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Optimization of Experimental Parameters in the Solvent Extraction of Trans-Resveratrol from Pruning Waste of *Vitis vinifera*, Fetească Neagră Variety

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Abstract: The past few decades have seen a marked expansion in market demand for food supplements with therapeutic value. Due to this demand, the recovery of vine waste for obtaining certain phytochemicals or plant synthesized compounds with health-promoting activities can be an important economic component, principally with the agreement of the European Union for resveratrol as a new food ingredient. For the sake of economic capitalization, it is necessary to determine optimum extraction parameters for maximum recovery. In this paper, we have determined the optimum parameters for the solvent extraction of trans-resveratrol from vine prunings. We tested different extraction conditions: 35 different types of solvents, 10 types of solid-to-liquid ratios, 10 extraction times, 10 types of granulosity of the ground material and 7 consecutive extractions on the same material. The optimal parameters determined were: solvent ethanol:diethyl ether 4:1 ratio, 1:35 solid liquid ratio g/mL, 4 days for extraction time, 500 µm–350 µm granulosity of powdered material and one extraction on the material. These findings are confirmed by optimization of extracting parameters according to Box–Behnken design.

Keywords: trans-resveratrol; pruning wastes; food supplements; HPLC; phytoalexin

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1. Introduction

Recently, there has been a rising interest in the identification, quantification and extraction of plant compounds with a beneficial effect on human health that can be utilized in the food, cosmetic or para-pharmaceutical industry. The identification of these compounds can generate new food supplements or food products [1].

Resveratrol (3,5,4′-trihydroxystilbene) is a natural phenol compound which is a part of the stilbene family [2] and it is synthesized by plants as a result of exposure to various stressful factors, such as exposure to ultraviolet radiation [3], damage and fungal parasites, etc. [4]. This compound offers potential positive therapeutic effects on slowing down the aging process [5], antioxidant effect against free radicals [6], anti-aging effects on skin by suppressing the responsible enzymes, antibacterial effects [7], antifungal effects [8], anti-inflammatory activity [9], anti-carcinogenic effect, cardio protective effects [10], protection against diseases of the nervous system [11] and beneficial effects on the symptoms of diabetes [7].

Due to the positive impact of resveratrol intake to the human body confirmed by research, resveratrol supplements have been commercialized since 2012 [12]. This antioxidant is further used in the cosmetic [13] and food industries, with the permission of the Commission Implementing Decision (EU) 2016/1190 for the use of resveratrol as a new food ingredient in the European Union [14]. In the food industry, resveratrol started to be used in many areas, such as in manufacturing edible films enriched with this compound [15,16] or in the chocolate industry as an antioxidant [17].

Although resveratrol has been successfully extracted from several types of waste from the wine industry (seeds [18], grape skins [19], grape marc [4,20–23], stems [24,25],

leaves [26]), the highest level of resveratrol was determined in the prunings resulting from the annual winter pruning of the vineyard [27].

A wide range of research has shown that vine punings contain a large amount of trans-resveratrol [28]. In pruning waste, the predominant stilbenoids identified were trans-ε-viniferin and trans-resveratrol [24].

Moreover, after the pruning, vine wastes still accumulate this compound [29], due to structural genes, 4CL, PAL, C4H, and STS (forming trans-resveratrol), which are still active, the content of trans-resveratrol rising by up to 40 times post-harvest [30]. Ji et al. (2014) analyzed the content of some parts of vine and grapes and reported that trans-resveratrol content starts rising in the spring months, meeting a maximum level in autumn, and then declines with the reduction in temperatures [27].

Both the plant growth conditions (climatic conditions and plant management) as well as the type and variety of *Vitis genus* significantly influence the composition and concentration of stilbenes in the shoots. The content of stilbene can fluctuate depending on the variety, although the climatic conditions of agricultural cultivation of vines are the same. This is because stilbenes are a part of the plant's protection to pathogen infecion, and varieties resistant to these infections will synthesize a greater amount of stilbenes [31].

Grapes (*Vitis* spp.) are one of the world's largest fruit crops, with 74.5 million tons cultivated in 2014 [16]. The cultivation of vines annually generates significant amounts of viticulture waste, such as shoots, tendrils, leaves and wood, with an estimated amount of 1–5 t per hectare per year [30]. These wastes can represent a source of bioactive substances, especially due to the fact that they are usually composted or burned for disposal [1]. Polyphenols were extracted often from pruning wastes using different techniques, supercritical fluid extraction method being used lately [32,33].

Lately, due to a rising widespread consumption of grapes and the masive waste generation, multiple uses were found for this waste. Vine prunings, pomace and grape seeds are valuable wastes, rich in polyunsaturated fatty acids, phenolics, vitamin E and phytosterols, with uses in the food industry for preservation or nutritional value, for the production of bio-fuel, aclohols, animal feed and fertilizers [34]. Extraction of resveratrol from puning wastes is one of the uses among many others.

Due to the promising health benefits of this antioxidant and the high amount contained by pruning wastes, an optimization of extraction parameters is necessary due to economic reasons and industrialization practice.

The abundance of trans-resveratrol in vine shoots such as routine pruning, has already been demonstrated in numerous studies over the last decade [31].

Statistical analysis was made with Box–Behnken design, which is a proficient and effective statistical method to obtain the optimized experimental conditions, despite the effect of various process variables such as solid–liquid ratio, type of solvent, maceration time, granulosity and number of extractions [35]. The aim of this study was to determine the optimal experimental parameters for the extraction of trans-resveratrol in order to obtain a higher yield from the material of the vine prunings due to the growing demand on the commercial preparation of compounds with therapeutic effects.

2. Materials and Methods

2.1. Vine Pruning Samples

Vine pruning samples Fetească Neagră variety were collected from region Cotnari, Iași on 7 November 2020.

2.2. Reagent and Chemicals

Diethyl ether and the trans-resveratrol standard (99% GC) was bought from Sigma-Aldrich (Hamburg, Germany). Acetonitrile, methanol and ethanol (LiChrosolv for HPLC) were purchased from Merk (Darmstadt, Germany). The PTFE membrane filters with 0.22 µm dimension were obtained from Phenomenex (Torrance, CA, USA).

2.3. Standard Solutions

A set of 11 standards diluted in methanol of different concentrations were prepared to obtain the calibration curve. Resveratrol standards were kept in brown glass jars at −20 °C, away from exposure to light to prevent the phenomenon of photochemical isomerization, which resveratrol is susceptible of.

