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ANTIOXIDANT ACTIVITY AND TOTAL PHENOLIC CONTENT OF GRAPE SEEDS AND PEELS FROM ROMANIAN VARIETIES

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Abstract: *Grape seeds and peels are valuable by-products from wine production industry that can be valorized in food production. Both grape seeds and peels have a great amount of antioxidants and polyphenolic compounds. The aim of this study was to investigate the total phenolic content of red and white grape seeds and peels and to establish the optimal solvent concentration for highest extraction yield. For this purpose, Folin Ciocâlteu method was used to achieve the phenols content and the antioxidant activity was estimated using 2,2 – diphenyl-1-1picrylhydrazyl (DPPH) reagent. The total phenolic content ranged from 81.13 mg GAE/g for red peels, 93.47 mg GAE/g for white peels, to 128.47 mg GAE/g for red seeds and 164.70 mg GAE/g for white seeds. The One-Way ANOVA method was used to see if there are differences between the antioxidant activities depending on the solvent concentration. The results showed that the methanol concentration significantly ($p < 0.05$) influences the antioxidants extraction. The inhibition percent IC_{50} ranged from 0,24 to 4,37 µg/mL for white peels, from 3,12 to 6,29 µg/mL for red peels, from 5,53 to 5,90 µg/mL for white seeds and from 4,59 to 6,14 µg/mL for red seeds. This study highlighted the possibility to use grape seeds and peels as food ingredients or natural antioxidant to extend the shelf life of food, especial of lipids and lipid-containing foods because of their high antioxidant activity and total phenolic content.*

Key words: *grape seeds, grape peels, antioxidant activity, total phenolic content.*

1. Introduction

Grapes, one of the most cultivated fruits in the world, are used in high proportion (80%) in wine-making production [1]. Wine industry has an adverse environmental impact because of residual phenols of by-products resulted from grapes processing. Relative high amounts of phenolic compounds in waste are a problem for flora and fauna from discharge zones due to their inhibition of germination properties [2].
Grape pomace generated in wine- making process is a valuable by-product for oil extraction and some active compounds, such as dietary fiber, polyphenols, anthocyanins, flavonols and resveratrol [3, 4]. The wine-making grape pomace includes as main components peels and seeds. The polyphenols and other compounds with antioxidant activity are present in grape peels and seeds. The quantitative and qualitative distribution of polyphenolic compounds in grape peels and seeds depends on grape variety, harvesting conditions, geographic location of the culture, soil, vinicultural techniques, winemaking process, etc. [5].
Many studies showed that the phenolic profile is related to the fruit maturity level: it increases with the ripening level increase [6]. During the winemaking process, part of the phenolic compounds in grapes is

transferred to wine, but a high amount still remains in the grape by-products, mainly in the grape pomace. More than 70% of phenols from grapes rest in pomace [5].
The phenolic compounds are present in plants and have many biological functions. The researchers revealed hundreds of polyphenols in edible plants [7]. In wine pomace there are flavonoid compounds such as anthocyanins, flavonols, flavan-3-ol, tannins and non-flavonoid substances, especially resveratrol [8] and phenolic acids [9]. In red pomace there are many antochianyns, while in white pomace the flavonols are in higher quantity [10]. The total polyphenolic content of grape pomace can be influenced by the differences between white and red wine making processes [11].
In grape peels there are elagic acid, resveratrol, myricetin, quercetin, kaempferol, epigalocatechine. The results showed by many researches revealed the fact that the phenolic content is higher in red peels than in white peels because the monomeric anthocyanins are absent from white peels [10].
In grape seeds catechin, epicatechin, malic acid and galocatechin are the most important phenols. The phenolic content of grape seeds is greater than of grape peels [12].
The most important operation for phenols recovery from grape seeds and peels is the extraction. Various solvents including ethanol, methanol, ethyl ether, etc. were used by researchers for the phenols extraction. Also, various techniques, such as ultra-sonication, stirring and shaking were applied. The most used solvent is methanol or methanol/water mixtures [13].
A higher total phenolic content gives a better antioxidant activity [14, 15]. The inclusion of antioxidants is a method to prolong shelf life of food products while they add extra health benefits. Polyphenolic compounds exhibits health benefits due to its cardioprotective and anti-inflammatory effects, anti-ulcer and anti-carcinogenic activity [16].
There are many researches about the phenolic compounds and antioxidant activity from grape peels and seeds, but there are very few reports that compare the total phenolic content distribution between peels and seeds among different varieties.
The aim of this study was to determine the antioxidant activity and the total phenolic content of grape seeds and peels from two wine pomace varieties, red and white. This study wants to underline the importance of grape seeds and peels with respect their potential as a source of natural antioxidants and as an alternative to the reuse of by-products from winemaking process.

2. Materials and methods

2.1. Materials

Grape seeds and peels from red and white wine grape pomace provided by the viticulture center Jaristea, Odobesti ecosystem were used in this study. Grape seeds and peels were separated manually from dried grape pomace and ground in a speed grinder to obtain grape seeds and grape peels flours.
As reagents of analytical grade, standards of gallic acid, ascorbic acid, methanol, calcium carbonate, 2,2 – diphenyl-1-1picrylhydrazyl (DPPH) reagent and Folin-Ciocâlteu phenol reagent purchased from Sigma-Aldrich and Merck were used.

