SOLID-LIQUID EXTRACTIONS EFFICIENCY IN DETERMINATION OF ANTHOCYANIN CONTENT OF THE OENOLOGICAL DEPLETED MATERIAL

RANDAMENTUL EXTRACŢIILOR SOLID-LICHID ÎN DETERMINAREA CONŢINUTULUI ÎN ANTOCIANI DIN MATERIAL OENOLOGIC EPUIZAT

FILIMON V. R.[1], NICULAUA M.[2], MIHALACHE ARION Cristina[1],COŢOVANU Roxana[1]
e-mail: razvan_f80@yahoo.com

Abstract. *The study aims to determine anthocyanin content of alcoholic extracts obtained by treating dried pomace resulted in the process of winemaking from the local variety of black grapes, Vulpea. Were conducted a total of 17 stages of extraction, the ratio between the plant material and solvent initially was 1:10, then dropping to 1:5 (extractions 2-17). Was determined spectrophotometrically, total content of anthocyanins and polyphenols, associated with each phase of extraction (392.89 mg/100g, in the first stage of extraction), up to a theoretical yield of 99.80% recovery (distribution coefficient which is considered as total yield of extraction), corresponding to the 9th phase of extraction. After the 17 extractions plant material was considered depleted, theoretically yield of recovery being 99.99% (5.62 mg/100 g in last phase of extraction).*

Key words: yield, anthocyanins, polyphenols, dried pomace

Rezumat. *Studiul are ca scop determinarea conţinutului în antociani din extractele alcoolice obţinute prin tratarea tescovinei uscate rezultate în urma procesului de vinificaţie a soiului autohton de struguri negri, Vulpea. Au fost realizate un număr de 17 etape de extracţie, raportul dintre materialul vegetal şi solvent fiind iniţial 1:10, apoi scăzând la 1:5 (extracţiile 2-17). A fost determinat spectrofotometric conţinutul total în antociani şi polifenoli corespunzător fiecărei etape de extracţie (392,89 mg/100g, în prima etapă de extracţie), până la obţinerea unui randament de recuperare teoretic de 99,80% (coeficient de repartiţie la care se consideră un randament total de extracţie), corespunzător celei de a 9-a etape de extracţie. După cele 17 extracţii, materialul vegetal a fost considerat epuizat, randamentul teoretic de recuperare fiind de 99,99% (5,62 mg/100 g, în ultima etapă de extracţie).*

Cuvinte cheie: randament, antociani, polifenoli, tescovina uscată

INTRODUCTION

Annually, worldwide are produced between 5 and 9 million tons of grape pomace (Oreopoulou V., Russ W., 2007). Of all the recoverable compounds from grape pomace, phenolic compounds are the most valuable. These include

[1] University of Agricultural Sciences and Veterinary Medicine Iaşi, Romania
[2] Research Centre for Oenology - Iaşi branch of Romanian Academy

anthocyanin pigments, flavonoids, tannins, phenolic acids, which can be used as antioxidants or functional food components.

Anthocyanin pigments, have a special significance in current research in oenology and food industry (obtaining natural food colorants, functional foods). They are present, in black grapes, in quantities ranging from 30 to 888 mg/100g (Horbowicz M. *et al.,* 2008, Gould K., 2009). Locate, usually in the skin of black grapes, anthocyanins are extracted only partially (30-40%) by the winemaking process, so the pomace resulting from the production of red wines, contains significant amounts of these phenolic compounds (Câmpeanu R. *et al.,* 1989). If the pomace is dried, the percentage of extracted anthocyanins is lower, influenced by physico-chemical degradation processes of some compounds (Rein Maarit, 2005).

Over 10 thousand tonnes of skins are processed annually in Europe, resulting in about 50 tonnes of anthocyanin colorant, and these numbers are in an upward trend (Davies K., 2004). In Romania, the production of wine grapes was, in 2009, 915.8 thousand tonnes (Monthly Statistical Bulletin, 9/2009). Extraction of anthocyanin pigments is the first step in the experiments concerning their total or individual determination. A suitable extraction procedure should maximize the recovery of anthocyanins, with minimal intervention and minimal degradation or alteration of their natural form (*in vivo*) (Brouillard R. and Dangles O., 1994).

MATERIAL AND METHOD

The study aimed to determine total monomeric anthocyanins (AC) and phenolic compounds content (TPC) of dried pomace, obtained from local black grapes variety, Vulpea. Sampling was carried out in September 2009, from the Ampelographic Collection of the Faculty of Horticulture Iasi, V. Adamachi farm, viticultural center Copou, Iasi. Wine technology applied to the grapes was classic, with crushing and declustering. Maceration took place in static plastic vessels, for 72 hours, followed by pressing (pneumatic press). After 3 weeks of storage at ambient temperature (14 ± 2°C) and dark, pomace was considered dried.

