Journal homepage: http://fens.usv.ro/index.php/FENS
Journal of Faculty of Food Engineering,
Ştefan cel Mare University of Suceava, Romania
Volume XX, Issue 3-2021, pag. 189 - 196

FOOD AND ENVIRONMENT SAFETY

EXTRACTION AND CHARACTERIZATION OF RESVERATROL FROM VEGETABLE WASTE FROM VINES

***Petronela Gabriela NISTOR[1], Sonia AMARIEI [2]**
[1]*Faculty of Food Engineering, "Stefan cel Mare,, University, Suceava, România,*
nistor.gabi.petronela.96@gmail.com,
*Corresponding author
Received 20th June 2021, accepted 25th September 2021

Abstract: *Vine shoots resulting from vine pruning can become valuable products of interest for many industries. Research conducted to date showed that resveratrol is found in many parts of the vine. The aim of the present study was to determine the resveratrol content in Romanian vine sprouts samples by direct injection into the HPLC instrument coupled with diode array detector after being filtered through 0.45 μm PTFE membrane filters. The resveratrol content varied from 5.44 mg/100 g to 10.27 mg/100 g in ethanolic extracts. For aqueous extract, the resveratrol was found in only two samples in very low concentration. Thise paper aimed to contribute to the research on the capitalization of residual products from viticulture industry.*

Keywords: *sprouts, antioxidant capacity, HPLC, polyphenols, trans-resveratrol*

1. Introduction

Resveratrol is one of the most well-known and studied polyphenols today. Resveratrol is a compound belonging to the stilbenoid class [1-2]. This compound generates so many benefits for the body that one can talk about a miracle of nature. Resveratrol has many biological properties, but the most common biological activity is antioxidant activity. These properties have been used to protect cells against oxidative stress [3-4].

This polyphenols has antibacterial activity, has an anti-inflammatory role, fights diabetes, has an anticancer effect and fights against aging [5]. The importance of this compound is highlighted in several industries, including the food industry, the pharmaceutical industry and the medical industry[6].With a very wide range of biological activities on human body, alternatives are currently being sought out to obtain resveratrol, even from vegetable waste resulting from pruning[7-8].

The waste resulting from the pruning of the vine is a rich source of compounds with phytochemical value, but these are often not recovered[9-10].During the summer cuttings, a considerable amount of green mass is removed, such as portions of different sizes of shoots and leaves[11-12].Resveratrol is available in various concentration in these by-products of pruning, and can be used in various fields of interest for this compound [13].Among the many benefits to human health the following are included: the fight against free radicals through its antioxidant activity, protecting the heart and cardiovascular system, activating the action of sirtuins that fight against aging, increasing nitric oxide production, preventing platelet aggregation, causing

DOI: https://doi.org/10.4316/fens.2021.021

blood clots in the blood vessels[14], decrease in the frequency of polydipsia and preliphagia in patients with diabetes [15].

2. Matherials and methods

The trans-resveratrol standard (99 GC) was purchased from Sigma-Aldrich (Germany), and methanol and acetonitrile were purchased from Merk (Merk KgaA, Darmstadt, Germany). In order to extract resveratrol, vine shoots harvested from a crop located in Mălini, Suceava Country, were used.The plant material was dried in an oven at 45 ºC and grounded with a centrifugal mill. To determine resveratrol, 1 g of vine powder was mixed in 6 vials with 20 ml of ethanol (for each vial separately), and in another 6 vials the mixture was made with 20 ml of water. The concentration of ethyl alcohol used was 80%.

The 12 vials were exposed to ultrasound treatment at diffrent amplitudes (30, 40, 50, 60, 70, 99%), at a temperature above 50 ºC, pulsation of 0:0, for 30 minutes. After the ultrasound treatment, the samples were centrifuged for 10 minutes at 3200 rpm and filtered using qualitative filter paper and stored at -18ºC.

2.1 Determination of the total polyphenol content by Folin Ciocâlteu method

Thus, in 12 test tubes labeled according to the established codes, the following substances were added: 0.2 ml of vine mixture; 2 ml of Folin Ciocâlteu reagent (1:10 g/g) and 1.8 ml of Na_2Co_3 (7.5%). The samples obtained were mixed very well and stored in the dark for 30 minutes. The absorbance was measured at a wavelength of λ=750 nm, using the UV- VIS- NIR 3600 Shimadzu spectrophotometer (Shimadzu Corporation, Japan).