2.2. Methods

2.2.1. Antioxidant activity

The antioxidant activity was achieved by DPPH method. The extracts were prepared according to Xuetao and collaborators method [17], with some modifications. 4 g of sample was extracted with 15 mL of solvent (methanol: distillated water, 0:100, 60:40, 70:30 and 80:20). For this purpose, the mixes were ultra-sonicated at 40 ºC, at 25 Hz for 30 minutes. Then, they were centrifuged at 3000 rpm for 5 minutes. The supernatant was collected and the residue was mixed with 15 mL solvent and centrifuged again. This operation was repeated 3 times. The supernatants were collected and used for analysis.

Each extract was diluted to 50%, 40%, 30%, 20% and 10% with the corresponding solvent (methanol: distillated water, 0:100, 60:40, 70:30 and 80:20). 2 mL of sample was mixed with 1 mL of DPPH reagent and the absorbance at 517 nm wavelength was read immediately by using UV-Vis-NIR spectrophotometer (Shimadzu Corporation, Japan). A blank sample was prepared for each solvent.

The free radicals inhibition percent IC_{50} was calculated using Eq.1 [18]:

$$IC_{50} = [1-(A_{517sample}-A_{517\ blank}] \times 100 \quad (1)$$

where $A_{517\ blank}$ is the absorbance value of the control sample and $A_{517sample}$ is the absorbance value in the presence of the extract sample and standard.

2.2.2. Total phenolic content

The phenolic content of grape seeds and peels were determined using the Folin Ciocâlteu method. 2 g of samples were mixed with 4 mL solvent (methanol: distillated water, 70:30) and shacked for 5 minutes on the orbital shaker. Then, the mixes were centrifuged at 6000 rpm for 10 minutes. The supernatant was collected and the residue was extracted again with 4 mL solvent, as it is described above. The two supernatants were mixed and used for determination. 0.5 mL of extract was mixed with 0.5 mL of Folin Ciocâlteu reagent and 3 mL of Na_2CO_3 20%.

The mixture was diluted with distillate water to final volume of 10 ml. The samples were allowed to stand for 30 minutes and the absorbance was read at 750 nm by using UV-Vis-NIR spectrophotometer (Shimadzu Corporation, Japan). A blank sample was prepared with distillate water. Each determination was made in triplicate. The calibration curve was made with different gallic acid concentrations. The results were expressed as milligrams of gallic acid equivalents (GAE) per g of extract (mg GAE / g).

2.2.3. Statistical analysis

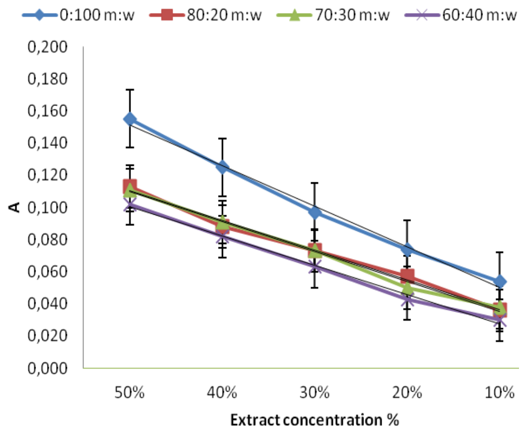
The results were expressed as the mean values ± S.D. of three parallel measurements. One-way ANOVA method from Microsoft Excel 2007 was used to test the effect of variation factors – different samples on variable IC_{50}. A difference was considered statistically significant at $p \leq 0.05$.

3. Results and discussion

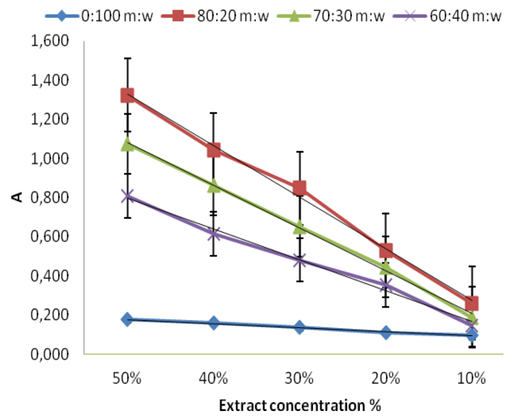
3.1. Antioxidant activity

Figure 1 shows the total phenols content, measured as absorbance at 517 nm, for the white and red grape peels extracts in water and methanol mixture at different proportion and at various extracts concentration. Statistical analysis of the data shows that the extraction solvent had a positive effect ($p < 0.05$) on the antioxidant activity by increasing it with concentration increase. According to the results obtained, there were significant differences ($p < 0.05$) between the total phenolic content of each seed and peel extract. White grape pomace had higher solvent extraction yield than the red varieties.

