Journal homepage: www.fia.usv.ro/fiajournal
Journal of Faculty of Food Engineering,
Ştefan cel Mare University of Suceava, Romania
Volume XX, Issue 4 - 2021, pag. 380 - 391

POTENTIAL OF ROMANIAN VINE WASTES AS A NATURAL RESOURCE OF TRANS-RESVERATROL

*** Alina Lenuța CRĂCIUN [1], Gheorghe GUTT [2]**
[1]Faculty of Food Engineering, "Ștefan cel Mare" University,
720225 Suceava, Romania, alina.craciun@usm.ro ,
*Corresponding author
Received 8th November 2021, accepted 22th december 2021

Abstract: *The methods for producing natural resveratrol are of big interest due to the numerous health benefits of this substance and its increasing use in functional foods, food supplements and para-pharmaceutical preparations, especially with the recent permission of resveratrol as a novel food ingredient in the European Union (Commission Implementing Decision (EU) 2016/1190).*
Since annually a large amount of waste is produced in vine pruning, the aim of this study was to evaluate the potential of vine shoots from ten grape varieties (Fetească Albă, Fetească Neagră, Muscat Ottonel, Muscat de Hamburg, Tămâioasă, Coarnă Neagră, Merlot, Cabernet Sauvignon, Fetească Regală and Burgund Mare) from two different locations to be used as a natural source of trans-resveratrol. To reach this goal, two different solvents were explored, two different storage periods, three different time of collecting of samples in two different years. Moreover, a correlation between the type of vine (red or white) and trans-resveratrol content was analyzed. Maceration solvent diethyl-ether:ethanol, 1:4 ratio, showed a higher yield of extraction of trans-resveratrol than methanol 80%. Increasing the storage time by another 6 weeks positively affected the concentration of resveratrol for 5 samples, and for another 7 negatively. The time of collecting the samples had a high influence on the resveratrol content on shoots samples, highlighting the fact that autumn is the optimal time for harvesting. No correlation could be established among the grape varieties (red or white) and the trans-resveratrol content. The highest concentration of resveratrol was recorded for the Fetească Neagră variety – 2367.37 mg/kg. D.W.
.

Keywords: *Trans-resveratrol; food supplements; phytoalexin; vine wastes; HPLC.*

1. Introduction

Resveratrol (3,4',5-trihydroxistilbene) is a phytoalexin synthesized by some plants in response to exogenous factors such as UV radiation, chemical stress and fungal parasites and its found in a wide variety of dietary sources including grapes, plums, berries and peanuts [1]. Among foodstuffs the main source of resveratrol is red wine, and its moderate dietary consumption is considered to explain the so-called "French paradox" that in some parts of France the death rate caused by heart diseases is remarkably low in spite of high consumption of animal fats. [2]. Resveratrol confers a variety of benefits to human health and well-being. This antioxidant has been widely explored as anti-inflammatory, anti-aging, cardio protection and anticancer agent [3].

Despite the many health benefits of trans-resveratrol and the attraction to achieving those benefits by drinking red wine, wine is a poor source of the compound. A recent study has estimated that a daily average of 111 glasses of wine (11.1 liters) has to be consumed in order to reach the minimum dose that would exert therapeutic effects, if wine is the only dietary source of trans-resveratrol [4]. Hence, the need to extract and purify the compound from other

DOI: https://doi.org/10.4316/fens.2021.040

sources to prepare trans-resveratrol formulations for research and medicinal purposes is apparent [3].
The impact of resveratrol intake to human body has been intensively study, with positive results, as a result resveratrol supplements are available on the market since 2012 [5], and it already used in cosmetic [6] and food industry as can be seen by the recent permission of resveratrol as a novel food ingredient in the European Union (Commission Implementing Decision (EU) 2016/1190) [7]. Japanese knotweed (*Polygonum Cuspidatum*), an invasive herbaceous plant species is currently the prime source for the production of trans-resveratrol [3,8]. Some studies suggest that additional compounds such as emodin present in unpurified or partially purified Japanese knotweed root extracts may have laxative effects [9]. Since the plant thrives even in heavily polluted environments and soils contaminated with heavy metals, cellular uptake of those contaminants by the root tissues poses further concerns regarding the safety of the commercial preparations of (*E*)-resveratrol made from root extracts [10].
Recently, pruned wood from the vineyard has been shown to accumulate a broad spectrum of stilbenoids ranging from E-resveratrol to complex stilbene oligomers [11]. Therefore, grape canes represent a promising bioresource containing valuable bioactive compounds with potential applications in medicine because of multiple pharmacological properties of stilbenoids and in agriculture as an alternative source of anti-phytopathogenic substances [12,13]. Moreover, the latest research in the field shows that resveratrol content in vine shoots can increase between 400% and 1400% by storage under determined conditions (at 20-25 °C for 6-8 weeks) [14].
Viticulture is one of the most important agricultural activities in the world, with approximately 7.4 million hectares cultivated globally in 2018, of which 4.3 million hectares are cultivated in Europe. The first country is represented by Spain, with an area of 969.000 hectares, followed by China with 875.000 hectares and France with 793.000 hectares. Romania ranks 10th in the world as an area cultivated with vines, approximately 180,000 hectares. A quarter of the counties where vines are grown account for over 65% of the total area, Vrancea being the county with the largest area [15]. The annual average of by-products resulting from the cleaning of the vine is 1.3 kg of wood/live log, which results in $2x10^7$ tons of wood harvested annually in the world. Currently, the only use of this waste is as fertilizer for vines, by grinding and distribution on vineyards [16].
The aim of this study was to determine the potential of vine shoots as a raw material for extraction of resveratrol, a supplement that can be used in food, cosmetic and pharmacological industry. Specifically, by improving the extraction and storage conditions, a significantly higher amount of resveratrol can be obtained. Thus, from an economic point of view, shoots can be of great interest in obtaining resveratrol-based supplements. This research comes in addition to existent studies as an attempt to determine optimal conditions to extract resveratrol, conditions such as solvent for maceration, season of samples collecting, variety of vine and time of storage samples.

