



HAL
open science

trans -Resveratrol and trans - ϵ -Viniferin in Grape Canes and Stocks Originating from Savoie Mont Blanc Vineyard Region: Pre-extraction Parameters for Improved Recovery

Marion Zwingelstein, Micheline Draye, Jean-Luc Besombes, Christine Piot,
Gregory Chatel

► To cite this version:

Marion Zwingelstein, Micheline Draye, Jean-Luc Besombes, Christine Piot, Gregory Chatel. trans -Resveratrol and trans - ϵ -Viniferin in Grape Canes and Stocks Originating from Savoie Mont Blanc Vineyard Region: Pre-extraction Parameters for Improved Recovery. ACS Sustainable Chemistry & Engineering, 2019, 7 (9), pp.8310-8316. 10.1021/acssuschemeng.8b06723 . hal-02133831

HAL Id: hal-02133831

<https://univ-smb.hal.science/hal-02133831v1>

Submitted on 9 Mar 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 *Trans*-resveratrol and *trans*- ϵ -viniferin in grape
2 canes and stocks originating from Savoie Mont
3 Blanc vineyard region: pre-extraction parameters for
4 improved recovery

5 *Marion Zwingelstein,^{†,‡} Micheline Draye,[†] Jean-Luc Besombes,[†] Christine Piot[†] and Gregory*
6 *Chatel^{†,*}*

7 [†] Univ. Savoie Mont Blanc, LCME, F-73000 Chambéry, France.

8 [‡] Agence de l'Environnement et de Maîtrise de l'Energie (ADEME), F-49004, Angers, France.

9

10 KEYWORDS. Stilbenes, viticultural by-products, particles size, vineyard age, storage duration.

11

12 ABSTRACT. Viticultural by-products are known to contain high-value compounds such as
13 stilbenes. Wine sector in Savoie Mont Blanc region (France) generates high amount of waste
14 including grape canes and grapevine stocks that remain unvalued. Herein, the potential of the most
15 wide-spread grapevine varieties in Savoie Mont Blanc territory, Jacquère and Mondeuse, as
16 sources of two stilbenes, *trans*-resveratrol (Rsv) and *trans*- ϵ -viniferin (Vf) was investigated for the
17 first time. Using the reported extraction technique, the present work focused on several pre-
18 extraction parameters influencing the extraction efficiency, including grapevine variety, age of

19 vines, time of material storage and particles size of the woody material. This work revealed
20 important Rsv and Vf contents in Jacquère residues of 1681-6124 mg.kg⁻¹_{dw} and 1620-6667 mg.kg⁻¹_{dw}
21 respectively, whereas for Mondeuse variety, the target molecules yields were 1724-4636
22 mg.kg⁻¹_{dw} and 1713-5322 mg.kg⁻¹_{dw} respectively. These results are promising when compared with
23 high well-known stilbenes levels in Pinot Noir and Gewurztraminer varieties but also highlight a
24 considerable variability depending on the applied pre-extraction parameters.

25

26 INTRODUCTION

27 A growing demand for natural products in the consumer society emerged in the last decade.¹ In
28 the same vein, agricultural biomass is of great interest because of its richness in high-value
29 compounds and its abundance.² For example, France was the first exporter and the second largest
30 producer of wine worldwide with a production of 36.7 million hectoliters and a vineyard surface
31 of 787,000 hectares in 2017.³ During the winter period, these surfaces produce high amounts of
32 wood-type waste, most of it resulting from the vineyards pruning and consisting in grape canes
33 and grapevine stocks. These residues remain unvalued, traditionally burned in vineyards or let on
34 the field, especially on Savoie Mont Blanc territory (France) where the fear of the spread of
35 diseases and the hilly vineyards encourage these practices. However, grapevine by-products are
36 known to contain high-value molecules.^{4,5} Among them, stilbenes are phenolic compounds having
37 considerable importance for the grapevine since they defend the plant against aggressions from
38 pathogenic microorganisms by acting as phytoalexins.⁶ They are derived from the shikimic-
39 polymalonic acid pathway and are biosynthesized by the combination of three malonyl CoA and
40 one *p*-coumaroyl-CoA by stilbene synthase (StSy) to form *trans*-resveratrol, the first brick of
41 stilbenoid compounds biosynthesis.^{7,8} *Trans*-resveratrol (5-[(1E)-2-(4-Hydroxyphenyl)ethenyl]-

42 1,3-benzenediol, CAS: 501-36-0) and its dimer *trans*- ϵ -viniferin ((3R)-6-hydroxy-2-(4-
43 hydroxyphenyl)-4-[(E)-2-(4-hydroxyphenyl)ethenyl]-2,3-dihydro-1-benzofuran-3-yl]benzene-
44 1,3-diol, CAS: 62218-08-0) are the most studied stilbenes due to their diverse benefits especially
45 in human health and showing anticarcinogenic, cardioprotective and antioxidant properties when
46 used alone or in cocktails of stilbenes.⁹⁻¹¹
47 Despite their occurrence in plant kingdom, *trans*-resveratrol (Rsv) and *trans*- ϵ -viniferin (Vf) are
48 mainly obtained by chemical synthesis.^{12,13} To a lesser extent, Rsv can be extracted from Japanese
49 knotweed (*Fallopia japonica*) roots and both compounds can be obtained from skin of grapes and
50 berries but with low purity or also by using biotechnologies (cells culture or enzymatic
51 fermentation).¹⁴⁻¹⁷ Pinot Noir and Gewurztraminer grape canes, widely studied in the literature,
52 are known to be rich in Rsv and Vf.¹⁸⁻²⁰ Extraction techniques used to extract the stilbenes from
53 grape canes are extensive: classical solid-liquid extraction, ultrasound-assisted extraction,
54 microwave-assisted extraction and pressurized solvent extraction for the most recurrent.²⁰⁻²³
55 However, data found in literature (Table S1, Supporting information) demonstrate that Rsv and Vf
56 extraction yields differ drastically for the same variety depending on other parameters than the
57 extraction technique.
58 Working from vegetal sources is often problematic because of consequent variations implied by
59 biological diversity. On the other hand, it enables adjustments of all the factors affecting the
60 targeted compounds accumulation in plant. Herein, we propose to investigate several pre-
61 extraction parameters impacting the extraction yields of Rsv and Vf from grape canes of the two
62 most widespread grapevine varieties on Savoie Mont Blanc territory. To the best of our knowledge
63 these typical Savoie Mont Blanc varieties have not been studied yet and could potentially contain
64 high amounts of stilbenes. The investigated parameters included grapevine variety (Jacquère or

65 Mondeuse), age of vines (5 and 20 years old), particles size of the woody material extracted (< 0.2
66 mm, < 0.5 mm, < 1 mm and < 4 mm) and time material storage duration (from 2 to 9 months). A
67 complementary study is provided on the Rsv and Vf contents in Jacquère and Mondeuse stocks
68 and the variability of both compounds contents induced by the vineyard of origin.

