TESTING CERTAIN METHODS OF EXTRACTING POLYPHENOLIC EXTRACTS FROM *VITIS VINIFERA* SEEDS

Ancuţa VASILE, C. SAVIN, Rodica PAŞA, Doina DAMIAN

Staţiunea de Cercetare Dezvoltare pentru Viticultură şi Vinificaţie Iaşi, Iaşi
e-mail: ancuta.vasile@yahoo.com

The efficiency of the methods of extraction of the vegetal principles is enssured by the relation extraction agent/vegetal mass, temperature, the number of extraction phases, pH, the size of the vegetal materil particles, performance rating.In the present study, two extractive methods were tested, the discontinuous shaking extracting method and Soxhlet continuous extractive method. In the first case the extractive process was followed durind its dynamic course for 24 hours. In order for the mentioned extractive methods to be assessed, the obtained vegetal extracts were analyzed to determine the total polyphenol content (g GAE/L) and the index of tannoid matters (ITM). As a vegetal material, Vitis vinifera seeds were used, from the varieties Cabernet Sauvignon, Merlot, Băbească neagră and Fetească neagră.

The purposes were to select the most efficient metho for the extraction of total polyphenolic compounds, and to select the vegetal material with the highest content of tatal polyphenolic compounds.

The importance of the studies regarding the grape polyphenols is the evaluation of the enological potential of the different varietiess of vines, as well as the evaluation of thei properties benefic for healthcare, due to their anti-oxidant, anti-neoplastic and anti-inflammatory actions. The richest vegetal extracts as to the total polyphenols, tanoid matter and anthocyns content were obtained from Băbească neagră and Merlot grape seed by using both extractive methods.

Keywords*: total polyphenol, tannoid matters, Vitis Vinifera.*

Until now, several data about the polyphenols of vegetal origin have been collected, 8000 compounds having been already discovered [7]. Further to the progress registered as to their multiple biological functions, different extractive methods have been experimented.

Obtaining polyphenolic extracts, however, still raises a series of technological problems, the most important of them being the ensuring of the maximum extraction capacity [4, 5, 6, 9]. This parameter depends especially on the physical and chemical factors which influence the extractive processes.

MATERIAL AND METHOD

The extraction of total polyphenols in the Vitis vinifera seeds was carried out though the discontinuous stirring method and the continuous Soxhlet method. The extractive process was carried out, in this first case, in glass decanters with worn in cork and flat bottom; the vegetal materials were put in and the solvent was then added (ethyl hydroxide heated at 40 ^{o}C), in a ratio of 1/10 (solid material/solvent). The decanters were maintained for 1440 minutes on the mechanical agitator, at 100 rpm. The extracts obtained after 15, 30, 60 and 1440 minutes as of the moment the solvent had been added, were separated from the vegetal materials by a 10 minutes centrifugation at 6000 rpm. In the continuous Soxhlet extractive process, the same vegetal materials were used, maintaining the 1/10 ratio. In order for the analyses to be carried out, the polyphenolic extracts were kept at 4 ^{o}C.

The assessment of the extractive process's effectiveness was made by determining the total polyphenol content (g GAE/L) using the Singleton and Rossi method – 1965 [11], the anthocyans number (mg/ml) though the R Gayon and Sonestreet method – 1965 [10] and the tanoid matter index (TMI) according to Bourizeix – 1986 [2].

RESULTS AND DISCUSSIONS

The results achieved through the discontinuous stirring extractive method are shown in *figure 1 - 3*. The total polyphenols concentrations of the polyphenol extracts, obtained after 60 minutes of contact between the vegetal material and the solvent (*figure 1*) had very similar values, being comprised between 0.44-0.52 g GAE/l.