2. Materials and Methods

2.1. Vine shoots samples

Fifteen samples of vine shoot were collected from two different regions. Four samples (*Vitis Vinifera* variety Merlot, Fetească Regală, Cabernet Sauvignon and Burgund Mare) were collected from region Ștefănești, Pitești

(47,6708864;26,2668288) on 30 May 2019, eleven samples were collected from region Cotnari, Iași (47,6708864;269361914), three of them on 07 October 2019 (*Vitis Vinifera* variety Fetească Albă, Muscat de Hamburg and Muscat Ottonel), four samples were colected on 20 May 2020 (Tămâioasă, Fetească albă, Coarnă neagră and Muscat Ottonel) and four were colected on 07 November 2020 (Muscat Ottonel, Fetească Albă, Fetească Neagră and Coarnă Neagră).

2.2. Reagent and chemicals

The trans-resveratrol standard (99% GC) and diethyl ether was purchased from Sigma-Aldrich Co. Methanol, Ethanol and Acetonitrile (LiChrosolv for HPLC) was obtained from Merk. For preparation of the aqueous solutions was used double distilled and demineralized water. The syringe filters with PTFE membrane of 0.22 µm dimension was purchased from Pehnomenex.

2.3. Standard solutions

First a stock solution of resveratrol with a concentration of 1g /L was prepared. The resveratrol standard was initially dissolved in a minimal volume of methanol to guarantee a complete dissolution. Before analysis, a set of standards were prepared from the stock solution, diluted in methanol 99%. Special care was taken in connection with the degradation of standard solutions, keeping them protected from exposure to air and light. Standards were stored in brown glass containers at -20^0C.

2.4. Sample collection and preparation

Four sets of plant material, marked as I, II, III and IV were used in the study, as it follows:

I. The samples collected on May 30, 2019 (*Vitis Vinifera* variety Merlot, Fetească Regală, Cabernet Sauvignon and Burgund Mare) were kept for 6 weeks at 20 °C to increase the concentration of resveratrol, optimal parameters recommended by the latest research in the field [14]. After the 6 weeks, the samples were dried in a thermostat at 45 °C for 24h. Then the samples were powdered in a laboratory grinder and macerated with a solution of 80% (v/v) methanol, 5 mL per g of the powdered material for 72 h at room temperature in darkness. After filtration through filter paper, the dry matter was macerated once more with the same amount of the solvent, and the two extracts were combined. Until use in analyses the extracts were kept for several days in a refrigerator at 4 °C.

II. The samples collected on 7 October 2019 (*Vitis Vinifera* variety Fetească Albă, Muscat de Hamburg and Muscat Ottonel) were kept for 12 weeks at 20 °C to increase the concentration of resveratrol. After the 12 weeks, the samples were dried in a thermostat at 45 °C for 24h. Then the samples were powdered in a laboratory grinder and the powdered material was split in two. The powdered material was macerated with the technique described above, with the difference that one sample was macerated with a solution of 80% (v/v) methanol, and one sample was macerated with a solution of diethyl-ether:ethanol 96% in the ratio of 1:4. After that the samples were filtered and stored in same conditions described at I.

III. The samples collected on 20 may 2020 (*Vitis Vinifera* variety Tămâioasă, Fetească albă, Coarnă neagră and Muscat Ottonel) were split in two and one part was kept for 6 weeks at 20 °C and one part was kept for 12 weeks on the same conditions. After that, the samples were grinded, macerated and filtered with the technique described above, at point I, with the difference that one part was macerated with a solution of 80% (v/v) methanol, and one part was macerated with a solution of diethyl-ether:ethanol 96% in the ratio of 1:4.

