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OPTIMIZATION OF THE EXTRACTION OF PHENOLIC COMPOUNDS FROM GRAPE POMACE USING SUPERCRITICAL LIQUID (CO2) METHODOLOGY

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Abstract

The extraction of phenolic compounds and antioxidant activity (AA) of winemaking by-products using supercritical liquid methodology has been investigated. In order to have a green product, CO_2 was used in extraction. Extraction by supercritical liquids on grape pomace and wine enriched with total polyphenolic compounds (TPC) from extracts and antioxidant activity were evaluated. Several grape varieties from two different vineyards were used Stefanesti and Murfatlar. The extract TPC ranged from GAE/L to 156.66 mg to 2536.89 mg GAE/l, and for fortified wine ranged from 1510 mg GAE/L to 7457 mg GAE/L. Antioxidant activity in fortified wine varied between the values of 54.45 mmol TE/L and 216.13 mmol TE/L. With the help of chromatography several phenolic compounds, such as gallic acid, were identified, shikimic acid, caffeic acid, catechin, epi-catechin, syringic acid, p-coumaric acid

Key words*: Chlorophyll fluorescence water deficit, Vitis vinifera L, environmental drought stress, photosystem II efficiency*

INTRODUCTION

Grapes resulting from the winemaking process lead to the generation of increasing quantities of pomace (Zemni, 2023), reaching about 8.5 million tons worldwide annually (Abreu, 2025). Due to the increasing amounts of waste, the increase in greenhouse gas emissions and the decrease in natural resources (Colnik, 2024), the policy and objectives of protecting the environment in the EU provide for finding measures and ways to transform wine waste into valuable components. Improper disposal of wine-making waste on the ground leads to serious problems of soil contamination, groundwater contamination, and the spread of vine-specific diseases, with risks even to the health of the human population (Abreu, 2025).
The utilization of wine pomace to obtain different compounds responds to the principles of the circular economy, also leading–to a sustainable economy of the wine industry (Abreu, 2025; Liu, 2024; Matos, 2019; Yang, 2009).
Grape pomace contains tannins, polyphenolic acids (hydroxybenzoic and hydroxycinnamic acids), catechins, and other compounds, such as phenolic compounds, anthocyanins, fibers, vitamins, lipids, lignocellulosic compounds and minerals (Abruu, 2025), which can serve in the food, pharmaceutical and cosmetic industry (Abru, 2025; Rodrigues, 2022; Matos, 2019).
Obtaining extracts from pomace with high contents of active compounds and minimal losses, depending on the non-conventional methods and techniques used, the amounts of organic solvents energy consumption processing times, etc. (Khaw, 2017). One of these technologies is the extraction of supercritical CO_2 fluids.

Supercritical fluid extraction (SFE) is an environmentally friendly technology based on reduced processing energy input, alternative use of solvents, the possibility of recovering the gaseous solvent (CO_2) which can be stored in cylinders and reused in future extraction, leading to an increase in the sustainability of the use of supercritical fluid extraction (SFE) technology.

This extraction method, uses little or no organic solvent (ethyl alcohol) and can utilize supercritical fluids in extractions. Due to the variation in density, liquid/gas, viscosity and high diffusion (Khaw, 2017; de Melo, 2014; Fornari, 2012) these supercritical fluids have been successful in their use in extractions, due to their durability (Khaw, 2017).

This study aims to obtain functional ingredients through a greener extraction method (extraction with supercritical CO_2 fluids) and enrich the wine with these bioactive compounds with high nutritional value.

MATERIALS AND METHODS

Raw material. Grape pomace (*Vitis vinifera* var. 'Cabernet Sauvignon', 'Burgund Mare' and 'Merlot') was collected from the winery in INCDBH Stefanesti and from the SCDVV Murfatlar winery ('Mamaia', 'Pinot Noir' and 'Băbească neagră'), the harvest 2023. The marigold samples were frozen and stored before the analysis. Grape pomace was dried at 45 ◦C for 5 minutes at tunnel microwave drying tape JW-MW-6 KW (Onache, 2022). The grape pomace samples before drying had humidity between 85-75%, and after drying they reached between 5-8%, after which the samples were shredded using the mill with Fritsch Pulverisetti 16 hammers.

