Influence of Common and Selected Yeasts on Wine Composition Studied Using \(^1\)H-NMR Spectroscopy

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The fermentations of some Romanian grape musts with Saccharomyces cerevisiae yeast were studied under different conditions: natural fermentations (without additional yeast), induced fermentation using different amounts of selected yeasts and industrial fermentation. The wines fermentations have been daily monitored using \(^1\)H nuclear magnetic resonance (\(^1\)H-NMR) spectroscopy. Based on the \(^1\)H-NMR spectroscopy and applying multivariate statistical analysis, we studied the changes in the chemical compositions of wines depending on the alcoholic fermentation conditions establishing the influence of yeast used in the process.

Keywords: wine composition, \(^1\)H-NMR, PCA, grape must

Wine quality is influenced by grape variety, yeast strains and winemaking technologies [1]. The fermentation of grape juice into wine is a constant concern for the scientists. Traditionally, wine has been produced by natural fermentation of grape must by common yeasts that originate from grapes skin. On the other hand, the induced fermentation with selected yeasts is used by the wine producers aiming to obtain a high quality wine each year [2-4].

In the wine making process, the type and intensity of the flavor is influenced mainly by: yeast, environmental factors (climate, soil), cultivar, fruit condition, winemaking process, must pH, amount of sulphur dioxide, amino acids present in the grape must and malo-lactic fermentation [5-6].

Wine has been produced for years by natural must fermentation caused by yeasts that originate from the grapes skin without deliberate inoculation to start the process. Spontaneous alcoholic fermentation of grape must is a complex process characterized by the presence of a large number of different yeast genera and species contributing to the flavor of wines [7]. The natural fermentation of grape must is usually started by low-alcohol-tolerant yeasts (Kloeckera/Hanseniaspora) that lead the first stages of fermentation. After 3–4 days, they are replaced by elliptical yeasts (Saccharomyces cerevisiae) that continue and finish the fermentation process [8-9].

Non-Saccharomyces yeasts improve the wine bouquet, but are not able to complete fermentation due to their low ethanol tolerance. For this reason, several authors have studied fermentation with mixtures of yeasts, either simultaneously applied or in sequential cultures [10-11].

Amino acids originate from the must, they are not formed during the fermentation process; but by using non-Saccharomyces yeasts such as Oenococcus oeni and Lactobacillus plantarum, the amount of the amino acids is different in wines compared to the corresponding musts [12-16].

Numerous researchers have used infrared spectroscopy to study the fermentation process and also to detect the fermentation problems that can occur. Infrared spectroscopy has been successfully used for monitoring fermentations [17-19].

In previous studies we established a method for monitoring the fermentative process using \(^1\)H-NMR spectroscopy. This method offers results in real time representing a useful tool for closely controlling the winemaking process [20].

The fermentations of Romanian musts “Muscat Ottonel”, “Burgund mare” and “Feteasca Alba” with Saccharomyces cerevisiae yeast were studied. These grape musts fermentations were conducted under different conditions: natural fermentations (without additional yeast), induced fermentation using different quantities of selected yeasts and industrial fermentation.

The wine fermentations have been daily monitored using \(^1\)H-NMR spectroscopy. Based on the \(^1\)H-NMR spectroscopy and applying multivariate statistical analysis, we studied the changes in the chemical compositions of wines depending on the alcoholic fermentation conditions in order to establish the influence of yeasts used in the process.

Experimental part

The musts and wines used in this study were acquired from Urlati vineyard (the geographic aria of Dealu Mare, Romania) in 2009. Three varieties of musts were studied: “Muscat Ottonel” - a variety of white grape originated from France, it is a member of the Muscat family of Vitis vinifera, “Feteasca Alba” - a traditional white Romanian variety and “Burgund mare” - a red grape variety which was developed from Pinot Noir variety a century ago. All these are varieties of grapes that are widely spread in Romania.

The temperature during alcoholic fermentation process was 25°C. The laboratory fermentation process was conducted in different ways: natural fermentation (without any additional yeast), induced fermentation using a normal amount (20 g yeast/100 L must) and an excess amount of yeast (100 g yeast/100 L must). Two commercial yeast strains were used to induce fermentation process Saccharomyces Cerevisiae (PREMIUM® ROUGE 30 R, Enologica Vason) - S.c.1 and Saccharomyces Cerevisiae (Falvor 2000, Enologica Vason) - S.c.2. We monitored the fermentations process for at least 20 days. We also studied the wines obtained from those grape musts, coming from industrial fermentations.
**1H-NMR spectroscopy analysis**

All musts and wines were analyzed directly, without any prior preparation before NMR analyses. The spectra were recorded using a concentration wine/D2O = 9/1 (v/v). The chemical shifts are reported in ppm, using the TSP as internal standard (10 mM/L).

1H-NMR spectra were recorded on a Varian INOVA 400 spectrometer (“C.D. Nenitescu” Institute of Organic Chemistry, Romanian Academy), operating at 9.4 Tesla, corresponding to the resonance frequency of 400.12 MHz for the 1H nucleus, equipped with a direct detection four nuclei probe head and field gradients on z axis. Samples were analyzed in 5 mm NMR tubes (Norell 507). Typical parameters for 1H-NMR spectra were: 35° pulse, 4s acquisition times, 6.4 KHz spectral window, 16 scans and 52 K data points. The average acquisition time of the 1H-NMR spectra was approximately 4 min.

**Statistical analysis**

The statistical analyses (Principal Component Analysis) used to investigate the compositional differences between samples were carried out using the XLSTAT program (from Addinsoft).