69

70 **EXPERIMENTAL SECTION**

71 **Chemicals.** Ethanol (96%) was purchased from VWR, methanol ($\geq 99\%$) from Honeywell, glacial
72 acetic acid ($\geq 99\%$) from Fisher Chemical, *trans*-resveratrol ($> 98\%$) from Evolva, *trans*- ϵ -
73 viniferin ($\geq 95\%$) from Extrasynthese and 2-hydroxybenzothiazole (98%) from Alfa Aesar. All
74 chemicals were used as received, without further purification.

75 **Plant material.** Jacquère, a white grapevine variety representing 50% of the viticultural surface
76 on Savoie Mont Blanc territory and Mondeuse a red grapevine variety representing 12%, were
77 selected according to their prevalence in Savoie (Alps, France). The first one was grafted on SO4
78 rootstock (*Vitis riparia* x *Vitis berlandieri*) and the second one on 3309 rootstock (*Vitis riparia* x
79 *Vitis rupestris*). Grape canes and stocks (composed of the upper trunk, the grafting zone and the
80 lower trunk of grapevine plant) of both varieties were collected during February 2018 in a vineyard
81 in agri-environmental and climatic measures at Cruet (entitled “Vineyard 1”).

82 **Evaluation of variety and age impacts.** Jacquère and Mondeuse grape canes were harvested from
83 vines of two types, one of 5 years old and one of 20 years old and were stored during 6 months at
84 room temperature (without access to direct sunlight). For each variety, stocks were also harvested
85 and consisted in a pool of 5 years old and 20 years old vines. They were stored during 9 months at

86 room temperature (without access to direct sunlight). After storage duration, all samples were dried
87 overnight at 50 °C and crushed (Pulverisette 19, Fritsch, < 0.5 mm).

88 ***Evaluation of particles size impact.*** 20 years old Jacquère and Mondeuse grape canes kept at room
89 temperature during (without access to direct sunlight) 6 and 9 months respectively were dried
90 overnight at 50 °C and then crushed at different particles sizes: < 0.2 mm, < 0.5 mm, < 1 mm or <
91 4 mm (Pulverisette 19, Fritsch). A complementary assay consisted in grinding the rest of the < 4
92 mm particles size batch at a size < 0.2 mm was also conducted. The impact of particles size was
93 also tested on stocks described above and those of “Vineyard 2” (described in “Evaluation of
94 vineyard origin” section).

95 ***Evaluation of storage duration.*** 20 years old Jacquère and Mondeuse grape canes were kept at
96 room temperature (without access to direct sunlight) during 9 months (from February 2018 and
97 November 2018) and batch of canes were collected at 2 months (in April 2018), 6 months (in
98 August 2018) and 9 months (in November 2018) before to be dried overnight at 50 °C and then
99 crushed (Pulverisette 19, Fritsch, < 0.2 mm).

100 ***Evaluation of vineyard origin.*** In the same time, 5 years old and 20 years old Jacquère grape canes
101 and Jacquère and Mondeuse stocks were harvested from another vineyard in biological measures
102 at Chignin (entitled “Vineyard 2”). The samples were treated as corresponding samples of
103 Vineyard 1 described in the section “Evaluation of variety and age impacts”.

104 **Extraction process.** All samples were extracted according modified conditions of Pineiro *et al.*²¹
105 1 g of powder was extracted with 30 mL of ethanol/water 80:20 (v/v) during 1 h at 60 °C under

106 gentle stirring. Extractions from a same batch were performed in 5 replicates. Crude extracts were
107 then filtered (0.22 μm , PTFE) without any concentration step prior to HPLC-UV analysis.

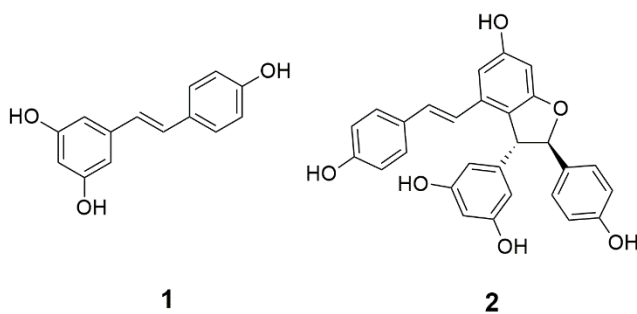
108 **High performance liquid chromatographic analysis.** Stilbenes characterization and
109 quantification in crude extracts were carried out on a HPLC system with UV detection (Perkin
110 Elmer, Series 200). Separation was performed at 40 $^{\circ}\text{C}$ on a column packed with 5 μm particles
111 (250 x 4.0 mm, Nucleosil 100-5 C18 PAH, Macherey Nagel). Stilbenes were analyzed using a
112 mobile phase gradient consisting of 0.1% v/v of acetic acid in water (solvent A) and 0.1% v/v
113 acetic acid in methanol (solvent B) pumped at 1 $\text{mL}\cdot\text{min}^{-1}$ into HPLC system with 5 μL as injection
114 volume. The gradient program was as follows: 25-45% B (from 0 to 5 min), 45-48% B (from 5 to
115 19 min), 48-99% B (from 19 to 21 min), 99% B (from 21 to 23 min), 99-25% B (from 23 to 28
116 min) and 25% B (from 28 to 38 min). Compounds were identified at 290 nm (λ) according to their
117 retention time and quantification was performed using a calibration curve of commercial *trans*-
118 resveratrol, *trans*- ϵ -viniferin and 2-hydroxybenzothiazole that was used as internal standard in
119 crude extracts. LOD and LOQ values were determined for each compounds according to the
120 following equations (from ICH guidelines): $\text{LOD} = 3.3\delta/s$ and $\text{LOQ} = 10\delta/s$ where δ is the standard
121 deviation of noise level and s is the slope of calibration curves. Purity of Rsv and Vf was evaluated
122 by HPLC-UV as follows: standard calibration was analyzed at different wavelengths: 220 nm, 260
123 nm, 290 nm and 320 nm. The ratios between peak area at 290 nm with peaks areas at other
124 wavelengths were determined (Table S4, Supporting information). Then, these ratios were
125 compared to those of compounds in crude extracts and a difference of $\pm 1\%$ was accepted to
126 validate the purity of the chromatographic peak.

127 **Statistical analysis.** Statistical analyses were performed using R software. All data (Rsv and Vf
128 yields of 5 replicates) were tested for normality and homoscedasticity with appropriate tests
129 (ANOVA, Kruskal-Wallis, t-test) with $\alpha = 0.05$ defined as threshold for statistical significance.
130 Different letters (a,b) were used to show the statistical differences between samples as a function
131 of the parameter studied. A same letter is used to shown data non significantly different. When
132 different letters are assigned to different data, it means that they are significantly different from
133 each other. Data with several letters are consequently non significantly different from data with
134 individual letter. In Figure S7 of Supporting information, asterisks were used to show significant
135 statistical differences with * for $p \leq 0.05$, ** for $p \leq 0.01$, *** for $p \leq 0.001$ and *ns* for not
136 significant.