2.4. Sample Collection and Processing

The prunings samples collected in November 2020 were stored for 12 weeks at 20 °C until they were dry. After that, the samples were dried in a thermostat at 45 °C for 24 h. Then, the samples were grinded in a laboratory grinder and the powder obtained was kept in a plastic container until maceration, away from direct sunlight and moisture.

2.4.1. Solvent Selection

The powder obtained was macerated with 35 different solvents, 5 mL/g of the powdered material for 72 h at room temperature in darkness. The selected solvents are as follows: 10% ethanol (Et), 20% Et, 30% Et, 40% Et, 50% Et, 60% Et, 70% Et, 80% Et, 90% Et, 96.6% Et, 10% methanol (Met), 20% Met, 30% Met, 40% Met, 50% Met, 60% Met, 70% Met, 80% Met, 90% Met, 99.9% Met, 10% acetone (Ac), 20% Ac, 30% Ac, 40% Ac, 50% Ac, 60% Ac, 70% Ac, 80% Ac, 90% Ac, 99.9% Ac, Met-HCl 99:1, Met-HCl-H_2O 1:88:19, ethanol-diethyl ether (Et-Diet) 1:4, Et-Diet 1:1 and Et-Diet 4:1. The extract obtained was then filtered through filter paper and kept at 4 °C until use in analysis.

2.4.2. Solid–Liquid Ratio

The solid-to-liquid ratio was varied to determine the optimal parameters of extraction. The powdered material was macerated with ethanol:diethyl ether 4:1 ratio for 72 h at room temperature in darkness with the ratio of solvent as follows: 1:5 (5 mL solvent/g of the powdered material), 1:10, 1:15, 1:20, 1:25, 1:30, 1:35, 1:40, 1:45 and 1:50. The extract obtained was then filtered through filter paper and kept in a refrigerator at 4 °C until use.

2.4.3. Maceration Time

The powdered material was macerated with ethanol:diethyl ether 4:1 ratio with 35 mL/g of the powdered material for 1, 2, 3, 4, 5, 6 and 7 days at room temperature in darkness. The extracts were collected every day, filtered through filter paper and stored at refrigeration temperature.

2.4.4. Granulosity Selection

The powdered material was sieved in stirring sieve system (Sieve shaker Retsch, Haan, Germany) and then was macerated with ethanol:diethyl ether 4:1 ratio with 35 mL/g of the powdered material for 4 days. The granulosities used were particles larger than 1000 µm, particles between 1000 µm and 710 µm, 710 µm–630 µm, 630 µm–500 µm, 500 µm–350 µm, 350 µm–250 µm, 250 µm–200 µm, 200 µm–180 µm, 180 µm–125 µm and smaller than 125 µm. A sample was taken from each type of powder, depending on the granulosity, and was macerated with ethanol:diethyl ether 4:1 ratio with 35 mL/g for 4 days. The extracts obtained were then filtered through filter paper and kept at 4 °C until use.

2.4.5. Number of Extractions on the Same Material

The powdered material with a granulosity of particles between 500 µm–350 µm was macerated with ethanol:diethyl ether 4:1 ratio with 35 mL/g of the powdered material for 4 days. After 4 days, the extract was filtered through filter paper and the remaining powder was then extracted in the same conditions 9 times. All the 10 extracts were kept at 4 °C until use in analyses.

2.5. Analytical HPLC Procedure

Prior to HPLC analysis, the extracts were partially purified. Using a rotary evaporator (Rotary evaporator with water bath HS-2005S-N HAHNSHIN-Korea) the extracts were evaporated closer to dryness and then were resolved in diethyl ether. The diethyl ether layer was extracted three times with 5% sodium bicarbonate solution in a separatory funnel and then was recovered, rotary evaporated closer to dryness and resolved in methanol. The final extract obtained was filtered through 0.22 µm PTFE membrane filters.

The analysis of the resveratrol content was carried out with the HPLC chromatograph (Shimadzu, Kyoto, Japan), with SPD-M-20A photodiode array detector equipped with a Phenomenex Kinetex 2.6 µm Biphenyl 100 Å 150 × 4.6 mm HPLC column, thermostated at 20 °C. The injection volume was 8 µL.

Elution was executed by a method described by Marshall et al. (2012) with some modifications, with a solvent system composed of two solvents: pure water (solvent A) and acetonitrile (solvent B). A binary gradient was used 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. The analysis conditions were a total running time of 49.5 min and a solvent flow rate of 0.5 mL/min [36].

The standard calibration curves were obtained from peak area and had a high degree of linearity ($R^2 > 0.99$). For data collection and processing, the version 1.21 version of LC solution software was used (Shimadzu, Kyoto, Japan). Analyses were performed in duplicate.

2.6. Statistical Analysis

The results with trans-resveratrol content of the extracts were entered to analysis of variance (ANOVA) using XLSTAT (trial version, Addinsoft Inc., New York, NY, USA) using the Fisher's test at the 95% confidence level ($p < 0.05$).

The second experiment was conducted in a three-factor full factorial design according to Box–Behnken design. Each independent variable (solid–liquid ratio, time, granulosity) had at least 3 levels, as follows: ratio (1:20, 1:35 and 1:50 g/mL), time (2, 4 and 6 days) and granulosity (>1000 µm, 500–350 µm and <125 µm). Design Expert 12 (trial version, Stat-Ease Inc., Minneapolis, MN, USA) was used for the full factorial design.

3. Results

3.1. Statistical Analysis of Variance

The analysis of variance (ANOVA) for trans resveratrol content of the extracts are presented in Table 1.

Table 1. Analysis of variance for trans-resveratrol content for the samples extracted with different solvents.

