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STUDIES ON THE EVALUATION OF THE PHENOLIC POTENTIAL OF GRAPE POMACE FOR RED WINES

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Abstract

The valorisation of phenolic compounds from grape pomace continues to remain a challenge, but also an opportunity to promote sustainable practices in wine industry. The objective of this study was to evaluate the phenolic potential of the red wine fermented-pomace obtained during 2023 harvest from the Feteasca Neagră and Cabernet Sauvignon varieties from three renowned Romanian vineyards: Murfatlar, Dealu Mare and Iaşi. The extraction method used was the classical solid-liquid method, that is the maceration of the pomace at ambient temperature for 24 hours, with discontinuous stirring. As extraction solutions there were used mixtures of ethyl alcohol and water in different concentrations: 25%, 50%, and 75% v./v. The polyphenolic content of the resulting extracts was evaluated by UV-VIS spectrophotometric determinations: total polyphenols (g GAE/L), anthocyanins content (mg/100 g grape pomace, colour intensity and antioxidant activity (DPPH%). A series of phenolic indices, such as the total polyphenol index and the Folin-Ciocalteu index, were also calculated. The obtained results highlighted that many factors influence the extraction process. The highest values for extracted polyphenols were recorded for the 50% hydroalcoholic solution, but also significant differences were induced by the grape variety and the viticultural terroir. The values obtained for the total phenolic compounds content fluctuated in the range of 7.1-31.9 g GAE/L and between 253-798 mg/100 g in the case of anthocyanin pigments. All phenolic extracts presented high antioxidant activity, confirming that they can be used as cheap and easily available sources of bioactive compounds for the pharmaceutical, cosmetic and food industries.

Key words*: grape pomace, hydroalcoholic extract, total phenolic compounds, antioxidant activity, anthocyanins.*

INTRODUCTION

The grape pomace is one of the main secondary products that result after pressing from the winemaking process. It consists mainly of skins, seeds and remains of the stems, overall representing 20-25% of the mass of the processed grapes (Vukusik et al., 2023). The grape pomace is an attractive natural resource due to its high content of phenolic compounds, which are well known for their antioxidant activity and nutraceutical benefits (Iliyas et al., 2021). It is estimated that 60-70% of the phenolic compounds of grapes remain in the pomace after winemaking and make up 4.8-5.4% of the dry matter of the pomace (Taladrid et al., 2023). The polyphenols in pomace include flavonoids, tannins and stilbenes (Nurgel & Reicher, 2020) but also anthocyanins. The variability of phenolic compounds and the extraction yield depend on the grape variety, the terroir, climate, soil conditions and winemaking process technology (Caldas et al., 2018). The choice of extraction technique for the recovery of bioactive compounds is essential because it significantly influences both the yield and the recovery of these compounds. One of the most used and traditional extraction techniques is the solid-liquid extraction by maceration (Rodrighez et

al., 2022). Although this method is laborious and time-consuming, it does not involve the use of expensive equipment. It is a widely used conventional technique for extracting bioactive compounds from plant materials, including grapes and grape pomace. Due to the polar nature of polyphenols, organic solvents such as ethanol, methanol, acetone, ethyl acetate, and chloroform are commonly used (Jara-Palacios et al., 2020). The typical process for solid-liquid extraction involves maceration and mechanical agitation, and the main factors influencing the efficiency of this method are: the type of solvent, the solvent-sample ratio, the particle size, the pH, the extraction temperature and the duration of the extraction process (Castelanos, 2022). According to the literature, the choice of solvent is one of the most important operational parameters. Thus, considering the polar nature of polyphenols, better extractive yields can be obtained using polar protic solvents, which can form hydrogen bonds, such as hydroalcoholic solutions (Fontana et al., 2013). The objective of this study was to evaluate the content of phenolic compounds in the pomace obtained from the vinification process of red grapes from the Fetească Neagră and Cabernet Sauvignon varieties from three renowned vineyards using a classic solid-liquid extraction method, simple, efficient and accessible.

