DETERMINATION OF TOTAL POLYPHENOLIC CONTENT AND ANTHOCYANINS OF DRIED POMACE OBTAINED FROM LOCAL GRAPE VARIETIES

DETERMINAREA CONȚINUTULUI POLIFENOLIC TOTAL ŞI AL ANTOCIANILOR DIN TESCOVINA USCATĂ OBȚINUTĂ DIN SOIURI AUTOHTONE DE VIȚĂ DE VIE

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Abstract. *Horticultural products are irreplaceable sources for obtaining natural compounds, with a nutraceutic and bioactive role (functional foods). Also, the use of waste from their processing has increased significantly in recent years. Pomace from red and black grapes, resulting from the technological process of wine (after maceration) was subjected to drying processes under natural conditions and then to the extraction of phenolic compounds. By analysis of the resulting fractions, obtained through repeated extractions, was determined total polyphenolic index (ITP or D_{280}) and the total content of anthocyanins (CA), of the dried pomace obtained from 15 local varieties of vines. Unlike the established nutrient classes (proteins, vitamins, minerals), phenolic compounds are not considered vital for survival, but instead, they have properties that promote optimum human health.*
Key words: waste, dried pomace, polyphenols, anthocyanins

Rezumat. *Produsele horticole constituie surse de neînlocuit pentru obţinerea unor compuşi naturali, cu rol nutraceutic şi bioactiv (alimente funcţionale). De asemenea, utilizarea produselor secundare provenite din prelucrarea acestora a crescut semnificativ în ultimii ani. Tescovina strugurilor roşii şi negri rezultată în urma procesului tehnologic de vinificaţie (după macerare), a fost supusă uscării, în condiţii naturale şi apoi extracţiei compuşilor fenolici. Prin analiza fracţiunilor rezultate din extracţii repetate a fost determinat indicele polifenolic total (ITP sau D_{280}), precum şi conţinutul total de antociani (CA) al tescovinei uscate provenită de la 15 soiuri autohtone de viţă de vie. Spre deosebire de clasele de nutrienţi consacrate (proteine, vitamine, minerale), compuşii fenolici nu sunt consideraţi vitali pentru supravieţuire, dar în schimb, într-o gamă largă de sensuri, au proprietăţi care promovează optimul de sănătate umană.*
Cuvinte cheie: deşeuri, tescovină uscată, polifenoli, antociani

INTRODUCTION

Phenolic compounds form one of the most important classes of compounds in all plant secondary metabolites. Their role, in both plants and humans, is evident by active character in the biological, physiological and environmental field.

Because of their bioactive properties and beneficial effects on human health, particular attention was given to attract new sources of valuable phenolic

compounds (tannins, phenolic acids, anthocyanins etc), and developing methods of extraction, isolation and characterization of them.

Anthocyanins play an important role in current research in oenology and industry of obtaining natural food colorants. Usually located in the skin of black grapes, anthocyanins are extracted only partially (30-40%) by the winemaking process, so pomace resulting from the production of red wines contains significant amounts of these phenolic compounds (Câmpeanu R. *et al*., 1989). They are glycosides of anthocyanidins (cyanidin, delphinidin, peonidin, petunidin, malvidin), which only in the case of *Vitis vinifera* species, are present as monoglycosides (Zănoagă C. *et al.*, 2010).

Grapes who reached the full maturity have a very complex chemically composition (table 1). This gradually changes with the processing, winemaking process, which they are subject to (Stoian V. *et al.*, 2000).

Table 1

Chemical composition (%) of grapes reached to the maturity
(Fregoni, 1998)

Substance	Cluster	Peel	Pulp	Seed
Water	30-45	60-70	70-80	31-45
Carbohydrate	1	1	10-35	34-36
Free organic acids	0,2-0,9	0,3-0,5	0,5-0,7	0,8-1,2
Related organic acids	1	-	0,3-1	-
Minerals	5-6	1-3	0,1-1	2-4
Polyphenols (tannins, anthocyanins etc)	1-3	1-5	trace	4-6
Nitrogenous substances	1-1,5	1-1,5	0,1	4-6
Odoriferous substances	-	-	trace	trace
Cellulose	45-55	28-32	0,4-0,6	35-45
Pectin	trace	trace	0,005-1	trace