Optimization process with experimental extraction equipment with supercritical liquids. Supercritical CO_2 extraction of polyphenolic compounds from the 6 varieties of grape pomace was performed on the SFT-110 SFE SYSTEM equipment at the following parameters: the solvent is pharmaceutical ethyl alcohol and the supercritical liquid is CO2; the ratio of supercritical CO2 liquid to pharmaceutical ethyl alcohol is 20:1; Extraction power is 3000 psi; Flow rate: 6 mL/min CO2; Static extraction: 10 Minutes (4 cycles); Dynamic extraction: 10 minutes (4 cycles). 30 G of grape pomace was used and 200 ml of extract was obtained.

Analytical Investigations. Quantitative UV-Vis Spectrophotometric Determinations

Total polyphenols in extract/wine (PFT). The phenolic compounds in wine are oxidized by the Folin-Ciocalteu reagent. In reaction with phenolic compounds, tungsten and molybdenum oxides are formed, which are blue and read at the spectrophotometer at the maximum absorbance with a wavelength of 750 nm (Onache, 2023). The optical density of the gallic acid standard solutions is drawn right by absorbent calibration - concentration and e calculates its parameters, slope and order at the origin.

The result is expressed in mg/l gallic acid and is calculated with the following formula:

$$(mg\ GAE/l)=Dilutie*(A760-b)/a \quad (1)$$

in which: *a* represents the slope of the calibration line; *b* is originally ordered for the calibration line, A760 is absorbance at 760 nm (DO 760 nm).

Determination of Anthocyanins (TA) in extract/wine is carried out with the method of determination by discoloration with SO_2 and is based on the property of anthocyanins to react with SO_2, forming colorless products. Prepare the mixture consisting of: 1 ml of filtered wine, 1 ml of HCl 0.1%, and 20 ml of HCl 2%. From this mixture are prepared two solutions, which are put in 2 test tubes having the following composition: Solution 1 of 5 ml mixture and 2 ml distilled water. Solution 2 of 5 ml mixture and 2 ml of soil 16% sodium metabisulfite.

After 20 minutes, the optical density of solutions 1 and 2 is measured against distilled water, at the wavelength of 520 nm, in the bowl of 1 cm. The result is expressed in mg/l and is calculated with the following formula:

$$TA(mg/l)= 875 * \Delta d \quad (2)$$

where Δd is the difference in optical density of the two solutions.

Determination of Catechins (TC). The method of determining catechins is the reaction with vanillin and is based on the reaction of the phloroglucinol cycle with vanillin, with the production of a red color, stable in concentrated acid solutions. The maximum absorption shall

be recorded at a wavelength of 500 nm. It is read according to the calibration curve at the wavelength of 500 nm.
The result shall be expressed in mg/l catechin and calculated using the following formula:

$$TC\ (mg/l)=Dilutie*(a\Delta d+b) \quad (3)$$

in which: a and b are the parameters of the calibration line Δd is reading to DO 500 nm.

The determination of Tannins (TT) in extracts/wines is the method of determination of tannins (Onache, 2022) is based on the property of tannins to turn hot (100°C) and strongly acidic environment (HCl concentrate) in cyanidin, which is red.
The optical density at the wavelength of 550 nm is directly proportional to the concentration of tannins. Dilute the red wine so that the optical density is in the range of 0.3-0.7. In red wine, the most common dilution is 1/50. Prepare in two test tubes the following solution: 2 ml diluted wine, 1 ml distilled water, 3 ml HCl concentrate.
One of the test tubes is boiled in the water bath at 100°C for 45 minutes. Cool the test tube with tap water. In both test tubes, add 0.5 ml of 96% ethanol to stabilize the color.
It is read at the wavelength of 520 nm, in the bowl of 1 cm, the optical density of the solutions in the two test tubes (boiled and uncooked). The reference is distilled water. Calculation formula:

$$TT\ (mg/l) = \Delta OD\ 520\ x 15.7 \quad (4)$$

(ΔOD 520 - the difference between the boiled and unboiled sample, read at the wavelength 520 nm).

