawberry candy
42	graphite	/	garlic	fruity
43	/	menthol like	pencil like, wood	/
44	fruity	menthol like	citrus, fresh	/
45	petrol	floral	paper, carboard	/
46	/	floral	anis, minty	/
47	solvent	floral	/	/
48	/	fruity	paper	/
49	/	fruity	paper	/
50	/	/	/	/

/: no odor perceived.

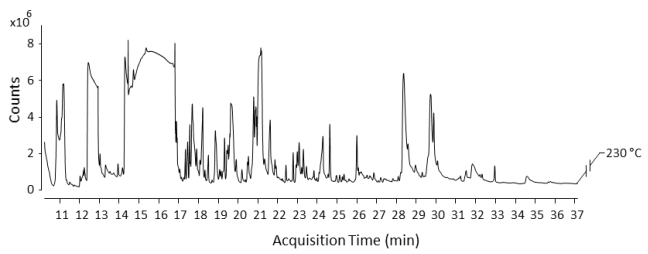

FIGURE S2. GC–EI–MS TIC of a concentrated LLE extract of a bag-in-box red wine. In order to avoid the saturations of the MSD, LLE extract was not concentrated with N_2. The TIC of nonconcentrated LLE extract is given in **Figure 1a-1** in the main manuscript.

Ag^+ SPE was performed according to a previously reported procedure[5] with some modifications.

The SPE was conducted with a SPE manifold due to the incompatibility between the cartridge format and the standard rack system of the automated SPE instrument (**Figure S3**).

After sample loading, the elute was collected and analyzed by GC–MS. During washing step, the elutes were collected. The reverse of the cartridge was operated by adopting a male-to-male luer. The elute from the last washing was collected. All elutes collected from washing steps were concentrated under N_2 to around 500 µL and analyzed by GC–MS. The results are shown in **Figure 1a** in the main manuscript.

GC–MS instrumentation The analyses were performed using an Agilent Intuvo 9000 GC coupled with an Agilent

5977B MSD (Agilent Technologies). Instrument parameters are given in **Table S2**.

Experiment details on procedural blanks. Re-distilled DCM was used as sample. Ag^+ SPE: - condition 10 mL of DCM; - loading 60 mL of DCM; - washing 10 mL of DCM, 20 mL of MeCN, and 10 mL od DCM; - elution for (1) 6 mL of 10 g/L 1-thioglycerol in DCM, for (2) 6 mL of 10 g/L DTT in DCM, for (3) 5 mL of H_2O, 20 mL of 10 g/L aq. L-cysteine, 10 mL of DCM; Clean-up for (1) LLE with 20 mL of saturated brine for 15 min at 900 rpm, for (2) SAFE twice. SAFE system was obtained from Glasbläserei Bahr (Manching, Germany). The vacuum (0.001 mBar) was maintained by a Pfeiffer Duo 2.5 pump and was monitored by a vacuum meter (Vacuu Brand, Wertheim, Germany). Water bath was kept at 40 °C, for (3) LLE by itself for 15 min at 900 rpm. Collected organic phases from clean-up step were dried over anhydrous Na_2SO4, concentrated to 100 µL under nitrogen, and stored at –20°C pending analysis. GC–MS instrumentation and parameters were the same as those reported in **Table S2**.

Discussion. The automation of the SPE was only possible using a customized SPE rack system (**Figure S3**). Moreover, the SPE automation process had to be disrupted to allow the cartridge to be manually reversed (**Figure S3**). The eluting reagent (1-thioglycerol) has acute toxicity[6] and was present at 10–15 g/L in the final extract, which raised potential safety concerns for it to be subsequently assessed by GC–O. In addition, large numbers of odorous artifacts at significant abundances were observed in the final extract (Figure 1b-1, Figure S4-1). Such artifacts may be considered irrelevant for selected reaction monitoring (SRM) mode in MS/MS quantitation. However, they are deemed to be detrimental for qualitative studies that usually rely on MS full scan and olfactometry detection, for which any interfering peaks/odors should be kept at a minimum level.[7] Optimizing the LLE clean-up of 1-thioglycerol step (using pure H_2O, different LLE duration, different volume of brine, or sourcing high purity 1-thioglycerol from other suppliers) did not attenuate the persistent artifacts (data not shown). Moreover, the overall sample preparation was still lengthy and labor intensive.