137 **RESULTS AND DISCUSSION**

138 **Identification of Rsv and Vf in extracts.** The retention times of Rsv and Vf (10.2 min and 12.1
139 min respectively) were compared to those of commercial standards solutions (**Figure 1**). In all
140 crude extracts,

141



143

144

145 **Figure 1.** Chemical structures of *trans*-resveratrol (1) and its dimer *trans*- ϵ -viniferin (2).

146

146 both Rsv and Vf were found at detectable and quantifiable levels independently of the type of
 147 resource studied (**Table 1** and Figure S2, Supporting information). No co-elution phenomenon
 148 with *cis*-resveratrol or other compounds was observed (Figure S3 and Table S4, Supporting
 149 information). Using HPLC-UV with a detection at 290 nm (λ), Rsv and Vf respond linearly in the
 150 range of 3.75-270 $\mu\text{g}/\text{mL}^{-1}$ and with a correlation coefficient $r > 0.99$. Quantified results were then
 151 expressed in milligrams of Rsv or Vf extracted per kilograms of dry weight ($\text{mg}\cdot\text{kg}^{-1}_{\text{dw}}$). At 290
 152 nm (λ) detection, the major compounds detected in Jacquère and Mondeuse grape canes extracts
 153 were Rsv and Vf whereas stocks extracts were more complex. Gabaston *et al.* also reported that
 154 Rsv and Vf were the main stilbenoid compounds in grape canes (mixture of Cabernet Sauvignon
 155 and Merlot) when Rsv oligomers (as *trans*- ϵ -viniferin, isohopeaphenol, *r*-viniferin, ampelopsin A
 156 and *r*²-viniferin) were more abundant in wood (mixture Cabernet Franc and Tannat) and roots
 157 (SO4 rootstock).²⁴

158

Compound	LOD ($\mu\text{g}/\text{mL}^{-1}$)	LOQ ($\mu\text{g}/\text{mL}^{-1}$)	t_R (min)
<i>trans</i> -resveratrol	0.119	0.362	10.2
<i>trans</i> - ϵ -viniferin	0.153	0.465	12.1

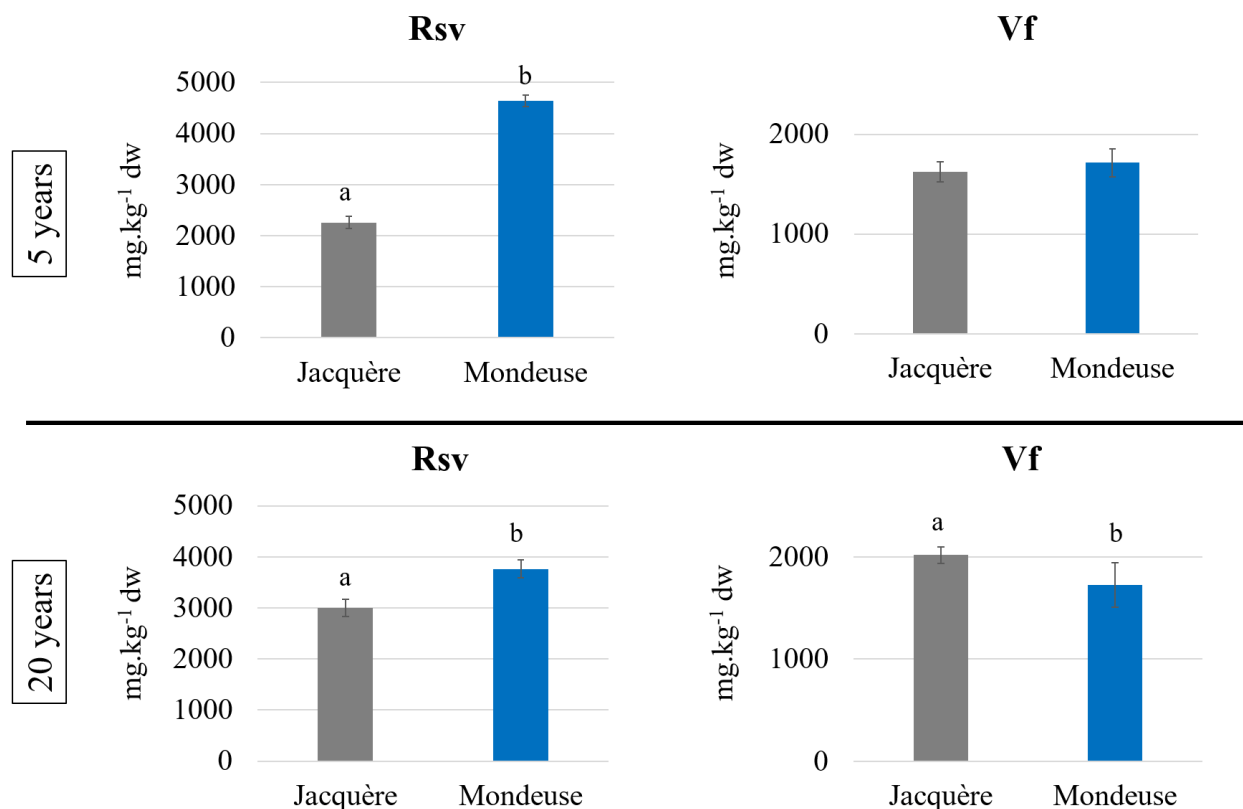
159

160 **Table 1.** Limits of detection (LOD), limits of quantification (LOQ) and retention times (t_R) of
 161 *trans*-resveratrol and *trans*- ϵ -viniferin using the HPLC gradient described in experimental section.

162

163 **Impact of vine variety and vineyard age.** Grape canes of Jacquère and Mondeuse treated in the
 164 same conditions (Vineyard 1, 6 months of storage and particles size < 0.5 mm) of 5 years old and
 165 20 years old vines were compared (**Figure 2**). Independently of age, Mondeuse grape canes

166 contained significantly higher levels of Rsv ($3759-4636 \text{ mg.kg}^{-1}_{\text{dw}}$) than Jacquère ($2259-2994$
 167 $\text{mg.kg}^{-1}_{\text{dw}}$) whereas Vf levels were similar for both varieties ($1620-2016 \text{ mg.kg}^{-1}_{\text{dw}}$). These
 168 quantities were in the same range than high levels of Pinot Noir and Gewurztraminer grape canes
 169 (Table S1, Supporting information). Another statistical analysis (Figure S5, Supporting
 170