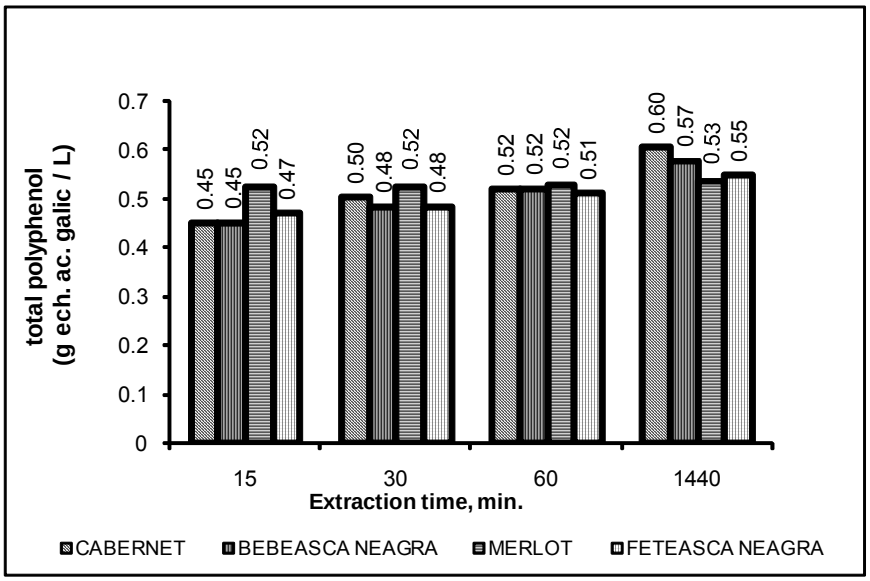

Figure 1. **The content of total polyphenols in the vegetal extracts obtained by discontinuous stirring extraction method**

In the extractive processes carried out for 1440 minutes the vegetal extracts that were obtained had total polyphenols concentrations that did not register a significant rise, the only exceptions being the vegetal extracts obtained from Cabernet Sauvignon and Băbească neagră vine seeds in which higher concentrations (by 15 % and 10 % respectively) were found.

The effectiveness of the extractive process in stirring condition was assessed by determining the tanoid matter index (TMI). The graphical representation of the data (*figure 2*) indicates a progressive rise of the TMI value further to the increase of the time of contact between the vegetal materials and the solvent. The highest values in the dynamic were registered in the case of the vegetal extracts from the Merlot vine seeds between the intervals of 15 to 30 minutes.

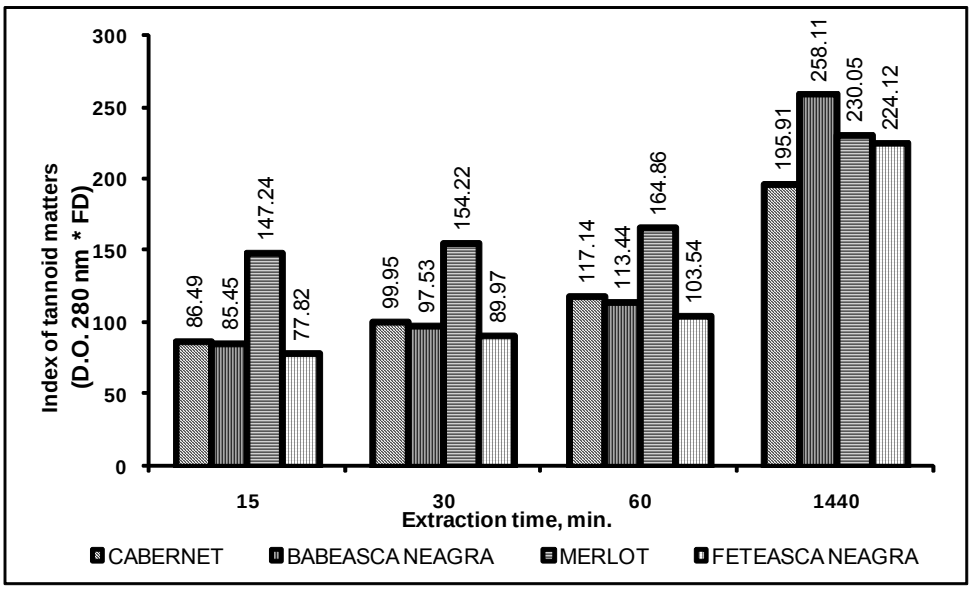

Figure 2. **The tanoid matter index of the vegetal extracts obtained by the discontinuous stirring extraction method**

In the extractive processes carried out for 1440 minutes, the TMI registered increased values in all polyphenolic extracts obtained, that is, 40 % in the case of the vegetal extract from the Cabernet Sauvignon seeds, 56 % in the case of the Băbească neagră seeds, 28 % for the vegetal extract from the Merlot seeds and by 54 % in the case of Fetească neagră seeds..

Figure 3 shows the data regarding the anthocyns content of the vegetal extracts obtained during the discontinuous extractive processes with stirring. The graphical presentation of the results shows that the vegetal extracts from the Băbească neagră seeds had an anthocyns concentration of 21.78 mg/ml after 30 minutes of contact with the solvent. In the extractive processes carried out for 1440 minutes, a differentiated rise of the anthocyns concentration was observed, according to the vegetal materials tested.