Table S2. GC–MS parameters

Parameter	Values
Injection volume	0.5 µL
Injection mode	Splitless
Inlet temperature	240 °C
Carrier gas	He
Constant flow	1.0 mL/min
Column	HP-5MS UI (30 m × 250 µm × 0.25 µm)
Oven program	45 °C (1 min) > 5 °C/min > 230 °C (15 min)
Guard chip	791 mm × 0.547 mm
Guard chip	Track oven
Bus temperature	230 °C
MSD connector	230 °C
MSD Transfer line	250 °C
MS source	230 °C
MS quad	150 °C
MS acquisition	Scan 35 – 350 *m/z*
Parameter	Values
Injection volume	0.5 µL
Injection mode	Splitless
Inlet temperature	240 °C
Carrier gas	He
Constant flow	1.0 mL/min

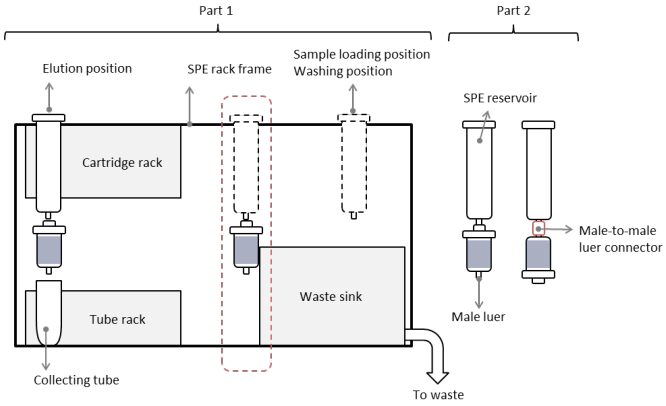

FIGURE S3. Schematic diagram illustrating the SPE automation system and two issues noticed during our trial. Part 1 shows the standard racking system and waste sink blocking the movement of the mini-compact type cartridge in use with a standard 6 mL SPE reservoir. Part 2 shows the operation of manually reversing the cartridge via a male-to-male luer connector.

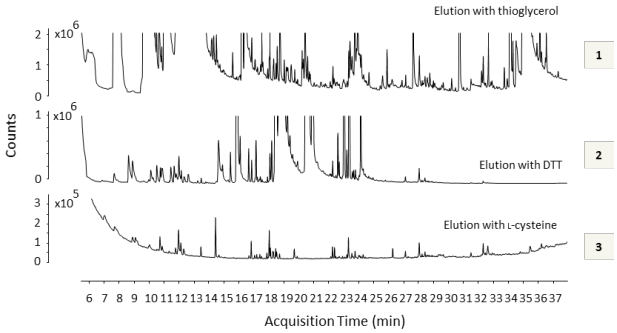

FIGURE S4. Comparison of the TICs of three procedural blank samples that were obtained using 1-thioglycerol (1), DTT (2), and L-cysteine (3). Note the differences in the scale of y-axis.

Section III. Ag^+ SPE method development

Ag^+ SPE cartridge screening Commercial Ag^+ SPE cartridges from four different suppliers were obtained and tested for their selectivity towards thiols. All Ag^+ SPE cartridges were conditioned with DCM (10 mL) and were loaded with DCM (20 mL) spiked with six known thiol standards (final concentration ~1 mg/L), followed by washing (with 10 mL of DCM, 20 mL of MeCN, 10 mL of DCM) and elution (with 6 mL of 10 g/L 1-thioglycerol). Elutes from washing and elution were collected. A small volume (5 µL) of final elutes was transferred to a clean cellulose smelling strip and subjected to olfactory evaluation for the presence and/or intensity of thiol-related odors. Strongest thiol odors were noted in the final elutes only when MetaSep IC-Ag was used (**Table S4**). The sensory data indicated that MetaSep IC-Ag performed better than other cartridges in current experiment setting. GC–MS analysis of the elutes (**Table S5**) confirmed the sensory results. Therefore, MetaSep IC-Ag was selected for the development of the Ag^+ SPE extraction protocol.

Cartridge wettability SPE cartridges need to be conditioned before use. Water was unable to penetrate the cartridges. By comparison, DCM went through cartridges freely just under gravity. This result was in line with previous reports.[5,12] After the cartridge being conditioned with DCM, wine sample was still unable to infiltrate the cartridge. It appeared that loading aqueous samples onto Ag^+ SPE cartridge was infeasible which was consistent with the previous observation.[5] Therefore, LLE had to be applied as a necessary pre-treatment for wine sam-

ple. However, during the later stage of our method development, we demonstrated that switching Ag^+ SPE from normal phase mode to reversed phase mode was not only operable but also crucial for a successful extraction.