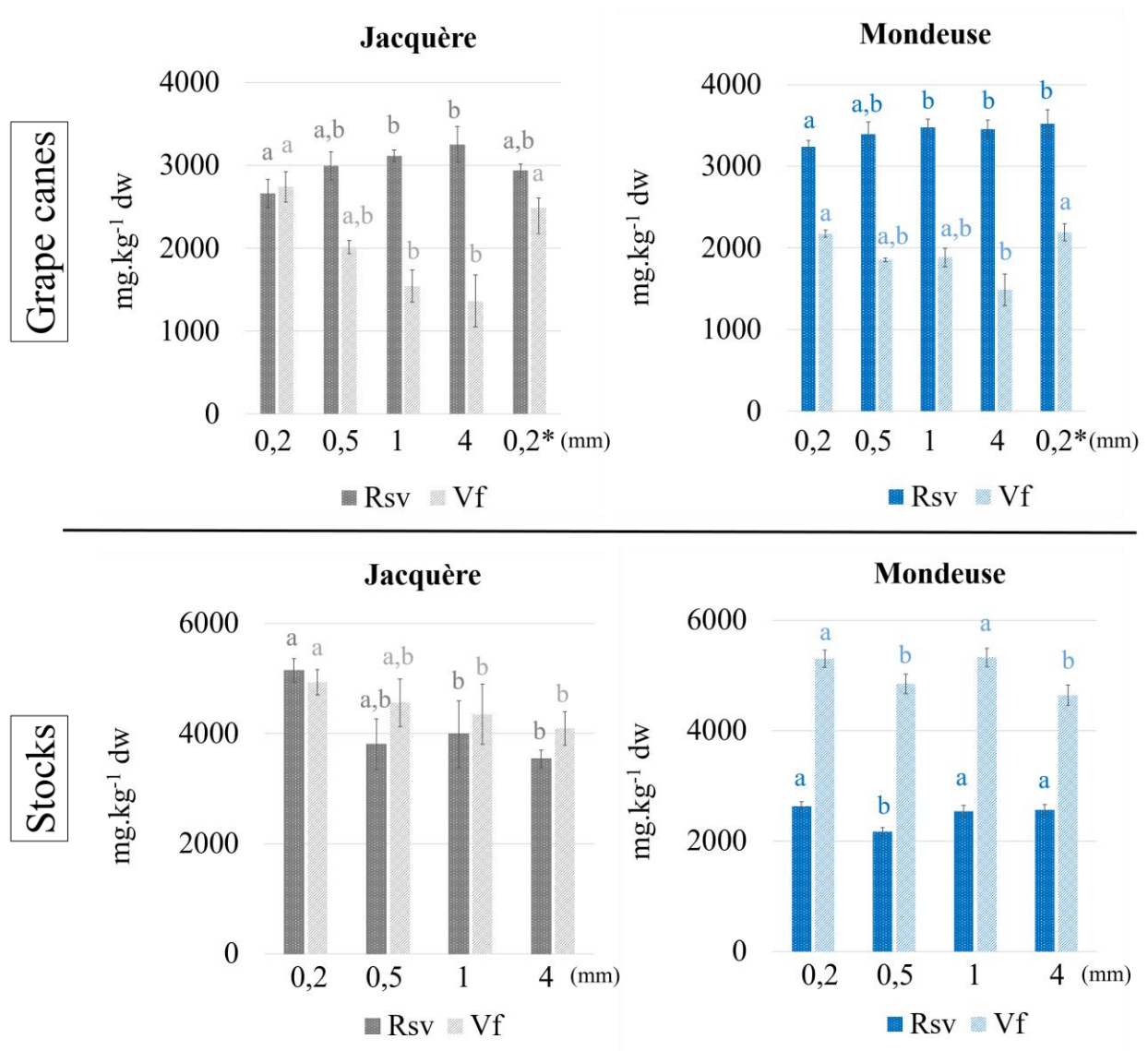
171
 172
 173 **Figure 2.** Rsv and Vf contents (expressed in milligrams per kilograms of dry weight, $\text{mg.kg}^{-1}_{\text{dw}}$)
 174 in Jacquère and Mondeuse grape canes for 5 years old vineyards and 20 years old vineyards. On
 175 each histogram, different letters were used to show significant statistical differences between
 176 varieties for a same compound and a same age ($\alpha = 0.05$).
 177

178 information) performed on the impact of age showed that aging of vineyards had a positive effect
179 on stilbenes contents of Jacquère since Rsv and Vf levels increased when the vine becomes older
180 (**Figure 2**). This tendency was also confirmed with Jacquère grape canes of an another vineyard
181 (Vineyard 2) with Rsv levels switching between 1838 mg.kg⁻¹_{dw} for 5 years old vines and 2912
182 mg.kg⁻¹_{dw} for 20 years old vines (Figure S6, Supporting information). Interestingly, the opposite
183 effect was observed for Mondeuse since Rsv levels were higher for young than for old vines
184 (**Figure 2**). To the best of our knowledge, it was the first time that a study was conducted on the
185 effect of ageing of vines on their stilbenes contents. Further studies (multiplying the sampling
186 vineyards) are needed to confirm these tendencies and investigate the mechanisms involved.
187 However, according to the results obtained, the selection of appropriate vine age could enhance up
188 to 35% the Rsv contents extracted.

189 In any case, if Mondeuse grape canes were richer in Rsv than Jacquère grape canes, the opposite
190 effect was observed for the vine stocks (**Figure 3**). Indeed, the amount of Rsv was 2171-2632
191 mg.kg⁻¹_{dw} for Mondeuse stocks and 3537-5148 mg.kg⁻¹_{dw} for Jacquère stocks (intervals depending
192 on the particles size used). At the same time, Vf contents remained similar in both varieties with
193 4640-5322 mg.kg⁻¹_{dw} for Mondeuse variety and 4091-4929 mg.kg⁻¹_{dw} for Jacquère variety as seen
194 on **Figure 4**. However, significant variability could be observed depending on the vineyard (Figure
195 S7, Supporting information). For Jacquère stocks of Vineyard 2, Rsv levels reached up to 6124
196 mg.kg⁻¹_{dw} and Vf levels up to 6667 mg.kg⁻¹_{dw} whereas for Mondeuse Rsv levels did not exceed
197 1953 mg.kg⁻¹_{dw} and Vf levels 4094 mg.kg⁻¹_{dw}. Németh *et al.* observed the opposite effect for Rsv
198 since the authors noted a higher concentration in grape canes than in roots of Merlot grafted on
199 TK5BB rootstock.²⁵ This divergence could be explained by several factors including year to year
200 variations and different rootstocks.²⁶⁻²⁹

201 Whether it is for grape canes or stocks, lots of factors could explain the differences observed
202 between the levels of the two exploitations. The UV-exposition of vineyards depending directly of
203 their location impacts the stilbenes accumulation in grape canes as well as the soil composition or
204 the fungal pressure.³⁰⁻³² Viticultural practices, as leaf removal, irrigation or nitrogen fertilization
205 and differing from a vineyard to another, could also be responsible for these variations.^{33,34}