Solvent Type	Resveratrol, mg/kg D.W.	F-Value
Et 10%	0.29 (0.007) ^{af}	1×10^7 ***
Et 20%	0.46 (0.024) ^{ae}	
Et 30%	1.27 (0.017) ^{z}	
Et 40%	1.58 (0.018) ^{w}	
Et 50%	3.11 (0.039) ^{t}	
Et 60%	3.28 (0.033) ^{s}	
Et 70%	18.51 (0.012) ^{o}	
Et 80%	66.86 (0.012) ^{h}	
Et 90%	39.00 (0.014) ^{l}	
Et 96%	55.11 (0.014) ^{k}	
Met 10%	0.44 (0.027) ^{ae}	
Met 20%	1.27 (0.028) ^{z}	
Met 30%	1.13 (0.024) ^{aa}	
Met 40%	0.95 (0.012) ^{ad}	
Met 50%	1.00 (0.010) ^{ac}	
Met 60%	1.93 (0.012) ^{v}	

Table 1. *Cont.*

Solvent Type	Resveratrol, mg/kg D.W.	F-Value
Met 70%	5.73 (0.031) ^{p}	
Met 80%	33.86 (0.012) ^{m}	
Met 90%	75.30 (0.020) ^{f}	
Met 99.9%	84.06 (0.015) ^{d}	
Acet 10%	1.34 (0.014) ^{y}	
Acet 20%	1.48 (0.012) ^{x}	
Acet 30%	1.05 (0.011) ^{ab}	
Acet 40%	2.02 (0.012)^{u}	
Acet 50%	1.94 (0.011) ^{v}	
Acet 60%	4.72 (0.018) ^{r}	
Acet 70%	4.90 (0.025) ^{q}	
Acet 80%	18.69 (0.018) ^{n}	
Acet 90%	66.46 (0.020) ^{i}	
Acet 99.9%	61.29 (0.012) ^{j}	
Met-HCl 99:1	92.06 (0.013) ^{c}	
Met-HCl-H_2O 1:88:19	67.84 (0.012) ^{g}	
Et-Diet 1:4	76.80 (0.009) ^{e}	
Et-Diet 1:1	92.12 (0.012) ^{b}	
Et-Diet 4:1	147.14 (0.011) ^{a}	

(Mean values ± standard deviation), a–z—different letters in the same column indicate significant differences between samples ($p < 0.0001$) according to the LSD test with $\alpha = 0.05$. *** $p < 0.001$. (Et = ethanol; Met = methanol; Acet = acetone; Et-Diet = ethanol-diethyl ether).

3.2. Solvent Selection

For appraising the efficacy of solvents to extract trans-resveratrol, the requirement was that the solvent efficiently extracts the intended compound from vine shoots material. The extraction yield of trans-resveratrol from pruning waste for 35 different solvents is presented in Figure 1.

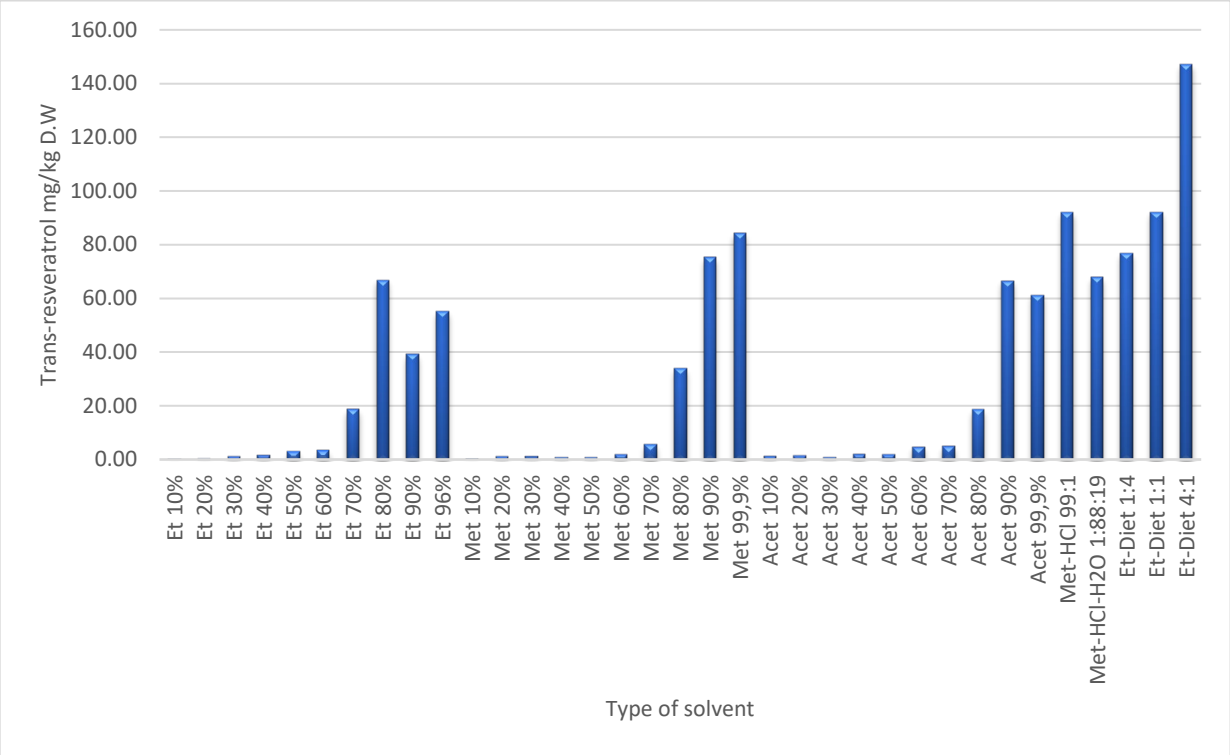

Figure 1. Extraction yield of resveratrol of different solvents from vine shoots of Fetească Neagră variety.

3.3. Solid–Liquid Ratio

Ten different solid–liquid ratio of maceration solvents was tested in order to identify the one who will offer a better extraction yield of resveratrol from vine shoots and the results are presented in Figure 2.