Antioxidant activity (AA) was determined in the extract/wine using 3.9 ml of DPPH solution - 1,1-diphenyl-2-picryl hydrazil 25 mg/1,000 ml of methanol together with 0.1 ml of grape pomace extract in methanol solution incubated for 45 minutes at t=25°C, and was also used with trolox (carboxylic acid-2 , 2 , 6-hydrozil 2,5,7,8-tetramethylchrom 0,05 mM in methanol solution used as standard to convert the inhibitory capacity of the solution of methanolic extract from grape pomace into trolox as equivalent or millimoles for antioxidant activity (Tarola A.M et al., 2019).
A spectrophotometer was determined at wavelength 515 nm and the initial absorbance after 45 minutes for each vine variety. The formula for calculation is:

$$AA = \left(1 - \frac{Ac-Aex}{Ac}\right) x\ 100,\ mmol\ TE/100\ ml \quad (5)$$

Polymerised tannins. The V/La ratio is the indicator of the degree of polymerization of tannins. V - represents the optical density of the colored combinations with vanillin, and La - is the optical density of the anthocyanidins formed by heating in acid medium.
The lower the value of the ratio, the higher the degree of polymerization of tannins.

Polyphenolic Profile by UHPLC-HRMS.
The quantitative analysis of individual polyphenols (phenolic acids, flavonoids, and stilbenes) was performed by UHPLC–ESI/HRMS (ultra-high-performance liquid chromatography-electrospray ionization tandem mass spectrometry) using a high-resolution Q Extractive mass spectrometer™ Focus Hybrid Quadrupole OrbiTrap equipped with HESI, coupled to a high-performance liquid chromatograph UltiMate 3000 UHPLC (Thermo Fisher Scientific, City, State, Country).

Chemicals and Reagents. Folin-Ciocalteu phenol reagent (pure) was purchased from Carl ROTH GmbH Co. (Karlsruhe, Germany),
radical scavenging assay reagents DPPH (95% 1,1-diphenyl-2-picryhidrazyl) were purchased from Acros Organics (Slovakia), Trolox 97% (6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid) was obtained from Alfa Aesar (Thermo Fisher GmbH Kandel, 76870 Kandel, Germany), gallic acid and vanillin were purchased from Carl ROTH GmbH Co. (Karlsruhe, Germany), and H_2SO_4 96% and HCl 37% were from Chemical Company (Bucharest, Romania). All chemicals and solvents used in chromatography were obtained from Carl Roth GmbH Co. (Karlsruhe, Germany) and Merck Co. (Darmstadt, Germany), and they were of HPLC or analytical grade (>99%) quality.
Wine Samples. The wine was obtained from the winemaking of the 6 varieties from which the grape pomace resulted, in the same year of production, 2023. The wine was enriched with 0.5% extract from the grape pomace of each variety.

RESULTS AND DISCUSSIONS

Table 1. Description of the varieties from which the extraction was made, the cultivation area, and their abbreviation

Crt No.	Sample of grape varieties	Origin	Extract/ Wine code	Wine+ 0,5% extract code
1	Burgund Mare	INCDBH Ștefănești	BM-St /w	BM-St-SFE
2	Cabernet_Sauvignon		CS-St /w	CS-St-SFE
3	Merlot		M-St_/w	M-St-SFE
4	Băbească neagră	SCDVV Murfatlar	BN-Mf_/w	BN-Mf-SFE
5	Mamaia		MM-Mf_/w	MM-Mf SFE
6	Pinot Noir		PN-Mf_/w	PN-Mf SFE

Figure 1 shows the bioactive compounds obtained from grape pomace extracts (the polyphenol content, anthocyanins) and antioxidant activity. It is noted that the extracts have a very low content of anthocyanins, by fermenting the wine on the breastbone and by the treatments suffered by the grape pomace, most anthocyanins are destroyed.

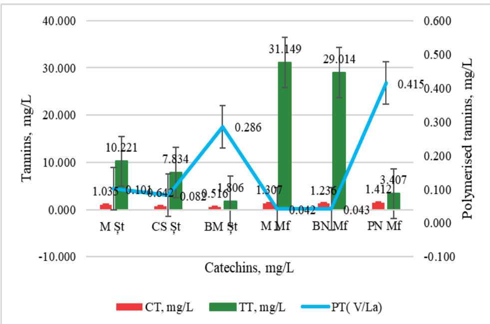

Figure 1. Content of flavonols in extracts obtained by supercritical liquids, *Notes* CT - total catechins, TT-total tannins and PT- polymerized tannins*

The total content of flavonols (abreviere, mg/l) obtained from grape pomace extracts was higher in the 'Mamaia' and 'Băbeacă neagră' varieties cultivated in the Murfatlar geographical area espectively 31.149 mg/l and 29.149 mg/l. At the opposite pole, the lowest concentrations of total flavonols were recorded in the extracts obtained from the 'Burgrund Mare' variety, cultivated in the Stefanesti wine region. The high concentrations of total flavonols recorded in the extracts obtained from the 'Mamaia' variety led to a decrease in tannins, a decrease in the content of polymerized tannins, and a low content of catechins.

