Influence of Maceration Enzyme Treatment on the Colour and Volatile Profile of Two Red Romanian Wines

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The effect of the addition of an extraction enzyme during the maceration stage of red wines made of two Romanian varieties was investigated in order to identify technological variants with positive results on the colour and volatile profile of the wines. Samples of grapes of the two varieties, harvested in four different viticultural regions, were vinified by classical red wine technology, except that the maceration-fermentation process was carried out both with and without the addition of extraction enzyme. The evolution of colour during maceration was monitored by determining the CIELAB parameters spectrophotometrically, while the differences in volatile profiles were evaluated by use of a dual-column gas-chromatograph working on the principle of an electronic nose. The study showed that the two varieties responded differently to the addition of the enzyme. The Babeasca neagra variety, which has a lower content of red pigments, benefited from the enzyme addition and showed an improvement of colour as a result of the enzymic treatment, while the volatile profile of the wine was not significantly modified. In case of Feteasca neagra the enzyme clearly affected the volatile profile, while the colour evolution during maceration and the CIELAB parameters did not display important modifications.

Keywords: wine colour, wine volatiles, maceration enzyme, electronic nose, Feteasca neagra, Babeasca neagra

The colour [1, 2] and the volatile profile of red wines [3] play a significant role in the perception of wine quality, having a big impact on the consumer preference for a certain wine. An improvement of the extraction of both colour and volatile compounds from the grape skins during the winemaking can be achieved by addition of enzymes [4-6]. Similar studies were performed by other researchers [7 - 9] on some local or international varieties.

The methods employed for the determination of the colour of wines are all spectrophotometric [10], the parameters measured varying in accordance to the colour system used for measurement and calculation [11]. The most used system for wine color measurements and comparison is the CIELab system [12-14], of which we have chosen to use for this work only the chromaticity coordinates *a* (colour placement in green-red space), *b* (colour placement in blue-yellow space), *c* (chromaticity) and *h* (colour hue).

Experience has shown that the evaluation and comparison of wines based solely on the results of physico-chemical parameters is not sufficient. The technology of winemaking, due to the complexity of the chemical and biochemical processes involved, induces many subtle differences in the composition and overall quality of the resulting wines, differences which require more complex equipment and methods in order to be distinguished. Some researchers monitored, with good results, the compositional changes during fermentation of the musts [14] or evaluated the final product by measuring and identifying some of the components in the resulted wines produced by different technological methods [16] by using advanced techniques such as proton nuclear magnetic resonance spectroscopy. Same ^{1}H-NMR technique was used to compare wines produced by different growing [17] or different winemaking techniques [18]. The IR spectroscopy [19] is also widely used for similar studies.

Regarding the aromatic profile of wines, the evaluation of the volatile compounds is faster and less expensive by the use of gas-chromatography. Some of these techniques, especially those coupled with mass spectrometry, are dedicated to the identification of the volatile compounds [20]. In this study we have used the gas-chromatographic technique only for fingerprinting the overall volatile profile of wines, in order to compare the resulted wines, evaluate the enzyme treatment effect and classify the experimental wines in categories.

In this work we studied the effect of the treatment with extraction enzymes on two indigenous grapevine varieties for red wines, Feteasca neagra and Babeasca neagra. The objective of the work was to evaluate some technological variants regarding the use of extraction enzymes during the making of red wines in order to identify positive influences on the colour and volatile profile of the wines. The results obtained showed that specific adapted technologies are required for each grape variety. The two varieties behaved differently in relation to the enzymic extraction, one displaying improvements in colour, while the other showed an enhancement of the aroma profile.

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Experimental part

Sampling and experimental protocol

Feteasca neagra and Babeasca neagra grapes were harvested in the autumn of 2009 from different Romanian vineyards and vinified in 6 technological variants in our laboratory. The Feteasca neagra variety originated in 4 different areas: Bucharest, Odobesti (Vrancea county), Valea Calugareasca (Prahova county) and Pietroasa (Buzau county), while Babeasca neagra originated in 3 different areas: Bucharest, Odobesti (Vrancea county) and Pietroasa (Buzau county). The grapes were harvested by hand and transported in the same day to our laboratory in plastic boxes of about 10 kg each, making sure that the berries of the grapes remained intact until the vinification. The grapes