2.2 Determination of the antioxidant activity of the vine extract by the DPPH method

The measurement of the antiradical capacity of the vine shoots was performed by using the stable free radical DPPH. The following substances were added to 13 tubes: 0.5 ml of extract, 0.5 ml of 80% methanol and 5 ml of DPPH reagent.The tubes were shaken very well and stored in the dark for 30 minutes.

The wabsorbance of the samples was measured using a UV-VIS-NIR 3600 Shimadzu spectrophotometer, at the wavelenght of λ=517 nm. The following formula was used to determine the antioxidant activity of vine shoots:

$$RSA\% = \frac{Ablank - Asample}{Ablank} \times 100$$

where *RSA* is the radical scavening activity, $A_{sample}$is the absorbance of the analyzed sample at 517 nm and $A_{blank}$is the absorbance of the blank at 517 nm.

2.3 Determination of resveratrol by high performance liquid chromatography

In order to perform the analysis, a set of dilutions were prepared from the stock solution: 0.025; 0.05; 0.075; 0.1; 0.5; 1; 2.5; 5; 10; 20 and 25 mg/l, respectively. The purified and filtered samples were injected into the HPLC device in an amount of 8 µl, and for analysis an SPD-M-20 A diode array detector was used. Separation was performed on a Phenomenez Kinetex 2.6 µm Biphenyl 100 Å HPLC column 15x4.6 mm thermostated at 200 ºC. Elution was performed with a solvent system consisting of pure water (A) and acetonitrile (B). The solvent flow rate was of 0.5 ml/min.

2.4 Determination of ash content of vine shoots

To determine the ash content, two samples were tested in parallel. In two clean, dry and previously weighed porcelain crucibles, a quantity of 5 g of vine grind was introduced, in a layer as uniform as possible. After weighing, the crucibles were placed in the calcination furnace. The calcination was performed at a temperature of 550 °C, for 9 hours, with the temperature being gradually raised in the calcination furnace. The ash content determined was expressed as percentage to two decimal places and calculated according to the formula:

$$Ash\ content, \% = \frac{m2 - m}{m1 - m} x100$$

where m_2 is the crucible weight with calcined sample, m_1 is the crucible weight with uncalcined sample and the *m* is the weight of the empty crucible.

2.5 Determination of mineral content by energy dispersive X-ray spectrometry

In order to performe this analysis, a Shimadzu X-ray energy dispersive spectroscope (EDX; Shimadzu Corporation, Japan) equipped with test tanks and a PC with the EDX Software application was used. To determine the mineral content of the vine shoots, two samples were calcined in parallel in the calcination furnace, thus finally as a percentage, the calcination being done automatically using the EDX Software application.

2.6 Determination of copper by flame atomic absorption spectroscopy

The device used for analysis was a flame atomic absorption spectrophotometer AA Shimadzu (Shimadzu Corporation, Japan), and the wave length at which the copper was read was 324.07 nm. After tracing the calibration curve, the absorbance of two samples of the vine extract was measured. A Cu cavity cathode lamp was used as the radiation source.The WinLab 32 application automatically calculated the copper concentrations. The concentration determined as mg/l was multiplied by the corresponding dilution.

3. Results and discussion

3.1 Total polyphenol content

The calculation of the total polyphenols in the vine shoots was performed using the calibration curve, and the results were expressed as mg gallic acid (GAE)/100 g vegetable product (sample) (Fig. 1).