IV. The samples collected on 07 November 2020 (*Vitis Vinifera* variety Muscat Ottonel, Fetească Albă, Fetească Neagră and Coarnă Neagră) were prepared with the technique described at III.

2.5. Analytical HPLC procedure

The extracts obtained were partially purified before HPLC analysis. The extracts were first evaporated using a rotary evaporator near to dryness and then redissolved in diethyl ether, and the solution was washed 3 times with 5% sodium bicarbonate solution in a separatory funnel. The diethyl ether layer was recovered, evaporated on a rotary evaporator and redissolved in methanol. The obtained extract was filtered through 0.22 µm PTFE membrane filters prior to HPLC analysis and then injected (with a volume of 8 µL) into the HPLC instrument (Schimadzu, Kyoto, Japan) for analysis using an SPD-M-20A diode array detector. The separation was carried out on a Phenomenex Kinetex 2.6 µm Biphenyl 100 Å HPLC Column 150 x 4.6 mm thermostated at 20°C. Elution was carried out with a solvent system consisting of pure water (solvent A) and acetonitrile (solvent B) as previously described by Marshall et al. (2012) with modifications. Binary gradient consisted of solvent A: pure water and solvent B: acetonitrile as follows: linear gradient from 0% to 10% B in 42 min, 10–40% B in 42.6 min, 40–90% B in 46.5 min. Run time was 49.5 min [17]. The solvent flow rate was of 0.5 mL/min. The determination of trans-resveratrol was carried out at 306 nm detection wavelength.

The obtained standard calibration curves showed high degrees of linearity ($R2 > 0.9996$). Data collection and subsequent processing were performed using the LC solution software version 1.21 (Shimadzu, Kyoto, Japan). Analyses were performed in duplicate and the quantification was made based on the peak area, using the external standard method.

3. Results and discussion

3.1. Solvent extraction yield

As it shown in Table 1, solvent diethyl-ether:ethanol 1:4 ratio have had a higher extraction yield in all cases. The increase in the amount of resveratrol extracted ranged from 5% (Fetească albă from 0.20 mg/kg D.W. with methanol 80% to 0.21 mg/kg D.W.) to 1569% (Tămâioasă from 0.36 mg/kg D.W. to 6.01 mg/kg D.W.). In most cases the amount of resveratrol extracted with this solvent was considerably higher. Most studies recommend methanol 80% [18] or ethanol 80% [19, 20, 21] for extraction of resveratrol from vine shoots samples. Gromova et al. (1975) used diethyl ether in the extraction of resveratrol from the bark of *Pinus sibirica* [22] and Piyaratne (2018) from the bark of spruce *Picea mariana* [3]. Although methanol is a polar organic solvent that provides a higher percentage of extractives, is not highly selective for polyphenols [23]. On the other hand, diethyl ether, who is a relatively nonpolar solvent, is more preferable because of the selectivity for polyphenols, including trans-resveratrol. This solvent facilitates the extraction methodology for the next steps of purification. [3].

Table 1.

Resveratrol content of the samples collected from the same area (Cotnari, Iași) extracted with methanol 80% and diethyl-ether:ethanol 1:4 ratio

Variety	Solvent extraction: Methanol (resveratrol mg/Kg D.W.)	Solvent extraction: Diethyl-ether:Ethanol 1:4 ratio (resveratrol mg/Kg D.W.)	Storage time of samples
Fetească Albă	107.09	178.65	12 weeks
Muscat Ottonel	58.96	106.66	
Muscat de Hamburg	51.27	106.99	
Tămăioasă	0.36	6.01	6 weeks
Coarnă Neagră	1.42	1.57	
Muscat Ottonel	1.51	12.52	
Fetească Albă	0.37	1.89	
Tămâioasă	0.35	1.15	12 weeks
Coarnă Neagră	0.21	0.28	
Muscat Ottonel	0.24	0.66	
Fetească Albă	0.20	0.21	
Fetească Neagră	374.03	1676.39	6 weeks
Fetească Albă	113.18	604.73	
Muscat Ottonel	525.04	735.76	
Coarnă Neagră	89.14	468.91	
Fetească Neagră	820.26	2367.37	12 weeks
Fetească Albă	92.98	524.37	
Muscat Ottonel	315.19	525.04	
Coarnă Neagră	157.19	261.21	

The research made by Aavikasaar et al. (2003) reported that dry extracts of vine stems obtained from its solution in 80% ethanol to be practically insoluble in diethyl ether. On the other hand, by adding diethyl ether to the solution extract 96.6% ethanol, at the diethyl-ether:ethanol ratio 1:4, the extract precipitate, resulting in a decrease of the content of dark brown matter, without the loss of stilbenoids [2]. In this study it was used this solvent as a solvent for maceration to extract resveratrol from vine shoots sample as an attempt to skip the precipitation step and to make the solvent more selective. Unexpectedly, this solvent generated considerably higher resveratrol yields.