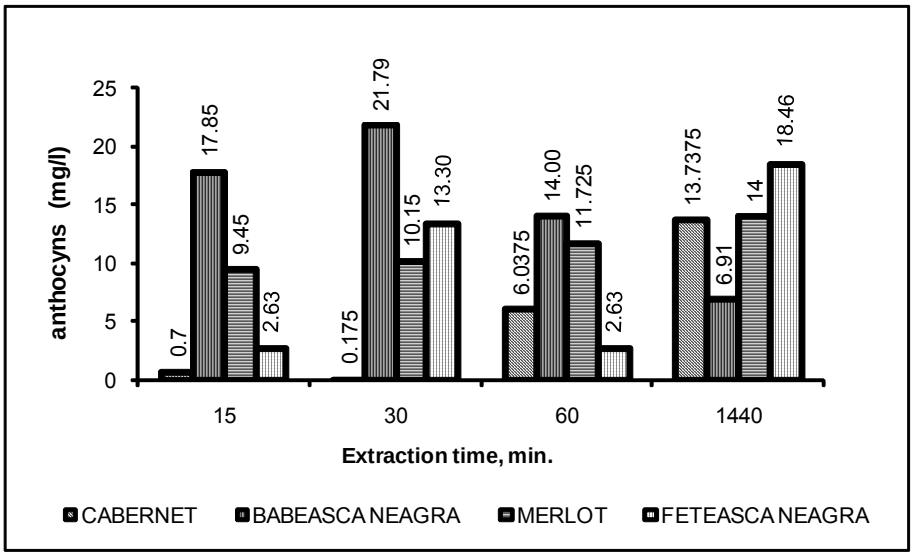

Figure 3. **The content of anthocyns in the vegetal extracts obtained by discontinuous extraction stirring method**

The features of the polyphenolic extracts obtained through the Soxhlet method are shown in *figures 4-6*. The graphical representation of the total polyphenol concentration – fig. 4 – shows that the richest extracts are those obtained from the Băbească neagră and Merlot grape seeds.

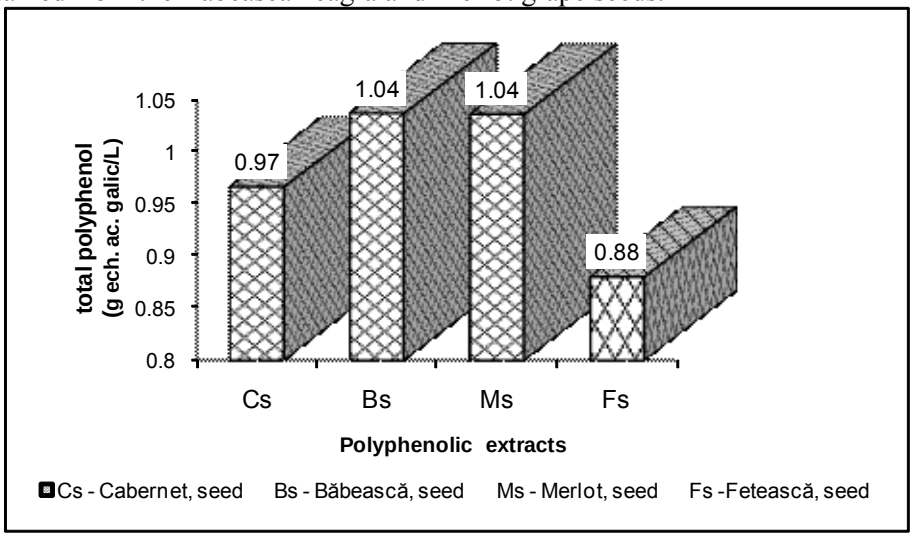

Figure 4. **The content of total polyphenols in the vegetal extracts obtained by the Soxhlet continuous extraction method**

The same profile was registered in the case of the TMI – figure 5; the values for Băbească neagră and Merlot were 690,4 and 463,5 respectively and 396,44 for Cabernet and 336 for Fetească neagră.