The total phenolic content for the white and red grape seeds extracts in water and methanol mixtures at different proportions (0:100, 60:40, 70:30 and 80:20, methanol: distillated water) and at various extracts concentration is showed in Figure 2.

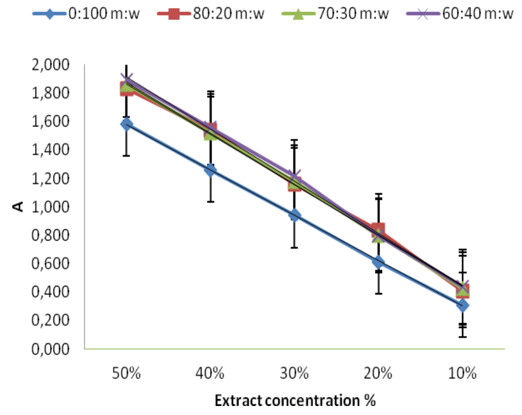


a)

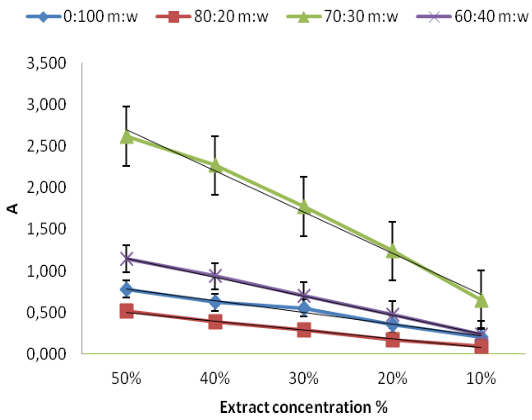


b)

Fig. 1. Absorbance curves for extracts diluted with aqueous methanol solutions (0:100, 60:40, 70:30 and 80:20, methanol: distilled water) from: a) white peels; b) red peels



a)



b)

Fig. 2. Absorbance curves for extracts diluted with aqueous methanol solutions (0:100, 60:40, 70:30 and 80:20, methanol: distilled water) from: a) white seeds; b) red seeds

The results obtained highlight that–the optimal mixture solvent for white grape peels and seeds is methanol/water in ratio 80:20, while the antioxidants from red peels and seeds are best extracted in water (0:100).

The parameter used to estimate the antioxidant activity is IC_{50} and it represents the antioxidant amount required to decrease the initial DPPH concentration by 50%. Low IC_{50} values indicated a high antioxidant activity [19].

As it is shown in Figure 3, grape peels antioxidants are best extracted with lower methanol concentrations as compared to grape seeds.

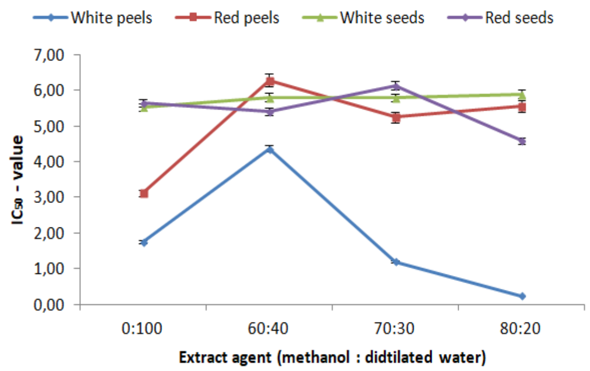


Fig. 3. IC_{50} values for grape seeds and peels extracts

The fact that the aqueous extracts from white peels and white seeds had the highest antioxidant activity underlines the possibility to use these by-products as natural additives in food products.

3.2. Total phenolic content

The variation of total phenolic contents of grape seeds and peels from white and red grape varieties are showed in Figure 4.

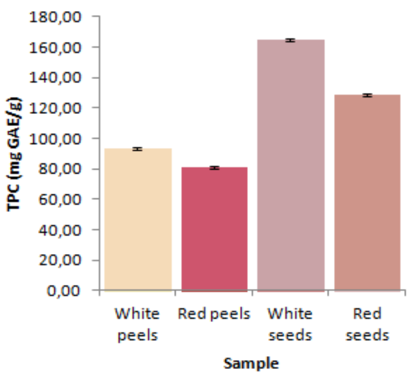


Fig.4. Total phenolics content in grape peels and seeds from red and white varieties

Regarding the total phenolic content, white peels have a higher amount of polyphenols than red peels, while white seeds have higher polyphenolic content than the red ones (Figure 3). The results revealed that grape seeds have more polyphenols than grape peels, in according to the results obtained in other studies [12, 20]. The main interest in phenolic compounds from grape by-products lies in their antioxidant capacity.

4. Conclusion

Solvent concentration is an important parameter that influences significantly the antioxidants extraction and consequently the antioxidant activity. The results for total phenolic content of grape seeds and peels confirmed other specialist's hypothesis: grape seeds are richer in polyphenols than grape peels.

In conclusion, grape seeds and peels are a natural source of phenolic compounds with high antioxidant activity. These by-products can be valorized in food industry as a source of natural phenols with the antioxidant power similar to that of synthetic antioxidants.

5. Acknowledgements

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