ABSTRACT

Key words: Typicity, Busuioacă de Bohotin variety, biotypes, maceration techniques, phenolic and volatile compounds

Busuioaca de Bohotin is one of the most known aromatic varieties in România that is part of the Muscat variety and the wines obtained from it express a unique flavour that reminds of the scent of rose flowers, basil, coriander, strawberries and wild strawberries. Due to limited ecological plasticity and higher susceptibility to diseases and pests, this variety is cultivated on rather narrow surfaces and the wines obtained from it have been frequently subject to adulterations.

The main purpose of the present study is to obtain data in order to determine the typicity of the Busuioacă de Bohotin variety and to establish a possible varietal marker that can help determine the authenticity of Busuioacă de Bohotin wines. To achieve these goals, the influence of many variables was taken into account. Thus, in the elaboration of the Busuioacă de Bohotin wines, two biotypes of the Busuioacă de Bohotin variety (pink biotype and dark violet biotype) of different geographic origins (Huși Viticulture Center and Averești Viticulture Center) were used, to which various techniques maceration were applied (V0−Maceration-fermentation-control sample; V1−Maceration-fermentation; V2−Thermomaceration; V3−Microwave maceration; V4−Ultrasound maceration; V5−Cryo-maceration; V6 Cryo-maceration+Microwave; V7−Carbonic maceration).

The doctoral thesis entitled "Study of phenolic and volatile compounds in Busuioacă de Bohotin wine in order to establish the typicity of the variety" is structured in two parts and six chapters.

Part I – Present State of Knowledge − includes Chapter 1 − "The Current State of Research on Phenolic and Volatile Compounds used in Wine Discrimination", which describes the new strategies/techniques and studies used in solving issues related to the typicity and authenticity of wines.

Part II entitled "Personal Contributions" is structured in five chapters and includes:
Chapter 2 − The purpose and objectives of the research and the description of the natural/organizational and institutional setting in which they were achieved;
Chapter 3 − Research Material and Methods − describes Huşi Viticultural Area, Busuioacă de Bohotin variety, the experimental protocol and the research methods used in the study;
Chapter 4 − Obtained Results and Discussions − presents the data obtained from the analysis of the physico-chemical composition of the Busuioacă de Bohotin musts and wines and their interpretation;
Chapter 5 − Statistical Analysis − Describes the application of statistical methods (PCA) to classify Busuioacă de Bohotin wines according to the variety biotypes;
Chapter 6 − Conclusions − makes a synthetic description of the data obtained from the analysis of the Busuioacă de Bohotin variety typicity.

Following the physico-chemical composition of the Busuioacă de Bohotin musts, the pink biotype was noted by a lower total acidity.

Examining the main parameters of the composition of Busuioacă de Bohotin wines, the samples obtained from the pink biotype were distinguished by a higher total acidity in contrast to the dark violet biotype. Analysing from the influence of terroir point of view, the Huși Viticulture Center led to the production of wines with higher values of the main physico-chemical parameters (alcohol concentration, total dry extract, non-reducing extract) compared to those obtained from the Avereşti Viticulture Center.

Of the maceration techniques used in the experiment, carbonic maceration (V7) was noted. Due to the specific aspects of this maceration technique − which meant keeping the whole grapes in CO_2 atmosphere - a deeper contact between the various constituent parts of the grape (skin, seeds, pulp) was avoided and the obtained wines had low values of the total dry extract and non-reducing extract, with a lower alcohol content and total acidity, and higher volatile acidity values. Also, it was remarked that, the samples obtained with microwave maceration (V3) had higher alcohol concentrations, the ones obtained via cryo-maceration (V5) had a lower total acidity, while ultrasound maceration (V4) led to lower values of total dry extract, non-reducing extract, alcoholic strength and total acidity.

