ABSTRACT

Key words: pre-fermentative treatments, oxalic acid, activated carbon, papain.

Currently, winemaking has the necessary technologies, oenological practices and products that enable the development of wines with higher compositional attributes. Among these, an important role in deciding the future of wines' quality, are the oenological treatments made before the fermentation stage of wine production. They are made in order to prevent, ameliorate or remove some defects caused by poor health of the crop, of the way in which the grapes are processed.

According to the directions for use, these treatments bring changes to the chemical composition of wines. The present study aims mainly at obtaining data on the influence of treatments applied before fermentation on the composition of white wine from Iaşi vineyard.

The paper is divided into eight chapters. The first two chapters shows aspects regarding the applied treatments before fermentation in wine industry and the current state of research on the content of acids and metals from white wines. In the next six chapters, the experimental part, are presented the institutional framework in which the research took place, the material, method and the analysis techniques used, results obtained and their interpretation, and general conclusions. The doctoral thesis contains 224 pages, which includes 29 tables, 41 figures and color photos and 151 references.

To assess the influence of the used treatments on the chemical composition of the wines, the main physical-chemical characteristics were determined (alcohol concentration, total acidity, volatile acidity, reductive substances, total dry extract, non-reductive extract, free SO_2, total SO_2, density, pH, conductivity), the total content of polyphenolic compounds, metals' and acids' content of the wine. The wine was also characterized in terms of colour using the CIELab76 method as well as by the identification and quantification of the main olfactory and gustatory characteristics, performed by organoleptic analysis.

In order to attain the required research objectives, two grape varieties and respectively wines, were studied: Aligoté and Tămâioasă românească.

The grapes were harvested at full technological maturity and processed according to white wines production protocol. The must obtained from destemming and crushing the grapes was transferred to glass vessels. Before alcoholic fermentation started, treatments were performed, using conventional and non-conventional products, obtaining the following variants: V1 - oxalic acid – 0,6 g/L, V2 - lactic acid – 3 g/L, V3 – succinic acid – 2 g/L, V4 – sodium silicate – 2,4 g/L, V5 - tannin – 5 g/hL, V6 - bentonite – 100 g/hL, V7 - graphen – 100 g/hL, V8 - chitosan – 100 g/hL, V9 – activated charcoal – 100 g/hL, V10 – proteinase (Bacillus neutral) – 0,05 g/L, V11 - α-amilase – 0,05 g/L,V12 - papain – 0,05 g/L, V13 – product obtained from vegetal proteins, cells' walls, bentonite – 0,05 g/L.

Control samples were also obtained (M), where no prefermentative treatments were applied, used for comparison with the treated variants. After finishing their alcoholic fermentation, the Aligoté and Tămâioasă românească wines were filtered and bottled using a Enomatic Tenco® filtering-bottling device. Immediately after, 40 mg/L sulphur dioxide per bottle was added. The bottles were corked using a Mini T.S® semi-automatic device. 6 month after, the samples were analysed.

It was registered that in the case of the 2011 Aligoté wines, the prefermentative treatments helped increase the alcoholic concentration of the wine, the values obtained being between 9.57% vol (M) and 10.49% volume (V12). The highest alcohol concentration values were recorded at variants treated with enzymatic products due to a further release of nutrients that appeared because of hydrolysis of proteins, favouring the initiation and conduct of alcoholic fermentation under optimal conditions (Cotea VD *et al.*, 2009). The wines can be classified as dry, from the point of view of reductive substances.

The 2012 Aligoté wines register lower values of the alcoholic concentration, in the case of chitosan (V8 - 12,12% vol.) and graphen treatments (V7 - 12,54% vol.). The enzymatic products also positively influenced the obtained wines, the maximum value of the alcoholic concentration (13,73%) being registered in the case of the papain treatment. Analysing the reductive sugars' content, the values ranged between 1 and 12,59 g/L; the wine samples M, V4, V5, V9, V10, V12 were classified as dry, V3,V4, V6, V7, V11, V13 were demi-dry while V1 and V8 were demi-sweet. The samples treated with sodium silicate (V4), graphen (V7) and activated charcoal (V9) underwent malolactic fermentation, fact that was registered as well due to the volatile acidity and pH increase, as well as in the decrease of the total acidity of wines.

The 2011 Tămâioasă românească wines registered high values of the alcoholic concentration in the case of graphen (13,48% vol.) and chitosan treatments (13,36% vol). The wines that were treated with activated charcoal (V9) had the lowest alcoholic concentration (12,64% vol.). The 2012 Tămâioasă românească wines have values of the alcoholic concentration ranging between 13,6 and 14,04% vol.