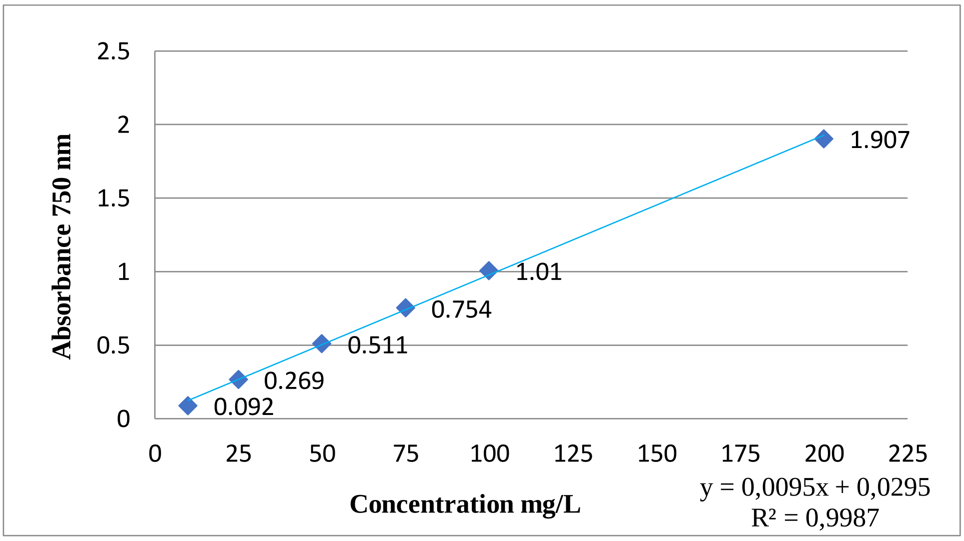

Fig. 1 Calibration curve for total polyphenol content in vine shoots

The results of the total polyphenol content of the first 6 vine samples in which water was used as solvent are shown in Fig. 2. The figure shows that the highest total polyphenol content was determined in sample 6, which was obtained by ultrasound treatment at 70% amplitude for 30 minutes, and the value of the resveratrol content was 62.80 mg GAE/100 g. Samples that were exposed to ultrasound at amplitudes of 50, 40 and 30% had average values of the content of total polyphenols of 30.84, 33.11 and 31.20 mg GAE/100 g vegetable product.Another value that was close to the maximum value of sample 6 was that determined in sample 2, which was extracted at an ultrasound amplitude of 60% and had a polyphenol content of 47.70 mg GAE/100 g plant product.Figure 2 shows that the sample that was extracted at 99% ultrasound amplitude had the lowest value of total polyphenol content, which was of 29.05 mg GAE/100 g plant product.Regarding the total polyphenols content that was determined in the case of ethanolic extractions, in was observed that these values were higher, as follows: for samples c (50:30), d (40:30), e (30:30), f (70:30) and b (60:30) the values of total polyphenol content were 49.50 mg GAE/100 g; 55.06 mg GAE/100 g; 57.36 mg GAE/100 g; 62.80 mg GAE/100 g and 67.52 mg GAE/100 g (Fig. 3).

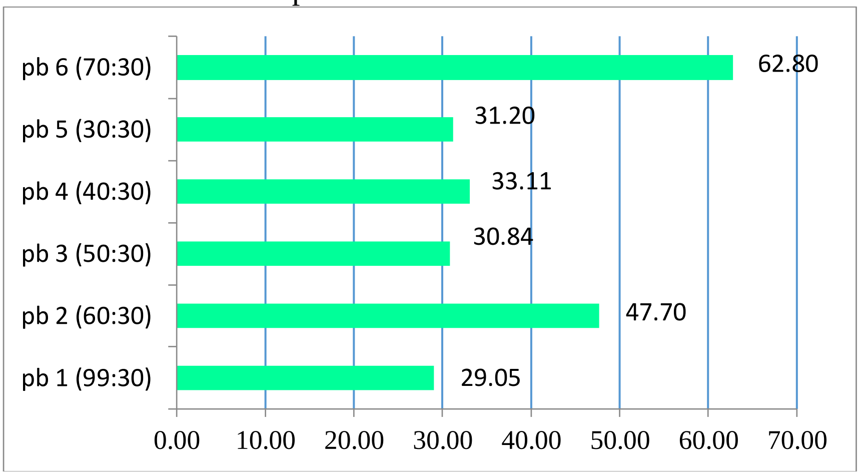

Fig. 2 Total polyphenol content of vine extracts (aqueous extraction)