The evaluation of the acids profile of Busuioacă de Bohotin wines by high performance liquid chromatography revealed the existence of quantitative differences, the pink biotype generally leading to the production of wines with a higher total content in acids while the dark violet biotype was remarked by a higher content in shikimic acid. Also, in addition to genetic variations of the variety, a significant influence on the acids content of the wines had carbonic maceration (V7); this maceration technique led to a high concentration in acetic, lactic, shikimic acids, and lower values of malic acid. Another maceration technique that caused alterations in the acids profile of Busuioacă de Bohotin wines was cryo-maceration + microwaves (V6), having a lower content of malic acid and higher acetic and lactic acid values.

Following the HPLC analysis of phenolic compounds in Busuioacă de Bohotin wines, the pink biotype had a higher total phenolic acids content versus dark violet biotype, with an average content of 178,06 mg/L (hydroxybenzoic acids) and 36,41 mg/L (hydroxycinnamic acids). The dark violet biotype produced wines with an average content of 128,94 mg/L (hydroxybenzoic acids) and 19,20 mg/L (hydroxycinnamic acids) for the Huși Viticulture Center, and 138,31 mg/L (hydroxybenzoic acids) and 20,17 mg/L (hydroxycinnamic acids) for the Avereşti Viticulture Center.

The determination of the anthocyanins profile of Busuioacă de Bohotin wines obtained from the two genetic variations of this variety allowed their classification on the basis of malvidin to peonidine ratio (Mv/Po).Thus, in the case of the dark violet biotype harvested from the Huşi Viticulture Center, the malvidin to peonidine ratio (Mv/Po) showed values that varied between 6,19 (V7) and 21,12 (V6), and in the case of Busuioacă de Bohotin samples obtained from the dark violet biotype of Avereşti Viticulture Center recorded values between 9,62 (V2) and 17,74 (V0) in 2014 and between 6,92 (V5) and 16,66 (V1) in 2015. Concerning the pink biotype, the obtained wines expressed much lower values of malvidin to peonidine ratio, which ranged between 3,09 (V2) and 6,37 (V1). In other words, in the case of Busuioacă de Bohotin wines obtained from the pink biotype, peonidine exhibited a higher proportion of participation in the anthocyanins

profile, respectively between 10,35% (V1) and 24,48% (V2), while the wines obtained from the dark violet biotype, peonidine recorded a participation rate of 4,15% to 11,38%.

Also, following the correlation of the anthocyanins profile of these two phenotypic variation with the biosynthesis pathway of these colour compounds, it was pointed out that the activity of enzymes from the anthocyanins synthesis pathway (flavonoid 3'-hydroxylase, flavonoid 3'5'-hydroxylase, dihydroflavonol-4-reductase) have been modified, leading to a higher proportion of maldivine, petunidine and delfinidine in the case of dark violet biotype while the pink biotype has a higher proportion of peonidine participation. In addition to the variety biotypes, an important influence on the anthocyanins profile of the obtained wines was the carbonic maceration (V7). Thus, in the case of the Busuioacă de Bohotin wines obtained from the pink biotype harvested from the Huși Viticultural Center (BBV−H), this maceration technique led to the lowest percentage of maldivine participation (70,43%) and also determined the highest percentages of delfinidine (18,19%) and peonidine (11,38%). The carbonic maceration also determined the lowest percentages of maldivine (38,25% in 2014 and 40,12% in 2015) and the highest percentages of acylated malvidine in 2014 (31,08% acetylated malvidine and 30,67% coumarylated malvinidine) and delfinidine (57,79%) in 2015 for wines obtained from the dark violet biotype from Avereşti Viticultural Center (BBV−A). Regarding the wines obtained from the pink biotype (BBR−H), anthocyanins were not detected, leading implicitly to the impossibility of determining the anthocyanins profile for carbonic macerated wines.

Evaluation of the anthocyanins content in Busuioacă de Bohotin wines, led to the conclusion that this variety did not record a high concentration in these colour compounds. Thus, the spectrophotometric determination of the anthocyanins content (the pH variation method) revealed the existence of very low amounts, ranging from 0 mg/L (BBR−H−V7−2014) and 2,84 mg/L (BBR−H−V1−2014) in the case of the pink biotype and between 0 mg/L (BBV-A-V7) and 11,86 mg/L (BBV−A−V2;V4) for the dark violet biotype. Moreover, by high performance liquid chromatography, the obtained concentrations of anthocyanins followed the same trend, also confirming the lack of these pigments in the case of wines obtained from the pink biotype in 2015.