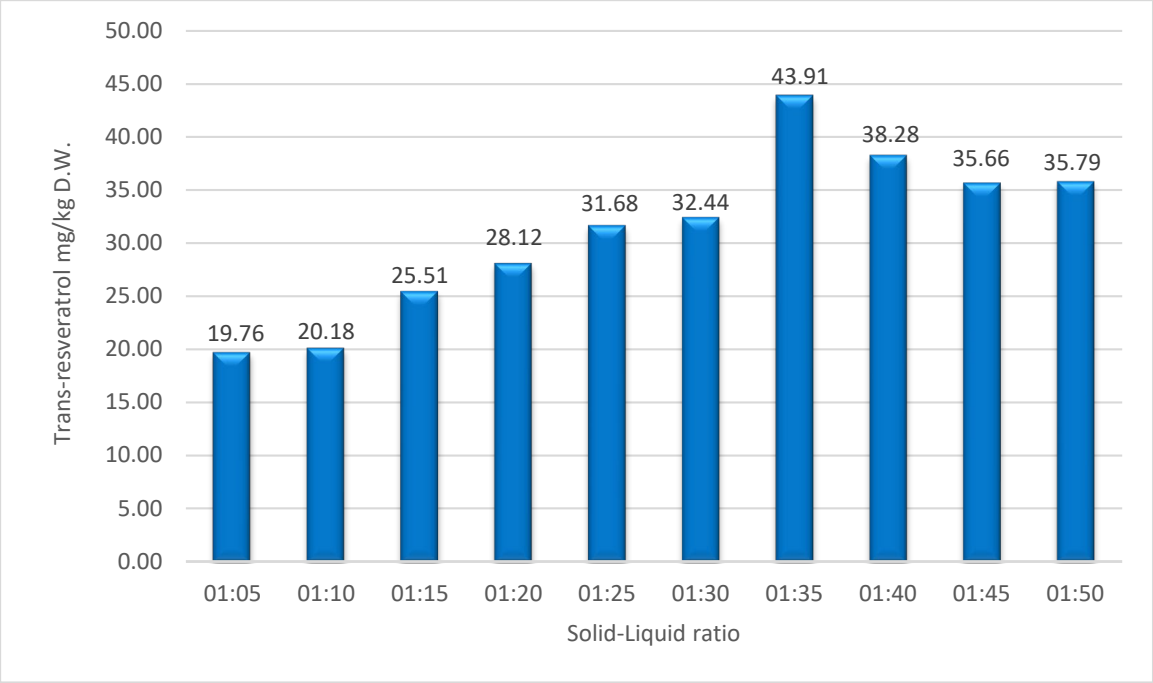

Figure 2. Extraction yield of resveratrol from vine shoots for different solid–liquid ratio.

3.4. Time of Maceration

Figure 3 shows the high difference in extraction yield of resveratrol depending on the extraction time.

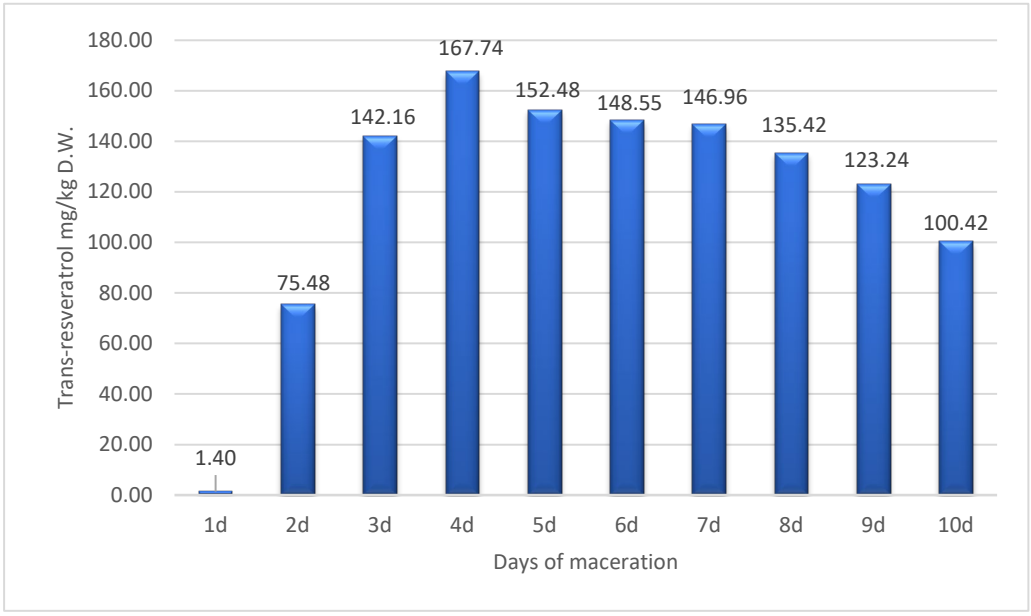

Figure 3. Extraction yield of resveratrol from vine shoots for different time of maceration.

3.5. Granulosity Selection

The extraction yield should increase by the reduction of the particle size because in that case, the superficial area available is larger and facilitates the mass transfer [37]. The results of resveratrol extraction are presented in Figure 4. and the grinded pruning waste were divided into 10 categories according to the size of the particles, as shown in Figure 5.

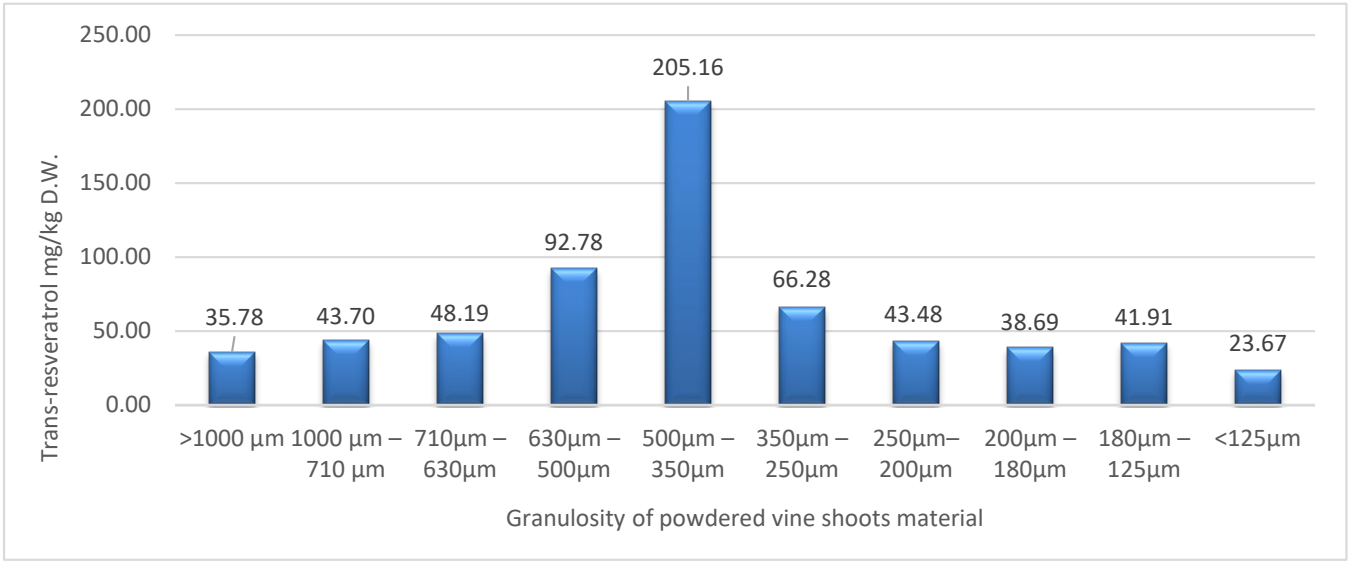

Figure 4. Extraction yield of resveratrol for different granulosity of the powdered material of vine shoots.