MATERIAL AND METHOD

This study aims to determine total amount of polyphenols and anthocyanins content (AC) of the dried pomace obtained from 15 local black grape varieties, of which 7 old grape varieties (Fetească neagră, Băbească neagră, Bătută neagră, Busuioacă de Bohotin, Negru de Căuşani, Negru vârtos, Vulpe) and 8 new Vinifera creations (Amurg, Arcaş, Balada, Codană, Negru aromat, Negru de Drăgăşani, Novac and Roz de Miniş).

Grape samples was harvested on 09 September 2009, from the Ampelographic collection of UŞAMV Iaşi, “V. Adamachi” farm, wine center Copou.

Wine technology applied was classic with destemming and crushing. Maceration was carried out in static plastic pots for 72 hours, followed by pressing (pneumatic press). Sugars content in the must, determined with refractometer, took an average of 18.7°Bx.

Fresh pomace of grape varieties was weighed, keeping about 1 kg of each variety and ready for drying. Marc drying space was previously prepared, clean, away from direct sunlight, wind and rain, at room temperature. After 3 weeks pomace was considered to be dry enough to prepare for storage (average moisture was 7.5%, determined by air oven drying samples of pomace, at 105°C for 4 hours). Preservation was done in paper bags, stored in dry, cool and dark place (refrigerator).

After 40 days of cold storage was passed to making extraction made in the Laboratory of Oenological Research of the Romanian Academy, Iaşi branch.

The solution used for the extraction was ethanol (alcohol), purity 96%, acidified with hydrochloric acid (HCl), having a concentration of 0.75% HCl. Thus resulted a pH = 1 - 1.5.

Seeds from each sample were further dried using microwave (1 minute at 750W, with mixed seed at 30 seconds).

Dried pomace (seeds and skins) was ground, using an electric grinder Goldy, thus achieving a high degree of comminution of plant material (particles less than 0.5 mm). From each sample was weighed 16g, using a balance with 2 decimal, then the samples were treated with 160 mL solution of extraction, resulting a ratio of 1:10. Extraction time was approximately 72 hours (3 days) at room temperature (25-28 ° C).

In grapes skin, anthocyanins without acyl group predominate, which can be removed very quickly, while acyl anthocyanins can be removed gradually. The second and third stages of extraction were made by treating pomace, recovered from the filter paper, for exhaustion of plant material. Before the third filter was applied a ultrasound treatment on vessels containing pomace treated with extraction solution. The ultrasonic device used was Pros Kit Digital Ultrasonic Cleaner SS-802, ultrasonic time: 480 seconds, for each sample. Using ultrasound, as a means of enhancing the process of transfer of property (mass, heat) and desorption, is reported in the literature since 1970. It was proven that ultrasonic can dislocate organic matter adsorbed on a specific area of sediments and increase significantly the bioavailability of sorbent.

Total polyphenolic index (IPT) or D_{280} index expresses the content of total phenolic compounds (phenolic acids, tannins and coloring substances) of the samples. This index has values between 20 and 100 at red wines.

The method principle for determining the index D280 is that, benzene nuclei, characteristic of phenolic compounds, strongly absorb ultraviolet light, with a maximum around λ =275-280 nm (Zamfir C., 2009).

D_{280} index is a spectral characteristic of all existing phenolic compounds (Ţârdea C., 2007).

Measurements were made using a UV-VIS spectrometer Analytik Jena Specord 200, as follows: absorbance was measured at λ = 280 nm in 1 cm quartz cell, in comparison with distilled water, the result being index D_{280}. For expressing the content of phenolic compounds in grams gallic acid, reported at gram of material, it was drew a calibration curve, using gallic acid solutions of different concentrations. It was necessary to determine the Folin-Ciocalteu index to obtain values of phenolic compounds at λ = 750 nm and applying formulas for calculating. This method is reliable, reproducible and can be used to any wine or alcoholic extract.