3.2. Influence of storage time on resveratrol content

Recent publications gave rise to the hypothesis that trans-resveratrol accumulate in vine wastes during postharvest storage [14, 24]. Houillé et al. (2015) reported a strong increase, approximately 40-fold, in the concentration of monomer trans-resveratrol during the first six weeks of storage at 20 °C of pruned grape canes. This phenomenon observed in eight different varieties was due to the induction of stilbenoid metabolism [14]. Table 2 shows the vine shoot concentrations of resveratrol after 6 and 12 weeks of storage. The increase in resveratrol concentration is recorded for 5 samples, varying between 64% (Merlot 17.82 mg /kg D.W. to 29.28 mg /kg D.W.) and 119% (Fetească Neagră 374.03 mg /kg D.W. to 820.26 mg /kg D.W.) The decrease in resveratrol concentration is recorded for 7 samples, the decrease varying between 2.78% (Tămâioasă 0.36 mg/kg D.W. to 0.35 mg/kg D.W.) and 98% (Muscat Ottonel 12.52 mg/kg D.W. to 0.24 mg/kg D.W.). Ewald et al. (2017) reported an enormous increase (400 to 1400%) of trans-resveratrol during the 6 months of storage. After another 6 moth of storage the trans resveratrol decresed in all grape canes, except for Regent variety [25]. The fact that post-pruning storage of vine shoots have a direct impact on the concentration of trans-resveratrol is confired by the study made by Cebrián et al. (2017), who reported a significant increase of this monormer on the first 3 month of storage for the variety Airén (77.10 to 151.60 mg/kg D.W.) and Cencibel (50.41 to 224.83 mg/kg D.W) [26].

Table 2.

Resveratrol content of vine shoots samples after 6 and 12 weeks of storage at 20 °C extracted with methanol 80%

Variety	Storage time		Sample set
	6 weeks (resveratrol mg/kg D.W.)	12 weeks (resveratrol mg/kg D.W.)	
Merlot	17.82	29.28	I
Cabernet Sauvignon	20.58	23.39	
Fetească Regală	17.74	3.13	
Burgund Mare	6.75	443	
Tămâioasă	0.36	0.35	III
Muscat Ottonel	12.52	0.24	
Coarnă Neagră	1.42	0.21	
Fetească Albă	0.37	0.20	
Fetească Neagră	374.03	820.26	IV
Fetească Albă	113.03	92.98	
Muscat Ottonel	149.98	315.48	
Coarnă Neagră	89.14	157.19	

Accumulation of trans-resveratrol during storage period is due to activity of key genes involved in the stilbene biosynthesis. Houille et al. (2015) reported that the presence of PAL, 4CL and C4H, the three genes of the general phenylpropanoid pathway, were constitutively expressed over the 6 weeks of storage, and in addition, STS (forming E-resveratrol) was induced during the first 4 weeks of storage. Therefore, vine shoots samples are still transcriptionally active during the storage period [14].

3.3. Influence of collecting period

Resveratrol concentration of the 3 sample sets of vine shoots varied considerably, as can be seen in Table 3., with the highest concentrations being recorded for the sample set collected in November 2020. The lowest concentrations were recorded for the sample set collected in May 2020.

The influence of the harvesting time can be seen clearly for the variety Fetească Albă and Muscat Ottonel. The most unfavorable harvest season would be spring, obtaining the lowest values of resveratrol. The most favorable season is autumn, especially November.

Resveratrol content may vary because of the difference in the microclimate and phytosanitary conditions in which vine grows [27]. Ji et al. (2014) studied the distribution and season regularity of resveratrol in different tissues of *Vitis armurensis* and shows that resveratrol contents are augmented gradually from January to September, and then decrease until January of the following year [28]. This study is in agreement with the result of this study regarding the fact that resveratrol reaches its maximum concentration in autumn.

Table 3.

Resveratrol content for vine shoots samples collected from the same area (Cotnari, Iași) extracted with diethyl-ether:ethanol 1:4 ratio after 12 weeks of storage

07 October 2019 (II)		22 May 2020 (III)		07 November 2020 (IV)	
Variety	**Resveratrol mg/kg D.W.**	**Variety**	**Resveratrol mg/kg D.W.**	**Variety**	**Resveratrol mg/kg D.W.**
Fetească Albă	178.65	Fetească Albă	0.51	Fetească Albă	524.37
Muscat Ottonel	109.66	Muscat Ottonel	0.66	Muscat Ottonel	525.04
Muscat de Hamburg	106.66	Coarnă Neagră	0.28	Coarnă Neagră	261.21
		Tămâioasă	1.15	Fetească Neagră	2367.37

The variation in trans-resveratrol concentration may also be influenced by the year of harvest, namely the climatic and growing conditions of that year. Thus, in 2020, the highest concentrations of trans-resveratrol were recorded in the extracts of vine shoots. This is also confirmed by Guerrero et. al (2016) who determined the trans resveratrol content for 4 vine shoots variety in 2014 and 2015 and showed that this was the most varying compound within the years. Trans-resveratrol was more abundant in 2015 than in 2014, in all varieties [29].