3.6. Number of Extractions on the Same Material

Seven extractions were performed on the same material in an attempt to improve extraction yield and to reduce the loss by recovering any resveratrol that could not be extracted on the first extraction and the results are shown in Figure 6.

3.7. Optimization of Extracting Parameters

The experimental and coded values presented in Table 2. were used to analyze the variance with Box–Behnken design. The measured response and the predicted response obtained are shown in Table 3 and the 3D graphs of trans-resveratrol content evolution after extraction are shown in Figure 7.

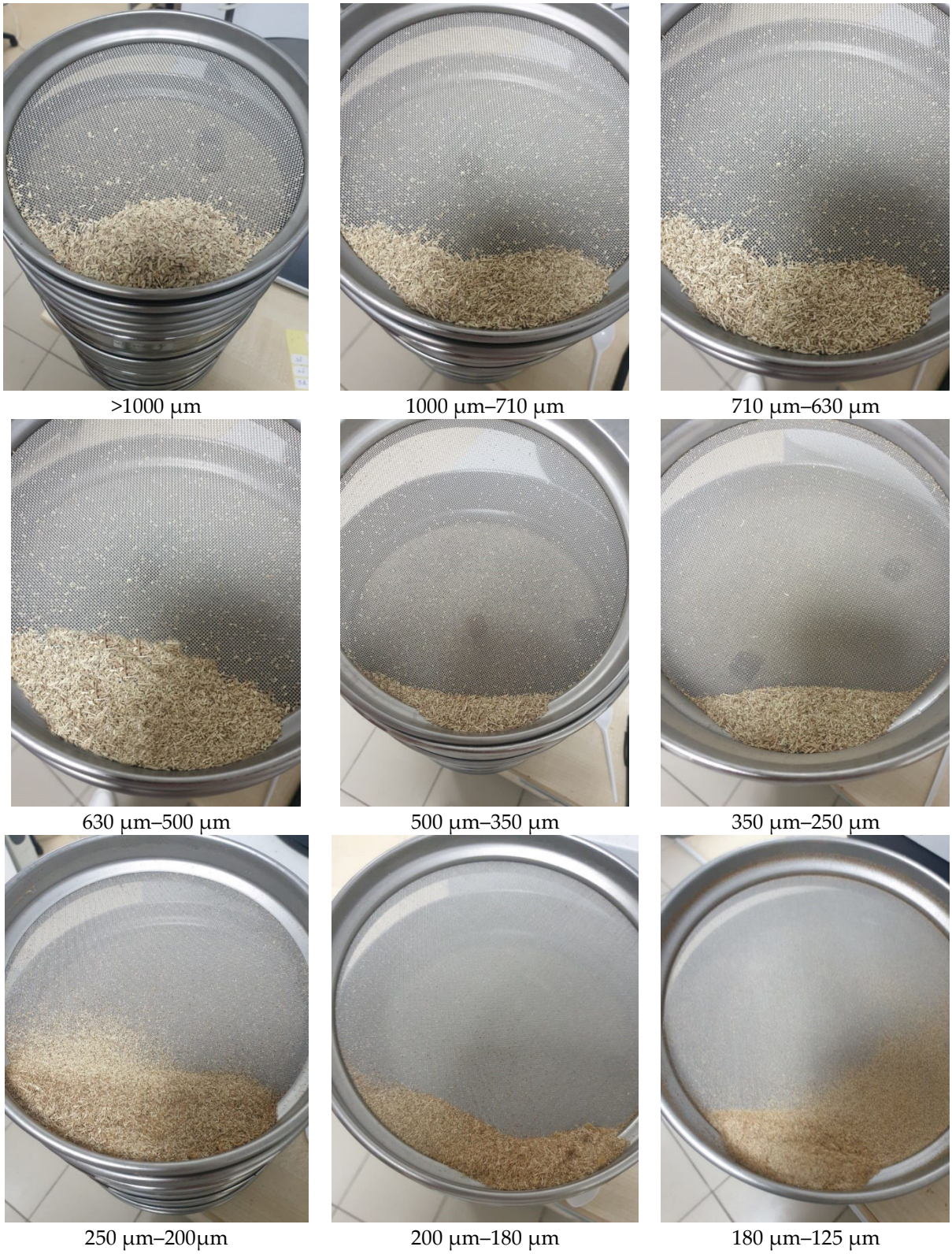

Figure 5. *Cont.*

<125 µm

Figure 5. Types of granulosity.

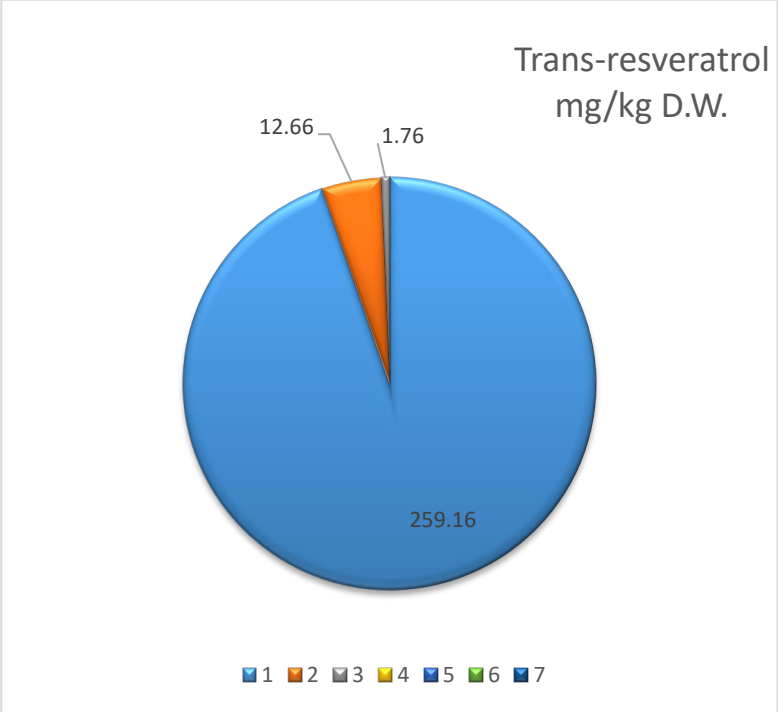

Figure 6. Extraction yield of resveratrol after 7 extractions on the same material.