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Table 1
TECHNOLOGICAL VARIANTS FOR WINES PRODUCED FROM FETEASCA NEAGRA (FN) FROM GRAPES ORIGINATING IN DIFFERENT AREAS

Variant	Area				Oenological product used	Dosage and time of addition
	Bucharest	Odobesti	Valea Calugareasca	Pietroasa		
v1		FNO1 r1 FNO1 r2 FNO1 r3	FNV1 r1 FNV1 r2 FNV1 r3	FNP1 r1 FNP1 r2 FNP1 r3	selected yeast	30 g/hl BM45 yeast (in the beginning of fermentation)
v2		FNO2 r1 FNO2 r2 FNO2 r3	FNV2 r1 FNV2 r2 FNV2 r3	FNP2 r1 FNP2 r2 FNP2 r3	selected yeast + oak chips	30 g/hl BM45 yeast + 250 g/hl oak chips (added after fermentation, kept in contact 4 weeks)
v3	FNB3 r1 FNB3 r2 FNB3 r3	FNO3 r1 FNO3 r2 FNO3 r3	FNV3 r1 FNV3 r2 FNV3 r3	FNP3 r1 FNP3 r2 FNP3 r3	selected yeast + maceration enzyme	30 g/hl BM45 yeast + 2 g/hl Lallzyme OE enzyme (in the beginning of fermentation)
v4	FNB4 r1 FNB4 r2 FNB4 r3	FNO4 r1 FNO4 r2 FNO4 r3	FNV4 r1 FNV4 r2 FNV4 r3	FNP4 r1 FNP4 r2 FNP4 r3	selected yeast + maceration enzyme + oak chips	30 g/hl BM45 yeast + 2 g/hl Lallzyme OE enzyme + 250 g/hl oak chips (added after fermentation, kept in contact 4 weeks)
v5	FNB5 r1 FNB5 r2 FNB5 r3	FNO5 r1 FNO5 r2 FNO5 r3	FNV5 r1 FNV5 r2 FNV5 r3	FNP5 r1 FNP5 r2 FNP5 r3	selected yeast + maceration enzyme + Limousin tannin	30 g/hl BM45 yeast + 2 g/hl Lallzyme OE enzyme + 2 g/hl Limousin tannin (added after fermentation)
v6	FNB6 r1 FNB6 r2 FNB6 r3	FNO6 r1 FNO6 r2 FNO6 r3	FNV6 r1 FNV6 r2 FNV6 r3	FNP6 r1 FNP6 r2 FNP6 r3	selected yeast + maceration enzyme + Tostato tannin	30 g/hl BM45 yeast + 2 g/hl Lallzyme OE enzyme + 2 g/hl Tostato tannin (added after fermentation)

of each variety and origin were crushed with a destemmer-crusher and the must obtained was equally distributed in vats of 10 L where the winemaking was performed by classical maceration on cap in the absence or in the presence of extraction enzymes, in accordance with the experimental protocol. One month after the fermentation was completed, oak chips and lyophilized tannins were added in both enzyme treated wines and non-enzyme treated wines, in order to reproduce some of the most widespread winemaking techniques used for these varieties and thus to obtain wines with colour and volatile profiles in the usual range specific to these varieties. All the experimental variants for Feteasca neagra (FN) wines and the technological treatments applied are presented in table 1. The variant code name is composed by the abbreviation of the grape variety, followed by the first letter designating the location of grapes origin and then by a number from 1 to 6, representing the technological treatment. Each technological variant was prepared in triplicate, therefore the last part of the variant codification contains the experimental replication (r1, r2 and r3). The Babeasca neagra (BN) wines variants were similar, with two differences: the abbreviated notation of the variety is in this case BN, and there were no Babeasca wines available from Valea Calugareasca.

Reagents

The oenological yeast BM45 used for the fermentation of all samples was provided by Lallemand and is mostly recommended for red wines, in which it enhances the varietal character, with a moderate fermentation speed at a temperature between 18-28°C. According to the producer's description BM45 yeast produces high levels of polyphenol-reactive polysaccharides, resulting in wines with great mouth feel and improved color stability.

