

# EXTRACTION, UNDER STATIC CONDITIONS, BY MEANS OF ETHANOL OF VEGETAL COMPOUNDS FROM THE VITIS VINIFERA SEEDS

## EXTRACȚIA COMPUȘILOR VEGETALI DIN SEMINȚELE DE VITIS VINIFERA CU ETANOL ÎN CONDIȚII STATICE

**SAVIN C., VASILE ANCUȚA, PAȘA RODICA, DAMIAN DOINA**

Research and Development for Wine Growing and Wine Making Iași

**Abstract:** *The specialized studies, published in the last years, report that there is not an optimum extraction protocol for all the vegetal sources containing polyphenols. The methods used in the polyphenols extraction from the vegetal materials are: (1) extraction by solvents, (2) solid phase extraction and (3) extraction by supercritical fluids. In the study herein, the method of extraction by solvents was tested (ethylic alcohol heated at a 40 °C temperature, 1:10 ratio) under static conditions, with periodical shunting for 24 hours. Vitis vinifera seeds pertaining to the Cabernet Sauvignon, Merlot, Băbească neagră and Fetească neagră varieties were used in the extractive process.*

*The intended purpose was to select the richest vegetal material in total polyphenol compounds. In order to assess the efficiency of the extractive process, analyses of the vegetal extracts were carried out, in dynamics (15', 30', 60' and 24 hours), determining the content in total polyphenols (g GAE/L), antocyanins mg/L, the tannin index (IMT).*

The polyphenol derivatives are components of the vegetal metabolic systems with vital functions in plants. The vegetal tissues of Vitis Vinifera contain various substances having functional phenol groups, pertaining to various classes of organic compounds.

Various studies on the polyphenols in grapes (Boureix M, 1976; Metche M. , 1986; Excaribano – Bailon, 2003; Boureix M și colab, 1986; Cheyner V. și colab., 1986) proved, on one side, essential in assessing the oenological potential of various varieties of vines and, on the other side, very important in assessing their benefic properties in maintaining the metabolic equilibrium and the human body health condition. Thus, the finding of the polyphenol compounds-rich vegetal sources as well as of the appropriate extraction methods were a major concern of the researchers in various countries.

### MATERIAL AND METHOD

In the current study, the solid phase extraction method was tested under static conditions. Vitis vinifera seeds pertaining to Cabernet Sauvignon, Merlot, Băbească neagră and Fetească neagră varieties were used in the extractive process. In order to provide for the reproducibility of the experimental results, the characterization of the grape seeds was carried out, observing the methods standardized by "International Seed Testing Association" (ISTA). In accordance with the said methods, the humidity of the vegetal materials was established below 0,05 %, the physical purity 99,5 %, and genetic purity 100 %.

The grapes seeds, washed and dried in humidity free room, at a temperature of 20 – 25 °C were ground to sizes between 1 – 2 mm. The extractive process was carried out in glass-ground stopper and flat bottom bottles in which the vegetal materials were introduced, then the solvent was added, namely ethylic alcohol heated to 40 °C, solid material /solvent 1/10 ration. The extracts obtained at 15, 30, 60 and 1140 minutes from the moment of adding the solvent were separated from the vegetal materials by centrifugation for 10 minute at 6000 rotations/minute and preserved at 4 °C for analyses.

The assessment of the extractive process efficiency was carried out by determining the content in total polyphenols (GAE grams/liter) by Singleton and Rossi method – 1965, of the antocyanans (mg/ml) by the R Gayon and Sonestreet method – 1965 and of the tanoid matters index (IMT) according to Bourzeix -1986

## RESULTS AND DISCUSSIONS

The polyphenol compounds are individualized by a series of properties influencing the extracts' conditioning, separation and analysis techniques. Among these, we name the spectral properties, the molecular mass and solubility in various polar organic solvents. The extractive process of the polyphenol compounds is influenced as well by the vegetal product (origin, humidity, grinding degree), by the solvent (its nature, vegetal product / solvent ratio, contact time, temperature) and extraction procedure (discontinuous, continuous) (Excaribano – Bailon, 2003).

Considering the information in the literature, in order to sort the high content polyphenol compounds vegetal materials, the ethylic alcohol-based extractions method under static conditions was chosen. The data achieved are presented in figures 1 – 3.

In the first part of the study, the vegetal extracts were characterized from the point of view of the content in total polyphenols. The data obtained are graphically represented in figure 1.