SPE reservoirs and housings The format of the selected Ag^+ SPE cartridge is compacted mini cartridge (**Figure S5**). Therefore, the use of a SPE reservoir was required. Initially, polypropylene SPE reservoir was used. However, many impurities that were originated from polypropylene SPE reservoir were detected in the elutes. The negative impact of unsuitable Ag^+ SPE cartridge tube materials and their leachable impurities on the trace analysis of fatty acids have been demonstrated previously.[12] With similar observations that were seen in our application, it was evident that using SPE reservoir made in glass was essential. No leachable impurities were detected thereafter, even when relatively large volumes of samples/organic solvents (~200 mL) were in contact with the glass reservoirs (data not shown). This is of great importance given that the expected detection signals of ultra-trace (ng/L) analytes are at low abundance and can be easily interfered by impurities even at low concentrations. Same consideration was given to the materials of SPE housings, which could be another source of leachable impurities. The SPE housing was also made of polypropylene, but the mini compact cartridge type only had very small surface area which led to much less noticeable impurities during the extraction process. Therefore, no adjustments were made on the SPE housing materials. Regardless, a simple modification method for changing Ag^+ SPE housing is given in **Figure S5**, should glass-only extraction environment be required for future applications.

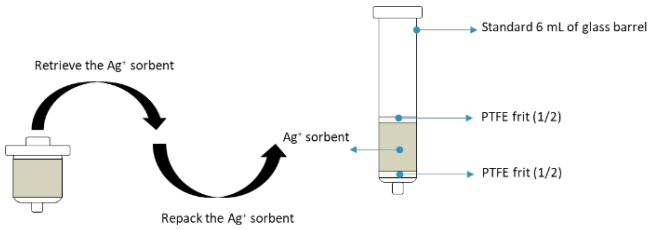

FIGURE S5. Schematic diagram illustrating the process of retrieving the Ag^+ sorbent from the original mini cartridge and repacking it in a glass barrel with two ends fitted with polytetrafluoroethylene (PTFE) frits.

Testing DTT as elution reagent for Ag^+ SPE **LLE** was performed for a bag-in-box red wine with the protocol mentioned in Materials and Methods. The pooled LLE extracts were used for spiking experiment. **Spiking experiment**: An aliquot of 10 mg/L of thiol standard solution was spiked into wine LLE extract to final concentration of 100 µg/L. **Ag^+ SPE** was performed according to the same procedure as that in **Section IV**, with the following modifications: 20 mL spiked LLE extract was used for sample loading, four concentrations of DTT was used for elution. **SAFE**: SAFE apparatus and parameters were the same as those in **Section IV**. **GC–MS**: GC–MS instrumentation and parameters were the same as those reported in **Table S2**.

Discussion

Ag^+ SPE cartridge selection

Commercial Ag^+ SPE cartridges from four different suppliers were obtained and tested for their selectivity towards thiols. All Ag^+ SPE cartridges were conditioned with DCM (10 mL) and were loaded with DCM (20 mL) spiked with six known thiol standards (final concentration ~1 mg/L), followed by washing (with 10 mL of DCM, 20 mL of MeCN, 10 mL of DCM) and elution (with 6 mL of 10 g/L 1-thioglycerol). Elutes from washing and elution were collected. A small volume (5 µL) of final elutes was transferred to a clean cellulose smelling strip and subjected to olfactory evaluation for the presence and/or intensity of thiol-related odors. Strongest thiol odors were noted in the final elutes only when MetaSep IC-Ag was used (**Table S4**). The sensory data indicated that MetaSep IC-Ag performed better than other cartridges in current experiment setting. GC–MS analysis of the elutes (**Table S5**) confirmed the sensory results. Therefore, MetaSep IC-Ag was selected for the development of the Ag^+ SPE extraction protocol.

Cartridge wettability

SPE cartridges need to be conditioned before use. Water was unable to penetrate the cartridges. By comparison, DCM went through cartridges freely just under gravity. This result was in line with previous reports.[5,12] After the cartridge being conditioned with DCM, wine sample was still unable to infiltrate the cartridge. It appeared that loading aqueous samples onto Ag^+ SPE cartridge was infeasible which was consistent with the previous observation.[5] Therefore, LLE had to be applied as a necessary pre-treatment for wine sample. However, during the later stage of our method development, we demonstrated that switching Ag^+ SPE from normal phase mode to reversed phase mode was not only operable but also crucial for a successful extraction.