As expected, the acids added at prefermentative stage, contributed to the increase of the wines total acidity, the maximum values being registered at the V3 sample, treatment with succinic acid, while the treatment with sodium silicate (V4) led to a decrease of the total acidity.

In order to accentuate the influence of the prefermentative treatments on the content of polyphenolic compounds, a series of specific analyses were done: D280 index or the total polyphenolic index (I.P.T.) and the Folin-Ciocâlteu index. The treatments with activated charcoal (V9) and bentonites (V6) have influenced the fining and discolouration of the wines, diminishing the quantity of polyphenolic compounds. The tannin treatment (V5) increased the values of this index. It was also noticed that, at the same time with the degradation of proteins as a result of proteinase activity (V10), a decrease of the polyphenolic compounds quantity appeared. An explanation is that the resulted protides form a precipitate with the tannins in wine much easier.

The analysis of organic acids in wines was done by HPLC. In 2011 Aligoté wines, the tartaric acid content had values between 2,02 g/L (V4) and 2,65 g/L (V1). In 2011 wines, the highest tartaric acid content was found in the variant treated with oxalic acid (V1– 2,33 g/L). The lowest quantities in 2011 wines were registered in the samples that were obtained through treatment with sodium silicate (V4 - 0,37 g/L), graphen (V7 - 0,94 g/L and charcoal (V9 - 0,4 g/L). In the same samples, the highest concentrations of lactic acid was found (not taking into consideration of course, the sample processed by lactic acid treatment), fact that proves that these wines suffered a malolactic fermentation.

2012 Aligoté wines showed high values of malic acid in samples where treatment with acids was used (V1, V2, V3), while the lowest quantities were registered in the sample where chitosan treatment was applied (V8 – 0,0014 g/L). The variants obtained by treatment with sodium silicate (V4), tannin (V5) and charcoal (V9) underwent malolactic fermentation, influencing thus the increase of lactic acid in wines. The process appears due to the metabolisation of malic acid into lactic acid and carbon dioxide. The lowest quantities of lactic acid were identified in samples where treatments with oxalic acid (V1), succinic acid

(V3) and graphen (V7) were applied. A part of the 2012 Aligoté wines had high values of acetic acid, reaching over the normal limits, with a maximum value in the sample obtained through treatment with bentonites (V6 – 0,77 g/L). Prefermentative treatments led to the decrease of fumaric acid concentration in 2011 Aligoté wines compared to the control sample, while, in 2012 variants, fumaric acid content was under the detection limit in samples processed by treatment with bentonite, tannin and proteinase.

In the case of 2011 Tămâioasă românească, the tartaric acid concentration was diminished due to prefermentative treatments from 1,85 g/L (M) up to 1,44 g/L (V4),. On the other hand, the malic acid content grew due to the applied operations (1,61 g/L – V6 compared to 1,24 g/L – M). For 2012 wines, the sodium silicate treatment had the same effect as for 2012 wines, contributing to the decrease of tartaric acid. The malic acid content presented no major variations, the values ranging between 1,51 g/L (V7) and 1,77 g/L (V4). The use of prefermentative treatments led to the decrease of citric acid content in wine from 0,88 g/L (M) down to 0,35 g/L (V9).

As treatments with oxalic acids, lactic and succinic acid were used in the prefermentative stage, the quantities of these organic acids are very high compared to the other treatments.

The metals' content in wines is influenced not only by soil composition, agricultural practices (fertilisations, phyto-sanitary treatments), used equipment, but also by applied oenological practices that are applied to must and wine (Eschnauer, H., 1982).

Except the variant where sodium silicate was used, the treatments with lactic acid (V1), oxalic acid (V2), proteinase - *Bacillus neutral* (V10) and α-amilase (V11) led to obtaining Aligoté wines with a higher sodium quantity in 2011.A reduction of the sodium quantity is registered in 2011 wines in V3, V7, V8, V9, V12 samples, where the values range between 0,16–8,87 mg/L; the treatments with organic acids (V1 and V3) as well as with enzymatic products (V10, V11, V12) decreased the sodium concentration in 2012 wines. Treatment with oxalic acid (V1) favoured the onset of a deposit of calcium oxalate that led to the decrease of calcium level until 32, 81 mg/L in the 2011 sample and 13, 49 mg/L in the 2012 sample. Another treatment that influenced the decrease of this metal was sodium silicate (V4). The maximum calcium limit in wine was overpassed in the case of the wine variant obtained through treatment with vegetal proteins.

