Environmental Engineering and Management Journal *April 2018, Vol.17, No. 4, 795-802*
http://www.eemj.icpm.tuiasi.ro/; http://www.eemj.eu

"Gheorghe Asachi" Technical University of Iasi, Romania

EVALUATION OF PHENOLIC COMPOUNDS CONTENT IN GRAPE SEEDS

Valeriu V. Cotea[2], Camelia Luchian[1]*, Marius Niculaua[2], Cătălin Ioan Zamfir[2], Ioan Moraru[1], Bogdan Constantin Nechita[2], Cintia Colibaba[1]

[1]The University of Agricultural Sciences and Veterinary Medicine "Ion Ionescu de la Brad", Faculty of Horticulture, 3 Mihail Sadoveanu Alley, 700490 Iasi, Romania
[2]Research Centre for Oenology - Romanian Academy - Iaşi Branch, 9 Mihail Sadoveanu Alley, 700505 Romania

Abstract

There is an increasing interest in the food industry and in protective health care for the expansion of natural antioxidants from plant materials. The solid wastes generated by the winemaking industry represent about 30% of the material used and it consists mainly of grape pomace (containing seeds, pulp, stem and skin). It is well known that high quantities of valuable compounds like dietary fibre, oils from the seeds, anthocyanins and phenolic compounds still remain within the grape marc. The phenolic compounds have great potential due to their antioxidant capacity and health benefits to prevent coronary problems and other chronic diseases: cancer, diabetes or neurodegenerative issues. Based on this evidence the evaluation of the polyphenols concentration of grape pomace, a by-product of winemaking, might be of great importance. The aim of this work was to determine by using a HPLC method, the amounts of phenolic compounds in six experimental and unconventional aqueous and ethanolic extracts, from grape marc and its components, and to evaluate the ability to obtain extracts for pharmaceutical uses. The results showed that all the grape marc extracts showed remarkable amounts of polyphenols and that supercritical fluid extraction method was the most efficient.

Key words: antioxidants, extraction methods, grape pomace, phenolic compounds

Received: May, 2017; Revised final: January, 2018; Accepted: March, 2018; Published in final edited form: April 2018

1. Introduction

Grapes are among the most cultivated fruits around the world, therefore the composition and properties have been extensively investigated, with significant results that confirm the presence of large amounts of phenolic compounds with beneficial effects on consumer health. Most of the phenolic compounds found in wine are able to act as antioxidants. In addition, the wine industry by-products are also characterized by a high content of phenolic compounds due to their incomplete extraction during winemaking (Ky et al., 2014; Maier et al., 2009; Teixeira et al., 2014). Solid waste originated from the wine industry represents 25% - 30% of raw matter used and consists mainly of grape pomace (containing seeds, pulp, stem and skin) (Dwyer et al., 2014). These by-products constitute a cheap source for the extraction of compounds with antioxidant effect, which can have a significant benefit for the pharmaceutical industry and the overall economy (González-Centeno et al., 2013).

The amount of soluble phenolic compounds found in grapes is unevenly distributed, as follows: 60-70% of total soluble phenolic compounds are present in seeds, 28-35% in skins and the rest of about 10% are found in pulp. The grape skins are rich in flavonols; the proanthocyanidins and the flavan-3-ols are the most predominant phenolics in grape seeds (Godevac et al., 2010). Also, red grape skins are

* Author to whom all correspondence should be addressed: e-mail: kamelia_luchian@yahoo.com; Phone: 0232407519

characterized by a high amount of anthocyanins, which is greatly influenced by the plant variety and other factors like *térroir* or viticultural practice (Rodríguez-Montealegre et al., 2006). Grape seeds, as other authors have previously reported, have the following general composition, expressed in g constituent/g seeds: 40% fiber, 16% volatile oil, 11%protein, 7% phenolic compounds and other substances (sugars, minerals etc.) (Campos et al., 2008).

The main scope of this study was to determine and quantify the amount of phenolic compounds found in six experimental aqueous and ethanolic extracts; these were obtained from grape seeds and their composition was analysed using a HPLC method, in order to evaluate the possibility of obtaining extracts with bioactive properties for pharmaceutical uses.

One of the methods used, the supercritical fluid extraction (SFE), is a well-known technique applied in the separation of bioactive compounds found in fruits and other plants. This method is valued because it uses a very high solvent power and, also, for the distinctive physicochemical properties of supercritical fluids. Due to relatively low viscosity and the high diffusivity of the fluids, these can be easily inserted into solid materials more efficiently than other liquid solvents, thus reducing the overall analysis time and increasing the method's efficiency (Qingyong and Wai, 2001).