SPE reservoirs and housings

The format of the selected Ag^+ SPE cartridge is compacted mini cartridge (**Figure S5**). Therefore, the use of a SPE reservoir was required. Initially, polypropylene SPE reservoir was used. However, many impurities that were originated from polypropylene SPE reservoir were detected in the elutes. The negative impact of unsuitable Ag^+ SPE cartridge tube materials and their leachable impurities on the trace analysis of fatty acids have been demonstrated previously.[12] With similar observations that were seen in our application, it was evident that using SPE reservoir made in glass was essential. No leachable impurities were detected thereafter, even when relatively large volumes of samples/organic solvents (~200 mL) were in contact with the glass reservoirs (data not shown). This is of great importance given that the expected detection signals of ultra-trace (ng/L) analytes are at low abundance and can be easily interfered by impurities even at low concentrations. Same consideration was given to the materials of SPE housings, which could be another source of leachable impurities. The SPE housing was also made of polypropylene, but the mini compact cartridge type only had very small surface area which led to much less noticeable impurities during the extraction process. Therefore, no adjustments were made on the SPE housing materials. Regardless, a simple modification method for changing Ag^+ SPE housing is given in **Figure S5**, should glass-only extraction environment be required for future applications.

Ag^+ SPE elution, clean-up, and artifacts

Elution with DTT and associated difficulties in clean-up

Elution was the crucial issue to be addressed for the Ag^+ SPE protocol. As an alternative to 1-thioglycerol, 1,4-dithiothreitol (DTT) was evaluated as elution reagent. DTT has been frequently used as a reducing reagent to release free thiols that are trapped onto organomercuric sorbents.[13–15] In

Table S3. Specifications of the selected Ag^+ SPE cartridges

	MetaSep IC-Ag[8]	Enviro-Clean Fusion Ag^+[9]	Discovery Ag-Ion[10]	Maxi-Clean IC-Ag[11]
Format	mini compact cartridge	6 mL open barrel cartridge	6 mL open barrel cartridge	mini compact cartridge
Cartridge housing material	Polypropylene	polypropylene	polypropylene	polypropylene
Sorbent	Ag^+	Ag^+	Ag^+	Ag^+
Sorbent bed mass	300 mg	1000 mg	750 mg	0.8 m Eq. of Ag^+
Retention	chloride ion removal	aromatic hydrocarbons	fatty acid methyl esters	chloride, iodide, and bromide removal
Retention mechanism	ion chromatography	normal phase charge transfer	normal phase charge transfer	ion chromatography
Retention capacity	4.8 mg/g	20–25 mg/L	less than or equal to 1 mg (total FAMEs)	not provided

Table S4. Sensory evaluation results of the elutes collected from Ag^+ SPE process using four types of SPE cartridges[a]

	MetaSep IC-Ag	Enviro-Clean Fusion Ag^+	Discovery Ag-Ion	Maxi-Clean IC-Ag
After sample loading	No odor detected	No odor detected	No odor detected	No odor detected
After washing with DCM	No odor detected	No odor detected	No odor detected	No odor detected
After washing with MeCN	No odor detected	No odor detected	No odor detected	No odor detected
After washing with MeCN	No odor detected	No odor detected	No odor detected	No odor detected
After washing with MeCN	No odor detected	No odor detected	No odor detected	No odor detected
After elution (thioglycerol)	Strong odor detected	No odor detected	No odor detected	No odor detected

[a] Sample: 10 mL of DCM spiked with six thiols at final concentration of 10 mg/L.

Table S5. GC–MS results (peak areas) of the elutes collected from Ag^+ SPE process using four types of SPE cartridges[a]

Sample	2M3FTS (*m/z* 114)	FFT (*m/z* 114)	4MSP (*m/z* 132)	BM (*m/z* 124)	3SH (*m/z* 132)	3SHA (*m/z* 116)
Original sample	88742	46965	37249	61504	4514	21473
	MetaSep IC-Ag					
After sample loading	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
After washing step 1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
After washing step 4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
After elution (replicate 1)	50812	8879	27176	52908	coelution	19267
After elution (replicate 2)	54398	9554	27976	54837	coelution	17724
	Enviro-Clean Fusion Ag^+					
After sample loading	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
After washing step 1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
After washing step 4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
After elution	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Discovery Ag-Ion					
After sample loading	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
After washing step 1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
After washing step 4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
After elution	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Maxi-Clean IC-Ag					
After sample loading	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
After washing step 1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
After washing step 4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
After elution	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

[a] Sample: 10 mL of DCM spiked with six thiols at final concentration of 10 mg/L. n.d.: not detected.