MATERIALS AND METHODS

The pomace used for extractions was obtained in 2023, following the winemaking process of two *Vitis vinifera* grape varieties for red wines, one autochthonous, Fetească Neagră, and one international variety, Cabernet Sauvignon, from three renowned wine regions in Romania: Murfatlar, Dealu Mare and Iaşi. The winemaking technology was classic with five-day maceration and fermentation, followed by pressing using a hydraulic press. The pomace was conditioned by drying in a thin layer at ambient temperature, being palletized every 24 hours to facilitate water evaporation and to prevent the development of bacteria and fungi in the wet layer. The extraction was carried out at ambient temperature, with discontinuous stirring, for 24 hours, the pomace/solvent ratio being 1/4. The extraction solution was a hydroalcoholic mixture of different concentrations, respectively 25%, 50% and 75%. The liquid extracts obtained were separated from the solid part by filtration (using filter paper) and then by centrifugation (1700 rpm for 15 minutes) and kept in a refrigerator at 4°C for approximately 1-2 days until the analyses were carried out by spectrophotometric determinations using the Helios Alpha UV-VIS spectrophotometer (Thermo Spectronic, Marea Britanie). ¸

The experimental extractions variants and procedure are chematically presented in Figure 1.

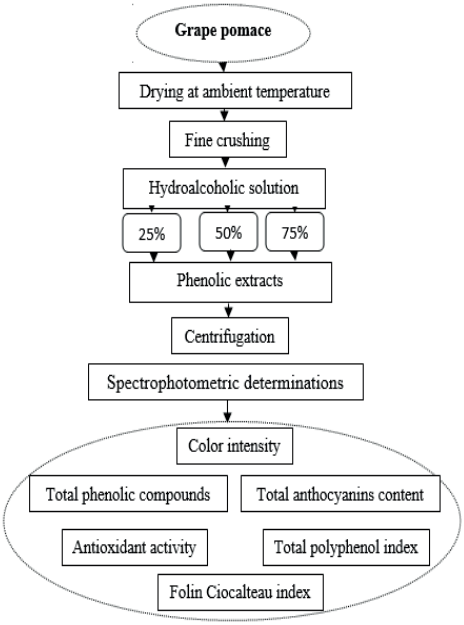


Figure 1. Experimental extraction scheme of pomace

The *total phenolic compounds*, expressed as (GAE g/L), were determined using the Folin-Ciocalteu method, by measuring the absorbance at 760 nm. The *anthocyanin content* (mg/100 g grape seed) was evaluated by the Ribèreau-Gayon method, based on the colour change of anthocyanins depending on pH; this consists of measuring the variation in absorbance at 520 nm of the colour of anthocyanins at pH 0.6 and 3.5 using distilled water as a control (Ribèreau-Gayon et al., 2017). The *antioxidant activity* (AA%) was analyzed according to the method proposed by Brand-Williams et al. (1995) using 0.1 ml of extract diluted 1:10. Thus 0.1 mL of each

sample was mixed with 3.9 mL of 4% DPPH diluted in 96% (v/v) ethanol and stirred. The homogenized mixture was incubated in the dark for 30 minutes at room temperature and subsequently, the absorbance was measured at 517 nm. The *colour intensity* was determined based on the sum of the absorbance values measured at 420 nm, 520 nm and 620 nm, expressed in absorbance units (Au). The *total polyphenol index* is based on the fact that the benzene rings, characteristic of phenolic compounds, absorb strongly the UV light, having an absorption maximum at a wavelength of 280 nm. The *Folin-Ciocalteu index* is based on the reaction of hydroxyl groups with a phospho-molybdenum reagent which is reduced to a mixture of blue tungsten oxide and molybdenum. The blue colour produced has a maximum absorption at 750 nm and is proportional to the total amount of phenolic compounds.

RESULTS AND DISCUSSION

The efficiency of the extraction process of bioactive compounds is influenced by a number of factors, including the type of solvent and its concentration (Schieber et al., 2017). Previous research has shown that milder extraction methods performed at ambient temperatures can reduce the degradation of bioactive compounds and improve extraction yield (Zhang et al., 2020).