Previous published work has shown that SFE technique is highly selective for phenolic compounds, such as gallic acid, epicatechin, catechin and quercetin, thus high amounts of polyphenols from grape marc were retained. This method cannot be applied for the extraction of polyphenols with larger molecule, such as proanthocyanidins (Massias et al., 2015; Pinelo et al., 2007). Therefore, the aim of this study is to investigate, using a chromatographic method, the phenolic content of the obtained extracts by different methods and to correlate the results with the possibility of using them for pharmaceutical purposes.

2. Material and methods

2.1. Extract preparation

Plant material used in the experiments is Fetească neagră grape seeds from 2014 harvest at 195 g/L total sugars from the Iași region Adamachi vineyard.

After 5 days of controlled maceration-fermentation at 18°C the seeds ware collected from the bottom of the fermentation tank. Immediately afterwards using type 1water (with resistivity18.2 MΩ×cm) the seeds were washed by hand three times. The wet seeds were dried overnight at room temperature. The next day using an Ika M20 universal mill the dry material was ground at less than 500 µm particles (the product was sieved with Bolin stainless steel wire mesh sieve). The powder material was dried out for 60 minutes in a vacuum oven (JP Selecta Vaciotem-T) at 50 °C and 100 mbar. The material was cooled and stored in a vacuum dessicator until extractions were performed.

Lipids were removed in five steps with 100 mL ethyl ether each time at room temperature using a magnetic stirrer Falc F30 at 300 rpm. For phenolic compounds analysis, a 5 g powdered material was introduced in a 250 mL Soxhlet apparatus. Extraction was carried out continuously with 130 mL of 96% ethanol at 80°C using a Falc F60 heating block.

2.2. Supercritical fluid carbon dioxide extraction (SFE)

In the case of supercritical extraction (SFE), the equipment used is manufactured by Jasco (Japan). The device has a 10 mL extraction cell (EV-2) for solid samples in a CO-2060 extraction oven. The chemical modifier is added in the mixing chamber with a PU-2089 quaternary gradient pump with a build-in degasser and is combined with the flow of CO_2 liquid from a PU-2080-CO_2 pump with cooled piston heads. The system is pressurised at 10 MPa by the back pressure regulator BP-2080 in order to maintain the liquid state in the system. All the modules are coordinated by a LC-NetII/ADC unit wich can manage physical parameters of the system (Colibaba et al., 2015). Five extractions were performed from 5 g of plant material (*Fetească neagră* grape seeds from 2014 harvest) each time, using liquid CO_2 at a rate of 1 mL/min and ethanol at a rate of 0.5 mL/min. The extraction lasted 60 min. each time and, in the end, 142 mL of liquid were collected.

2.3. Subcritical water extraction (SWE)

Subcritical water extraction was made at 3 bar (SWE 3 bar) and 15 bar (SWE 15 bar) using two commercial espresso machines. Two samples were made by using water and a 75% ethanol solution for each two pressure equipment's (SWE 15 bar 75% EtOH and also SWE 3 bar 75% EtOH). The amount of 7 g of grounded material was used in extractions and the collected volume of 200 mL was obtained at the end of each extraction.

All the extracts were concentrated to dryness using a rotary evaporator (Heidolph Laborota 4003) at 40 mbar, constant 40°C bath temperature and a rotational speed of 80 rpm. During the distillation, the system was stopped in order to remove the different solvents whenever the process is not progressing.

All extraction was done in triplicate so it can evaluate the influence of the methodology upon the complexity of bio-organic phenolic compounds.

2.4. Phenolic compounds analysis

The flask was washed five times with 2 mL of water. The samples was filtered through 0.45 µm nylon 25 mm syringe filters prior to LC analysis in order to remove colloids. The LC analysis was performed using a monolithic column (Castellari et al., 2002). For the analysis of phenolic acids, samples

were processed on a Shimadzu HPLC (LC-DAD) consisting of: quaternary pump Shimadzu Prominence series LC-20AD with five-channel degasser DGU-20A5 Shimadzu Prominence series, autoinjector SIL-20AC Shimadzu Prominence series (injection volume: 10 µL, sample temperature 20ºC), column oven CTO-20AC Shimadzu Prominence series, diode array detector SPD-M20A Shimadzu Prominence series (200-440 nm), fluorescence detector (Shimadzu FLD RF-10Axl) in order to achieve a double spectral certification for substances, chromatographic system controller CBM-20A Shimadzu Prominence series PC connectivity via LAN.

The gradient was optimized using trifluoroacetic acid (TFA) as an eluent for 1% methanol MeOH (A channel) and 50% MeOH (B channel). The column system is composed of a pre-column Chromolith Guard Cartridge 5×4.6 mm and two Chromolith Performance RP-18 endcapped 100×4.6 mm columns manufactured by Merck.

Phenolic compounds were characterized by their UV spectra which were recorded at 256, 280, 324, and 365nm. The different chemical compounds were identified according to their order of elution and retention times of pure standard compounds.

The chromatogram represented in Fig. 2 it was plotted after HPLC system calibration using the method published by Castellari (Castellari et al., 2002), in order to analyze the obtained samples.