206 **Impact of particles size.** A patent published in 2015 mentioned that particles size of Melon de
207 Bourgogne grape canes influences Rsv and Vf yield in a classical maceration in ethanol/water
208 80:20 (v/v) during 15 min followed by an extrusion of the mixture.³⁵ According to the patent, 4
209 mm was better than 8 and 1 mm to recover both compounds (respectively 915, 387 and 764 mg.kg⁻¹
210 _{dw} for Rsv and 8170, 6440 and 5482 mg.kg⁻¹_{dw} for Vf). In the present study, grape canes of
211 Jacquère variety (Vineyard 1, 20 years old, 6 months of storage) and Mondeuse (Vineyard 1, 20
212 years old, 9 months of storage) were crushed at different particles sizes (< 0.2 mm, < 0.5 mm, < 1
213 mm and < 4mm) to investigate the effect of particles size on Rsv and Vf extraction. Results on
214 **Figure 3** showed higher yields while increasing particles size for Rsv whereas the opposite effect
215 was observed for Vf. These results were observed for both varieties. For Jacquère, Rsv yields were
216 improved up to 20% when particles size < 4 mm was used instead of < 0.2 mm and Vf yields
217 decreased of 50%. Gorená *et al.* firstly demonstrated that intact canes are required to allow
218 stilbenes accumulation (mainly due to Rsv contents increase) during storage while in < 20 mesh
219 (i.e. < 0.84 mm) samples no accumulation occurs.³⁶ Similarly, Billet *et al.* recently mentioned that
220 cutting grape canes in small sections of 5 and 10 mm before storage causes a faster Rsv



221
 222
 223 **Figure 3.** Rsv and Vf contents (expressed in milligrams per kilograms of dry weight, mg.kg⁻¹_{dw})
 224 in Jacquère and Mondeuse grape canes and stocks in function of particles size (mm). On each
 225 histogram, different letters were used to show significant statistical differences between particles
 226 sizes for a same compound and a variety ($\alpha = 0.05$). 0.2*: batch of < 4 mm powder crushed at <
 227 0.2 mm.

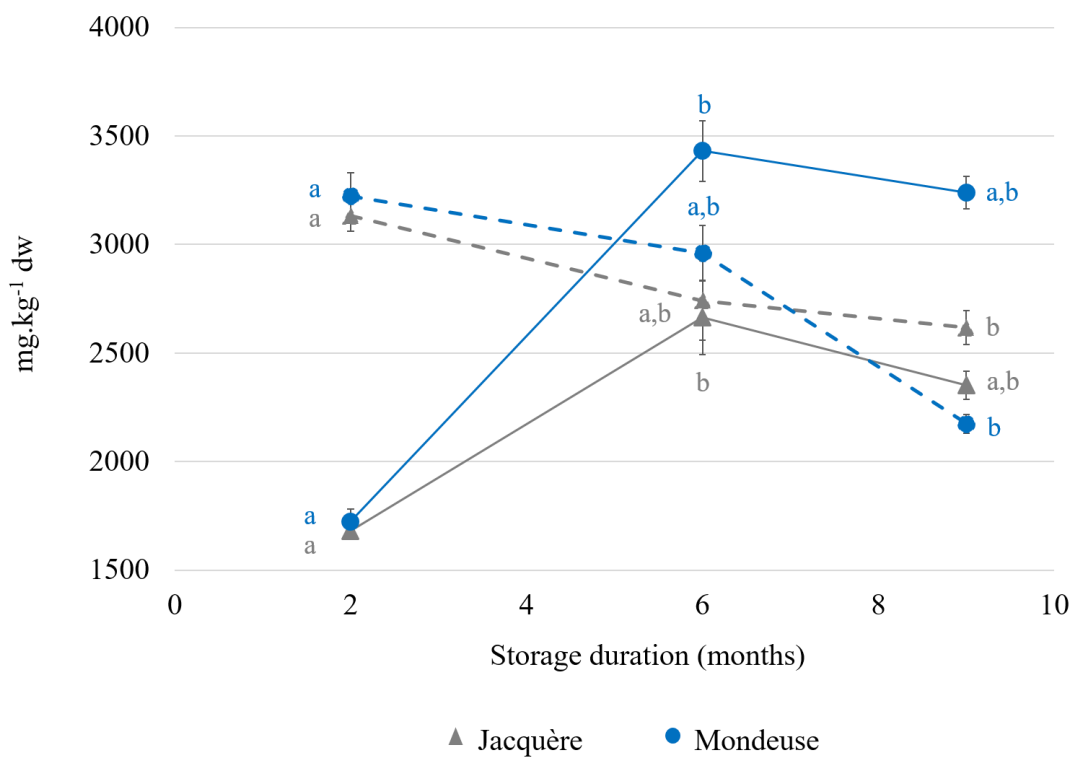
228

229 accumulation in comparison with 2 mm sections.³⁷ In the present work, it was shown that larger
230 was the particles size (in the range of 0.2-4 mm), better was the plant response to mechanical
231 wounding in terms of Rsv accumulation. This behavior is really unexpected since grape canes were
232 stored during 6 and 9 months and were almost dry (2-3 grams of water per 100 grams of grape
233 canes). However, Ewald *et al.* also noted a biological response to drought stress of grape canes
234 stored up to 30 months, especially in the case of Rsv.²⁸
235 In addition, it was demonstrated for the first time that the biosynthesis of Vf is not impacted by
236 grinding. Extraction efficiency of this compound is only ruled by mass transfer limitation since a
237 secondary grinding of the particles size < 4 mm batch at < 0.2 mm allowed to recover yields
238 obtained for the first < 0.2 mm batch (0.2* on **Figure 3**).^{38,39}
239 Interestingly, extraction efficiency of targeted compounds in stocks decreased when the particles
240 size increased probably showing the absence of biological response under mechanical stress in this
241 part of the plant. This tendency was also observed in stocks of Vineyard 2 (Figure S7, Supporting
242 information).

243

244 **Impact of storage duration.** There are numerous studies on the evolution of stilbenes contents in
245 grape canes during their storage.^{12,18,19} The reported works admit that there is a peak of Rsv
246 accumulation in grape canes between 6 weeks and 6 months of storage. In the present work, impact
247 of storage on compound accumulation was evaluated on grape canes of Jacquère and Mondeuse
248 varieties of the same exploitation (Vineyard 1) and the same age (20 years). Shoots were stored
249 for 9 months at room temperature with collections at 2, 6 and 9 months and crushing at < 0.2 mm.
250 The results presented on **Figure 4** showed similar levels for Rsv and Vf for both varieties at 2
251 months of storage. At 6 months, Rsv levels increased significantly up to 1.5 fold for Jacquère and

252 up to 2 fold for Mondeuse whereas Vf contents decreased slightly. After 9 months of storage, Rsv
 253 levels started to decrease and Vf levels continued to decrease, specially for Mondeuse with a
 254 significant loss of 30% when compared to 2 months levels (16% for Jacquère). Rsv increase for
 255 the first 6 months was also observed by Cebrián *et al.* whereas Gorena *et al.* and Houillée *et al.*
 256 highlighted Rsv increase only until 2-3 months followed by a decay for various grapevine varieties
 257
 258



259
 260
 261 **Figure 4.** Rsv (solid line) and Vf (dashed line) contents (expressed in miligrams per kilograms of
 262 dry weight, mg.kg⁻¹ dw) in Jacquère and Mondeuse grape canes depending on their storage duration
 263 at room temperature. Different letters were used to show significant statistical differences between
 264 durations for a same compound and a same variety ($\alpha = 0.05$).
 265

266 showing that it is not a phenomenon proper to Jacquère and Mondeuse varieties.^{36,41,40} The
267 divergence between the Rsv accumulation durations could be due to several storage specific
268 factors. Indeed, temperature, relative humidity and UV exposition of storage location could also
269 affect stilbenes accumulation.^{40,42,43} Concerning Vf contents evolution over time, to the best of our
270 knowledge, it was the first time that a considerable and constant decrease during storage was
271 mentioned.