The results regarding the recovery of phenolic compounds from the grape pomace collected in the three wine regions studied - Murfatlar, Dealu Mare and Iaşi - are highlighted in Table 1. The parameters evaluated include total phenolic compounds, anthocyanins, colour intensity, antioxidant activity, Folin-Ciocalteau index, and total polyphenol index. The values of total phenolic compounds were lower in the case of the Fetească Neagră variety (7.81-24.4 GAE g/L) compared to the Cabernet Sauvignon variety (16.67-31.89 GAE g/L), the differences being given by the source of pomace and concentration of the solvent mixture (ethanol/water). The highest efficiency in the recovery of phenolic compounds was observed with the 50% hydroalcoholic solution, recording the highest values of phenolic compounds (24.4 GAE g/L for Dealu Mare and 23.7 GAE g/L for Iaşi), values significantly higher compared to those obtained with solutions of 25% concentration. In the case of the Cabernet Sauvignon variety, the values of phenolic compounds were also higher in the case of a hydroalcoholic concentration of 50%, registering 31.89 GAE g/L in Murfatlar and 21.7 GAE g/L in Iaşi. These differences can be explained by the higher polyphenol content of the Cabernet Sauvignon variety compared to Fetească Neagră, as well as by the influence of terroir on the chemical composition of the pomace. Moderate concentrations of alcohol have been shown to be more effective in extracting phenolic compounds (González et al., 2010), fact also demonstrated by our results. Regarding the anthocyanin content of the analysed phenolic extracts, a significant variability of the values was observed, which were located in a fairly wide range, between 253-798 mg/100 g of grape pomace. The values were lower in the case of the 25% ethanol solvent for the Cabernet Sauvignon variety from Murfatlar. An increase in values was observed with the increase in the concentration of the hydroalcoholic solution, at 50% reaching a maximum of 588-646 mg/100 g of pomace in the case of the Fetească Neagră variety and 790 mg/100 g of pomace in the case of Cabernet Sauvignon. These results suggest that a medium concentration of ethanol is optimal for the efficient extraction of anthocyanins from the cellular structures of the grape pomace, without damaging them. In the case of the 75% hydroalcoholic solution, the anthocyanin content decreased slightly, on average by 9.2% for Fetească Neagră and 4.3% for Cabernet Sauvignon. Regarding the colour intensity of phenolic extracts, in the case of the Fetească Neagră variety, the value was 8.27 AU at 25% alcohol and increased significantly at concentrations of 50% and 75% alcohol, to 22.45 AU and 18.23 AU, respectively. In this case, the most efficient extraction was with the solution of 50%. In Cabernet Sauvignon, the colour intensity was highest at extractions with 75% alcohol (38.9 AU), followed by the extraction with alcoholic solution of 50% (20.23 AU), the lowest being at 25% alcohol (10.07 AU). More concentrated solvents can cause faster extraction of some compounds, but also an incomplete solubilization of others,

which can lead to the loss of important fractions of polyphenols, especially the most delicate or poorly soluble ones. The antioxidant activity, an essential indicator of the ability of grape pomace to neutralize free radicals, is a key factor in assessing its potential to protect the body against oxidative stress. The antioxidant activities of the extracts were relatively constant for both varieties extracted with solutions of 50% and 75% alcohol, exceeding 90%. These high antioxidant activities are mainly due to the content of the extracted flavonoid compounds, including catechins and anthocyanins, highlighting the potential of these extracts to combat oxidative stress (Nakamura & Okada, 2020). The research of Zhang et al. (2020) confirms that the antioxidant activity of grape pomace is directly correlated with the content of polyphenols and that the high alcohol concentrations are able to efficiently extract these compounds from the cellular structures of the grape pomace. The Folin-Ciocalteau index measures the total polyphenol content, being an essential tool in the analysis of bioactive compounds in phenolic extract (Kumar & Goel, 2019). In the case of the Fetească Neagră variety, the highest values were obtained with the 50% hydroalcoholic solution, with 22.78 AU in Dealu Mare and 17.89 AU in Iaşi. Similar results were recorded for the Cabernet Sauvignon variety with 15.42 AU in Iaşi and 26.16 AU in Murfatlar. These data suggest higher extraction efficiency at moderate alcohol concentrations. The total polyphenol index, also known as the 280 nm index, is based on the absorption characteristic of phenolic benzene rings present in (+)-catechin and its polymeric derivatives (tannins), the former being identified as the main monomer in grape skins (Riberau Gayon, 2000; Spigno et. al., 2007). For this reason, the index is correlated with the total phenolic compound values. Due to the particularities of the extraction procedure (extraction method, solid/solvent ratio, variety, temperature, time, hydroalcoholic solution), different from the models presented in the specialized literature, the data obtained could not be compared with those in the existing literature.

Tabelul 1. The phenolic potential of extracts obtained from the varieties Fetească Neagră and Cabernet Sauvignon in the wine regions of Dealu Mare, Iaşi and Murfatlar obtained with ethanolic solutions of 25, 50 and 75%