2.5. Statistical analyses

Statistical analyses were performed using Statgraphics Centurion XVI® software, (StatPoint Technologies, Inc, U.S.A.). In this study, we applied a one-way ANOVA procedure that was designed to construct a statistical model describing the impact of one categorical factors X_j (different variants of extraction method) on a dependent variable Y (some phenolic compounds from Feteasca neagră grape seeds). The statistic displayed Fisher's LSD (Least Significant Difference) it is the way that we can select a single pair of samples and declare their means to be significantly different. While the chance of incorrectly declaring two samples to be different with this method is fixed at 5%, making comparisons amongst many pairs of means may result in an error on at least one pair with a considerably higher probability (Zamfir et al., 2014).

3. Results and discussion

The first set of phenolic acids that have eluted for the LC analysis, usually are benzoic acids and in the Fig. 2 the results were presented for a group of compounds with medium concentration. Vanillic acid is well known for its "sweet mouthfeel" characteristics and has a very high extraction rate in polar type environments like alcohols. By comparing with static Soxhlet extraction, in the case of SFE method, the amount extracted was doubled and, by applying high pressure, an improvement in the extraction process can be observed.

CO_2 exerts a small influence on *p*-hydroxybenzoic acids, mainly due to the solubility of these compounds in lipid-like substances (logP=1.58) and also, these acids are marginally influenced by the techniques applied.

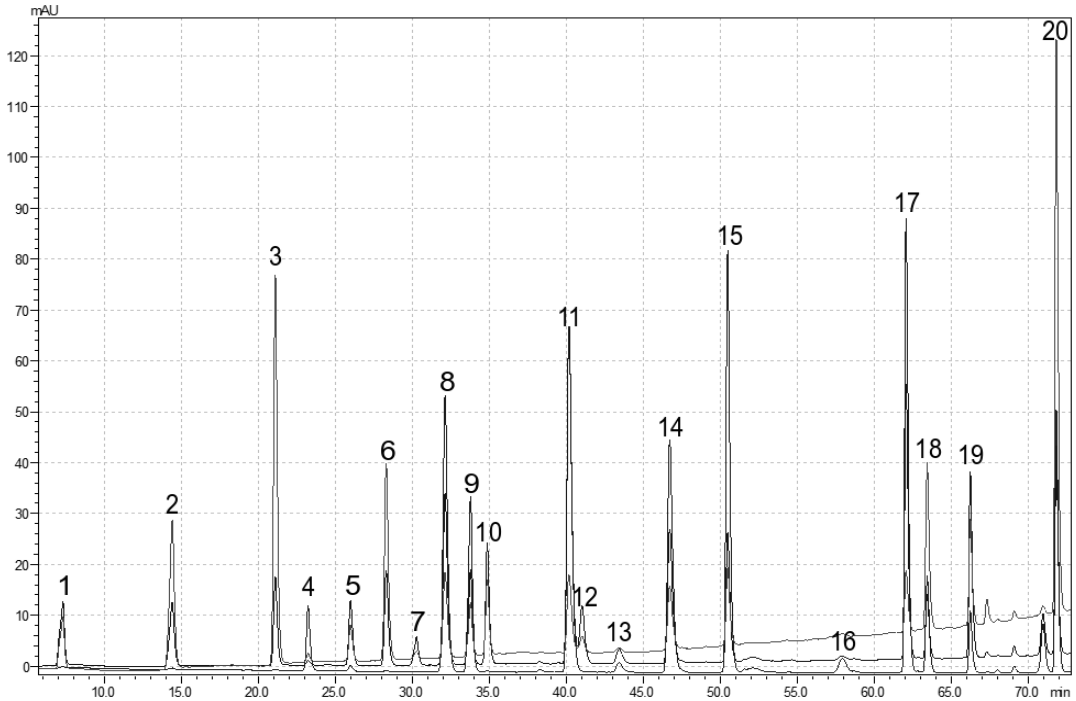

Fig. 1. Chromatogram of standards separated by LC-DAD (1 – gallic acid; 2 – protocatechuic acid; 3 – p-hydroxybenzoic acid; 4 – gentisic acid; 5 – catechin; 6 – m-hidroxybenzoic acid; 7 – vanillic acid; 8 – caffeic acid; 9 – chlorogenic acid; 10 – syringic acid; 11 – epicathechin; 12 – p-coumaric acid; 13 – ferulic acid; 14 – salicylic acid; 15 – sinapinic acid; 16 – ellagic acid; 17 – trans-resveratrol; 18 – rutin; 19 – cis-resveratrol; 20 – quercitine)

The amount of syringic acid, a minor compound, is not influenced by the experimental protocol, because its concentration in wines is usually below 30 mg/L.

Salicylic acid is considered to have great value in the pharmaceutical applied field as a painkiller and it was found in high concentrations in berry like fruits, including grapes. The increased pressure effect from the force applied in the experiment and also some solvent interactions double the efficiency (2.06 logP), but marginally due to the techniques applied at normal atmospheric pressure.