Were conducted several extraction stages of phenolic compounds from the plant material, to depletion. Extraction was performed with ethanol:HCl:water system (96:1:3), resulting a pH = 1.5, in which, the chemical composition of anthocyanins is stable. Acids are very important in maintaining stability of anthocyanins, being necessary in the formation of flavylium cation, the most stable form (at pH 1.5 - 2) and to improve the extraction efficiency (Socaciu Carmen, 2008). The ratio of plant material and solvent was initially (first extraction) 1:10 (w/v), then decreased to 1:5 (w/v). Appearing the need to obtain extracts for food use, which should not contain toxic reagents (methanol, acetone), was preferred the extraction system with ethanol, although recoveries are not as important as those obtained by extraction with methanol (methanol is 20% more effective than ethanol and 73%, than water) (Socaciu, Carmen, 2008).

The containers were stored at low temperature and dark (6±1°C). Before the last filtering, was applied, a treatment with ultrasound, as a means of increasing the property transfer process and desorption.

In acidic medium, there is a balance between the colored and colorless forms of anthocyanins. This balance is in function of pH (Lee J. *et al.*, 2008). Was chosen pH 0.6 and pH 3.5, and measured the absorbance (optical density), at 520 nm wavelength, spectrophotometrically. Coloring intensity variation between the two pH

values, is proportional to the anthocyanin content. Measurements were made by means of a UV-VIS spectrometer Analytik Jena Specord 200, being measured also the absorbance at wavelength 750 nm, for the Folin-Ciocâlteu colorimetric method (Of. J. of EU, 2010). Expression of anthocyanin content was made in mg/100 g dried pomace and phenolic compounds, in grams gallic acid equivalents (g GAE)/100 g plant material.

RESULTS AND DISCUSSIONS

Grape pomace moisture content, after the storage period, was 7.5 %, determined by the oven drying method, four hours at 105°C (Afusoae Iulia *et al.*, 1988). Sugars in the grape must, refractometrically determined at 20°C, had an average value of 22°Bx (211 g/L).

In the grape skins, predominate simple forms of anthocyanins (Zănoagă C. *et al.*, 2010), which can be extracted in the first stages of extraction, while acylated anthocyanins can be gradually extracted (Ţârdea C. *et al.*, 2007). This extraction took place in several stages, up to a theoretical extraction coefficient of 99.99 %, resulting, in the final, 17 stages of extraction. AC and TPC values of the 17 extractions realised, were summarized in table 1.

Table 1

AC and TPC values at Vulpea variety

Number of extraction	AC (mg/100g)	TPC (mg GAE/100g)
1	**392.89**	**1280.17**
2	158.21	588.52
3	73.64	258.83
4	65.05	166.49
5	64.09	149.68
6	25.57	110.03
7	16.76	60.19
8	15.81	44.04
9	**13.47**	**38.68**
10	9.33	27.16
11	8.05	26.76
12	*4.13*	*19.81*
13	*15.06*	*20.41*
14	9.01	15.31
15	7.52	12.80
16	6.04	12.20
17	**5.62**	**11.47**
Total	**890.32**	**2842.63**

At first extraction, the AC value was maximum, 392.89 mg/100g, followed at the next steps by a decrease of this value, reaching, at the ninth extraction, 13.47 mg/100g. Theoretical yield of recovery, according to the ninth stage of extraction was calculated as 99.80%, coefficient of distribution considered as a total yield of extraction. Thereafter, further extractions are no longer effective,

economically speaking, due to very small quantities of anthocyanins extracted, reported to the amount of solvent used.

To deplete effectively the oenological material, we realized another eight rounds of extraction, performed by resuming solid part with solvent. In the last phase of extraction, anthocyanin content was 5.62 mg/100g.