272

273 CONCLUSIONS

274 Jacquère and Mondeuse grape canes and stocks from Savoie Mont Blanc territory represent
275 valuable sources of *trans*-resveratrol (Rsv) and *trans*- ϵ -viniferin (Vf) with contents up to 6124
276 mg.kg⁻¹_{dw} for Rsv and up to 6667 mg.kg⁻¹_{dw} for Vf. However, it must be not forgotten that
277 considerable variations are observed from year to year.^{26,28,29,44} Nevertheless, this study also
278 highlighted the variability that could be induced by several pre-extraction parameters influencing
279 recovery of Rsv and Vf, some of which could be optimized. Age of vineyards seems to have an
280 effect on the stilbenes content of grape canes and further investigations are needed to confirm this
281 tendency. Remarkably, the increase of particles size of the woody material entailed an
282 enhancement of Rsv extraction in Jacquère and Mondeuse grape canes with an optimum for
283 particles size < 4 mm in comparison with < 0.2 mm, < 0.5 mm and < 1 mm. This resulted from an
284 accumulation of Rsv by plant response to a mechanical stress whereas Vf biosynthesis was not
285 impacted. Further investigations are needed to better understand this phenomenon. Comparably to
286 other works, Vf contents in Jacquère and Mondeuse grape canes decreased considerably during 9
287 months of storage while Rsv accumulated effectively during 6 months and then decreased. The
288 common practice of open-air burning justified by the fear of diseases propagation in viticultural

289 community (especially in the case of stocks) will be no longer possible on Savoie Mont Blanc
290 territory. Indeed, a prefectural decree implemented in 2017 prohibits open-air burning of
291 agricultural waste including viticultural waste. This decision will bring an important amount of
292 waste with high contents of Rsv and Vf available for chemical valorization.

293

294 **ASSOCIATED CONTENT**

295 **Supporting Information:** Table of Rsv and Vf contents in Pinot Noir and Gewurztraminer grape
296 canes from the literature; HPLC-UV chromatograms from experiments; Rsv and Vf contents in
297 Jacquère and Mondeuse grape canes in function of the age of vines, the vineyard of origin and
298 plant material particles size.

299

300 **AUTHOR INFORMATION**

301 **Corresponding Author**

302 * Dr. Gregory Chatel. E-mail: gregory.chatel@univ-smb.fr Twitter: [@gregory_chatel](https://twitter.com/gregory_chatel)

303 ORCID: [000-0001-8814-812X](https://orcid.org/000-0001-8814-812X)

304 **ORCID Authors:**

305 Marion ZWINGELSTEIN ORCID: 0000-0002-6267-9130

306 Micheline DRAYE ORCID: 0000-0002-8216-0184

307 Jean-Luc BESOMBES ORCID: 0000-0002-3793-3256

308 Gregory CHATEL ORCID: [000-0001-8814-812X](https://orcid.org/000-0001-8814-812X)

309

310 **Notes**

311 The authors declare no competing financial interest.

312

313 ACKNOWLEDGMENT

314 The authors gratefully acknowledge the Agence de l'Environnement et de Maitrise de l'Energie
315 (ADEME) and the Conseil Savoie Mont Blanc (CSMB) for the awarding of the PhD scholarship
316 to Marion Zwingelstein. They also thank the Université Savoie Mont Blanc and its foundation for
317 their final supports. They also thank participating winegrowers for plant material providing and
318 their willingness. Finally, they thank Thibault Masset (Laboratoire de Chimie Moléculaire et
319 Environnement, Univ. Savoie Mont Blanc, France) for helpful discussions on statistical analysis.

320

321 REFERENCES

322

- 323 (1) ADEME. Rapport de tendances à l'horizon 2017 pour l'ADEME. 2012.
324 <https://www.ademe.fr/rapport-tendances-a-lhorizon-2017-lademe> (accessed October 26, 2018).
- 325 (2) Abosedo, I. A.; *et al.* Biomass Valorization: Agricultural Waste in Environmental Protection,
326 Phytomedicine and Biofuel Production. In *Biomass Volume Estimation and Valorization for Energy*;
327 Tumuluru, J. S., Eds.; IntechOpen, 2017.
- 328 (3) Organisation Internationale de la Vigne et du Vin. Eléments de conjoncture mondiale. 2018.
329 <http://www.oiv.int/public/medias/5952/fr-oiv-note-de-conjoncture-avril-2018.pdf> (accessed
330 October 23, 2018).
- 331 (4) Angelov, G.; Boyadzhiev, L.; Georgieva, S. Useful Bioactive Substances from Wastes: Recovery of
332 Trans-Resveratrol from Grapevine Stems. *Open Chem. Eng. J.* **2016**, *10* (1), 4-9.
- 333 (5) Sánchez-Gómez, R.; Zalacain, A.; Alonso, G. L.; Salinas, M. R. Vine-Shoot Waste Aqueous Extracts
334 for Re-Use in Agriculture Obtained by Different Extraction Techniques: Phenolic, Volatile, and
335 Mineral Compounds. *J. Agric. Food Chem.* **2014**, *62* (45), 10861–10872.
- 336 (6) Jeandet, P.; Delaunois, B.; Conreux, A.; Donnez, D.; Nuzzo, V.; Cordelier, S.; Clément, C.; Courrot,
337 E. Biosynthesis, Metabolism, Molecular Engineering, and Biological Functions of Stilbene
338 Phytoalexins in Plants. *BioFactors Oxf. Engl.* **2010**, *36* (5), 331–341.
- 339 (7) Rivière, C.; Pawlus, A. D.; Mérillon, J.-M. Natural Stilbenoids: Distribution in the Plant Kingdom
340 and Chemotaxonomic Interest in Vitaceae. *Nat. Prod. Rep.* **2012**, *29* (11), 1317–1333.
- 341 (8) Roubelakis-Angelakis, K. A. *Molecular Biology & Biotechnology of the Grapevine*; Kluwer
342 Academic Publishers, 2001.
- 343 (9) Billard, C.; Izard, J.-C.; Roman, V.; Kern, C.; Mathiot, C.; Mentz, F.; Kolb, J.-P. Comparative
344 Antiproliferative and Apoptotic Effects of Resveratrol, ϵ -Viniferin and Vine-Shots Derived
345 Polyphenols (Vineatrols) on Chronic B Lymphocytic Leukemia Cells and Normal Human
346 Lymphocytes. *Leuk. Lymphoma* **2002**, *43* (10), 1991–2002.
- 347 (10) Romain, C.; Gaillet, S.; Carillon, J.; Vidé, J.; Ramos, J.; Izard, J.-C.; Cristol, J.-P.; Rouanet, J.-M.
348 Vineatrol and Cardiovascular Disease: Beneficial Effects of a Vine-Shoot Phenolic Extract in a
349 Hamster Atherosclerosis Model. *J. Agric. Food Chem.* **2012**, *60* (44), 11029–11036.
- 350 (11) Baur, J. A.; Pearson, K. J.; Price, N. L.; Jamieson, H. A.; Lerin, C.; Kalra, A.; Prabhu, V. V.; Allard,
351 J. S.; Lopez-Lluch, G.; Lewis, K.; *et al.* Resveratrol Improves Health and Survival of Mice on a
352 High-Calorie Diet. *Nature* **2006**, *444* (7117), 337–342.