The second most important benzoic acid concerning its concentration in wine, the protocatechuic acid (5-100 mg/L), is highly soluble in water, ethanol and also ether. It was adequately extracted by adding some ethanol to the water and also by SFE method, as shown in Fig. 3. In the case of the SFE method, a rather low concentration of *m*-hydroxybenzoic acid can be observed, when using an ethanol solution at low pressure, although other proposed techniques did not extract *m*-hydroxybenzoic acid. This was possibly due to the fact that some other substances might have been transformed into this compound during the extraction process.

According to Yilmaz and Toledo, the gallic acid has the highest concentration among all benzoic acids (around 15-100 mg/g dry matter) (Yilmaz and Toledo, 2004) and it is considered to be a "backbone" for tannins (as monomeric structure) in the majority of the Romanian grape varieties' seeds. The results show a great improvement in all extraction techniques in comparison with the classical Soxhlet solvent reflux method. The solvent and the pressure applied are important to overall process yield, therefore, and by combining the two factors, the entire extraction process can be improved.

The lack of influence on ellagic acid content, which has the same concentration for all tested extraction protocols, is presented in Table 1. A small amount of catechin was detected in the extract obtained by using supercritical carbon dioxide, but epicatechin was not present. These monomeric procyanidins are found in large amounts in grape seeds, but their low water solubility explains the small extracted quantity. In the cases of Soxhlet, SFE and ethanol assisted extraction methods, one can expect to obtain higher concentrations (Nechita et al., 2015). *Trans*-resveratrol, has been extracted using the SFE method and also with subcritical water, in the presence of ethanol at both high and low pressure. This molecule have certified biological activity and increasing interest in the pharmaceutical industry.

B1 and B2 procyanidins are dimeric tannins that have been extracted using all proposed methods, except the classical Soxhlet method (Fig. 4). As in wines, the amounts of sinapic acid found in grape seed extracts has low values because it is transformed into hydroxycinnamic acids, like ferulic acid, which has a good solubility and extractability in aquatic medium. The largest quantity of ferulic acid is present in the sample obtained using the SFE method, but also in the ones obtained under high pressure by using water and ethanol; these methods showed a higher extractability than the Soxhlet technique.