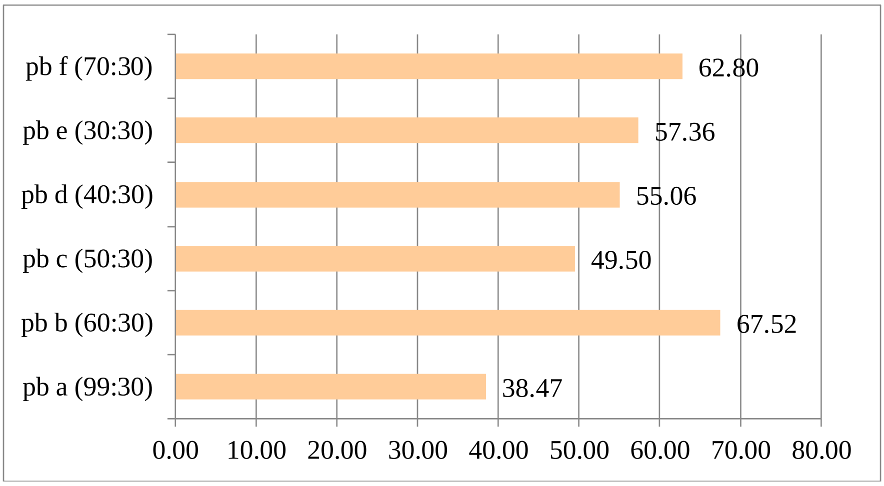

Fig. 3 Total polyphenol content of vine extracts(ethanolic extraction)

By analyzing the results obtained for the two types of extraction, it can be concluded that the samples in which ethanol was used as solvent, had higher values of the total polyphenol content than the samples in which water was used as solvent. Results closer to the total polyphenol content determined in Romanian vine shoots were obtained by Llobera, in 2012 [6].Following the extractions performed with ethanol/water solvent and acetone/water, the highest value was identified in the shoots samples coming from a vine crop on the island of Mallorca, Spain (38,4 mg GAE/100 g vegetable product). The total polyphenol content of 29.4 mg GAE/100 g plant product was identified in the 80% ethanol extraction of red grape shoots, and 22.9 mg GAE/100 g was determined in the acetone extractions 70% and 80% ethanol of the shoots, respectively.

3.2 Antioxidant activity by the DPPH method

Regarding the antioxidant activity, in the case of aqueous extraction the highest percentages were identified for samples 4 and 5 (27.56%), followed by sample 3 (27.01%). The lowest value was obtained for sample 2, and it was 25.46% (Fig. 4). The antioxidant activity of the samples in which ethanol was used as solvent showed the highest value in sample c (28.18%). Samples d and f also presented high values, which were of 28.11% and 28.14%. The lowest antioxidant activity was determined in the case of sample b, and it was 27.97% (Fig. 5).

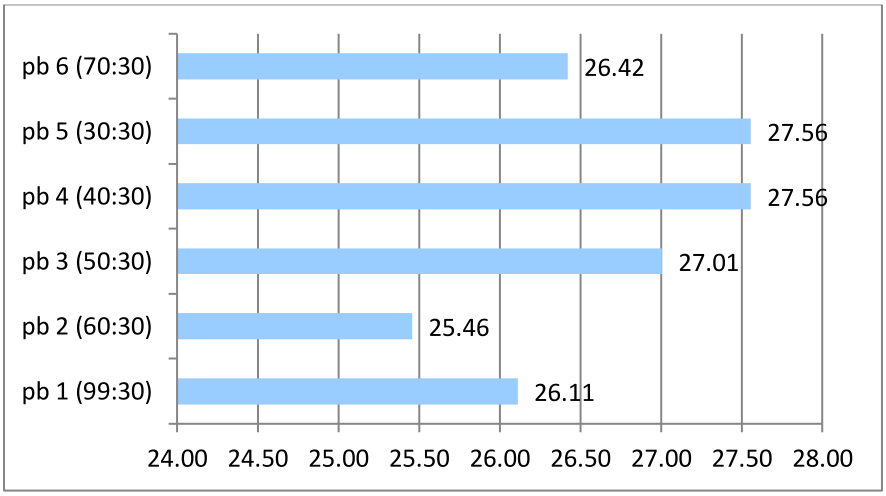

Fig. 4 Antioxidant capacity of vine shoots studied using the DPPH method, %(aqueous extract)