The polyphenolic profile of extracts (Table 2) made by UHPLC-HRMS is shown below.

Table 2 presents the results obtained from grape pomace extracts using supercritical liquid CO2, for all the compounds found. Flavonols are found in fresh grape pomace, but degrade as they undergo treatments, drying and extraction with organic solvents. Catechin is present in three values 0.01mg/L (CS_Sf) - 0.31mg/L (MM_Mf).

Epicatechin had values between 9.04 mg/L in the 'Băbească Neagră' variety and 0.03 in the 'Burgund Mare' variety. Shikimic acid is an important polyphenolic acid in plant synthesis and was highlighted by large quantities in the grape pomace extract. The highest values were recorded in the extracts obtained from the 'Mamaia' variety (9.29 mg/L), cultivated in the Murfatlar region, and 4.49 mg/l as was recorded in the extracts obtained from the 'Merlot' variety, cultivated in the Stefanesti wine region. Phenolic acids are found in high łquantities, for example gallic acid, which is found in the grape pomace extract between 0.05mg/L (BM-St) and 15.64 mg/L (MM_M). It has a low content for CS_St (0.09 mg/L) compared to the reference 5.49 mg/100 g DW (Rockenbach, 2011) for the same variety, but it falls with other 'Mamaia' variety, compared to the 'Bordeux' variety in reference (Rockenbach, 2011). Another high phenolic acid is caffeic acid, ranging from 0.03 mg/L (CS_St) to 38.87 mg/L (MM_Mf).

The wine obtained at the two winemaking centers (Stefanesti and Murfatlar) was fortified with 5% extract, obtained by the CO2 supercritical fluid extraction method. The fortification of the wines was done as follows: for one liter of wine, 5 ml of extract obtained by the SEF-CO2 method were used.

Table 2. Individual phenolic compounds of pomace extracts obtained by supercritical liquid CO_2

Polyphenold profiles/grape pomace varieties mg/L	BM-St	CS-St	M-St	BN-Mf	MM-Mf	PN-Mf
shikimic acid	0.87	3.63	4.49	4.40	9.29	3.06
gallic acid	0.05	0.09	0.11	15.64	14.82	2.02
3,4-DHB acid	1.05	2.29	2.66	1.14	3.65	3.00
2.5 DHB acid	n.d.	0.04	0.02	0.05	0.11	0.04
4 HDB acid	0.30	0.31	0.52	0.46	0.60	0.40
caffeic acid	0.06	0.03	0.04	28.70	38.87	0.52
catechins	0.03	0.01	0.02	0.13	0.31	0.00
epicatechins	0.03	0.04	0.03	9.04	8.10	0.15
siringic acid	1.77	7.59	13.56	13.49	19.44	15.03
oenin	n.d.	n.d.	n.d.	4.57	4.42	n.d.
p-cumaric acid	0.09	1.65	2.79	0.40	0.47	0.11
polydatin	n.d.	0.46	0.47	0.31	0.43	0.41
t-resveratrol	n.d.	0.91	0.00	0.81	2.53	n.d.
taxifolin	n.d.	0.01	0.04	0.16	0.34	0.07
suberic acid	2.25	1.47	2.04	0.03	6.34	0.35
vitexin	n.d.	0.03	0.06	0.59	0.42	0.01
hesperidin	0.29	n.d.	0.28	0.42	0.44	0.28
ferulic acid	1.08	n.d.	0.00	0.08	0.65	n.d.
rutin	0.29	n.d.	0.28	0.42	0.44	0.28
plorizin	0.23	0.27	0.33	n.d.	0.47	0.33
elagic acid	0.79	1.04	0.94	0.99	1.90	1.37
azelaic acid	4.41	3.42	6.09	2.01	14.95	2.46
quercetin	0.82	1.07	0.97	1.02	1.93	1.40
abscisic acid	0.01	0.05	0.10	0.26	0.30	0.17
sebacic acid	4.04	0.08	0.32	0.05	1.31	0.08
ploretin	n.d.	0.02	0.02	n.d.	0.07	0.02
kaempferol	0.14	0.14	n.d.	0.56	1.76	0.14
EGCG	n.d.	0.32	n.d.	0.34	0.34	n.d.