Table S6. GC–MS results on elutes collected from Ag^+ SPE process using four types of SPE cartridges

Peak area (quantifier ion)	Standard solution[a]	Extracts obtained after elution with DTT at different concentrations (g/L, in DCM) and SAFE clean-up[b]			
		0.125	0.25	0.5	1
3M2FS (114)	131303	n.d.	n.d.	52329 (39.9%)	67834 (51.7%)
FFT (114)	126379	n.d.	n.d.	12042 (9.5%)	19482 (15.4%)
4MSP (132)	41590	n.d.	2284 (5.5%)	26522 (63.8%)	48352 (116.3%)
BM (124)	60032	n.d.	2937 (4.9%)	79543 (132.5%)	148227 (246.9%)
3SH (134)	2697	n.d.	n.d.	7547 (278.9%)	11886 (440.7%)
3SHA (116)	19072	n.d.	711 (3.7%)	57357 (300.7%)	94301 (494.4%)
DTT (102)	n/a	n.d.	9655	1.97×107	2.04×107
3M2FS (114)	131303	n.d.	n.d.	52329 (39.9%)	67834 (51.7%)
FFT (114)	126379	n.d.	n.d.	12042 (9.5%)	19482 (15.4%)
4MSP (132)	41590	n.d.	2284 (5.5%)	26522 (63.8%)	48352 (116.3%)

[a] concentrations of thiol standards were around 10 mg/L.

[b] number in parentheses represents the ratio of the measured peak area against that detected in the standard solution.

n/a: not applicable. n.d.: not detected.

the initial test, a DTT solution (10 g/L in DCM, 6 mL) could liberate thiols that were fixed onto Ag^+ SPE cartridge, as indicated by the presences of strong thiol odors in the collected final elutes. However, excessive amounts of DTT in the final elutes had to be removed to permit GC analyses.

Three clean-up techniques, low pressure column chromatography, SPE, and SAFE were evaluated for their ability to remove DTT from the elutes. As DTT has relatively high polarity (based on thin layer chromatography results, data not shown), separation using silica gel as stationary phase to pair with less polar solvents seemed logical. This was evaluated in two different formats: low pressure column chromatography and SPE. For SPE, both commercially available and self-packed cartridges were tested. Low pressure column chromatography and SPE using silica gel as stationary phase efficiently removed the majority of DTT when in use with DCM and DCM/n-hexane solvent system. However, silica gel SPE or column chromatography would give poor separation results for analytes which have similar retention behavior as DTT. This was the case for 3SH. Significant loss of 3SH was noticed because it eluted too close to DTT (data not shown). Another concern for these silica gel-based separations was the significant amounts of contaminations, likely to be originated from silica gel. Prewashing and baking silica gel multiple times did not decrease the contamination to a satisfactory level. The separation difficulties and contaminations issues suggested this approach was problematic for DTT removal.

SAFE (solvent assisted flavor evaporation)[16] was assessed for its ability to remove DTT. SAFE efficiency depended on the initial DDT concentration which significantly impacted the elution of thiol analytes (**Table S6**). Using a relatively high concentration of DTT (0.5 g/L, 1.0 g/L, or higher, 6 mL) was crucial for sufficient elution. When DDT was required to be at high concentrations, SAFE demonstrated moderate ability for DTT removal, agreeing with previously reported data.[13,14]

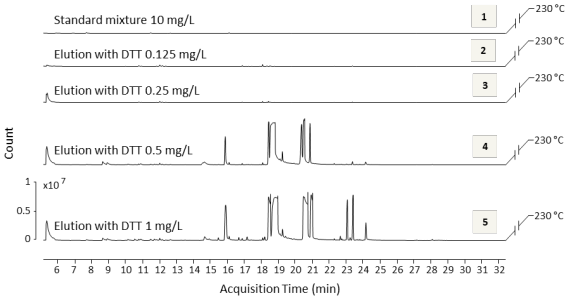

FIGURE S6. GC–EI–MS TICs of (panel 1) a thiol standard solution (10 mg/L) and of wine extracts (panel 2–5) obtained through Ag^+ SPE that were eluted with different concentration of DTT, followed by SAFE clean-up.

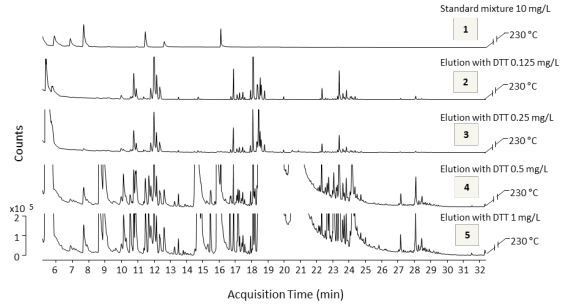

FIGURE S7. Expanded GC–EI–MS TICs of that presented in Figure S6.