3.4. Variety influence

Red wines, in generally, contain a high amount of trans-resveratrol, while in white wines trans-resveratrol occurs mostly in low levels [30 - 32]. The analysis was made to see if this ascertainment applies also to vine shoots samples from different varieties.

The shoot samples were divided into 2 categories, depending on the variety of grapes, white or red to see if the resveratrol content can be correlated with the grape variety. More samples of both varieties, red and white, collected and processed under the same conditions needed to be studied to establish a correlation on the difference of trans- resveratrol content. The average concentration of trans-resveratrol is 82.3 mg/kg D.W. for white varieties and 221.73 mg/kg D.W. for red varieties. Anastasiadi et al. (2012) found the average concentration of *trans*-resveratrol to be 149 mg/kg D.W. for red and 113 mg/kg D.W. for white varieties in vine shoots [33]. Piñeiro et al. (2016) reported a trans-resveratrol content for vine shoots extracts who ranged between

37.4 - 1529 mg/kg D.W. for white varieties and 45.6 - 664.9 mg/kg D.W. for red varieties [34]. The results are much higher for red varieties in our study because the red variety who had the higher resveratrol content, Fetească neagră (820.26 mg/kg D.W.), had 2.6 times more than the white variety Muscat Ottonel (315.48mg/kg D.W.), who had the higher resveratrol content among the white varieties.

Table 4.

Resveratrol content for vine shoots samples collected from the same area (Cotnari, Iași) extracted with methanol 80% after 12 weeks of storage

White grape vines		Red grape vines		Sample set
Variety	**Resveratrol mg/kg D.W.**	**Variety**	**Resveratrol mg/kg D.W.**	
Fetească Albă	107.9	Muscat de Hamburg	51.27	II
Muscat Ottonel	58.96			
Tămâioasă	0.35	Coarnă Neagră	0.21	III
Muscat Ottonel	0.24			
Fetească Albă	0.20			
Muscat Ottonel	315.48	Fetească Neagră	820.26	IV
Fetească Albă	92.98	Coarnă Neagră	157.19	

3.5. Potential of vine shoot wastes as a source for resveratrol extraction

Agricultural wastes represent a largely ignored source of high-value phytochemicals substances and value-added industrial products that could contribute to sustainability objectives. Summer and autumn trimming of vine is performed to maintain desired canopy shape best suited for optimal grape production. During this process considerable amount of green mass (portions of shoots, leaves and tendrils) is removed [35]. The global wine and table grape industry, with annual sales of >US$ 100 billion, generates large quantities of waste each year that is generally composted or burned for disposal. Grapevines are pruned annually, and these wastes represent a potentially important global source for extraction of *trans*-resveratrol [36].

The resveratrol content of vine waste is between 0.20 – 2367.37 mg/kg D.W. for the varieties researched in this study. By optimizing extraction parameters and sample processing after collection the resveratrol extraction yield could be significantly improved. These findings are in agreement with other studies who reported a resveratrol content between 1300 and 4100 mg/kg D.W. in cane extracts [37]. In the work of Cetin et al. (2011) resveratrol content varied between 9.5 and 39.4 µg/100g D.W [38]. Aaviksaar et al. (2003) determined the presence of *trans*-resveratrol in Estonian grape cultivars in concentrations between 100 and 4700 mg/kg D.W [2]. In the survey of *trans*-resveratrol contents of grape cluster rachis from nine *Vitis vinifera* varieties, Melzoch et al. (2001) found levels ranging from 7 to 480 mg/kg D.W [39].

Higher levels of trans-resveratrol that is approaching to the results of this study were reported in China by Zhang et al. (2011), who found concentrations between 570 and 1751 mg/kg fresh matter in one-year-old canes [40]. Püssa et al. (2006) reported a resveratrol content between

1100 and 3200 mg/kg D.W. for hybrid cultivars from Estonia [41]. Piñeiro et al. (2017) reported a resveratrol content for Spanish vine shoots samples between 122 – 5361 mg/kg D.W [20].

This results confirmed that production of stilbene by grapevine largely depends on the grape cultivar, climate, extraction conditions and the expose to fungal parasites and physiological stresses such as UV light and heavy metals [42].

Zhang et al. (2011) analyzed the trans-resveratrol content for 165 grape cane samples from three major grape production regions. Of the cultivars analyzed, *Vitis vinifera* (937.9 mg/kg FW –fresh weight) have had the higher amount of trans-resveratrol than hybrids of *V. vinifera* and *V. lambrusca* (571 mg/kg FW). Among the grape vine varieties, the grape cane extracts coming from wine grapes vine (64.7 to 1,751.6 mg/kg FW) had a higher content of trans-resveratrol than table grapes vine (671.2 to 841.8 mg/kg FW) [40].