Table 2. Experimental and coded values according to Box–Behnken design.

Factor	−1	0	1
Ratio S/L (g/mL) A	1:20	1:35	1:50
Time (days) B	2	4	6
Particle size intervals (µm) C	<125	500–350	>1000

Table 3. Measured response and predicted response according to Box–Behnken design.

No.	Ratio S/L (g/mL)	Time (Days)	Particle Size Intervals (μm)	Measured Response	Predicted Response
1	1:35	6	1 mm	148.55	147.43
2	1:20	2	500	67.13	91.65
3	1:20	4	1	43.91	42.95
4	1:20	6	500	43.70	45.78
5	1:50	2	500	128.12	126.04
6	1:50	4	125	89.17	90.13
7	1:35	2	125	108.64	109.76
8	1:20	4	125	92.35	66.71
9	1:50	4	1	158.98	184.62
10	1:20	2	1	184.39	160.83
11	1:20	6	125	104.2	127.76
12	1:50	6	500	201.02	176.50
13	1:35	4	500	205.16	205.16

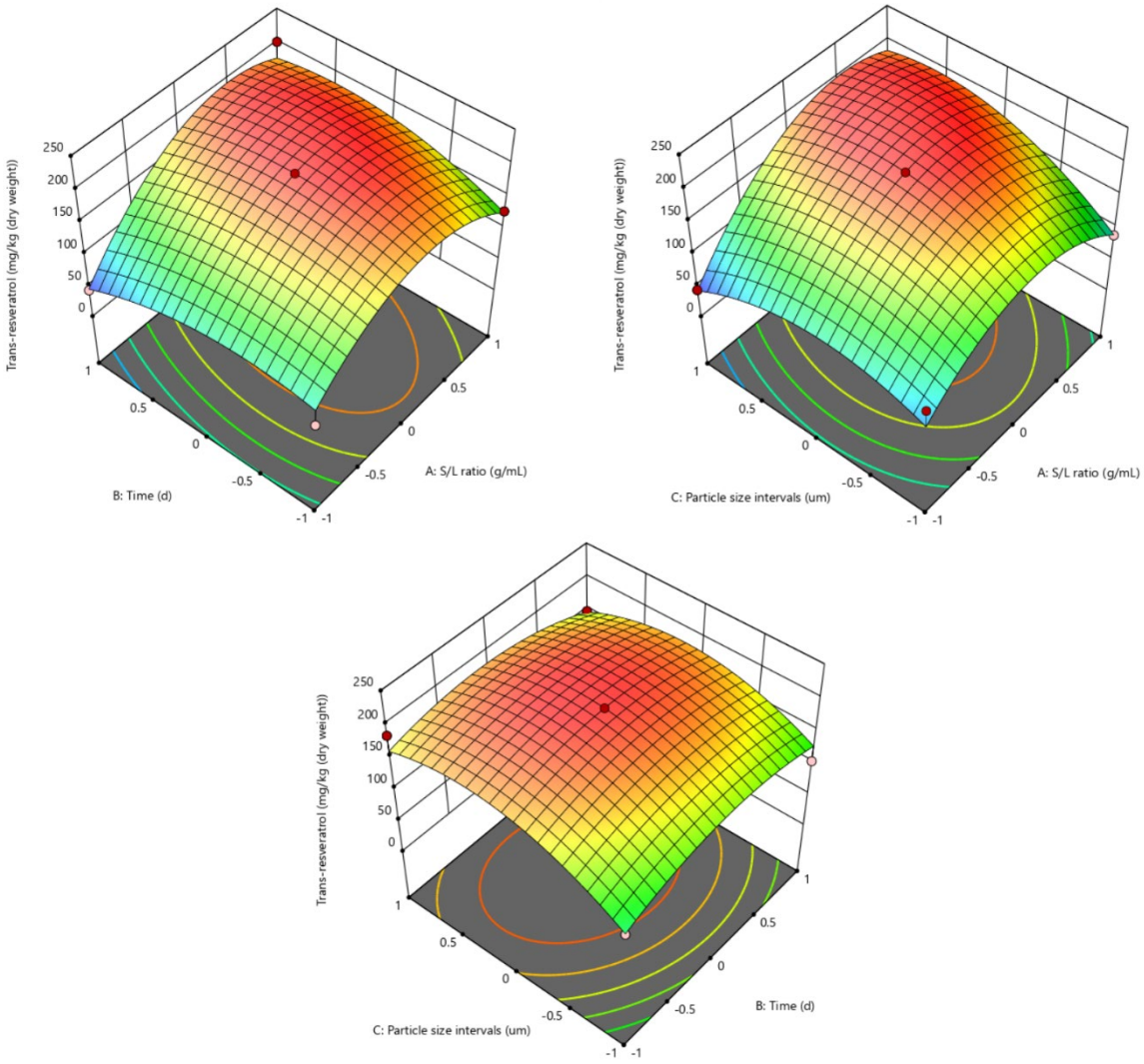

Figure 7. Three-dimensional graphs of trans-resveratrol content evolution after extraction with ethanol:diethyl ether 4:1 ratio.

4. Discussion

4.1. Solvent Selection

Figure 1 presents the result of the extraction of resveratrol by different solvents. Among the extractions with different concentrations of ethanol, the 80% ethanol solvent stood out with the highest content of resveratrol extracted (66.86 mg/kg dry weight (D.W.)). Of the methanol solvents, 99.9% methanol extracted the highest concentration of resveratrol (84.06 mg/kg D.W.), exceeding 80% ethanol solvent. Series 10–99.9% acetone solvents had the lowest concentration of resveratrol after extraction. By adding 1% HCl to the 99.9% methanol solvent positively improved the extraction yield, reaching 92.06 mg/kg D.W. By adding 19% water to this solvent, the extraction yield dropped. Among the solvents with ethanol–diethyl ether in different ratio, the ethanol–diethyl ether 4:1 ratio stood out, obtaining the highest concentration of resveratrol (147.14 mg/kg D.W.), exceeding all solvents.