**Results and discussions**

Grape juice is a complex mixture of many compounds, contains mainly water, mono-saccharides and smaller amounts of different compounds, including amino acids and some organic acids. In the fermentation process, the mono-saccharides are consumed and the ethanol is formed in the alcoholic fermentation reaction, but also, in a parallel reaction, some other compounds are formed such as organic acids (acetic, succinic) and some polyols like 2,3-butanediol and glycerol. The method used for marker identification in 1H-NMR spectrum was presented in earlier studies [21-22]. Some of the markers identified for wine, which have been used for quantitative measurement of different compounds in 1H-NMR spectra are exemplified in figure 1.

![Figure 1. Section of 1H-NMR spectra of Muscat Ottonel wines obtained in different fermentation conditions: A-natural fermentation; B-induced fermentation, C-induced fermentation (yeast excess), D-industrial fermentation](image-url)
In induced fermentation case of "Burgund mare" must both types of the _Sacharomyces cerevisiae_ yeasts were used (S.c.1 and S.c.2). Figure 2 illustrates the Principal Component Analyses score plots derived from compounds quantitatively measured based on 1H-NMR spectra of all "Burgund mare" wines. The PCA results demonstrate that fermentation process induces variation in wine metabolite profile and type of yeast stain contributes to the variation. A tendency of grouping can be noticed for the industrially obtained "Burgund mare" wine and the wines obtained in the laboratory by induced fermentation with the S.c.2 yeast. This result was predictable since similar yeast is used in the industrial fermentation.

Table 1 shows the absolute concentration of some compounds measured in "Burgund mare" wines using 1H-NMR spectroscopy. One can notice that the larger amount of ethanol is formed in case of induced fermentation with a larger amount of S.c.1 yeast. In the meanwhile the amino acids amount is decreasing in case of induced fermentation. It can be noticed that the largest amount of glycerol is formed through industrial fermentation and the induced fermentation generated more acid wines.

For all types of grape musts analyzed, during the fermentation process, the glucose and fructose were consumed in 10-15 days of fermentation. First the yeasts start consuming glucose, and when the amount of glucose is decreasing, they start to consume fructose. This issue is related with the fact that _Saccharomyces cerevisiae_ prefers glucose.

The white grape musts were subject to induce fermentation using the S.c.2 yeast. Ethanol absolute concentration in "Feteasca Alba" and "Muscat Ottonel" wines obtained in different fermentation conditions varies between 1.99 M/L for natural fermentation and 2.46 M/L in case of induced fermentation. In the case of glycerol in the "Fetesca Alba" and "Muscat Ottonel" wines the amount of glycerol in the produced wine decreases in the case of induced fermentation.

Figure 3 illustrates the absolute concentration of acetic acids in "Feteasca Alba" and "Muscat Ottonel" wines. The larger amount of acetic acids is formed by induced fermentation with excess of selected yeast. Comparable results were recorded in the case of succinic acid.

The compositional changes in the metabolite profile measured, using 1H-NMR spectroscopy, during alcoholic fermentation process of musts "Feteasca Alba", "Muscat Ottonel" and "Burgund mare" were subjected to Principal Component Analyses (PCA).

In figure 4 a grouping tendency according with the wine grape variety is noticeable. The PCA results illustrates that the variation in wine metabolite profile induced by fermentation process and different _Sacharomice cerevisiae_ yeast is important, but not critical in case of authentication studies.

**Table 1**

<table>
<thead>
<tr>
<th>Type of fermentation</th>
<th>Ethanol (M/L)</th>
<th>Glycerol (M/L)</th>
<th>Acetic acid (mM/L)</th>
<th>Lactic acid (mM/L)</th>
<th>Succinic acid (mM/L)</th>
<th>Amino acids (mM/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural fermentation</td>
<td>2.56</td>
<td>0.24</td>
<td>9.50</td>
<td>19.35</td>
<td>13.20</td>
<td>19.70</td>
</tr>
<tr>
<td>Induced fermentation (S.c.1)</td>
<td>2.58</td>
<td>0.20</td>
<td>19.40</td>
<td>18.35</td>
<td>15.40</td>
<td>16.76</td>
</tr>
<tr>
<td>Induced fermentation (yeast excess S.c.1)</td>
<td>2.70</td>
<td>0.22</td>
<td>21.10</td>
<td>15.53</td>
<td>17.40</td>
<td>14.54</td>
</tr>
<tr>
<td>Induced fermentation (S.c.2)</td>
<td>2.3</td>
<td>0.23</td>
<td>17.90</td>
<td>17.30</td>
<td>11.35</td>
<td>12.79</td>
</tr>
<tr>
<td>Induced fermentation (yeast excess S.c.2)</td>
<td>2.64</td>
<td>0.24</td>
<td>19.80</td>
<td>15.98</td>
<td>11.71</td>
<td>10.66</td>
</tr>
<tr>
<td>Industrial fermentation</td>
<td>2.17</td>
<td>0.24</td>
<td>6.99</td>
<td>16.26</td>
<td>8.51</td>
<td>18.27</td>
</tr>
</tbody>
</table>
Conclusion
The compositional changes during alcoholic fermentation process of grape musts “Feteasca Alba”, “Muscat Ottonel” and “Burgund mare” were monitored on a daily basis using 1H-NMR spectroscopy. The largest amount of glycerol is formed during the natural and the industrial fermentations. The induced fermentation, conducted in the laboratory, produced wines with an increased acidity. This method offers results in real time and it represents a useful tool for closely controlling and conducting the winemaking process.

The PCA method shows that the variation in terms of wine composition, induced by fermentation process and the use of different yeast is important, but not critical in case of authentication studies.

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