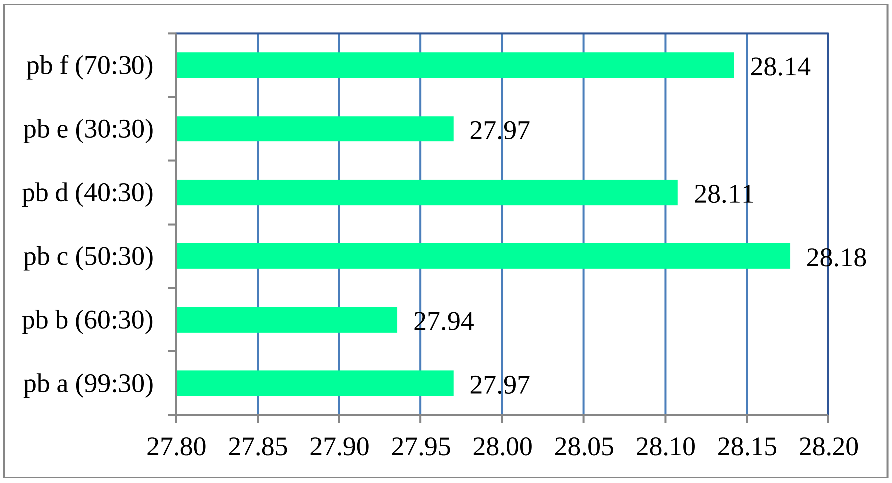

Fig. 5 Antioxidant activity of vine shoots studied using the DPPH method, %(ethanolic extract)

3.3 Resveratrol content determined by HPLC

The standard calibration curve obtained showed high degrees of linearity (R>0.99). The concentration was determined from the equation of the curve in the samples of vine shoots: Y=117661x, and the absorbance at which the resveratrol content was measured was 306 nm (Fig. 6).

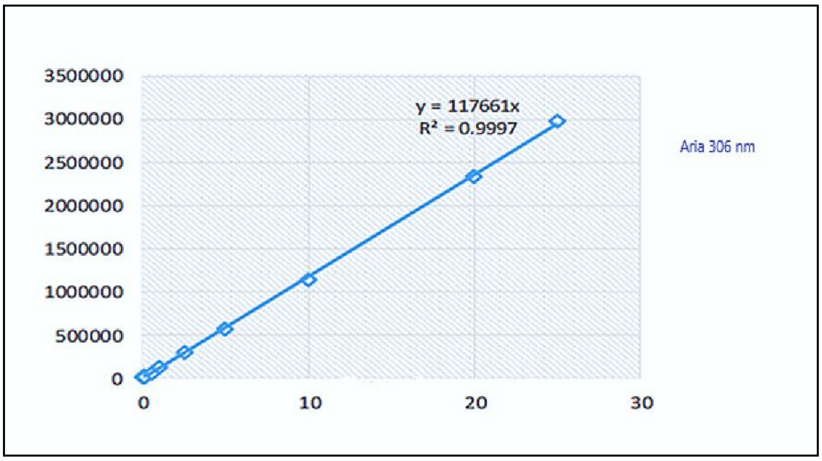

Fig. 6 Calibration curve for resveratrol content expressed as mg/l

The concentration of trans-resveratrol varied from 5.44 mg/100 g to 10.27 mg/100 g for ethanolic extracts, but in the case of aqueous extractsresveratrol was determined only in two samples, where its concentration was of 0.11 mg/100 g(sample 6) and 0.67 mg/100 (sample 2). The highest trans-resveratrol content was cuantified in sample a (10.27 mg/100 g plant product), followed by sample f (9.36 mg/100 g).

Mean values of trans-resveratrol content were identified in samples b (5.44 mg/100 g), c (5.80 mg/100 g), d (6.09 mg/100 g), and e (5.50 mg/100 g).Resveratrol concentration similar to those determined in the analyzed vine shoots was also obtained by Ghaewalova et al., 2018 [7]. The trans-resveratrol content of the shoots in the Czech Republic varied between 3.13 mg/100 g and 13.23 mg/100 g.