The CIE Lab analysis of the chromatic characteristics highlighted the existence of differences between these two biotypes, resulting in high values of clarity (L) and lower values for the red component (a +) with low chromatic purity (C−Chroma) and high tonality (H) for wines made from the pink biotype. Among the maceration techniques used that have been shown to have an impact on the chromatic characteristics of the obtained wines, were noted the variants: V7 (Carbonic maceration), V2 (Thermomaceration), V3 (Microwave maceration), V5 (Cryo-maceration) and V6 (Cryo-maceration + Microwave).

The gas-chromatographic evaluation of Busuioacă de Bohotin wines revealed the existence of a large number of terpene compounds such as linalool (0,45−2,61 mmol/L), citronellol (0,07−1,15 mmol/L), nerol (0,06−0,88 mmol/L), geraniol (0,04−0,77 mmol/L), hotrienol (0,09−0,34 mmol/ L) and α-terpineol (0,1-0,33 mmol/L). Also, from this category of terpenoids, the polyoxygenated forms of these compounds were also detected,

such as the furanic and pyranic oxides of linalool (*cis* and *trans*), nerol oxide and rose oxide.

In terms of ester content, there was a prevalence of ethyl esters of fatty acids that generally express fatty, fruity odorous notes and in a lesser proportion of acetylated esters (fruity odorants) and lactones (creamy, milk and fruity odorant notes). Thus, from the category of ethyl esters of fatty acids, the highest predominance was having ethyl caprylate, ethyl caprate, ethyl lactate, ethyl 9-hexadecenoate, ethyl palmitate, ethyl laurate and ethyl myristate. Among the maceration techniques used were noted: carbonic maceration (V7) with a low content in terpenic compounds, higher alcohols and higher ester concentration; cryo-maceration (V5) showed high concentrations in terpenic compounds; ultrasound maceration (V4) recorded high values in higher alcohols and lower concentrations in esters, and the variant that was applied cryo-maceration + microwaves (V6) was distinguished by high concentrations in terpenoids and higher alcohols.

The Busuioacă de Bohotin biotypes also exerted an influence on the content of the volatile compounds in the obtained wines, the dark violet biotype being noted by high concentrations in terpenic compounds, higher alcohols and esters relative to the pink biotype.

Following the sensory analysis of the Busuioacă de Bohotin wines, the samples obtained by carbonic maceration (V7) and cryo-maceration (V5) were noted. Thus, carbonic maceration has generally led to a minimum score for most olfactory and tasting descriptors. However, there was an exception (BBV−A−V7−2015), this sample recording the highest scores from the assessment of olfactory descriptors (coriander, ripe fruits, exotic fruits, dried fruits, green fruits, hay, rose) and taste descriptors (sweet, unctuosity, structure and persistence), in which the high values of reducing sugar content (73,06 g/L) had an important role.

Wines obtained by cryo-maceration (V5) were highlighted by obtaining high scores for olfactory descriptors such as coriander, ripe, exotic, dry and green fruits, hay, wild flowers, spices, honey, rose and taste descriptors: sweet, unctuosity, structure, and persistence.

In addition to maceration techniques, an important role in the sensory evaluation of Busuioacă de Bohotin wines was given by the biotypes of this variety. Thus, the samples obtained from the dark violet biotype (BBV) showed the most intense olfactory sensations of coriander, ripe, exotic, dry and green fruits, wild flowers, spices, honey, rose, with a lasting persistence, specific to Busuioacă de Bohotin variety.

In the statistical evaluation of the obtained data, the principal components analysis (PCA) was used as a chemometric method and the results obtained from the analysis of the phenolic compounds, volatile compounds and organic acids represented the variables used in the statistical model.