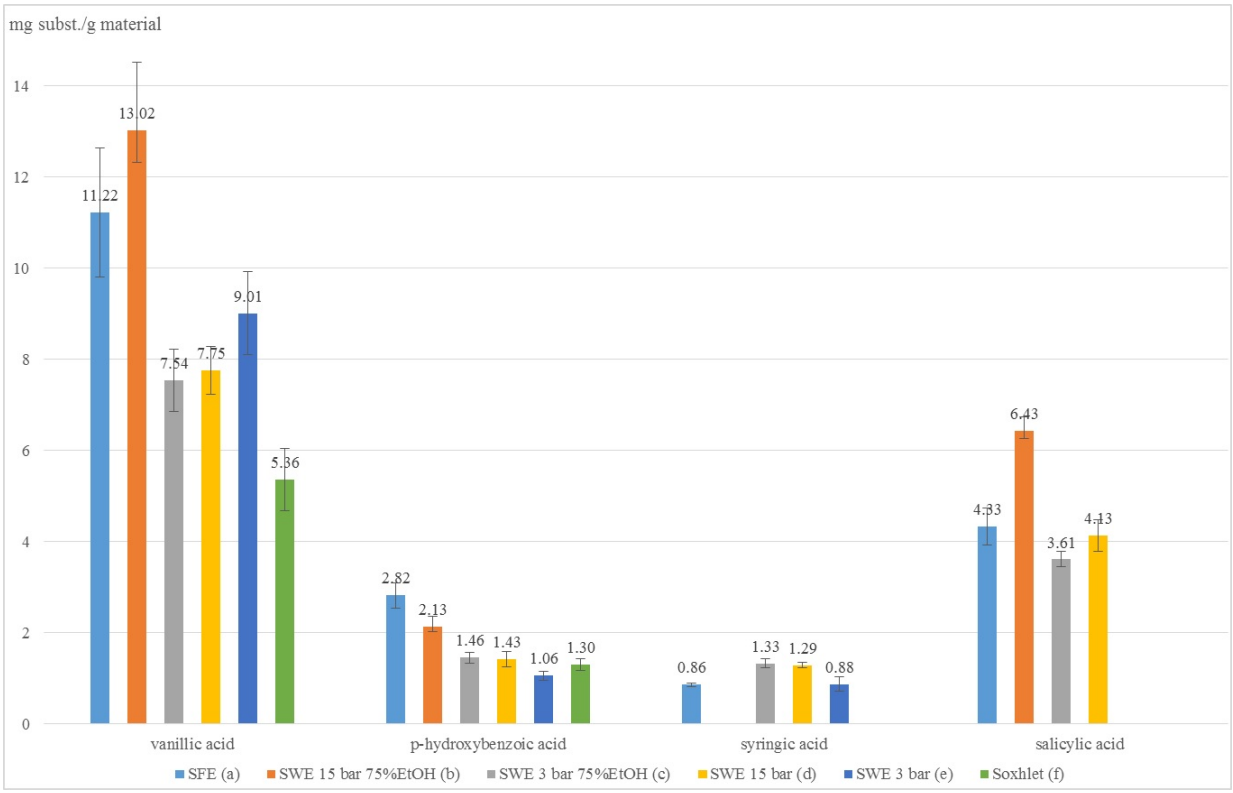

Fig. 2. Effect of separation techniques upon some minor benzoic acids

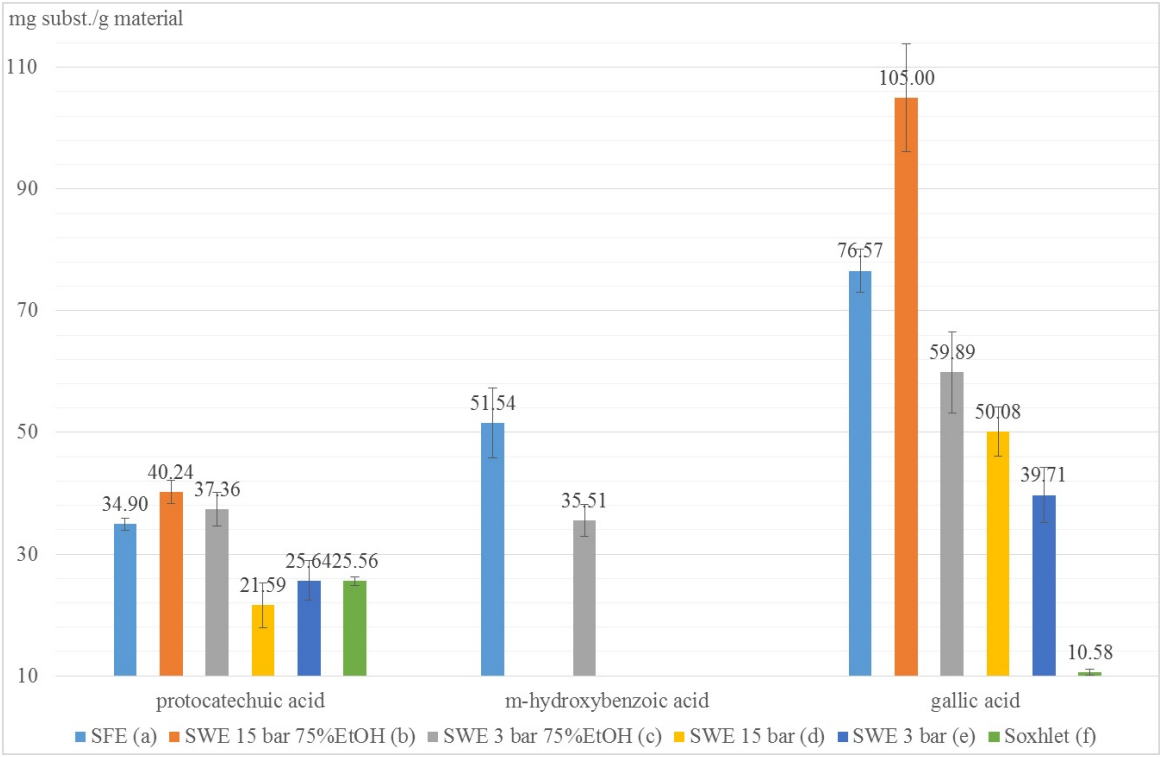

Fig. 3. Effect of separation techniques upon some major benzoic acids

Table 1. Other minor phenolic compounds extracted

mg substance / g grape seed	***SFE(a)***		***SWE 15 bar 75%EtOH (b)***		***SWE 3 bar 75%EtOH (c)***		***SWE 15 bar (d)***		***SWE 3 bar (e)***		***Soxhlet (f)***	
	mg/g	±SD	mg/g	±SD	mg/g	±SD	mg/g	±SD	mg/g	±SD	mg/g	±SD
ellagic acid	0.19	0.01	0.18	0.01	0.18	0.02	0.16	0.02	nd	nd	0.12	0.01
catechin	0.82	0.14	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
trans-resveratrol	4.99	0.23	4.37	0.44	4.1	0.35	3.94	0.50	nd	nd	nd	nd

**nd - not detected*

The results obtained from the statistical analysis are presented in Table 2. These information present the estimated difference between each pair of means. A superscript letter has been placed next to the means pairs, indicating that these pairs show statistically significant differences at the 95.0% confidence level. We used Fisher's least significant difference (LSD) procedure for discriminate among the means that represent different kind of extraction method of these compounds. With this method, there is a 5.0% risk of calling each pair of means significantly different when the actual difference equals 0. In Table 3 are displayed the samples arranged into homogeneous groups, shown as columns of X's. A homogeneous group is a group within which there are no significant differences. For understanding in the protocatechuic acid case, sample comes from SFE method is in a group with samples from SWE 3 bar 75%EtOH method. Samples from SWE 15 bar 75%EtOH method are in a group with samples from SWE 3 bar 75%EtOH method that is mean need more data to distinguish which group sample SWE 3 bar 75%EtOH actually belongs to.

