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

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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

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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

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

INTRODUCTION

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

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

EXPERIMENTAL SECTION

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

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

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

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

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

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

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

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

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

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

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

RESULTS AND DISCUSSION

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

CONCLUSIONS

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

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

ASSOCIATED CONTENT

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

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Notes

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REFERENCES

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

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

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

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.

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