n.d. - not determined

The wine obtained at the two wine centers (Stefanesti and Murfatlar) was fortified with 5% extract, obtained by the extraction method of supercritical fluids CO_2. The Figure 2 shows the total polyphenol content in wine, before and after fortification with the extracts obtained.

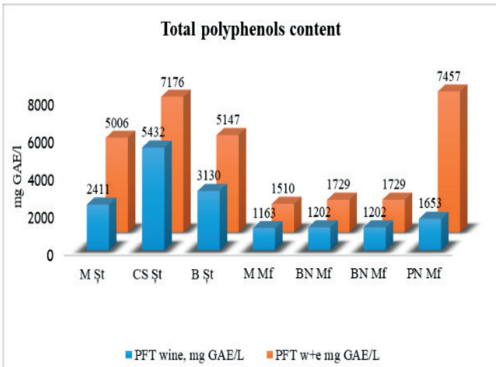

Figure 2. Total polyphenols content of wine varieties before and after fortification with pomace extract biocompounds (**Notes PFT wine - represents total polyphenolic compounds in the initial wine, PFT w+e - represents total polyphenolic compounds in fortified wine with extract*)

In Figure 2, it is noted that the highest amount of total polyphenols, existing in fortified wine, is found in 'PN_Mf' SFE (7457 mg GAE/L), even though the initial wine had a rather low concentration (1653 mg GAE/L). The same difference in terms of total polyphenol concentrations was also evident in SFE 'CS_St' (7176 mg GAE/L).

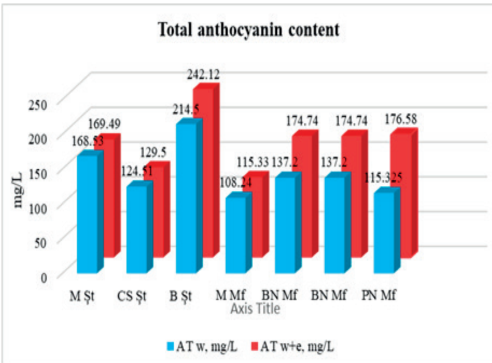

Figure 3. Total content of anthocyanins from wine varieties before and after fortification with biocompounds pomace extracts

By drying the grape pomace are destroyed from anthocyanins (Figure 3), as well as by extraction of SFE-CO_2, for this reason fortified wines do not have very large quantities of anthocyanins. A significant increase is in fortified wine by 'PN_St'_SFE (288.58 mg/l) compared to wine without extract 'PN_St' (254.19 mg/l).

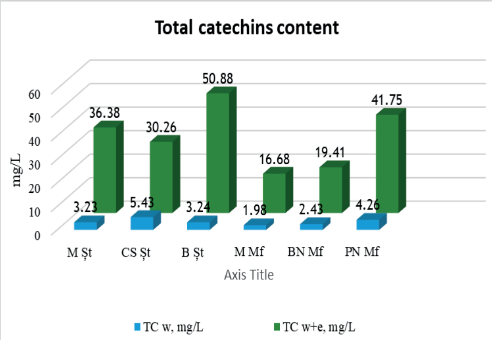

Figure 4. Total content of catechins of wine varieties before and after fortification with pomace extracts

The content of catechins (Figure 4) in the fortified wine was high than in the original wine, it increased with the of addition of grape pomace. Almost all fortified wines have an increase of about 10 times higher than the original wine. The highest concentration is in the 'BM_St' SFE wine (50.8831 mg/l), followed by 'PN_Mf' SFE (41.7521 mg/l).

The tannin content is lower in the fortified wines (Figure 5). 'PN_Mf' SFE (68.61 mg/l) is higher than the original 'PN_Mf' wine (14.0929mg/l).