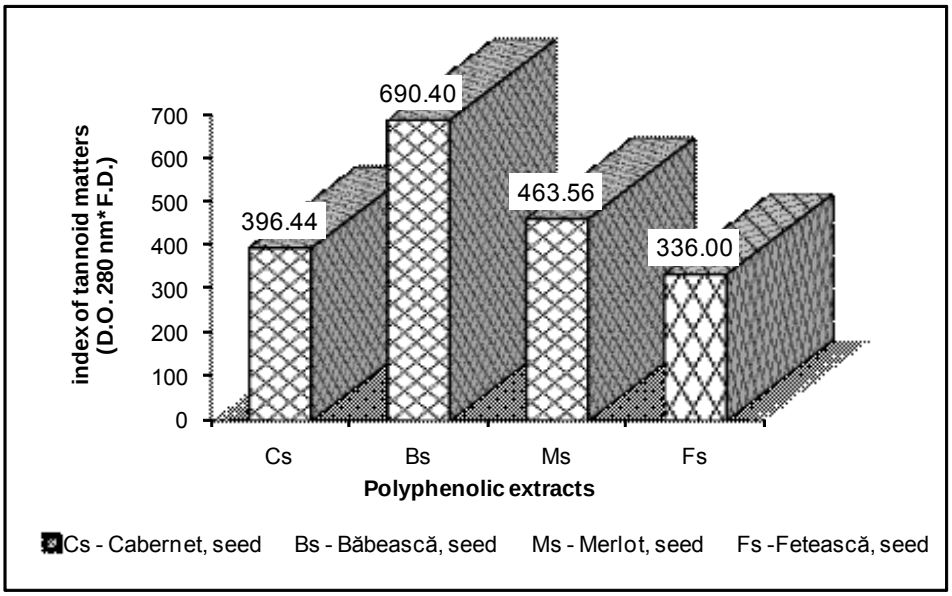

Figure 5, **The tanoid matter content in the vegetal extracts obtained by the Sexhlet continuous extraction method**

As to the anthocyns content, figure 6 shows that the highest values were registered in the case of the vegetal extracts obtained from Cabernet Sauvignon and Merlot seeds, with concentrations of 16,8 mg/L and 14,35 mg/L respectively.

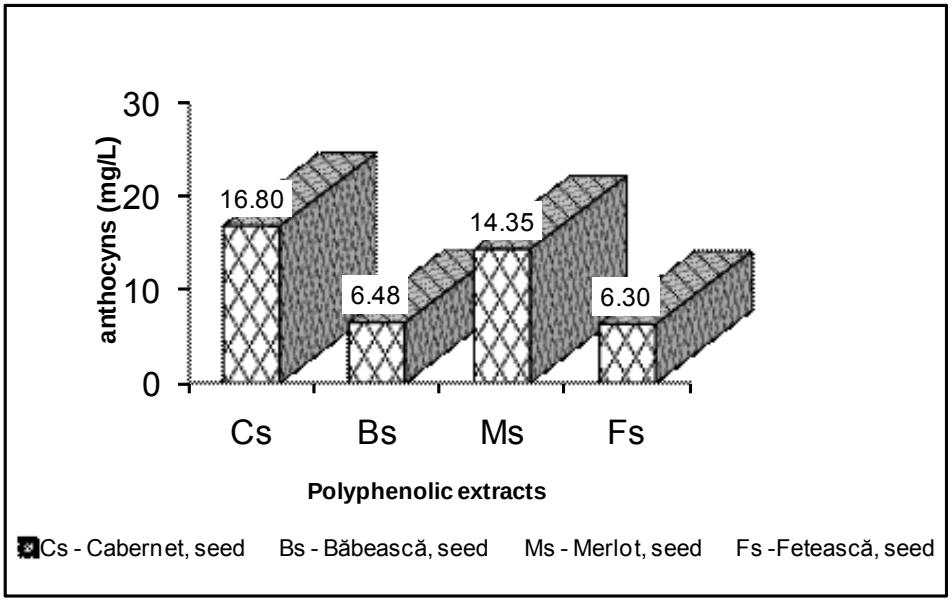

Figure 6. **The content of anthocyns in the vegetal extracts obtained by the Sexhlet continuous extraction method**

CONCLUSIONS

1. By the discontinuous extraction method with stirring, 90 per cent of the total polyphenols are extracted in the solvent after 60 minutes while the tanoid matters and the antocyns need 1440 minutes.

2. The vegetal extracts obtained though the continuous Soxhlet extractive method are richer in total polyphenols, anthocyns and tanoid matter while the extraction is much higher , varying according to the vegetal material used.

3. The richest vegetal extracts as to the total polyphenols, tanoid matter and anthocyns content were obtained from Băbească neagră and Merlot grape seed by using both extractive methods.

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