In acidic medium, there is a balance between colored and colorless forms of **anthocyans**. This balance is a function of pH (Zamfir C., 2009). It was chosen pH 0.6 and pH 3.5 and measured the absorbance (optical density) at λ = 520 nm for both sample and blank, using 1 cm glass cuvette optical path, compared with distilled water. Coloring intensity variation between pH values is proportional to the anthocyanin content. With this change, phenolic function is not affected and it is recognized that other phenolic compounds (tannins) do not interfere the determination (Zamfir C., 2009).

RESULTS AND DISCUSSIONS

Observations made during drying pomace:

- yield samples of pomace at drying: from1 kg of fresh pomace resulted 200 g dry pomace (seeds, skins and parts of rachides).

- has been found that grape varieties with lighter colour were more susceptible to mold, than most intensely colored.

Following interpretation of the absorption spectra obtained at the wavelength of each compound analyzed and applying formulas were obtained the results for the total phenolic compounds and anthocyanin content, data were centralized in table 2.

Table 2

IPT (total phenolic compounds) and CA (anthocyanin content) values of the studied samples

No.	Variety	D_{280}	IPT (g gallic acid /g mp)	CA (mg/g mp)
1	Arcaş	**0,2234**	**33,458**	17,0908
2	Băbească neagră	0,2293	34,334	**4,5801**
3	Roz de Miniş	0,2271	34,011	8,7744
4	Bătută neagră	0,2272	34,017	6,7723
5	Busuioacă de Bohotin	0,2275	34,071	19,0929
6	Amurg	0,2286	34,231	30,5280
7	Negru vârtos	0,2286	34,232	7,8119
8	Fetească neagră	0,2289	34,284	19,7090
9	Negru aromat	0,2289	34,283	11,7391
10	Balada	0,2252	33,714	24,3677
11	Codană	0,2297	34,397	20,6715
12	Negru de Căuşani	0,2304	34,509	21,5956
13	Novac	0,2312	34,626	52,3585
14	Negru de Drăgăşani	0,2325	**34,821**	21,7881
15	Vulpe	**0,6851**	34,201	**170,8209**

Values were expressed with 4 decimal to reveal very small differences that occur at the total amount of polyphenols in the studied varieties.

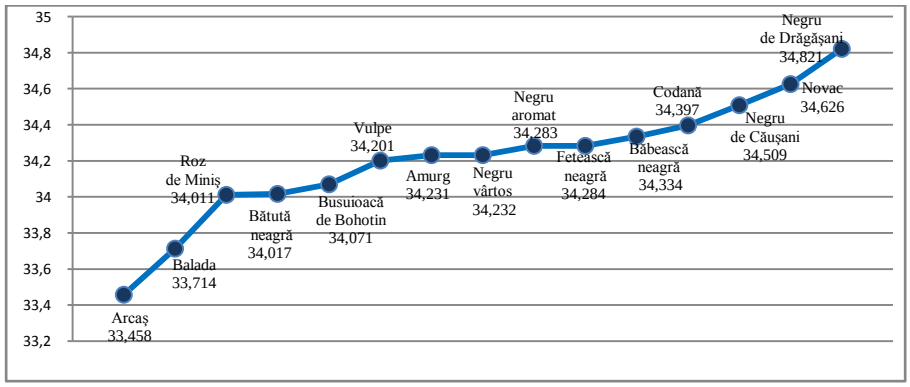

Fig.1. D_{280} index values of the studied varieties

Index D_{280} (fig. 1) has assigned values to specific parameters of red wines, which in literature are in the range 20-100. Can be observed the location of this index, close to the theoretical lower limit, but still with significant values, ranging from: 33.458, Arcaş variety and 34.821 at variety Negru de Dragăşani.

Regarding the content of total phenolic compounds (fig. 2), determined quantitatively, it is noted that it has very similar values, except Vulpe variety with a higher value, 0.6851 g/g, phenolic compounds expressed as gallic acid.

From the data, we can notice the existence of local varieties of grapes with potential for accumulation of phenolic compounds higher than established varieties, such as Fetească neagră or Băbească neagră, like Vulpe, Novac, Balada şi Negru de Drăgăşani, although were not harvested at technological maturity of each, but were harvested at the same time.