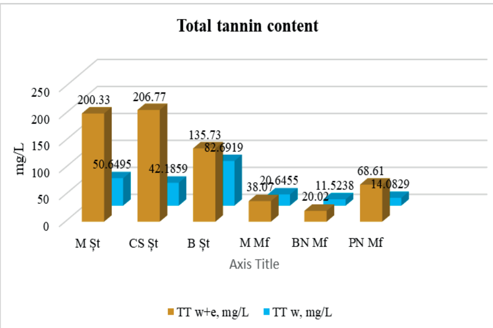

Figure 5. Total content of tannins of wine varieties before and after fortification with pomace extracts

In Figure 6, the antioxidant activities of Stefanesti wines are noted, the highest concentration is observed in fortified wine 'CS_St' SFE (216.13 mmol TE/100 ml), followed by fortified wine 'M_St' SFE (125.98 mmol TE/100 ml). The highest antioxidant activity of a fortified wine from Murfatlar is 'PN_Mf' SFE (101.56 mmol TE/100 ml).

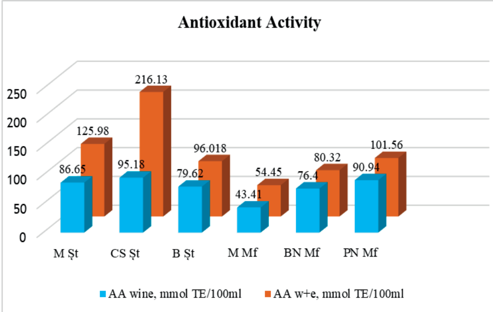

Figure 6. Antioxidant activity for wine varieties before and after fortification with pomace extracts

Table 3 presents the correlations established between the polyphenolic compounds in the original wine and the fortified wine.

A positive correlation was established between the total antioxidant activity of the polyphenolic compounds and the content of catechins

A negative correlation was established between the content of polymerised tannins and tannins (-0.828).

Positive correlations were also established between the antioxidant activity, anthocyanins (0.586) and catechins (0,790).

Table 3. Correlation matrix (Pearson)

Variables	TPF mg GAE/L	TA, mg/L	TC, mg/L	TT, mg/L	PT mg/L	AA, mmol TE/g
TPF mg GAE/L	**1**	0.476	**0.958**	0.504	-0.102	**0.765**
TA, mg/L	0.476	**1**	0.266	-0.130	0.388	**0.586**
TC, mg/L	**0.958**	0.266	**1**	**0.631**	-0.191	**0.790**
TT, mg/L	0.504	-0.130	**0.631**	**1**	**-0.828**	0.390
PT mg/L	-0.102	0.388	-0.191	**-0.828**	**1**	0.170
AA, mmol TE/g	**0.765**	**0.586**	**0.790**	0.390	0.170	**1**

The influence of grape pomace extract on the bio-compound content of fortified wines is outline in Figure 7. The 85.45% PCA score is a very good score.

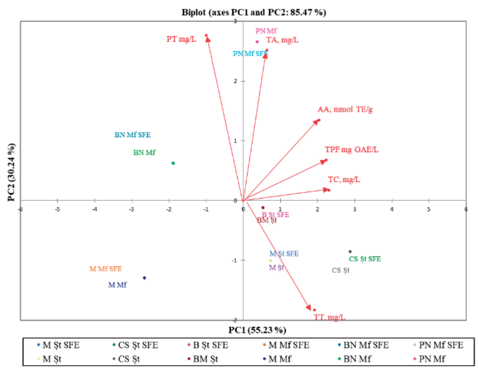

Figure 7. Distribution and principal component analyzed (PCA), according to the composition of polyphenolic compounds in the original and fortified wines

The samples were also analysed with HPLC-HRMS to identify individual polyphenolic compounds in wine and fortified wine.

Table 4 shows the polyphenolic profile of wine varieties and wines fortified with grape pomace extract.

The influence of grape pomace extract on the content of individual polyphenols in fortified wines is underlined in Figure 8.