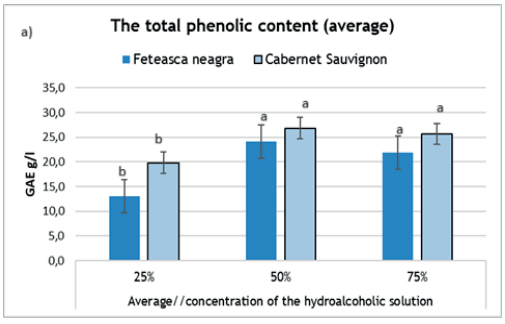
Measured parameters	Fetească Neagră					
	Dealu Mare			Iaşi		
	25%	50%	75%	25%	50%	75%
Total phenolic compounds g GAE /L	7.81±1.8b	24.4±9.6a	20,67±4.2a	18,27±2.1a	23,7±4.6a	23,01±3.7a
Anthocyanins (mg/100 g pomace)	331±22d	588±30ab	521±28c	537±40bc	646±36a	632±45a
Colour intensity (AU)	8.27±1.5c	22.45±3.2b	18.23±3.2b	21.61±2.0b	29.54±3.4a	22,3±2.6b
Antioxidant activity (%)	-	-	-	90,23±7.9a	91,83±10.6a	90,06±8.5a
Folin-Ciocalteu index, 750 nm (AU)	7.84±1.5d	22,78±2.4a	19.48±2.0ab	13,85±1.6c	17.89±1.9b	17,38±2.1b
Total polyphenols index, 280 nm (AU)	78.93±15.2c	233,24±25.4b	219.51±23.2b	206.21±20.1b	301.53±27.2a	290.96±22.4a
Measured parameters	Cabernet Sauvignon					
	Murfatlar			Iaşi		
	25%	50%	75%	25%	50%	75%
Total phenolic compounds g GAE /L	22.98±8.5a	31.89±10.2a	30.69±11.0a	16.67±5.9a	21.7±4.7a	20.6±4.5a
Anthocyanins (mg/100 g pomace)	253±18c	798±48a	790±41a	559±35b	790±49a	797±53a
Colour intensity (AU)	10.07±2.2d	20,23±2.5bc	18,35±2.1c	24.49±2.8b	36,77±3.0a	38.96±3.5a
Antioxidant activity (%)	88.21±3.9a	90.35±4.7a	91.02±4.3a	91.85±4.8a	92.53±5.0a	92.93±3.8a
Folin-Ciocalteu index, 750 nm (AU)	18.21±2.2b	26,16±2.8a	24.3±3.0a	12,82±1.8c	15.42±1.9bc	14.66±2.0bc
Total polyphenols index, 280 nm (AU)	199.1±11.2b	193.3±15.2b	278,9±17.9a	217.55±20.5b	284.7±24.3a	280.7±20.2a

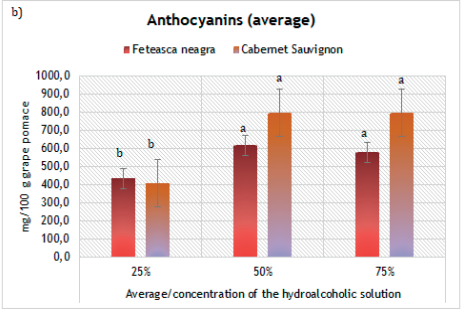
Average values ± standard errors (n = 3). The different letters show the statistical difference among results for $p<0.05$. For the same compound, a common letter for 2 or more variants means no significant difference among them.

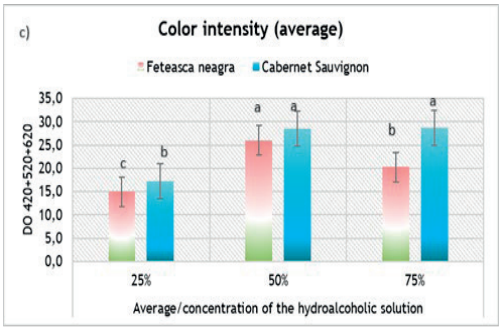
Figure 2 presents the averages obtained for each of the two varieties studied: Fetească Neagră and Cabernet Sauvignon, using three hydroalcoholic solutions: 25%, 50%, 75% for