However, the concentrations of residual DTT in some final extracts remained high, even with repeated SAFE operations. For instance, the residual DTT in one final extract which underwent two consecutive SAFEs still yielded a flat-top MS peak with an intensity at 2×10^7 (**Table S6** and **Figure S6**). Additionally, large numbers of artifacts were detected when DTT was used (demonstrated by a procedural blank, **Figure 2b-2** and **Figure S4**). Moreover, SAFE was still rather time consuming and labor intensive. Therefore, DTT was not chosen as the elution reagent for Ag^+ SPE because of the artifacts as well as the issues related to its removal from the elutes, despite its good elution ability.

Ag^+ SPE elution with L-cysteine and its artefact resistance

Initially, L-cysteine was considered as a candidate. L-Cysteine is freely soluble in H_2O but poorly soluble in tested organic solvents (DCM, 10% DCM in acetic acid, MeCN, or MeOH), which discouraged its use in Ag^+ SPE that was operated in normal phase mode. However, given the limitations observed with the use of DTT and 1-thioglycerol, we reconsidered L-cysteine as elution reagent. Based on our trial, aq. L-cysteine solution could slowly penetrate Ag^+ SPE cartridges, only when a certain level vacuum was applied (> 2.5 psi). This unexpected result suggested that operating Ag^+ SPE in both normal phase and reversed phase mode was possible, offering the opportunity of using aq. L-cysteine solution as elution reagent. A quick sensory evaluation of the collected aqueous elutes after eluting with L-cysteine (10 g/L in H_2O, 6 mL) showed the presence of strong thiol odors in the elutes. This warranted further investigations. After optimization, 5 mL of H_2O, 20 mL of aq. L-cysteine solution (10 g/L), and 10 mL of DCM were used in sequence for the final elution. All the elutes were collected and subjected to a 15 min LLE clean-up. The addition of H_2O was aimed to prime the sorbent ready for aq. L-cysteine solution. DCM in the last step was intended to wash off residual thiols from the cartridge and to facilitate the subsequent LLE clean-up. To eliminate some unpleasant sulfur odors that were presented in the pure L-cysteine, L-cysteine solution was freshly prepared and purged with N_2 through a gas dispenser. The procedural blank obtained using the proposed elution protocol showed significantly less amounts of artifacts in comparison to those when either DTT or 1-thioglycerol was selected for elution (**Figure 1b-3** and **Figure S4**). Moreover, L-cysteine is a nontoxic chemical and can be fully removed by LLE clean-up which made the final extracts safe for GC–O or other intended sensory evaluations.

Section IV. Spiking experiment

Spiking experiment:

Pre-extraction spiking: To an aliquot of a bag-in-box red wine (750 mL), a 75 µL of 10 mg/L thiol standard solution that contains six thiols was added to reach thiol final concentration at 1000 ng/L.

Post-extraction spiking: To the organic extract of a non-spiked bag-in-box wine (750 mL) that was collected after LLE, Ag^+ SPE, and LLE clean-up, a 75 µL of 10 mg/L thiol standard solution that contains six thiols was added.

Post LLE spiking: To the organic extract of a non-spiked bag-in-box wine (750 mL) that was collected after LLE sample pre-treatment, a 75 µL of 10 mg/L thiol standard solution that contains six thiols was added.

SPE: experiment details as described in the Materials and Methods in the main manuscript.

Collected extracts were dried over anhydrous Na_2SO_4, concentrated under N_2 to 100 µL and stored at −20 °C pending GC–MS analysis.

GC–MS: GC–MS analyses were performed using the MDGC–MS/O system (bypass mode, 1D GC–MS/O) described in the Materials and Methods in the main manuscript. GC–MS parameters are given in Table S7.

Table S7. GC–MS parameters

Parameter	Values
Injection volume	2.0 µL
Injection mode	Splitless
Inlet temperature	240 °C
Carrier gas	He
Constant flow	1.0 mL/min
Column 1	Agilent DB–5 (30 m × 250 µm × 0.25 µm)
Column 1 flow rate	1.5 mL/min
Oven program for column 1	45 °C
Column 2	Agilent DB–35MS (30 m × 250 µm × 0.25 µm)
Column 2 flow rate	2.5 mL/min
Oven program for column 2	55 °C (1 min) > 3 °C/min > 180 °C (0 min) > 150 °C/min > 300 °C (15 min)
Host oven temperature	240 °C
Backflush	OFF
Deans switch H/C	OFF
CTS Initial temperature	OFF
CTS Rate 1	OFF
CTS Final temperature 1	OFF
CTS Rate 2	OFF
CTS Final temperature 2	OFF
MSD Transfer line	260 °C
MS source	230 °C
MS quad	150 °C
MS acquisition mode	Scan 35 – 350 *m/z*