The extraction method is also critical to ensure a good recovery of stilbenoids and the range of extraction methods tends to bias the results [43]. Furthermore, an accurate quantification of stilbenoids relies on calibration curves with pure stilbenoid standards because the quantification of stilbenoids as a resveratrol equivalent leads to an underestimation of the concentrations of resveratrol oligomers [11]. Additionally, a disparity in stilbenoid composition of vine shoot samples has been observed during storage, the time elapsed between vine pruning and the phytochemical analyses. An increase in the trans-resveratrol concentration from 6 weeks to 12 weeks was observed.

Temperature of the storage of shoot samples have a high influence on the accumulation of trans- resveratrol. Houille et al. (2015) reported a delay in accumulation for temperatures below 20 °C (15 °C and 5 °C). After a short heat-shook treatment (2h at 65 °C) applied to vine shoot samples with the intent to denature proteins, inhibits the accumulation of trans-resveratrol during the storage period and suggests the involvement of active enzymes in this phenomenon. Similarly, when canes were stored at −20 °C, trans-resveratrol was not induced [14].

4. Conclusion

Results of this study show that solvent diethyl-ether:ethanol 1:4 ratio used for extraction of resveratrol from vine shoots is better than methanol 80%, obtaining greater yields, up to 1569%. Storing the shoots for another 6 weeks influenced both positively and negatively the amount of resveratrol. The increase or decrease in the amount of resveratrol may be due to the variety, cultivation conditions or chemical composition of the shoots at time of harvest.

Harvesting time of the shoots can significantly influence the amount of resveratrol available in the shoots. Our findings show that November is the optimal harvesting time the vine waste as a prime material for resveratrol extraction, the variety Fetească Neagră having a resveratrol content of 2367.37 mg/kg D.W. The processing of waste in optimal conditions can increase the extraction efficiency much compared to their processing at the time of harvest.

It could not be established a correlation between the color of grape variety and resveratrol content, as in the case of wines.

These results suggest that Romanian vine waste can be an excellent source for resveratrol extraction. Further improvements can be done on establishing the optimal parameters for extracting resveratrol from vine shoots and purification methodologies.

5. Acknowledgment

This paper has been financially supported within the project entitled “DECIDE-Development through entrepreneurial education and innovative doctoral and postdoctoral research”, project code POCU/380/6/13/125031, project co-financed from the European Social Fund through the 2014-2020 Operational Program Human Capital.

6. References

[1]. AL-JUMAILY, E. F., HAMID, G. S., & ALI, K. F. Synthesis and total phenol content of new resveratrol derivative. *Open J Adv Drug Deliv*, *2*(3), 320-329**, (2014)**

[2]. AAVIKSAAR, A., HAGA, M., PUSSA, T., ROASTO, M., & TSOUPRAS, G. Purification of resveratrol from vine stems. Proceedings-estonian academy of sciences chemistry (Vol. 52, No. 4, pp. 155-164). **(2003)**

[3]. PIYARATNE, P. S. Extraction and Purification of (E)-Resveratrol from the Bark of Maine's Native Spruces. **(2018)**

[4]. LACHENMEIER, D.W., GODELMANN, R., WITT, B., RIEDEL, K., REHM, J. Can resveratrol in wine protect against the carcinogenicity of ethanol? A probabilistic dose response assessment. Int. J. Cancer 134:144-153. **(2014)**

[5]. HEGER, A., FERK, F., NERSESYAN, A., SZEKERES, T., KUNDI, M., WAGNER, K. H., & KNASMÜLLER, S. Intake of a resveratrol-containing dietary supplement has no impact on DNA stability in healthy subjects. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, *749*(1-2), 82-86. **(2012)**

[6]. JEANDET, P., CLÉMENT, C., TISSERANT, L. P., CROUZET, J., & COUROT, É.. Use of grapevine cell cultures for the production of phytostilbenes of cosmetic interest. *Comptes Rendus Chimie*, *19*(9), 1062-1070. **(2016)**

[7]. Commission Implementing Decision (EU) 2016/1190 of 19 July 2016 authorising the placing on the market of trans-resveratrol as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council (notified under document C(2016) 4567) C/2016/4567, available online on: https://eur-lex.europa.eu/legal-content/GA/TXT/?uri=CELEX:32016D1190

[8]. WANG, D. G., LIU, W. Y., & CHEN, G. T. A simple method for the isolation and purification of resveratrol from Polygonum cuspidatum. *Journal of pharmaceutical analysis*, *3*(4), 241-247. **(2013)**

[9]. SRINIVAS, G., BABYKUTTY, S., SATHIADEVAN, P.P., SRINIVAS P. Molecular mechanism of emodin action: transition from laxative ingredient to an antitumor agent. Med. Res. Rev. 27(5):591-608 **(2007)**