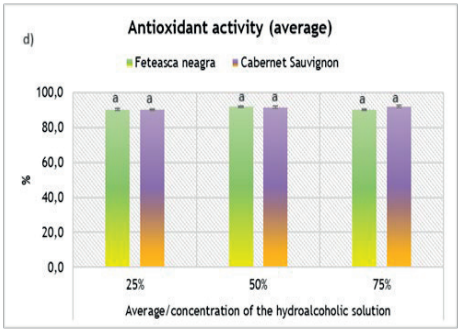
the following analysed parameters: a) total phenolic compounds, b) anthocyanins, c) colour intensity, d) antioxidant activity, e) total polyphenol index and f) Folin-Ciocalteu index. It is observed that the most effective hydroalcoholic extraction solutions are those of 50%, a result also supported by the specialized literature which mentions that at moderate alcohol concentrations (50%), there is a favourable balance between the solubility of phenolic compounds and the properties of the solvent (Liu et al., 2020). In the case of total phenolic compounds, the average obtained for the 50% hydroalcoholic solution was 24.1 GAE g/L for the Feteasc Neagră variety and 26.8 GAE g/L for Cabernet Sauvignon. A significant increase in values for both varieties compared to the 25% hydroalcoholic solution is noted, by 84.4% for Feteasc Neagră and by 35.2% for Cabernet Sauvignon. These results are consistent with the literature, which suggests a higher efficiency in the extraction of phenolic compounds from grape pomace at moderate alcohol concentrations (Sánchez & Rodríguez, 2020). Regarding the extraction of anthocyanins from the pomace, the highest values were obtained at a concentration of 50%, with an average value of 675 mg/100 g pomace for Feteasc Neagră and 794 mg/100 g pomace for Cabernet Sauvignon. The higher anthocyanin content obtained in the extract obtained from the 50% alcohol solution for the Cabernet Sauvignon variety correlates with a higher colour intensity of the phenolic concentrate (28.5 AU), this value being 8.7% higher compared to that obtained from the Feteasc Neagră variety. The antioxidant activity is slightly higher at 50% alcohol (91.83%) compared to the other solvent concentrations, and in the case of the Cabernet Sauvignon variety, the maximum value of 91.98% is also obtained at 50% alcohol.

The increase in the values obtained for the two analysed phenolic indices (total polyphenol index and Folin-Ciocalteu index) at a concentration of 50% alcohol suggests an efficient and complete extraction of a wide spectrum of phenolic compounds, highlighting the essential role of moderate alcohol concentrations in optimizing the polyphenol extraction process. These results emphasize the importance of the balance between solvent and solubility of phenolic compounds, which allows for their maximum extraction without compromising the integrity of the bioactive structures.









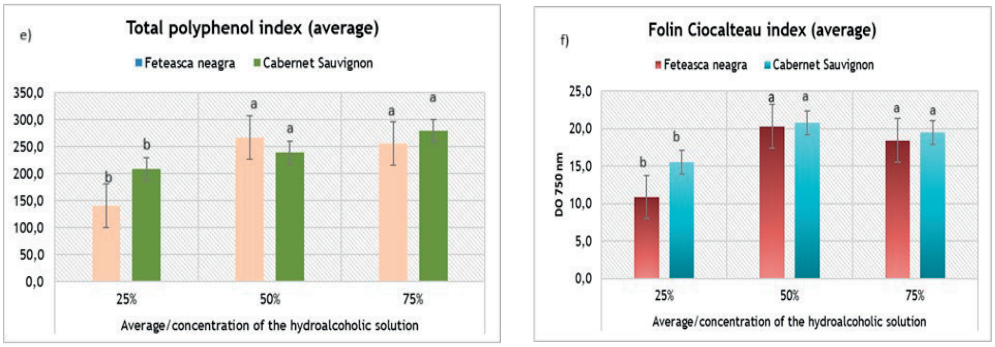


Figure 2. The average values obtained for: a) total phenolic compounds, b) anthocyanins, c) colour intensity, d) antioxidant activity, e) total polyphenol index, f) Folin-Ciocalteu index. Average values ± standard errors (n = 3). The letters show the statistical difference among results for $p<0.05$. For the same compound, a common letter for 2 or more variants shows no significant difference among them

CONCLUSIONS

The grape pomace represents a valuable source of bioactive compounds, having significant potential for use in various industries, especially in the food and cosmetic industries, due to its antioxidant and nutritional properties.

The classical solid-solvent extraction method, using hydroalcoholic solutions of different concentrations, has proven to be an effective technique for extracting phenolic compounds from grape pomace.

The results obtained showed that hydroalcoholic solutions with a concentration of 50% were the most effective in extracting bioactive compounds from both the Fetească Neagră and Cabernet Sauvignon varieties, indicating a favourable balance between the solubility of phenolic compounds and the properties of the solvent at this concentration.

Higher alcohol concentrations (75%) resulted in a slight decrease in extraction efficiency, possibly due to the reduced solubility of phenolic compounds in solutions with higher alcohol content, suggesting that a medium alcohol concentration is optimal for efficient extraction. Also, the differences between the studied varieties (Fetească Neagră and Cabernet Sauvignon) revealed that the thicker grape skin of the Cabernet Sauvignon variety allows for a more efficient extraction of phenolic compounds, which is reflected in the higher values of antioxidant activity and colour intensity.

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