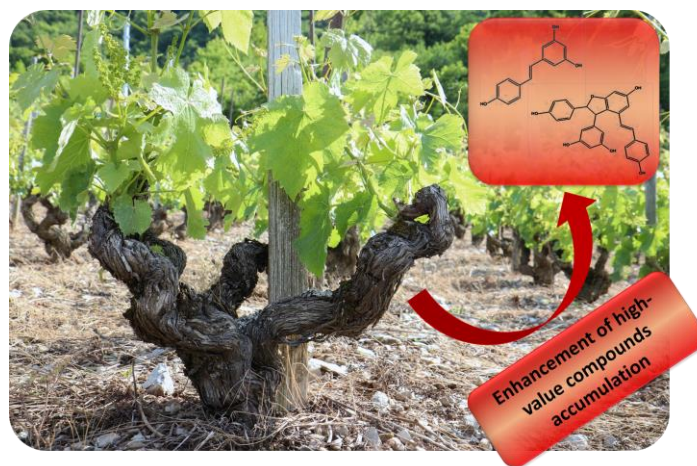
- 353 (12) Farina, A.; Ferranti, C.; Marra, C. An Improved Synthesis of Resveratrol. *Nat. Prod. Res.* **2006**, *20*,
354 247–252.
- 355 (13) Lindgren, A. E. G.; Öberg, C. T.; Hillgren, J. M.; Elofsson, M. Total Synthesis of the Resveratrol
356 Oligomers (\pm)- Ampelopsin B and (\pm)- ϵ - Viniferin. *Eur. J. Org. Chem.* **2016**, *2016* (3), 426–429.
- 357 (14) Chen, H.; Deng, Q.; Ji, X.; Zhou, X.; Kelly, G.; Zhang, J. Glucose Oxidase-Assisted Extraction of
358 Resveratrol from Japanese Knotweed (*Fallopia Japonica*). *New J. Chem.* **2016**, *40* (9), 8131–8140.
- 359 (15) Singh, C. K.; Liu, X.; Ahmad, N. Resveratrol, in Its Natural Combination in Whole Grape, for Health
360 Promotion and Disease Management. *Ann. N. Y. Acad. Sci.* **2015**, *1348* (1), 150–160.
- 361 (16) Li, M.; Kildegaard, K. R.; Chen, Y.; Rodriguez, A.; Borodina, I.; Nielsen, J. De Novo Production of
362 Resveratrol from Glucose or Ethanol by Engineered *Saccharomyces Cerevisiae*. *Metab. Eng.* **2015**,
363 *32*, 1–11.
- 364 (17) Chastang, T. Etude de la synthèse du resvératrol et de ses dérivés (viniférines) par des suspensions
365 de cellules de vigne et optimisation de la production en bioréacteur. Ph.D. Dissertation, Ecole
366 Centrale Paris, 2014.
- 367 (18) Lambert, C.; Richard, T.; Renouf, E.; Bisson, J.; Waffo-Tégou, P.; Bordenave, L.; Ollat, N.;
368 Mérillon, J.-M.; Cluzet, S. Comparative Analyses of Stilbenoids in Canes of Major *Vitis Vinifera*
369 *L.* Cultivars. *J. Agric. Food Chem.* **2013**, *61* (47), 11392–11399.
- 370 (19) Vergara, C.; von Baer, D.; Mardones, C.; Wilkens, A.; Wernekinck, K.; Damm, A.; Macke, S.;
371 Gorena, T.; Winterhalter, P. Stilbene Levels in Grape Cane of Different Cultivars in Southern Chile:
372 Determination by HPLC-DAD-MS/MS Method. *J. Agric. Food Chem.* **2012**, *60* (4), 929–933.
- 373 (20) Guerrero, R. F.; Biais, B.; Richard, T.; Puertas, B.; Waffo-Teguo, P.; Merillon, J.-M.; Cantos-Villar,
374 E. Grapevine Cane's Waste Is a Source of Bioactive Stilbenes. *Ind. Crops Prod.* **2016**, *94*, 884–892.
- 375 (21) Piñeiro, Z.; Guerrero, R. F.; Fernández-Marin, M. I.; Cantos-Villar, E.; Palma, M. Ultrasound-
376 Assisted Extraction of Stilbenoids from Grape Stems. *J. Agric. Food Chem.* **2013**, *61* (51).
- 377 (22) Piñeiro, Z.; Marrufo-Curtido, A.; Vela, C.; Palma, M. Microwave-Assisted Extraction of Stilbenes
378 from Woody Vine Material. *Food Bioprod. Process.* **2017**, *103*, 18–26.
- 379 (23) Karacabey, E.; Mazza, G.; Bayındırlı, L.; Artık, N. Extraction of Bioactive Compounds from Milled
380 Grape Canes (*Vitis Vinifera*) Using a Pressurized Low-Polarity Water Extractor. *Food Bioprocess*
381 *Technol.* **2012**, *5* (1), 359–371.
- 382 (24) Gabaston, J.; Cantos-Villar, E.; Biais, B.; Waffo-Teguo, P.; Renouf, E.; Corio-Costet, M.-F.;
383 Richard, T.; Mérillon, J.-M. Stilbenes from *Vitis Vinifera L.* Waste: A Sustainable Tool for
384 Controlling Plasmopara Viticola. *J. Agric. Food Chem.* **2017**, *65* (13), 2711–2718.
- 385 (25) Németh, G.; Hegyi, O.; Dunai, A.; Kocsis, L. Stilbenes in the Different Organs of *Vitis Vinifera Cv.*
386 Merlot Grafted on Teleki Kober 5BB Rootstock. *OENO One* **2017**, *51* (3), 323–328.
- 387 (26) Németh, G.; Molnár, Z.; Podmaniczky, P.; Nyitrai-Sárdy, D.; Kállay, M.; Dunai, A.; Kocsis, L.
388 Trans-Resveratrol Content in Grape Cane and Root of Different Scion-Rootstock Combinations.
389 *Mitteilungen Klosterneubg. Rebe Wein Obstbau Früchteverwert.* **2017**, *67* (4), 256–264.
- 390 (27) Gabaston, J.; Leborgne, C.; Waffo-Teguo, P.; Valls, J.; Pinto, A. P.; Richard, T.; Cluzet, S.;
391 Mérillon, J.-M. Wood and Roots of Major Grapevine Cultivars and Rootstocks: A Comparative
392 Analysis of Stilbenes by UHPLC-DAD-MS/MS and NMR. *Phytochem. Anal.* *0* (0).
- 393 (28) Ewald, P.; Delker, U.; Winterhalter, P. Quantification of Stilbenoids in Grapevine Canes and Grape
394 Cluster Stems with a Focus on Long-Term Storage Effects on Stilbenoid Concentration in Grapevine
395 Canes. *Food Res. Int.* **2017**, *100*, 326–331.
- 396 (29) Bavaresco, L.; Civardi, S.; Pezzutto, S.; Gatti, M.; Mattivi, F. Role of the Genotype on Stilbene
397 Synthesis in Grapevine. *Acta Hort.* **2009**, No. 827, 347–350.
- 398 (30) Guerrero, R. F.; Cantos-Villar, E.; Puertas, B.; Richard, T. Daily Preharvest UV-C Light Maintains
399 the High Stilbenoid Concentration in Grapes. *J. Agric. Food Chem.* **2016**, *64* (25), 5139–5147.
- 400 (31) Acuña-Avila, P. E.; Vásquez-Murrieta, M. S.; Franco Hernández, M. O.; López-Cortéz, M. del S.
401 Relationship between the Elemental Composition of Grapeyards and Bioactive Compounds in the
402 Cabernet Sauvignon Grapes *Vitis Vinifera* Harvested in Mexico. *Food Chem.* **2016**, *203*, 79–85.