The extraction enzyme used, Lallezyme OE, also from Lallemand, is a high concentration pectinase from *Aspergillus niger* recommended for red grapes maceration. It also displays medium cellulase and hemicellulase secondary activities, allowing for a smooth extraction effect.

The oak chips were Pronektar medium toast from American oak provided by Tonnellerie Radoux USA. Generally the American oak chips release in wines less tannin than French oak chips, but they confer slightly more aroma of vanilla and cocoa.

The tannins used were both provided by Enologica Vason. The Premium Limousin is a granulated tannin obtained from oak wood from the Limousin region of France through a special hydro-alcoholic extraction technique that allows the extraction of substances similar to those released in the wines during aging in wooden casks. This tannin contributes to the stabilization of the colour fractions of red wines, improving the aging potential and its resistance to oxidation. The Premium Tostato is a granulated tannin obtained from toasted French cask oak through a special extraction and desiccation process. This product is a very soft tannin, supposed to increase the woody toasty hints tending towards cocoa.

NaOH from Chimopar S.A. and bromothymol blue from Merck were used for the determination of total acidity. No other reagents were required, as both spectrophotometry and gas-chromatography measurements were performed using unaltered samples of the wines.

Experimental part

Methods and equipment

The wines were analyzed 4 months after the completion of fermentation, by performing several classical physico-chemical determinations (alcoholic strength, dry extract and total acidity), together with measurements of colour parameters and evaluations of the volatile profile by a special technique of gas-chromatography based on the principle of electronic nose. For the colour measurement the CIELab system was applied and parameters calculated by using the software package Chroma Ver. 2.0.

Triplicate repeated measurements were performed for each variant on the electronic nose and spectrophotometer, the average being used where appropriate. Statistical analysis on the results was done using Microsoft Excel as well as the gas-cromatograph software AlphaSoft from AlphaMOS.

The colour of the wines was determined with a computer-controlled double beam spectrophotometer Specord 250 from Analytik Jena AG running the software WinAspect version 2.2.7, with which the CIELab colour parameters [14] were automatically calculated.

A Heracles electronic nose based on dual-column flash gas-chromatography from Alpha Mos company, was used

to differentiate variants based on their volatile profiles [21-24]. The analytical methods and parameters used for wines were developed in our laboratory [21-25].

Results and discussions

The possible effects of the use of extraction enzymes during maceration were investigated in three directions: physico-chemical analyses (ethanol concentration, total acidity, dry content), spectrophotometric analysis of colour (CIELab colour parameters) and evaluation of the volatile profile by use of the electronic nose.

Some relevant results for the ethanol concentration and total acidity of Feteasca neagra and Babeasca neagra wines are presented in figure 1, where values are the means of 3 repetitions. Standard deviation of means is also indicated. The final ethanol concentration of wines was, in general, not influenced by the technological treatments, being basically determined by the sugar concentration in grapes at harvesting time. However, for Feteasca neagra wines from Valea Calugareasca, a significant difference in the alcoholic concentration of samples macerated in the presence of enzyme is observed. The same was valid for the total acidity. Feteasca neagra from Odobesti was, as in previous years of research (data not shown here) the most acid of the wines, but was also the most alcoholic, with an ethanol content around 14% v./v. Feteasca neagra from Bucharest also displayed high acidity, but a content of only 12% ethanol, suggesting that Bucharest is a region with lower potential for red wines, at least from this viewpoint. As expected, the dry content for the samples produced with maceration enzyme slightly increased in all wines, irrespective of the region of origin or grape variety (data not shown). The wines of Feteasca neagra from Valea Calugareasca and both Feteasca and Babeasca neagra from Pietroasa were the most chemically-balanced of all, with chemical parameters in the normal range.

Regarding the effect of the enzyme treatment on the colour of the wines obtained, figure 2 shows clearly that the main factor that dictated the differences in colour was not the enzyme treatment or any other variation in the technological process during winemaking – but the region of origin of the grapes. The differences among technological variants are also visible in figure 2, but the variants of the same grape of the same origin are closely grouped.