3.4 Ash content of vine shoots

Following the calculation performed for the two samples of vine shoots, an ash content of 2.7035 was obtained in the case of the first sample and 2.894 g for sample number 2.

3.5 Minerals concentration in vine shoots

A light-dispersive X-ray spectrometer, the Shimatzu 900 HS model, was used to analyze the ash samples. The concentrations of minerals determined are presented in Table 1. By analyzing the results it can be observed that in the two samples the following elements were found: calcium (Ca), potassium (K), phosphorus (P), sulfur (S), iron (Fe), scandium (Sc), manganese (Mn), strontium (Sr), zinc (Zn), chromium (Cr) and copper (Cu). Among the studied elements, calcium (Ca) had the highest value of 52.37% for sample 1 and 55.70% for sample 2. Another element that predominated in vine shoots was potassium (K) with the values of 35.53% and 33.08% respectively, followed by phosphorus (P) and sulfur (S), for whichthe recorded values were 6.86% and 3.00% and 6.29%, 2.56% for sample 2.

Table 1

Concentration of metals selected from samples 1 and 2 of vine shoots

Analyte	Sample 1	Sample 2
Ca	52.375 %	55.701 %
K	35.537 %	33.081 %
P	6.861 %	6.290 %
S	3.002 %	2.564 %
Fe	0.688 %	0.658 %
Sc	0.680 %	0.811 %
Mn	0.363 %	0.385 %
Sr	0.253 %	0.250 %
Zn	0.130 %	0.127 %
Cr	0.062 %	0.080 %
Cu	0.049 %	0.054 %

3.6 Copper content of vine shoots

The calculation of the copper content in the vine shoots was performed using the calibration curve, and the results were expressed as mg Cu/l. The absorbance of copper was read at the wavelength λ=324,87 nm (Fig. 8). For the first sample a copper content of 0.5675 mg/l was determined, and for the second sample the copper content was 1.2569 mg/l. The copper concentration obtained from the analysis of the vine shoots from Suceava County proved to be relatively lower than the concentration from the shoots from other areas of the country. The values recorded for the copper concentration in the vine shoots coming from the Iași vineyard varied in a rather large range (25.17 mg/kg – 37.47 mg/kg), by comparisson to those obtained in the Murfatlar vineyard, where in the vine shoots it was determined a copper content of 45.84 mg/kg for the variety Sauvignon Blanc, and the Ștefănești Argeș vineyard (Fetească Regală - 38.47 mg Cu/kg) and Târnavelor vineyard where a copper content of 31.84 mg Cu/kg was determined in shoots of the variety Sauvignon Blank[8].

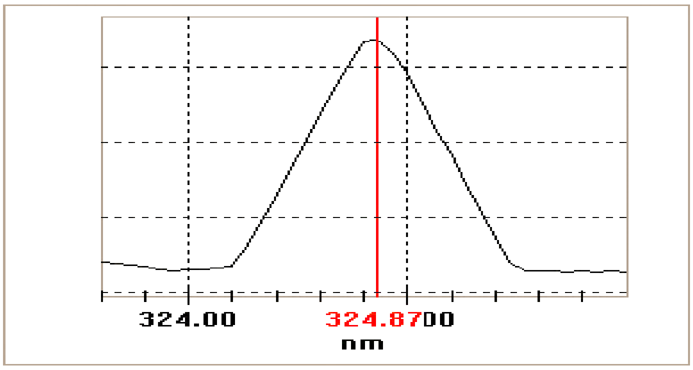

Fig. 7 – Absorption spectrum for copper

4. Conclusion

The experimental results showed that the extraction of total polyphenol was influenced by the type of solvent used and the conditions of the ultrasound treatment applied.