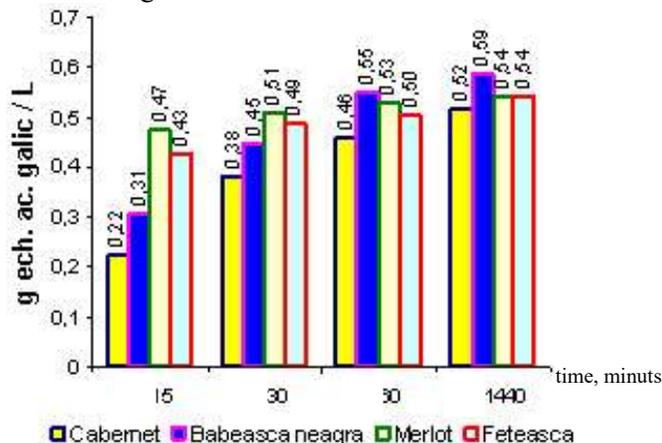


Figure no. 1. - Content of total polyphenols in vegetal extracts

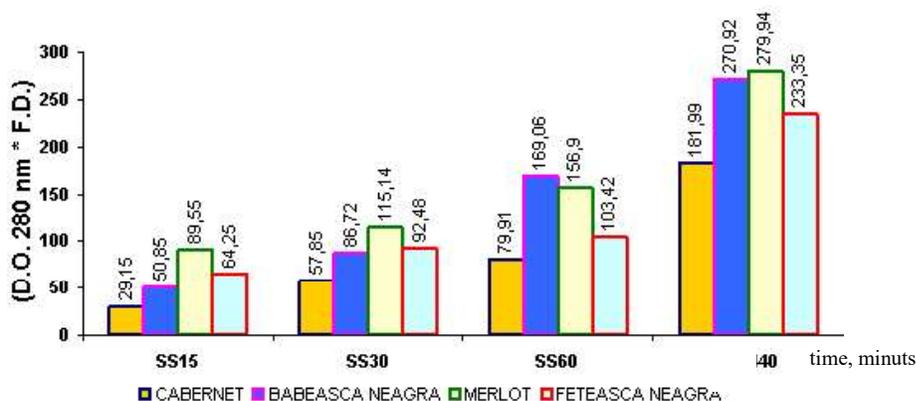
After 15 minutes of contact between the solvent and the tested vegetal materials, the highest concentration in total phenols was determined in the grapes seed extract of the Merlot variety and namely 0,47 g GAE/L. In order, there followed the vegetal extracts of Fetească neagră with 0,43 g GAE/L, Băbească neagră with 0,31 g GAE/L and Cabernet Sauvignon with 0,22 g GAE/L.

In the extractive process carried out for 30 minutes, the concentrations of total polyphenols were, from the value point of view, very close in the vegetal extracts obtained from the grapes seeds of the Merlot and Fetească neagră varieties, registering an increase, compared to the experiment conducted for 15 minutes of 8,5 % and 3,9 %, respectively. A significant increase of the total polyphenols content, however, registered in the case of vegetal extracts obtained from the grapes seeds pertaining to Cabernet Sauvignon and Băbească neagră varieties. Thus, in percentage, in the vegetal extracts, the concentrations in total polyphenols increased by 72 and 45 %, respectively.

During the experiment in which the extractive processes were conducted for 60 minutes, vegetal extracts with a concentration in total polyphenols higher only by 2 and 3,7 % , respectively in the case of Fetească neagră and Merlot grapes seeds and by 21 and 22 % in the case of de Cabernet Sauvignon and Băbească neagră grapes seeds were obtained.

In the extractive processes carried for 1440 minutes, vegetal extracts in which the concentrations in total polyphenols did not register a significant increase were obtained, except for the vegetal extract obtained from the grapes seeds of Băbească neagră variety, in which a concentration higher by 7,2 % was determined.

The efficiency of the extractive process under static conditions was also assessed by the determination of the tanoid matters index (IMT) and of the content in antocyanans. The data graphic representation – figure 2, shows a progressive increase of the IMT values along with the increase of the contact time of the solvent-tested vegetal materials.



**Figure no. 2 - Tanoid matters index in the vegetal extracts**

The highest values were registered in the vegetal extracts obtained in the extractive processes conducted for 1440 minutes, those obtained from the grapes seeds of the Băbească neagră and Merlot varieties distinguishing themselves.

Figure 3 shows the data regarding the antocyan content of the obtained vegetal extracts. The graphic representation of the results shows, distinguishingly, the grapes seeds vegetal extracts of the Băbească neagră and Merlot varieties in which the antocyan content increased even after 15 minutes of contact between the vegetal materials and the solvent, registering increasing values at 30 and 60 minutes. As for the IMP values in the vegetal extracts of the Băbească neagră and Merlot variety grapes seeds, the highest antocyan concentrations and namely 36,45 mg/ml and 35,03 mg/ml respectively at 1440 minutes were determined.

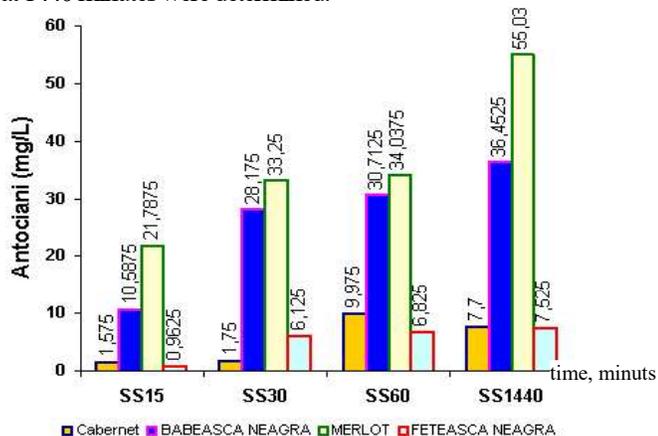


Figure no. 3 - Antocyan content in vegetal extracts

## CONCLUSIONS

1. The total polyphenols in the tested vegetal materials are extracted in solvent in proportion of solvent 90 % for 60 minutes, and the tanoid matters and antocyan after 1440 minutes.
2. The richest vegetal extracts in total polyphenols, tanoid matters and antocyan were obtained by the extraction in ethylic alcohol of the grapes seeds pertaining to the Băbească neagră and Merlot varieties.

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