The wines of Feteasca neagra obtained from grapes from Odobesti displayed a red colour with the highest blue component (negative values of parameter *b*), while the Feteasca neagra from Pietroasa and Bucharest had more yellow component. As for the red component, this was similar in samples from Bucharest, Pietroasa and Valea Calugareasca (values of 20-29 for parameter *a*), while

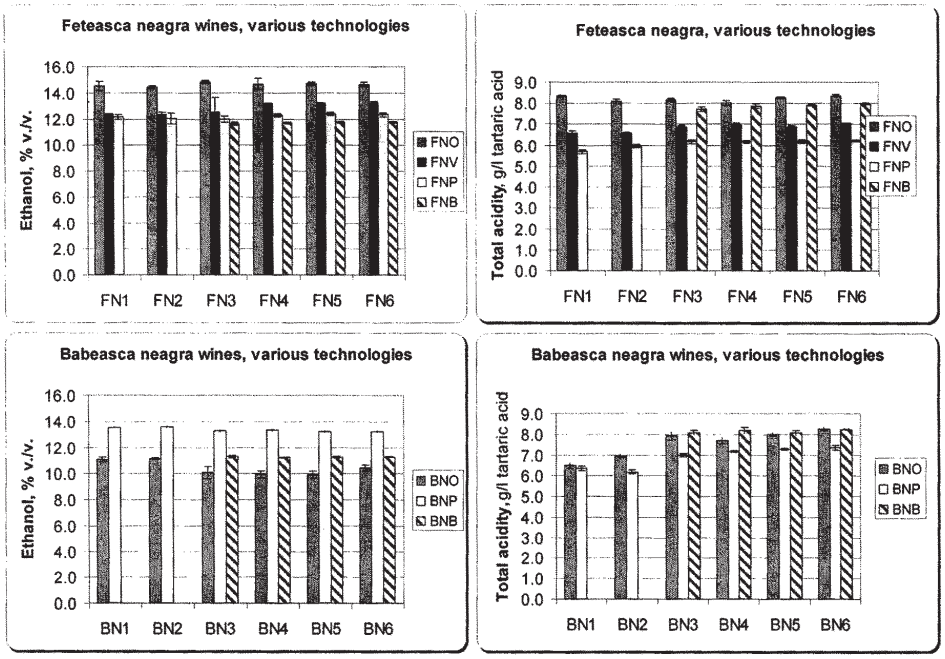

Fig. 1. Values of ethanol concentration and total acidity for the Feteasca neagra and Babeasca neagra wines produced from grapes from 4 different regions in 6 technological variants

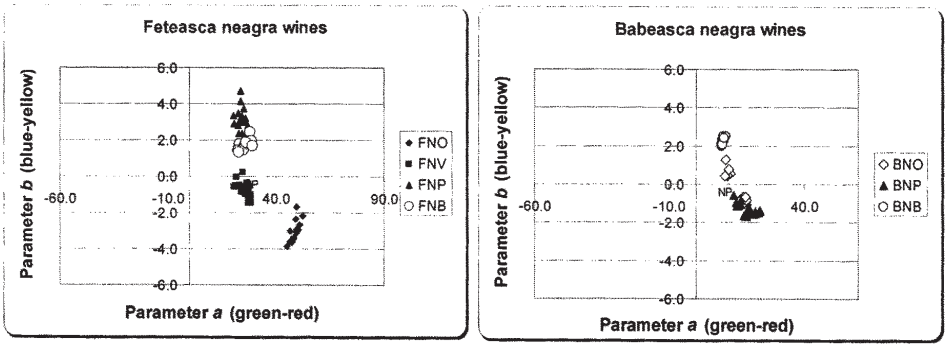

Fig. 2. The placement of Feteasca neagra (left) and Babeasca neagra (right) wine samples in the colour space a (green-red) versus b (blue-yellow)

Odobesti distinguished itself through a higher value (values of 45-52 for parameter *a*). The parameter *a* (green to red) for BN increases towards red due to enzyme addition at maceration, while for FN this improvement is minimal. The increase of parameter *a* towards positive values is correlated with an increase in the monomeric anthocyanins extraction [26].