For protocatechuic acid, m-hydroxybenzoic acid, vanillic acid, syringic acid and trans-resveratrol, 3 homogenous groups are identified. Within each column, the levels containing X or Y's form a group of means within which there are no statistically significant differences, which means that the extraction methods can provide similar results.

It can be observed that the SFE method has generated the highest values in eight of of the studied compounds. In the case of vanillic acid, ellagic acid and ferrulic acid, homogenous groups were formed, from a statistic point of view, for a significance level of 95%. The method SWE 15 bar 75%tOH generated the highest values in the case of five compounds. protocathechuic acid, vanillic acid and ellagic acid generate homogenous groups from a statistical point of view.

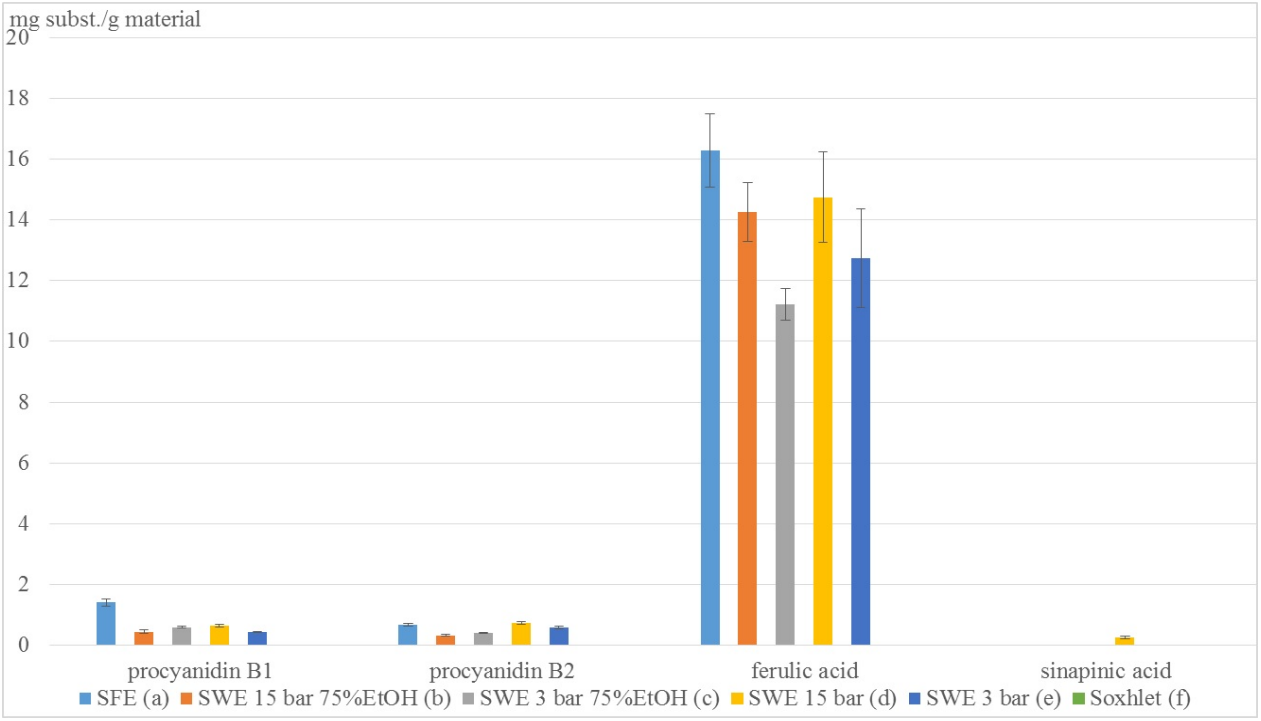

Fig. 4. Effect of separation techniques upon some other phenolic compounds identified

Table 2. The mean values and standard deviations of the analysed phenolic compound and the significant statistical differences