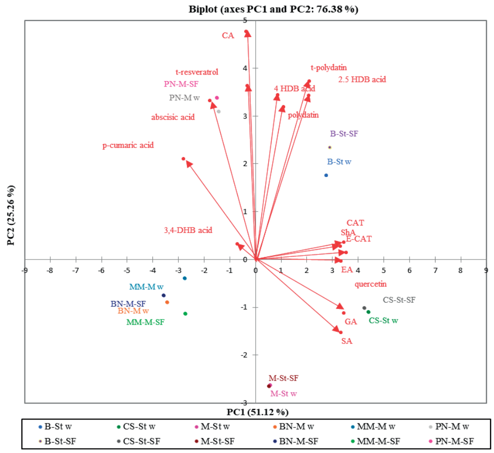

Figure 8. Distribution and principal component analyzed, according to the concentrations of compounds highlighted in the original and fortified wines

Table 4 Individual phenolic compounds of wine and fortified wines

Individual Polyphenols / Varieties	B-St w	CS-St w	M-St w	BN-M w	MM-M w	PN-M w	B-St-SF	CS-St-SF	M-St-SF	BN-M-SF	MM-M-SF	PN-M-SF
ShA	31.69	38.54	30.41	21.46	17.67	27.91	35.18	39.69	31.38	22.96	17.98	27.99
GA	76.55	110.73	67.87	5.78	14.81	14.63	78.39	112.75	65.97	6.52	17.12	15.20
3,4-DHB acid	6.02	4.53	5.85	7.32	4.31	5.02	7.03	4.48	5.79	7.19	4.37	5.30
2.5 HDB acid	1.36	1.02	1.01	0.58	0.72	1.42	1.39	1.33	0.94	0.49	0.66	1.57
4 HDB acid	0.89	0.79	0.44	0.86	0.42	0.92	0.97	0.80	0.47	0.85	0.44	0.85
CA	8.91	4.06	3.70	4.33	6.39	13.63	9.68	3.98	3.42	3.85	6.41	13.85
CAT	50.76	76.05	30.45	10.10	15.68	27.93	51.86	64.91	33.78	9.98	16.41	29.35
E-CAT	19.18	25.56	12.98	1.24	3.11	7.27	19.57	21.33	11.96	1.21	3.23	7.70
SA	250.11	406.84	315.82	33.03	78.85	86.61	253.73	415.50	266.38	34.72	79.26	84.86
p-cumaric acid	2.29	1.18	1.11	4.74	3.02	3.75	2.84	1.37	1.33	5.07	3.24	3.87
polydatin	2.06	0.98	0.75	0.44	1.60	1.23	2.24	0.85	0.76	0.60	1.29	1.32
t-resveratrol	3.03	1.22	0.92	1.08	3.10	1.95	3.11	1.05	0.71	1.72	2.05	2.66
t-polydatin	535.73	494.52	264.85	266.46	219.77	625.74	536.83	474.26	250.31	194.60	180.88	501.23
EA	0.87	0.92	0.37	0.23	0.19	0.15	0.89	0.92	0.44	0.24	0.23	0.20
quercetin	0.87	0.92	0.37	0.23	0.19	0.15	0.89	0.92	0.44	0.24	0.23	0.20
abscisic acid	0.34	0.40	0.30	0.50	0.45	0.71	0.36	0.39	0.32	0.53	0.42	0.78

CONCLUSIONS

The grape pomace has a complex chemical composition, with many biocompounds needed in human nutrition. In this study were determined total polyphenolic compounds and individual bioactive compounds, among which: phenolic acids, stilbene and flavonoids. According to the chromatographic analysis using UHPLC-HRMS, phenolic compounds are individually identified. The main component analysis (PAC) demonstrated that antioxidant activity may be associated with the phenolic composition of the grape pomace. The extracts from grape pomace showed high antioxidant activity in all grape varieties, the highest is seen in the Pinot Noir variety from Murfatlar (131.606 mmolTE/100 ml). Due to the significant concentration of polyphenolic compounds found in the grape pomace of the Mamaia variety from Murfatlar (1261.11 mg GAE/L), this waste can be reused in the food industry, in functional foods, as in our study was wine. The fortified wine with the highest total polyphenol content is Cabernet Sauvignon (7176 mgGAE/L) and Burgund (5147 mgGAE/L) from Stefanesti, and the wine with the highest antioxidant activity is Cabernet Sauvignon from Stefanesti (216.13 mmolTE/100 ml).

From the study it is noted that the CO_2 supercritical liquid extraction method is a sustainable management method for wine waste. It can be used as a method of fortifying wines. The sanogenic value of fortified wines can be mentioned.

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