As also shown in figure 2, Babeasca neagra, with a lower pigment concentration in the skins of the grapes comparing to the Feteasca neagra, produced wines with lower values of the red parameter. Moreover, in Fig. 2 we can see that the Babeasca samples produced with maceration enzyme always displayed more red colour than the rest of the samples produced without enzyme. It can be noticed that the BNP (Pietroasa) and BNO (Odobesti) samples split in two groups each on this figure, the groups with the lower values for parameter *a* always including the wines obtained without extraction enzyme (exemplified further in figure 3 for BNO). This probably holds true for Bucharest Babeasca wines, but the effect cannot be seen on this diagram, because there were no samples produced without enzymes from Bucharest grapes.

In accordance to these observations we can conclude that Babeasca neagra variety benefits from the pectinase addition during the maceration process, the colour of the resulting wine being thus enhanced.

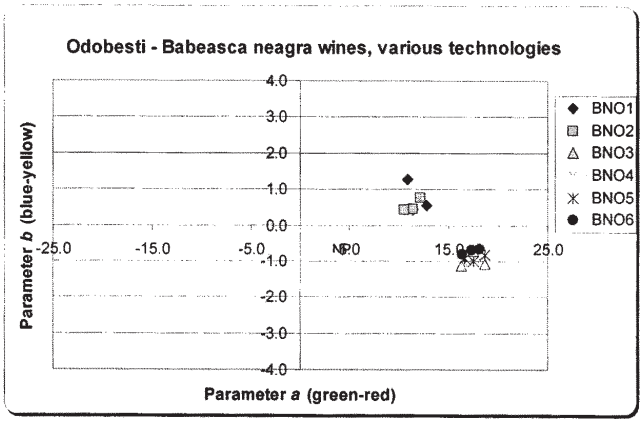

Fig. 3. The placement of Babeasca neagra wine samples of Odobesti produced in 6 technological variants in the colour space *a* (green-red) versus *b* (blue-yellow)

The diagram displayed in figure 3 includes, for exemplification purpose, only the Babeasca neagra wines from Odobesti, produced with various technologies, as described in table 1. Of all technological variants, the samples with enzymic treatment are distinct in the colour space from the samples not treated, due to a higher value of parameter *a* (more red colour) and a lower value of parameter *b* (more blue colour), the samples in both groups (with and without enzyme) being statistically similar irrespective of the addition of tannins or oak chips.

This shift towards bluer hues in both varieties due to the presence of the enzyme makes the colour for all wines treated with enzyme to be more purple than their non-treated counterparts; incidentally, this colour is in fact more appreciated by the consumers. The shift of parameter *b* towards more negative values (towards blue) also means an increase in the monomeric anthocyanic pigments due to enzyme extraction. The chromaticity (fig. 4) is improved by the enzyme only in Babeasca neagra wines (BN3-BN6).

In Feteasca neagra wines the chromaticity did not significantly differ due to maceration in the presence of enzyme, but differed from one area to another, the highest accumulation of pigment being, unexpectedly, in Odobesti, a region mostly dedicated to white wine production.

The hue (fig. 4) is also influenced by the enzymic treatment in Babeasca neagra wines, while in the Feteasca neagra wines hue is not affected. The differences observed between wines of different areas are mostly caused by the initial pigment accumulation in grapes and not determined by the technological treatments, with the exception of Babeasca wines from Odobesti, where the enzymic treatment modified the hues towards purple. A decrease in hue means an increased extraction of colour pigments and a shift toward bluer coordinates of colour.

A partial conclusion of the results presented until now might be that the enzyme is worth using for the extraction on grapes with lower anthocyan accumulation. However, one must bear in mind that the extraction enzyme can also influence the aromatic profile, and this is also worth taking into consideration.

To compare the overall aroma of wines, the flash gas-chromatography with dual columns working on the principle of an electronic-nose is more suitable for a general evaluation, and in this study we used the electronic nose Heracles to analyze and compare the volatile profiles of all the wines studied. Based on the gas-chromatographic analysis of the headspace of the wine samples, the