In the work of Rayne et al. (2008) a broad spectrum of protic and aprotic solvents were examined for extracting trans-resveratrol. Aprotic solvents gave optimum yields at solvent dielectric constants ($\varepsilon/\varepsilon_0$) between 36 and 42. That indicates that any solvent mixture giving this volumetrically averaged ($\varepsilon/\varepsilon_0$) range would give an optimum analyte yield. This optimal polarity range matches up with solutions of 70:30 and 80:20 ethanol:water (v/v) for protic systems. Changes in the protic solvent polarity from this optimum quickly reduced trans-resveratrol yields [38]. This could explain the higher yields of trans-resveratrol for 80:20 ethanol:water among the ethanolic solvents.

Soural et al. (2015) compared methanol and acetone as extraction solvents for resveratrol from vine shoots. Their findings are in agreement with the results of this study, as they conclude that the extraction yield is decreasing when acetone is used. The highest difference between methanol and acetone resulted when extractions were performed on cut vine shoots at laboratory temperature, in which case the extracts obtained with acetone solvents had a very low content of trans-resveratrol in comparison with the extracts obtained with methanol [39].

A few studies are focused on establishing the optimal solvents for trans-resveratrol extraction, and the majority tend to search for the optimal solvents for extracting all polyphenols or stilbenes from grape cane [40] and grape pomace [41]. Therefore, all of them recommend ethanol and methanol as extracting solvents in different concentrations (60–80%). Romero-Perez et al. (2001) established in their study that the optimal solvent for extracting trans-resveratrol from grape berry skins is ethanol 80% and the lowest concentration was obtained using acetone 99.9% [42].

The result that shows resveratrol is more soluble in solutions with a higher content of alcohol were confirmed by Angelov et al. (2016), who separated the vine stems extract in two fractions, containing either ethanol or water-soluble compounds. The aqueous fraction was essentially free of resveratrol and the ethanol fraction had 284.6 mg/kg trans-resveratrol [2].

The most frequent solvents used for the extraction of resveratrol from pruning wastes were ethanol 80% [1,42,43] and methanol 80% [39]. Although methanol offers high extraction yields due to the fact that it is a polar organic solvent, it is not a selective solvent for polyphenols [37]. Resveratrol has also been extracted from the bark of *Pinus sibirica* [44] and from the bark of spruce *Picea mariana* [45] by using diethyl ether with great results.

Furthermore, despite the fact that it is a fairly nonpolar solvent, diethyl ether is more recommended for extraction due to the selectivity for polyphenols, including trans-resveratrol. In addition, due to the selectivity for polyphenols, this solvent eases the further steps of the purification methodology [46].

In the attempt to partially purify the dry extracts of vine shoots obtained by maceration with 80% ethanol solvent, Aavikasaar et al. (2003) reported that these were insoluble in diethyl ether. To overcome this issue, diethyl ether was added to the extraction solvent in a 1:4 ratio, resulting in the appearance of a precipitate and facilitating the decrease in brown colored impurities, without the loss of stilbenoids [28]. Although the solvent diethyl

ether was used in the prepurification procedure to precipitate the impurities, in this study it was used as a solvent for maceration to establish the selectivity for trans-resveratrol. Surprisingly, this solvent managed to extract the largest amount of resveratrol when it was used together with ethanol in a ratio of 1:4. The solvent used, 80/20 (*v*/*v*) ethanol/diethyl, is in the range of 70–80% ethanol, a range that has been reported as having a high extraction affinity to resveratrol [38].

4.2. Solid–Liquid Ratio

As it can be seen in Figure 2, 1:35 liquid–solid ratio seemed to suit the resveratrol extraction perfectly, providing the highest concentration. This fact is confirmed by the results obtained for the other extractions, who showed an increase for resveratrol content from 1:5 liquid–solid ratio to 1:35 liquid–solid ratio, and decreased after that, until 1:50 liquid–solid ratio.

Preliminary studies have established that, to provide an effective interphase contact for an optimal wetting of raw material, a ratio of 5:1 solvent/solid is necessary (hydro module). From this value upwards the solubility constraints because of insufficient quantity of solvent are prevented [2].

Garcia et al. (2016) reported an optimal solid–liquid ratio of 1:40 in their attempt to extract resveratrol from peanut grass *Aracis repens* with the highest resveratrol content of 3.93 ± 0.35 mg/L. This result is near to the result obtained for vine shoots [47].

4.3. Time of Maceration

After one day, the degree of resveratrol extraction was the lowest. The resveratrol content of the extract obtained on day 3 was double that of the previous day. The highest concentration of resveratrol was obtained on day 4—167.74 mg/kg D.W. After the 4th day, the content of resveratrol extracted decreased, continuing to decline in the other days.

Soural et al. (2015) tested different temperatures and different maceration times for the extraction of resveratrol from vine shoots with different solvents. The time intervals tested for maceration at laboratory temperature were 8 h, 2.4 and 7 days. The results obtained after this test showed an increasing slope for the concentration of resveratrol until day 4, the day when the maximum amount of resveratrol extracted was reached, after which it started to decrease slightly. This was observed for both solvents used in the extraction, methanol and acetone [39]. These results are in agreement with the result of this study.

The effect of the contact time (respectively 1, 4, 7 and 10 days) and variation of ethanol concentration of the solvent (0, 4, 7.5 and 13%) on extraction of the phenolics was tested by Gambuti et al. (2009) on the skins and seeds of the grapes of Uva di Troia and Aglianico variety (*Vitis vinifera*). The results indicate that many compounds reach a maximum concentration on 4th day of maceration, compounds such as anthocyanins, vanillin reactive flavones, phenolics and proanthocyanidins [48]. These results validate the findings of this study.

4.4. Granulosity Selection

The material of the grounded vine shoots was sieved in 12 separate sieves and was macerated under the same conditions and with the same solvent to determine whether the granulosity of the material has an effect on the extraction content of trans-resveratrol (Figure 5).