Section V. Sensory results of pHPLC subfractions after Ag^+ SPE

Table S8. Odor descriptors of pHPLC subfractions of an Ag^+ SPE extract from a red wine (W1)[a]

	Participant			
	1	2	3	4
1	/	/	/	/
2	/	/	moisty	/
3	/	/	moisty	flower
4	/	/	/	/
5	/	/	/	/
6	/	fruity	vinegar	/
7	/	fruity	rancid	/
8	/	fruity	moisty	/
9	/	alcohol	moisty	grease
10	/	alcohol	salty	/
11	/	/	vinegar	/
12	/	/	yogurt	/
13	/	/	yogurt, buttery	/
14	/	buttery	fruity, fresh	flower
15	/	buttery	butter	flower
16	/	fresh fruity	wine	
17	/	fruity	strawberry yogurt	
18	Meat	meat	meat	meat
19	/	meat	meat	meat
20	/	meat	meat	leather
21	/	/	/	/
22	/	/	/	/
23	/	meaty	/	/
24	Solvent	acid like	/	/
25	/	thiol-like (fruity)	/	/
26	/	thiol-like (fruity)	/	/
27	Solvent	/	rancid, yogurt	/
28	/	thiol-like (fruity)	freshy minty	/
29	/	grilled	/	/
30	/	solvent	onion	/
31	/	/	/	flower
32	/	solvent	/	fruity
33	grassy, solvent	/	fruity	/
34	Alcohol	/	fresh, eucalyptol	/
35	/	grilled	garlic	cooked vegetables
36	/	grilled	meaty	cooked vegetables
37	/	roasted coffee	cooked garlic	smoke
38	/	fruity	raw garlic, chemical	/
39	/	fruity	fruity	/
40	/	fruity	fruity	/
41	/	grilled	chips	/
42	/	grilled	/	/
43	/	grilled	raw garlic	mushroom
44	/	grilled	raw garlic	mushroom
45	Dusty	/	/	flower oil
46	/	/	/	flower
47	/	/	/	/
48	/	/	/	/
49	/	/	meaty	/
50	/	/	/	/

[a] Same panelists who participated the sensory session in Table S1. /: no odor perceived.

Section VI. Case study

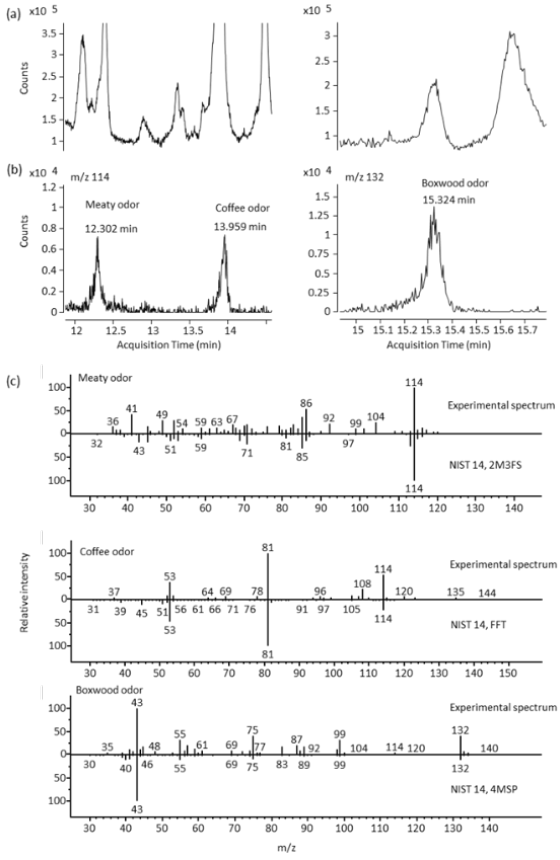

FIGURE S8. (a, b) 1D GC–MS/O chromatograms of a bag-in-box red wine (W0, spiked with 100 ng/L 2M3FS, FFT, 4MSP) Ag^+ SPE extract. (c) Comparison between three experimental spectra with NIST database.

REFERENCES

(1) de-la-Fuente-Blanco, A.; Ferreira, V. Gas chromatography olfactometry (GC-O) for the (semi)quantitative screening of wine aroma. *Foods* **2020**, *9* (12). https://doi.org/10.3390/foods9121892.