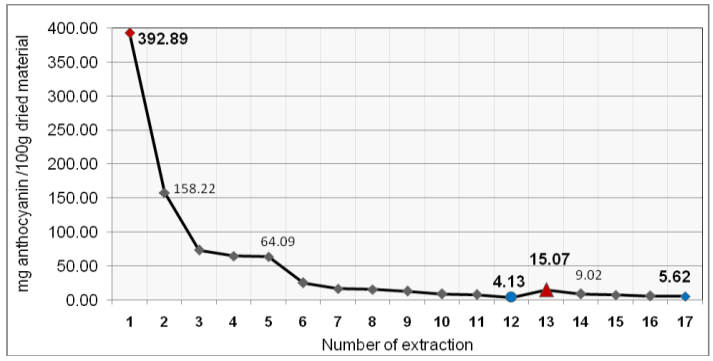

Fig. 1 - AC values trend during extractions

The general trend of AC amount was to decreasing. The twelfth stage of extraction, had the lowest values of the entire experience (4.13 mg/100g), at the next step, anthocyanin content increasing again (15.06 mg/100g), and then tend to decrease until the end (fig. 1). This increase of the AC values was possible due to the long progress of experience and the occurrence of acid hydrolysis, which takes place in a weak acid medium and at warm (Adams J.B., 1973). Proanthocyanidins have a higher trend of polymerization (in the presence of oxygen), the polymers formed are insoluble in water. In acidic medium, dimers (polymers) become anthocyanidins, single or accompanied by catechins (Cercasov Cornelia *et al.,* 2005) (fig. 2).

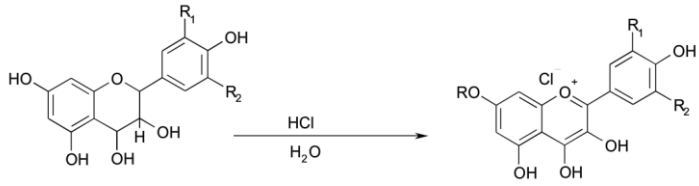

Fig. 2 - Transformation reaction of proanthocyanidins in anthocyanidins

The content of phenolic compounds, had also a decreasing trend during the extraction, with maximum value at the first stage of extraction (1280.17 GAE/100g mg) and a minimum at a last stage (11.47 mg GAE/100g). TPC values fluctuation, at the twelfth and thirteenth stages of extraction, was not as intense as at AC, here maintaining very close values (19.81 and 20.41 mg GAE/100g).

The percentage of anthocyanins, from total phenolic compounds, was maintained between 23 and 42 % at first nine extractions, considered sufficient to

depletion of dried pomace (fig. 3). At the thirteenth stage, the proportion of anthocyanins increase massively to 73.8%, confirming the assumptions regarding the transformation of phenolic compounds by polymerization reactions and the occurrence of acid hydrolysis, which converts a part of proanthocyanidins in anthocyanidins. The TPC values were not affected, but was an increase in the percentage of anthocyanins, spectrophometrically detected.

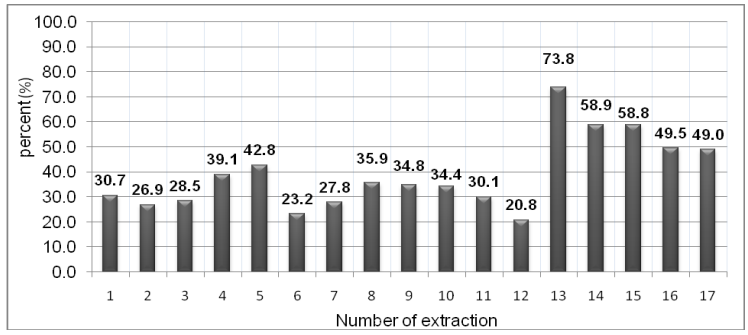

Fig. 3 - The percentage of anthocyanins from total phenolic compounds

Total AC and TPC values at the first nine extractions, when the yield was considered optimal, was 825.53 mg/100g, respectively 2696.63 mg GAE/100g, finally AC reaching the total amount of 890.32 mg/100g and TPC, 2842.63 mg GAE/100g, values equivalents to a completely dry extract. Percentage of anthocyanins, from total phenolic compounds, was, at the total quantities, 31%.

CONCLUSIONS

1. Dry pomace of local grapevine variety, Vulpea, was subjected to repeated extractions, using acidified ethanol, being determined the content of anthocyanins (AC) and total phenolic compounds (TPC), at the seventeen stages of extraction, realised to depletion of plant material.

2. The highest values of AC and TPC were determined in the first round of extraction, 392.89 mg/100g, respectively 1280.17 mg GAE/100g, at the second stage, the quantities being less than half of the initial values.

3. At the ninth stage of the extraction, theoretical recovery yield was 99.80 %, considered as a total yield of extraction, the AC value obtained at this stage being 13.47 mg/100g.

4. The general trend of TPC and AC values has been decreasing, with a growth in the thirteenth step of extraction, from 4.13 to 15.6 mg/100g, due to the conversion of colorless proanthocyanidins, in colored anthocyanidins, simple or accompanied by catechins, trough acidic hydrolysis, in the last step resulting 5.62 mg anthocyanins/100 g of dried pomace.

5. The total amount of anthocyanins at dried pomace of Vulpea variety was 890.32 mg/100g, representing an average of 31% from total phenolic compounds, amounts equivalent for a completely dried extract.

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