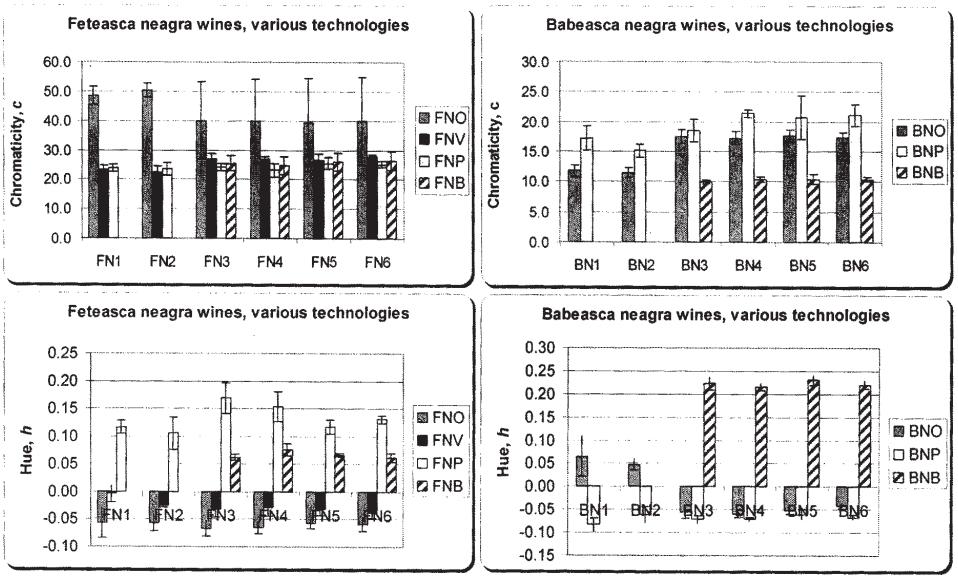

Fig. 4. The chromaticity and hue of Feteasca neagra (left) Babeasca neagra (right) wine samples of produced in 6 technological variants

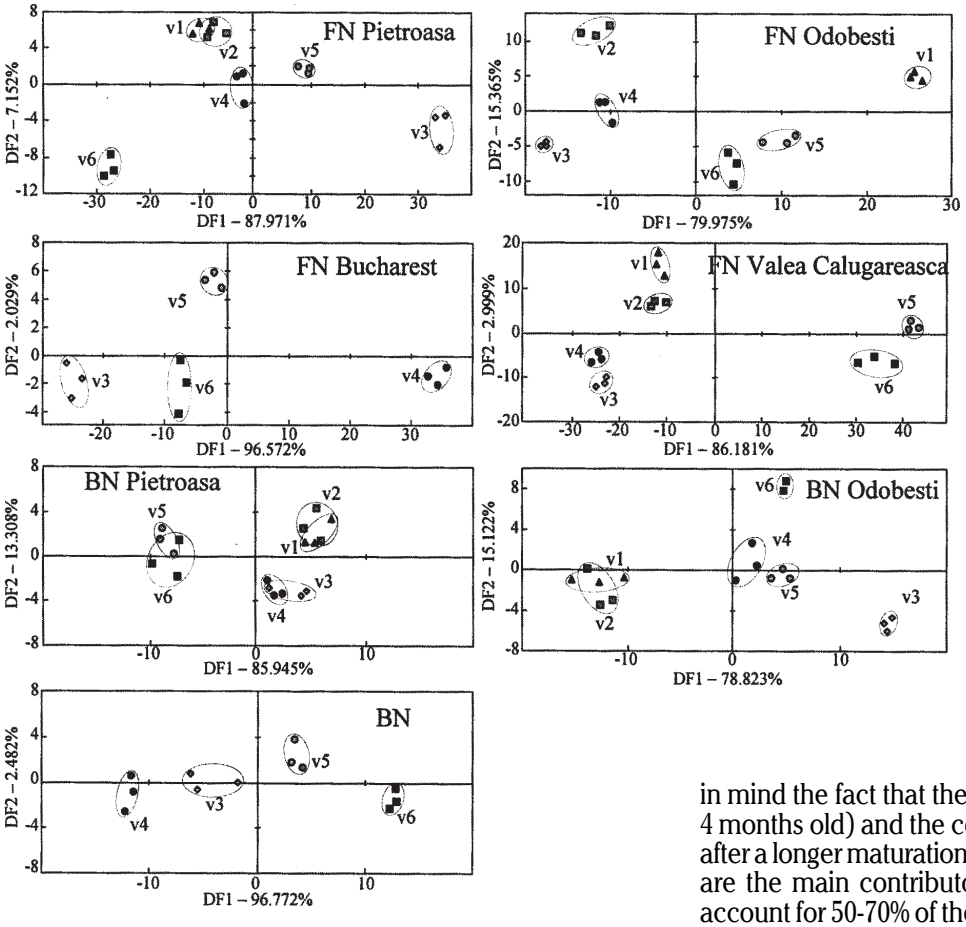

Fig. 5. Discriminant Factor Analysis diagrams showing the differentiation of the Feteasca neagra (FN) and Babeasca neagra (BN) wines produced in 6 technological variants (v1-v6) for each site of origin (only v3-v6 in case of Bucharest)