Regarding the anthocyanins content (fig. 3) of dried pomace, can notice Vulpe variety with high-value of CA index, 170.8 mg/g. It is noted that are local varieties with high potential for accumulation of anthocyanins, such as varieties: Novac, Amurg, Balada, Negru de Drăgăşani, Vulpe. At the opposite pole are varieties: Băbească neagră, Roz de Miniş, Negru vârtos, Bătută neagră, with low values of this parameter.

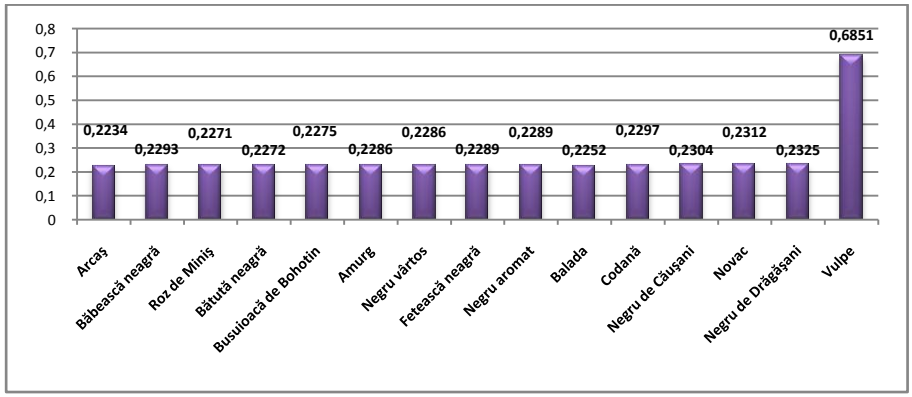

Fig.2. Total phenolic content values (g gallic acid /g dry pomace)

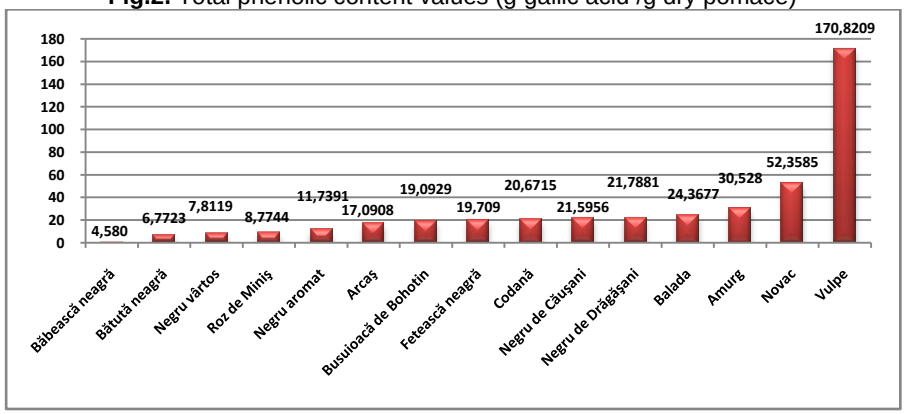

Fig.3. CA values (mg anthocyanins/g dry pomace)

CONCLUSIONS

1. Content of phenolic compounds in grapes is very variable, depending on variety, so higher values have Vulpe variety, 0.6851 g/g and the lowest value have Arcaş variety, 0.2234 g/g phenolic compounds expressed as gallic acid.

2. Dry pomace extracts, from local grape varieties of red and black grapes, are rich in phenolic compounds, with values of D_{280} index between 33 458, the Arcaş variety and 34.821, Negru de Drăgăşani variety, being thus a material with a real recovery potential in the sector of functional food and to obtain compounds with nutraceutic role.

3. Large quantities of anthocyanins, obtained after the extractions, with a maximum value at Vulpe variety, 170.8 mg/g and a minimum of 4.58 mg/g, Băbească neagră variety, show that pomace, even in the dry state, is a valuable source of vegetal pigments, with an significant utilization in food and pharmaceutical industry, replacing synthetic compounds.

4. The high values of indices examined supports the extraction of these compounds from the material considered and for the future arise the question of using smaller quantities of solvent and/or its recovery and recycling.

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