Extraction Method	***SFE (a)***	***SWE 15 bar 75%EtOH (b)***	***SWE 3 bar 75%EtOH (c)***	***SWE 15 bar (d)***	***SWE 3 bar (e)***	***Soxhlet (f)***
protocatechuic acid	34.90 ± 0.97 b,d,e,f	40.24 ± 1.91 a,d,e,f	37.36 ± 2.77 d,e,f	21.59 ± 3.72 a,b,c	25.63 ± 3.25 a,b,c	25.56 ± 0.71 a,b,c
m-hydroxybenzoic acid	51.54 ± 5.71 c	*nd*	35.50 ± 2.63 a	*nd*	*nd*	*nd*
gallic acid	76.57 ± 3.58 b,c,d,e,f	105 ± 8.91 a,c,d,e,f	59.88 ± 6.63 a,b,d,e,f	50.07 ± 4.04 a,b,c,e,f	39.70 ± 4.56 a,b,c,d,f	10.58 ± 0.49 a,b,c,d,e
vanillic acid	11.22 ± 1.42 c,d,e,f	13.02 ± 1.49 c,d,e,f	7.53 ± 0.69 a,b,f	7.75 ± 0.52 a,b,f	9.00 ± 0.90 a,b,f	5.36 ± 0.68 a,b,c,d,e
p-hydroxybenzoic acid	2.82 ± 0.28 b,c,d,e,f	2.13 ± 0.23 a,c,e,f	1.45 ± 0.11 a,b,e	1.42 ± 0.16 a,b,e	1.06 ± 0.09 a,b,c,d	1.30 ± 0.13 a,b
syringic acid	0.86 ± 0.04 c,d	*nd*	1.33 ± 0.09 a,e	1.29 ± 0.06 a,e	0.87 ± 0.15 c,d	*nd*
salicylic acid	4.33 ± 0.40 b,c	6.42 ± 0.30 a,c,d	3.60 ± 0.16 a,b,d	4.13 ± 0.35 b,c	*nd*	*nd*
trans-resveratrol	4.99 ± 0.23 b,c,d	4.37 ± 0.44 a	4.10 ± 0.34 a	3.94 ± 0.50 a	*nd*	*nd*
ellagic acid	0.19 ± 0.01 d,f	0.18 ± 0.01 d,f	0.17 ± 0.02 d,f	0.15 ± 0.02 a,b,c,f	*nd*	0.12 ± 0.01 a,b,c,d
catechin	0.82 ± 0.14 b,c,d,e,f	*nd*	*nd*	*nd*	*nd*	*nd*
procyanidin B1	1.41 ± 0.12 b,c,d,e	0.44 ± 0.05 a,c,d	0.59 ± 0.02 a,b,e	0.64 ± 0.04 a,b,e	0.42 ± 0.02 a,c,d	*nd*
Procyanidin B2	0.67 ± 0.05 b,c,d,e	0.31 ± 0.02 a,c,d,e	0.39 ± 0.01 a,b,d,e	0.73 ± 0.03 a,b,c,e	0.58 ± 0.04 a,b,c,d	*nd*
Ferulic Acid	16.29 ± 1.21 b,c,e	14.26 ± 0.96 a,c	11.21 ± 0.52 a,b,d	14.74 ± 1.48 c,e	12.73 ± 1.61 a,d	*nd*
Sinapinic Acid	*nd*	*nd*	*nd*	0.25 ± 0.04 a,b,c,e,f	*nd*	*nd*

The superscript letter (^{a,b,c,d,e,f}) indicates that these pairs show statistically significant differences at the 95.0% confidence level between each pair of means

Table 3. Distribution of samples obtained from different kind of extraction methods on statistically homogeneous groups

Extraction method	*SFE*	*SWE 15 bar 75%EtOH*	*SWE 3 bar 75%EtOH*	*SWE 15 bar*	*SWE 3 bar*	*Soxhlet*
protocate-chuic acid	**X**	**Y**	**XY**	**X**	**X**	**X**
m-hydroxy benzoic acid	**Y**	**X**	**X**	**X**	**X**	**X**
gallic acid	**X**	**Y**	**X**	**X**	**X**	**X**
vanillic acid	**Y**	**Y**	**X**	**X**	**X**	**X**
p-hydroxybenzoic acid	**Y**	**X**	**X**	**X**	**X**	**XX**
syringic acid	**X**	**X**	**Y**	**Y**	**X**	**X**
salicylic acid	**X**	**Y**	**X**	**X**	**X**	**X**
trans-resveratrol	**Y**	**X**	**X**	**X**	**X**	**X**
ellagic acid	**Y**	**Y**	**Y**	**X**	**X**	**X**
catechin	**Y**	**X**	**X**	**X**	**X**	**X**
procyanidin B1	**Y**	**X**	**X**	**X**	**X**	**X**
procyanidin B2	**X**	**X**	**X**	**Y**	**X**	**X**
ferulic acid	**Y**	**XX**	**X**	**XY**	**XX**	**X**
sinapinic acid	**X**	**X**	**X**	**Y**	**X**	**X**

Y - homogeneous means groups that exhibit the highest extraction values
X - homogeneous means groups exhibiting lower extraction values

4. Conclusions

By using a HPLC method to obtain the polyphenolic profile of grape seed extracts, can be concluded that the recommended extraction technique, which employs supercritical fluids (SFE), is an alternative for the classical Soxhlet procedure.