(2) Nikolantonaki, M.; Darriet, P. Identification of ethyl 2-sulfanylacetate as an important off-odor compound in white wines. *J. Agric. Food Chem.* **2011**, *59* (18), 10191–10199. https://doi.org/10.1021/jf201047u.

(3) Siebert, T. E.; Stamatopoulos, P.; Francis, I. L.; Darriet, P. Sensory-directed characterisation of distinctive aromas of Sauternes and Viognier wines through semi-preparative liquid chromatography and gas chromatography approaches. *J. Chromatogr. A* **2020**, *1637* (25), 461803. https://doi.org/10.1016/j.chroma.2020.461803.

(4) Stamatopoulos, P.; Frérot, E.; Tempère, S.; Pons, A.; Darriet, P. Identification of a new lactone contributing to overripe orange aroma in Bordeaux dessert wines via perceptual interaction phenomena. *J. Agric. Food Chem.* **2014**, *62* (12), 2469–2478. https://doi.org/10.1021/jf405397c.

(5) Takazumi, K., Takoi, K., Koie, K., & Tuchiya, Y. Quantitation method for polyfunctional thiols in hops (*Humulus lupulus* L.) and beer using specific extraction of thiols and gas chromatography–tandem mass spectrometry. *Anal. Chem.* **2017**, *89* (21), 11598–11604. https://doi.org/10.1021/acs.analchem.7b02996.

(6) National Center for Biotechnology Information. PubChem Compound Summary for CID 7291, 3-Mercapto-1,2-propanediol. https://pubchem.ncbi.nlm.nih.gov/compound/7291 (Accessed on 2021-9-28).

(7) Chen, L.; Darriet, P. Strategies for the identification and sensory evaluation of volatile constituents in wine. *Compr. Rev. Food Sci. Food Saf.* **2021**, *20* (5), 4549–4583. https://doi.org/10.1111/1541-4337.12810.

(8) GL Sciences. Ion Chromatography Sample Preparation MetaSEP IC Series | Products | GL Sciences https://www.glsciences.com/product/spe_columns/meta_sep/01147.html (Accessed on 2021-9-30).

(9) UCT. Enviro-Clean Fusion®Ag+ Archives https://www.unitedchem.com/product-category/spe/environmental/enviro-clean-fusionag/ (Accessed 2021-9-30).

(10) Merck. Discovery® Ag-Ion SPE Tube bed wt. 750 mg, volume 6 mL, pk of 30 | Sigma-Aldrich https://www.sigmaaldrich.com/FR/en/product/supelco/54225u (Accessed on 2021-9-30).

(11) S*Pure. IC-Ag and IC-Ag Plus Devices https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=&ved=2ahUKEwi1rMGin6bzAhVPCGMBHWx3B3EQFnoECAgQAw&url=https%3A%2F%2Fwww.sstarpure.com%2Fdata-sheets%2FIC-AG%2520SPv2.0.pdf&usg=AOvVaw2cgiNlFTEbI42Kl4OhOfqD (Accessed on 2021-9-30)

(12) Belaunzaran, X.; Bravo-Lamas, L.; Kramer, J. K. G.; Morales, R.; Aldai, N. Silver ion solid-phase extraction cartridges employing glass housings overcome the limitations observed in the GC analysis of animal lipids with low *trans* fatty acid content. *Eur. J. Lipid Sci. Technol.* **2017**, *119* (4), 1600124. https://doi.org/10.1002/ejlt.201600124.

(13) Reglitz, K.; Steinhaus, M. Quantitation of 4-methyl-4-sulfanylpentan-2-one (4MSP) in hops by a stable isotope dilution assay in combination with GC×GC-TOFMS: Method development and application to study the influence of variety, provenance, harvest year, and processing on 4MSP concentrations. *J. Agric. Food Chem.* **2017**, *65* (11), 2364–2372. https://doi.org/10.1021/acs.jafc.7b00455.

(14) Schoenauer, S.; Schieberle, P. Screening for novel mercaptans in 26 fruits and 20 wines using a thiol-selective isolation procedure in combination with three detection methods. *J. Agric. Food Chem.* **2019**, *67* (16), 4553–4559. https://doi.org/10.1021/acs.jafc.9b01242.

(15) Full, G.; Schreier, P. Covalent chromatography. A valuable method for the aroma analysis of thiols at trace levels. *Lebensmittelchemie* **1994**, *48* (1), 1–4.

(16) Engel, W.; Bahr, W.; Schieberle, P. Solvent assisted flavour evaporation – A new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices. *Eur. Food Res. Technol.* **1999**, *209* (3), 237–241. https://doi.org/10.1007/s002170050486.