Figure 4 shows that there were significant disparities in yield depending on the granulosity of the material, highlighting the material with 500 µm–350 µm granulosity, with a yield of 205.16 mg/kg D.W. Unexpectedly, the lowest yield (23.67 mg/kg D.W.) was recorded for the least granular material—125 µm. This is unexpected, because the surface area for mass transfer is larger than that of other materials. An amount of 55.1% of the chemical composition of vine prunings is represented by holocellulose (31.9% α-cellulose and 23.2% hemicellulose), followed by lignin, with 38.5% [49]. These differences in the extraction yield can be attributed to the non-uniformity of the chemical composition in the

composition of the shoots. This result highlights the fact that it is not necessary for the solid material to be grinded to fine particles in order to obtain a superior extraction yield. Thus, in the process of obtaining trans-resveratrol from vine shoots, the grinding of the material can be carried out through an energy-saving process by grinding the material up to 350 µm.

Soural et al. (2015) compared the resveratrol content of extracts for cut material (~1 cm) and powdered material (>1 mm) from vine shoots using methanol and preceding the extraction at laboratory temperature. Although the dimensions of the extraction material used in this study are different, a slight positive difference was recorded (4189.0 µg/g D.W. for the cut material and 4109.0 µg/g D.W. for the powdered material) [39].

4.5. Number of Extractions on the Same Material

The second extraction managed to extract another 5% resveratrol content compared to the first extraction, and the third only 0.67%. The next four extracts did not contain resveratrol (Figure 6).

From an economical point of view, further extractions on the same material are not advantageous. The use of solvents, time and manufacture practice are not economical for the second and third extraction, which together add up to only 5.67% out of the total content of trans-resveratrol from vine shoots. This attempt to increase the amount of trans-resveratrol extracted from the same material highlights the fact that the optimal parameters of the first extraction are well chosen, thus extracting 94.33% of the amount of resveratrol available.

Soural et al. (2015) conducted multiple Soxhlet extractions with methanol on vine shoots and compared the extraction yield of stilbene for each extraction. The yield of stilbene for the second extraction is under 5%, and the yields of the third and further extractions were under 1% [39]. These results are the same with the result obtained by conventional maceration with diethyl ether–ethanol 1:4 ratio.

4.6. Optimization of Extracting Parameters

The experimental results of trans-resveratrol content at different solid–liquid ratios (1:20, 1:35 and 1:50 g/mL), time (2, 4 and 6 days) and granulosity (<125, 500–350 µm and >1000 µm) were placed to the quadratic equation using the response surface analysis. The equation for trans-resveratrol content is displayed in Equation (1):

$$\begin{aligned}\text{Trans} - \text{resveratrol} &= 205.16 + 41.27 \times A + 1.15 \times B + 17.68 \times C + 24.08 \times AB + 29.56 \times AC - 7.85 \times BC \\ &-67.75 \times A^2 - 27.41 \times B^2 - 41.30 \times C^2\end{aligned} \quad (1)$$

where A—solid/liquid ratio g/mL, B—Time, C—particle size interval.

The analysis of variance indicated that the quadratic regression model is statistically significant ($p < 0.05$) with a coefficient of regression $R^2 = 0.90$. Figure 7 displays the 3D evolution of trans-resveratrol content in the function of the parameters employed (Table 2).

According to Figure 7, the highest trans-resveratrol content is recorded at the correlation of the following parameter values 1:35 g/mL solid:liquid ratio, 4 days with 500–350 µm (148.55 mg/kg D.W.), having as extraction solvent ethanol:diethyl ether in a ratio of 4:1. At these conditions, the predicted response for the optimized value is the same as the measured response, namely a resveratrol content of 205.16 mg/kg dry weight. The lowest value was observed at 1:20 g/mL solid–liquid ratio, 6 days with 500–350 µm (43.70 mg/kg D.W.). These results are in compliance with those reported by Soural et al. (2015) on the extraction of resveratrol from vine shoots [39] and Gambuti et al. (2009) on the extraction of resveratrol from skin and seeds of grape [48], regarding obtaining a maximum yield on day 4 of extraction. Additionally, Garcia et al. (2014) reported an optimum solid–liquid of sample/solvent ratio of 1:40 for extracting resveratrol from peanut grass *Aracis repens* [47], results that are near the results obtained, namely an optimal ratio of 1:35 solid/liquid.

The predicted optimum conditions for trans resveratrol extraction (Table 3) were very similar to the experimental concentration achieved under these conditions. Any increase or decrease in the value of the extraction parameters negatively affected the extraction yield.

5. Conclusions

Numerous studies have highlighted the evidence of the therapeutic effect of trans-resveratrol on the human body. Due to the fact that vine wastes are recently considered a valuable source of this compound through the high amount that they contain, the optimization of the extracting parameter is needed. Various extracting conditions were applied to determine the optimal method.

The results of the present study showed that the solvent ethanol:diethyl ether in a ratio of 4:1 is recommended for the extraction of trans-resveratrol from vine shoots, obtaining by its use the highest yield compared to other solvents (147.14 mg/kg D.W.). By increasing the diethyl ether quantity, the extraction yield was negatively affected. Acetone was a weak solvent for extracting trans-resveratrol, resulting in lower quantities extracted. Among the ethanol and methanol series, Et 80% (66.86 mg/kg D.W.) and Met 99.9% (84.06 mg/kg D.W.) had a good yield, but it was almost half that of the solvent with diethyl ether. Adding HCl to Met 99.9% positively improved the extraction yield, being the second solvent by the quantity extracted (92.06 mg/kg D.W.).

Among the different reports of the solid liquid, 1:35 liquid:solid ratio had extracted the highest content of trans-resveratrol (43.91 mg/kg D.W.). The optimal time of extraction was 4 days (167.74 mg/kg D.W.) and the optimal granulosity of the grinded vine shoots was 500 µm–350 µm (205.16 mg/kg D.W.).

With the extraction method described above, the yield of trans-resveratrol extraction was 94.33%. Economically, another extraction on the same material is not recommended.

The result obtained was confirmed by optimization of extracting parameters according to Box–Behnken design, in which the predicted response was similar to the obtained response. The highest trans-resveratrol content was recorded at the association of the following parameter values: 1:35 g/mL solid:liquid ratio, 4 days with 500–350 µm (148.55 mg/kg D.W.).

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