The maximum admitted level for zinc vas overpassed in 2012 wines in the case of tannin treatment (V5) and papain (V12). A low level of zinc is found in 2012 wines, especially in V3 (0, 06 mg/L), V4 (0,20 mg/L) and V8 (0,22 mg/L).

The copper content was under the detection limit in 2011 wines and in 2012 variants obtained by treatment with oxalic acid (V1), sodium silicate (V4), bentonites (V6), graphen (V7) and activated charcoal (V9).

In the case of Tămâioasă românească wines, the treatment with sodium silicate led to the increase of sodium content over allowed limits, the values reaching 303,75 mg/L in 2011 wines and 158,81 mg/L in 2012 wines; The allowed limit in wines ranges between 3–60 mg/L. In 2011 wines this limit was overpassed by variants V2, V5, V6, and V8. The wine sample that was obtained by treatment with a product based on vegetal proteins + cellular walls + bentonite (V13) showed an increase of the calcium content over the allowed limits.

An important quantity of iron, over the allowed limits, is registered in 2012 wine samples obtained through treatments with oxalic acid (V2 - 10,04 mg/L), succinic acid (V3 – 9,58 mg/L), chitosan (V8 – 11,86 mg/L), proteinase - *Bacillus neutral* (V10 15,27 mg/L) and bentonite (V6 – 7,19 mg/L). For 2011 Tămâioasă românească wines, the highest values are those obtained in the control sample (V – 12,99 mg/L), as well as in the variants treated with bentonites (V6 – 17,19 mg/L) and with graphen (V7 – 12,73 mg/L). The allowed maximum limit of zinc was overpassed in 2012 samples obtained in the control sample (9,35 mg/L), treatment with tannin (7,57 mg/L) and activated charcoal (6,11 mg/L). Copper was under the detection limit in all 2011 wine samples and in the 2012 tannin treatment variant.

The results of the L parameter show that the Aligoté and Tămâioasă românească wines obtained through activated charcoal treatments are the most clear. In the case of 2011 Aligoté wines, the colour differences compared to the control sample appear in the variants processed through treatments with tannin, bentonites and charcoal. In 2012 Aligoté wines, the differences appear in the samples treated with chitosan, charcoal and vegetal proteins. A hue difference appears in the 2011 wine variants treated with tannin, bentonite and proteinase; chitosan and papain in 2012 samples. Parameter **a** in Tămâioasă românească wines registers positive values, proof that, besides the green compounds, there also are present some red compounds. Colour differences compared to the control sample are found in the wine variants obtained through treatment with activated charcoal.

The sensorial profile of Aligoté wines was influenced by prefermentative treatments: treatments with acids brought to the wines a green fruity note, while enzymatic products used

added notes of wild flowers, ripe fruits and exotic fruits; the green character had a higher intensity in the case of wines where treatments with succinic acid and sodium silicate were applied. The sensorial evaluation, a more pronounced acidity was perceived in samples where acids were used; a better texture was identified in wines where enzymatic treatments were applied. The aroma profile of Tămâioasă românească wines was influenced by the applied prefermentative treatments ; strong notes of ripe fruits as well as exotic fruits were identified in the control sample as well as the wines obtained through treatments with oxalic acid ; powerful aromas of wild flowers were sensed in wines where treatments with α-amilase, papain and vegetal proteins were used. The treatment with oxalic acid produced wines with a good persistence.

Following the statistical analysis results, it was observed that, for the wines made in 2011, the prefermentative treatments had a distinctly significant influence on the content of tartaric acid, calcium and iron while a very significant influence was found on the amounts of lactic, acetic, succinic, oxalic acid and sodium. The grape variety significantly influenced levels of potassium and zinc; a distinctly significant influence was found on malic acid content and a very significant influence appeared on the contents of tartaric, acetic, citric and shikimic acid.

The 2012 wines, prefermentative treatments had a distinctly significant impact on the concentration of calcium and highly significant on the content of lactic acid, succinic acid, oxalic acid and sodium. The grape variety had a distinctly significant influence on the concentration of lactic acid and very significant influence on the concentrations of acids: malic, acetic, citric, fumaric and shikimic acid. The metal content of the wines was not influenced by the variety except for the calcium, where the influence was significant as well as for copper, where the influence was very significant.