[10]. BERCHOVÁ-BÍMOVÁ, K., SOLTYSIAK, J., VACH, M. Role of different taxa and cytotypes in heavy metals absorption in knotweeds (*Fallopia*). Sci. Agric. Bohem. 45(1):11-18. **(2014)**

[11]. LAMBERT, C.; RICHARD, T.; RENOUF, E.; BISSON, J.; WAFFO-TEGUO, P.; BORDENAVE, L.; OLLAT, N.; MERILLON, J.-M.; CLUZET, S. Comparative analyses of stilbenoids in canes of major Vitis vinifera L. cultivars. J. Agric. Food Chem., 61, 11392−11399 **(2013)**

[12]. SCHNEE, S.; QUEIROZ, E. F.; VOINESCO, F.; MARCOURT, L.; DUBUIS, P.-H.; WOLFENDER, J.-L.; GINDRO, K. Vitis vinifera canes, a new source of antifungal compounds against Plasmopara viticola, Erysiphe necator, and Botrytis cinerea. J. Agric. Food Chem. 2013, 61, 5459−5467. **(2013)**

[13]. MOREIRA, M. M., BARROSO, M. F., PORTO, J. V., RAMALHOSA, M. J., ŠVARC-GAJIĆ, J., ESTEVINHO, L., & DELERUE-MATOS, C. Potential of Portuguese vine shoot wastes as natural resources of bioactive compounds. *Science of the Total Environment*, *634*, 831-842. **(2018)**

[14]. HOUILLÉ B., BESSEAU S., COURDAVAULT V., OUDIN A., GLÉVAREC G., DELANOUE G., GUÉRIN L., SIMKIN A.J., PAPON N., CLASTRE M., GIGLIOLI-GUIVARC’H N., LANOUE A., *Byosynthetic origin of resveratrol acummulation in grape canes during pastharvest storage*, J. Agric. Food Chem. 63, pg. 1631-1638, **(2015)**

[15]. 2019 Statistical Report on World Vitiviniculture, available online on: https://www.oiv.int/public/medias/6782/oiv-2019-statistical-report-on-world-vitiviniculture.pdf

[16]. ANGELOV, G., BOYADZHIEV, L., & GEORGIEVA, S. Useful bioactive Substances from Wastes: Recovery of trans-resveratrol from grapevine stems. The open chemical engineering Journal, 10(1) **(2016)**

[17]. MARSHALL, D.A., STRINGER, S.J., SPIERS, J.D. Stilbene, ellagic acid, flavonol, and phenolic content of muscadine grape (*Vitis rotundifolia Michx.*) Cultivars. Pharm Crops 3:69-77. **(2012)**

[18]. SOURAL, I., VRCHOTOVÁ, N., TŘÍSKA, J., BALÍK, J., HORNÍK, Š., CUŘÍNOVÁ, P., & SÝKORA, J. Various extraction methods for obtaining stilbenes from grape cane of Vitis vinifera L. *Molecules*, *20*(4), 6093-6112. **(2015)**

[19]. JESUS, M. S., GENISHEVA, Z., ROMANÍ, A., PEREIRA, R. N., TEIXEIRA, J. A., & DOMINGUES, L. Bioactive compounds recovery optimization from vine pruning residues using conventional heating and microwave-assisted extraction methods. *Industrial Crops and Products*, *132*, 99-110. **(2019)**

[20]. PIÑEIRO, Z., MARRUFO-CURTIDO, A., VELA, C., & PALMA, M. (2017). Microwave-assisted extraction of stilbenes from woody vine material. *Food and Bioproducts Processing*, *103*, 18-26. **(2017)**

[21]. ROMERO-PÉREZ, A. I., LAMUELA-RAVENTÓS, R. M., ANDRÉS-LACUEVA, C., & DE LA TORRE-BORONAT, M. C. Method for the quantitative extraction of resveratrol and piceid isomers in grape berry skins. Effect of powdery mildew on the stilbene content. *Journal of Agricultural and Food Chemistry*, *49*(1), 210-215. **(2001)**

[22]. GROMOVA, A. S., TYUKAVKINA, N. A., LUCKIY, V. I., KALABIN, G. A. & KUSHNAREV, D. F. Oxystilbenes in the bark of *Pinus sibirica*. *Khim. prir. soedin.*, 1975, 677–682 (in Russian). **(1975)**

[23]. SPIGNO, G., TRAMELLI, L., & DE FAVERI, D. M. Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. *Journal of food engineering*, *81*(1), 200-208. **(2007)**

[24]. GORENA, 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 Chemistry, 155, 256–263. **(2014)**

[25]. EWALD, P., DELKER, U., & WINTERHALTER, P. (2017). 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 Research International*, *100*, 326-331 **(2017)**

[26]. 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. *Industrial Crops and Products*, *109*, 730-736 **(2017)**