- 403 (32) Houillé, B.; Besseau, S.; Delanoue, G.; Oudin, A.; Papon, N.; Clastre, M.; Simkin, A. J.; Guérin, L.;
404 Courdavault, V.; Giglioli-Guivarc'h, N.; et al. Composition and Tissue-Specific Distribution of
405 Stilbenoids in Grape Canes Are Affected by Downy Mildew Pressure in the Vineyard. *J. Agric.*
406 *Food Chem.* **2015**, *63* (38), 8472–8477.
- 407 (33) Keller, M.; Steel, C. C.; Creasy, G. L. Stilbene Accumulation in Grapevine Tissues: Developmental
408 and Environmental Effects. *Acta Hort.* **2000**, *514*, 275–286.
- 409 (34) Cavaliere, C.; Foglia, P.; Marini, F.; Samperi, R.; Antonacci, D.; Laganà, A. The Interactive Effects
410 of Irrigation, Nitrogen Fertilisation Rate, Delayed Harvest and Storage on the Polyphenol Content
411 in Red Grape (*Vitis Vinifera*) Berries: A Factorial Experimental Design. *Food Chem.* **2010**, *122* (4),
412 1176–1184.
- 413 (35) Soum, S.; Piccirilli, A.; Bataille, F. Method for Obtaining Polyphenols from a Vegetable Raw
414 Material Containing Same. FR2998296 (B1), March 6, 2015.
- 415 (36) Gorena, T.; Saez, V.; Mardones, C.; Vergara, C.; Winterhalter, P.; von Baer, D. Influence of Post-
416 Pruning Storage on Stilbenoid Levels in *Vitis Vinifera* L. Canes. *Food Chem.* **2014**, *155*
417 (Supplement C), 256–263.
- 418 (37) Billet, K.; Houillé, B.; Besseau, S.; Mélin, C.; Oudin, A.; Papon, N.; Courdavault, V.; Clastre, M.;
419 Giglioli-Guivarc'h, N.; Lanoue, A. Mechanical Stress Rapidly Induces E-Resveratrol and E-
420 Piceatannol Biosynthesis in Grape Canes Stored as a Freshly-Pruned Byproduct. *Food Chem.* **2018**,
421 *240*, 1022–1027.
- 422 (38) Özkal, S. G.; Yener, M. E.; Bayındırlı, L. Mass Transfer Modeling of Apricot Kernel Oil Extraction
423 with Supercritical Carbon Dioxide. *J. Supercrit. Fluids* **2005**, *35* (2), 119–127.
- 424 (39) Molero Gómez, A.; Pereyra López, C.; Martínez de la Ossa, E. Recovery of Grape Seed Oil by Liquid
425 and Supercritical Carbon Dioxide Extraction: A Comparison with Conventional Solvent Extraction.
426 *Chem. Eng. J. Biochem. Eng. J.* **1996**, *61* (3), 227–231.
- 427 (40) Houillé, B.; Besseau, S.; Courdavault, V.; Oudin, A.; Glévarec, G.; Delanoue, G.; Guérin, L.; Simkin,
428 A. J.; Papon, N.; Clastre, M.; et al. Biosynthetic Origin of E-Resveratrol Accumulation in Grape
429 Canes during Postharvest Storage. *J. Agric. Food Chem.* **2015**, *63* (5), 1631–1638.
- 430 (41) Cebrián, C.; Sánchez-Gómez, R.; Salinas, M. R.; Alonso, G. L.; Zalacain, A. Effect of Post-Pruning
431 Vine-Shoots Storage on the Evolution of High-Value Compounds. *Ind. Crops Prod.* **2017**, *109*, 730–
432 736.
- 433 (42) Sáez, V.; Gayoso, C.; Riquelme, S.; Pérez, J.; Vergara, C.; Mardones, C.; von Baer, D. C18 Core-
434 Shell Column with in-Series Absorbance and Fluorescence Detection for Simultaneous Monitoring
435 of Changes in Stilbenoid and Proanthocyanidin Concentrations during Grape Cane Storage. *J.*
436 *Chromatogr. B* **2018**, *1074–1075*, 70–78.
- 437 (43) Guerrero, R. F.; Puertas, B.; Fernández, M. I.; Palma, M.; Cantos-Villar, E. Induction of Stilbenes in
438 Grapes by UV-C: Comparison of Different Subspecies of *Vitis*. *Innov. Food Sci. Emerg. Technol.*
439 **2010**, *11* (1), 231–238.
- 440 (44) Tříska, J.; Vrchotová, N.; Balík, J.; Soral, I.; Sotolář, R. Variability in the Content of Trans-
441 Resveratrol, Trans- ϵ -Viniferin and R2-Viniferin in Grape Cane of Seven *Vitis Vinifera* L. Varieties
442 during a Three-Year Study. *Molecules* **2017**, *22* (6), 928.

443
444
445
446
447
448
449
450
451

452 **SYNOPSIS**

453 The present work provides new data on the impact of pre-extraction parameters in terms of high-
454 value compounds recovery from typical Savoie Mont Blanc territory grape canes and grapevine
455 stocks.



456