A superior extraction of the phenolic acids, non-hydrolysed tannins, stilbenes and flavones, can be obtained by using ethanol solutions as solvents. These extracts can be converted into different physiological formulations, thus making it possible to be used in the pharmaceutical field.

Acknowledgments

This research was carried out by the Partnership in priority areas - PN II, developed with the support of MEN - UEFISCDI, project no. 183/2014 (PN-II-PT-PCCA-2013-4-0333) "Technology of capitalization of the bioactive elements from the grape seed waste with usefulness in the food and pharmaceutical industry, plant and environmental protection" (Acronym: PROVITIS).

References

Campos L.M.A.S., Leimann F.V., Pedrosa R.C., Ferreira S.R.S., (2008), Free radical scavenging of grape pomace extracts from Cabernet Sauvingnon (Vitis vinifera), *Bioresource Technology*, **99**, 8413-84.

Castellari M., Sartini E., Fabiani A., Arfelli G., Amati A., (2002), Analysis of wine phenolics by high-performance liquid chromatography using a monolithic type column, *Journal of Chromatography A*, **973**, 221-227.

Colibaba L.C., Cotea V.V., Rotaru L., Nechita B., Niculaua M., Tudose-Sandu-Ville S., Luchian C., (2015), Volatiles in *Tămâioasă Românească* via supercritical fluid extraction (SFE) analysis, *Environmental Engineering and Management Journal*, **14**, 297-302.

Dwyer K., Hosseinian F., Rod M., (2014), The market potential of grape waste alternatives, *Journal of Food Research*, **3**, 91-106.

Godevac D., Těsević V., Veličković M., Vujisić Lj., Vajs V., Milosavljević S., (2010), Polyphenolic compounds in seeds from some grape cultivars grown in Serbia, *Journal of the Serbian Chemical Society*, **75**, 1641-1652.

González-Centeno M.R., Jourdes M., Femenia A., Simal S., Rosselló C., Teissedre P.L., (2013), Characterization of polyphenols and antioxidant potential of white grape pomace by-products (*Vitis vinifera* L.), *Journal of Agricultural and Food Chemistry*, **61**, 11579-11587.

Ky I., Lorrain B., Kolbas N., Crozier A., Teissedre P.L., (2014), Wine by-products: Phenolic characterization and antioxidant activity evaluation of grapes and grape pomaces from six different French grape varieties, *Molecules*, **19**, 482-506.

Maier T., Schieber A., Kammerer D.R., Carle R., (2009), Residues of grape (*Vitis vinifera* L.) seed oil production as a valuable source of phenolic antioxidants, *Food Chemistry*, **112**, 551-559.

Massias A., Boisard S., Baccaunaud M., Leal Calderon F., Subra-Paternault P., (2015), Recovery of phenolics from apple peels using CO_2 ethanol extraction: kinetics and antioxidant activity of extracts, *The Journal of Supercritical Fluids*, **98**, 172-182.

Nechita B.C., Niculaua M., Cotea V.V., (2015), Assessment and adaptation of methods of extraction of grape seed polyphenol compounds, *Scientific Papers Journal, Horticulture Series*, **58**, 195-200.

Pinelo M., Ruiz-Rodríguez A., Sineiro J., Señorans F.J., Reglero G., Núñez M.J., (2007), Supercritical fluid and solid-liquid extraction of phenolic antioxidants from grape pomace: A comparative study, *European Food Research and Technology*, **226**, 199-205.

Qingyong L., Wai C.M., (2001), Supercritical fluid extraction in herbal and natural product studies- A practical review, *Talanta*, **53**, 771-782.

Rodríguez-Montealegre R., Romero-Peces R., Chacón-Vozmediano J.L., Martínez-Gascueña J., García-Romero E., (2006), Phenolic compounds in skins and seeds of ten grape *Vitis vinifera* varieties grown in a warm climate, *Journal of Food Composition and Analysis*, **19**, 687-693.

Teixeira A., Baenas N., Dominguez-Perles R., Barros A., Rosa E., Moreno D.A., Garcia-Viguera C., (2014), Natural bioactive compounds from winery by-products as health promoters: A Review, *International Journal of Molecular Sciences*, **15**, 15638-15678.

Yilmaz Y., Toledo R.T., (2004), Major flavonoids in grape seeds and skins: antioxidant capacity of catechol, epicatechin and gallic acid, *Journal of Agricultural and food Chemistry*, **52**, 255-260.

Zamfir C.I., Cotea V.V., Luchian C.E., Niculaua M., Odăgeriu G., (2014), The influence of different prefermentative maceration processes and tartaric stabilization treatments on the color, cation content and other physico-chemical parameters of 'Băbească neagră' rosé wines, *Vitis*, **53**, 45-52.