apparatus performs a multivariate statistical analysis which allows the differentiation of the wines on the basis of their overall volatile profile. This differentiation is expressed using the multivariate analysis method known as Discriminant Factor Analysis or DFA. The results of the DFA analysis performed on all experimental variants in this study are shown in figure 5.

Because of the influence of the place of origin of the grapes as well of all the variations in technology (addition of oak chips, maceration enzyme and two types of oenological tannins) the results vary from one case to another, but what can be said for all cases is that the apparatus was able to distinguish, in general, each type of wine (and technological treatment) from the others. There were several exceptions. For example, in figure 5, wines v1 and v2 of Feteasca neagra from Pietroasa and Valea Calugareasca had almost similar volatile profiles, showing that the influence of oak chips on the volatile profiles of these wines, at 4 months after fermentation, was not significant. By contrast, the two types of tannins added seemed to have a significant effect on the volatile profiles, samples v5 and v6 being always well differentiated from the other types of wines.

Similar observations can be derived from figure 5 regarding the wines of Babeasca neagra, with one slight qualitative difference compared to those of Feteasca neagra. While the apparatus is still capable, in general, of distinguishing the various variants, the separation is less good in case of the variety Babeasca neagra (all samples fit into a smaller area of the diagram). Again, the samples v1 and v2 in Pietroasa and Odobesti seem to have very similar volatile profiles, so apparently the addition of oak chips to variant v2 had no significant effect compared to the control variant v1.

In order to draw a conclusion regarding the influence of various technological treatments on colour one should bear in mind the fact that the wines were still very young (only 4 months old) and the colour stabilization usually appears after a longer maturation period. In this phase anthocyanins are the main contributors to wine colour. Anthocyanins account for 50-70% of the colour values in young red wines [27], but Han *et al.* [26] further showed that the monomeric anthocyanins detected accounted for 64.56-81.57% of parameter *a* value, 59.37-76.23% of parameter *L* value, 0.73-31.34% of parameter *b* value, 64.05-81.97% of chromaticity *C*, 21.11-60.13% of hue *h* value. Monagas *et al.* [28] also demonstrated, using Principal Component Analysis, that for each variety there is a high degree of interrelation between the colour parameters and the anthocyanin pigments, indicating the importance of the grape variety factor in the definition of the wine chromatic characteristics.

As for the effect of the various technological treatments on the volatile profiles of the resulting wine the experiments and the analyses made using the electronic nose have shown that the apparatus is capable, in general, of distinguishing the wines samples based on the type of treatment they have received – the degree of separation being better in case of the variety Feteasca neagra. An exception was found with the v2 variant, indicating that the particular type of oak chips used in this case did not have a significant effect on the volatile profile of the wine.

Conclusions

In this study an investigation was made on the effect of the treatment with extraction enzyme on the colour and volatile profile of red wines made of two indigenous grapevine varieties – Babeasca neagra and Feteasca neagra.

The results showed that the wines made of Babeasca neagra variety benefit from the pectinase addition during the maceration process, the colour of the resulting wine being thus enhanced and improved. In the case of wines made of Feteasca neagra the effect of the enzyme treatment on the colour is not significant, irrespective of whether oenological tannins, liophylised or as oak chips, were used or not during the winemaking.

As for the effect of enzyme on the volatile profiles of the resulting wines, the data obtained with the electronic nose have shown that a good discrimination is possible among the technological variants of Feteasca neagra variety. This means that the enzyme addition, coupled or not with oenological tannins addition, had a significant effect on the Feteasca neagra wines volatile profiles. The discrimination among the volatile profiles of Babeasca neagra wines obtained with various technological treatments was not sufficient to justify the application of enzyme for the aroma improvement.

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