[27]. VERSARI, A., PARPINELLO, G. P., TORNIELLI, G. B., FERRARINI, R., & GIULIVO, C. Stilbene compounds and stilbene synthase expression during ripening, wilting and UV treatment in grape cv. Corvina. Journal of Agricultural and Food Chemistry, 49, 55315536. **(2001)**

[28]. JI, M., LI, Q., JI, H., & LOU, H. Investigation of the distribution and season regularity of resveratrol in Vitis amurensis via HPLC–DAD–MS/MS. *Food chemistry*, *142*, 61-65. **(2014)**

[29]. 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. Industrial Crops and Products, 94, 884–892. **(2016)**

[30]. Ratola, N., Luís Faria, J., & Alves, A. Analysis and quantification of trans-resveratrol in wines from Alentejo region (Portugal). *Food Technology and Biotechnology*, *42*(2), 125-130. **(2004)**

[31]. CVEJIC, J. M., DJEKIC, S. V., PETROVIC, A. V., ATANACKOVIC, M. T., JOVIC, S. M., BRCESKI, I. D., & GOJKOVIC-BUKARICA, L. C. Determination of trans-and cis-resveratrol in Serbian commercial wines. *Journal of chromatographic science*, *48*(3), 229-234. **(2010)**

[32]. PAJOVIĆ-ŠĆEPANOVIĆ, R., WENDELIN, S., & EDER, R. Phenolic composition and varietal discrimination of Montenegrin red wines (Vitis vinifera var. Vranac, Kratošija, and Cabernet Sauvignon). *European Food Research and Technology*, *244*(12), 2243-2254. **(2018)**

[33]. ANASTASIADI M., PRATSINIS H., KLETSAS D., SKALTSOUNIS A.-L., HAROUTOUNIAN S.A.: Grape stem extracts: Polyphenolic content and assessment of their in

vitro antioxidant properties. LWT – Food Science and Technology, 48: 316–322. **(2012)**

[34]. PIÑEIRO, Z., MARRUFO-CURTIDO, A., SERRANO, M. J., & PALMA, M. Ultrasound-assisted extraction of stilbenes from grape canes. *Molecules*, *21*(6), 784 **(2016)**

[35]. LACHMAN, J., KOTÍKOVÁ, Z., HEJTMÁNKOVÁ, A., PIVEC, V., PŠENIČNAJA, O., ŠULC, M., & DĚDINA, M. Resveratrol and piceid isomers concentrations in grapevine shoots, leaves, and tendrils. *Horticultural Science*, *43*(1), 25-32. **(2016)**

[36]. RAYNE S., KARACABEY E., MAZZA G. : Grape cane waste as a source of *trans*-resveratrol and *trans*-viniferin: High-value phytochemicals with medicinal and anti-phyto-pathogenic applications. Industrial Crops and Products, 27: 335–340. **(2008)**

[37]. Karacabey E., Mazza G.: Optimization of solid-liquid extraction of resveratrol and other phenolic compounds from milled grape canes (*Vitis vinifera*). Journal of Agricultural and Food Chemistry, 56: 6318–6325 **(2008)**

[38]. ÇETIN, E. S., ALTINÖZ, D., TARÇAN, E., & BAYDAR, N. G. Chemical composition of grape canes. *Industrial Crops and Products*, *34*(1), 994-998 **(2011)**

[39]. MELZOCH K., HANZLÍKOVÁ I., FILIP V., BUCKIOVÁ D., ŠMIDRKAL J.: Resveratrol in parts of vine and wine originationg from Bohemian and Moravian vineyard regions. Agriculturae Conspectus Scientificus, 66: 53–57 **(2001)**

[40]. ZHANG, A., FANG, Y., LI, X., MENG, J., WANG, H., LI, H., ... & GUO, Z. (2011). Occurrence and estimation of trans-resveratrol in one-year-old canes from seven major Chinese grape producing regions. *Molecules*, *16*(4), 2846-2861. **(2001)**

[41]. PÜSSA T., FLOREN J., KULDKEPP P., RAAL A.: Survey of grapevine *Vitis vinifera* stem polyphenols by liquid chromatography-diode array detection-tandem mass spectrometry. Journal of Agricultural and Food Chemistry, 54: 7488–7494. **(2006)**

[42]. GONZALEZ-BARRIO R., BELTRAN D., CANTOS E., GIL M.I., ESPIN J.C., TOMAS-BARBERAN F.A.: Comparison of ozone and UV-C treatments on the postharvest stilbenoid monomer, dimer, and trimer induction in var. “Superior” white table grapes. Journal of Agricultural and Food Chemistry, 54: 4222–4228. **(2006)**

[43]. KARACABEY, E.; BAYINDIRLI, L.; ARTIK, N.; MAZZA, G. Modeling solid−liquid extraction kinetics of trans-resveratrol and trans-ε-viniferin from grape cane. J. Food Process Eng., 36, 103−112. (**2013**)