It was found that following aqueous extraction, the values recorded for the content of total polyphenols were much lower than those obtained in the case of ethanolic extraction.The antioxidant activity of the studied vine shoots proved to be relatively lower, with the values recorded for aqueous extraction varying in a range between 25.46 and 27.56%, and those determined for ethanolic extractions from 27.96% to 28.18%.When compared to previous studies, these values were found similar to those obtained for vine shoots in the Czech vineyard.The highest tras-resveratrol content was identified in sample a (10.27 mg/100 g plant product), followed by sample 7 with a content of 9.36 mg/100 g, and the mean values were identified in samples b (5.44 mg/100 g), c (5.80 mg/100 g), d (6.09 mg/100 g) and e (5.50 mg/100 g). The conclusion drawn from this analysis was that even in the case of resveratrol the, ethanolic extraction was much more advantageous than water extraction.The mineral content determined indicated that the samples of vine shoots represented a matrix rich in calcium, potassium and phosphorus, the most abundant being calcium with a value between 52.37% and 55.70%. The copper concentration obtained from the analysis of the vine shoots from Suceava County proved to be relatively lower by comparison to the concentrations detected in the vine shoots from other areas of the Romania.

5. References

[1]. TRISKA J., HOUSKA M., Physical Method of Resveratrol Induction in Grapes and Grape Products –A Review,*Czech I. Food Sci.*, 30: 489-502, (2012).

[2]. KEYLOR M. H., MATSUURA B. S., STEPHENSON C. R. J., Chemistry and Biology of Resveratrol-Derived Natural Products, *Chemical reviews*, 115: 8976-9027, (2015).

[3]. FABJANOWICZ M., PLOTKA-WYSYLKA J., NAMIESNIK J., Detection, identification and determination of resveratrol in wine. Problems and challenges, *Trends in Analytical Chemistry*, 103: 21-33, (2018).

[4]. KISELEV K. V., Perspectives for production and application of resveratrol, *Applied Microbiology and Biotechnology*, 90: 417-425, (2011).

[5]. SALEHI B., MISHRA A. P., NIGAM M., SENER B., KILIK M., SHARIFI-RAD M., FOKOU P. V. T., MARTINS N., SHARIFI-RAD J., Resveratrol: A Double-Edged Sword in Health Benefits, *Biomedicines*, 6: 2-13, (2018).

[6]. LLOBERA A., Study on the antioxidant activity of grape stems (Vitis vinifera). A preliminary assessment of crude extracts, *Food and Nutrition Sciences*,3: 500-504, (2012).

[7]. GHAERWALOVA L., HUTAR D., MASAK J., KOLOUCHOVA I., Antioxidant activity and phenolic content of organic and conventional vine cane extracts, *Food analysis, food quality and safety*, 36 (4): 289-295, (2018).

[8]. https://docplayer.ro/145228002-Microsoft-powerpoint-prezentare-faza-v-ader-pptx.htmlAcccessed June. 24, 2021.

[9]. YANG T., WANG L., ZHU M., ZHANG L., Properties and molecular mechanisms of resveratrol: a review, *Pharmazie*, 70: 501-506, (2015).

[10]. SILVA P., SUREDA A., TUR J., A., ANDREOLETTI P., CHERKAOUI-MALKI M., Latruffe N., How efficient is resveratrol as an antioxidant of the Mediterranean diet, towards alterations during the aging process?, *Free radical reasearch*, 53 (1): 1101-1112, (2019).

[11]. SENILA B., NEAG E., TOROK I., CADAR O., KOVACS E., ȚENU I., ROMAN C., Vine shoots waste – new resources for bioethanol production, *Romanian Biotechnological Letters*, 25 (1): 1253-1259, (2020).

[12]. RATZ-LYKO A., AND ARCT J., Resveratrol as an active ingredient for cosmetic and dermatological applications: a review, *Journal of cosmetical laser therapy*, 2-8, (2018).

[13]. MIRONEASA I., and MIRONEASA S., Application of heat moisture treatment in wheat pasta production, *Food Control*, 128: 2-18, (2021).

[14]. LAI J., CHIU J., M., CHIOU R. Y. Y., Fresh preservation of alfa sprouts and mushroom slices by soaking with thymol and resveratrol solution, *Food Sciences and Nutrition*, 5: 776-783, (2017).

[15]. DAVID G., MICHEL J., GASTALDI E., GONTARD N., ANGELLIER-COUSSY H., How vine shoots as fillers impact the biodegradation of PHBV-based composites, *International Journal of Molecular Science*, 21 (228): 2-13, (2020).