THE MANAGEMENT OF SELECTED YEAST STRAINS IN QUANTIFYING TERPENE FLAVOURS IN WINE

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ABSTRACT: This paper aims at studying the accumulation of free volatile terpene flavours (FVT) and bound precursor terpene flavours (BPT) under the action of 12 selected wine yeast strains (S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S11, S12). The importance of these flavours results from the fact that they confer wines a savoury, floral aroma; their amount is closely related to a series of factors, of which one makes the object of this analysis. The must under alcoholic fermentation comes from the Gârbova area (Sebeș-Apold Vineyard), obtained from the variety Muscat Ottonel. The 12 strains used in the alcoholic fermentation resulted in the determination of considerable amounts of terpene flavour compounds, between 370 µg/L and 1100 µg/L, which recommends their use in making quality wines.

KEY WORDS: wine yeasts, Muscat Ottonel, BPT, FVT, UV-VIS spectrophotometer

1. INTRODUCTION

Wine yeasts play a big part in the formation and establishment of wine sensory properties. Nowadays, great emphasis is placed on using selected yeasts of superior biotechnological properties, so as to ensure quality wines. Human perception of wine flavours results from an entire complex of sensations conferred by various components and substances, of which some can be determined, and others not so much (Lawless and Heymann, 1998, Pacala et al.2009, Begea et al. 2009). Volatile and non-volatile compounds interacting in this process already exist or form in the grape, and depend on the processes employed in primary and secondary fermentation (Parley et al. 2001; Du Toit et al. 2006). During primary fermentation, carbohydrates in the must turn into alcohol and derivative substances, carbon dioxide and hundreds of components that contribute to the formation of wine flavour. Throughout this process, yeasts actively contribute to all the transformations, significantly improving the flavour palette of the wine (Dubourdieu et al., 2006; Tate et al., 2006; Zhang et al., 2010). Terpene compounds also play a part. Thus, linalool is a colourless liquid with a fresh floral scent reminding of spices and lemons. Under the influence of acids, linalool turns into geraniol, nerol and α-terpineol. In grapes, linalool is accompanied by oxides. The odor detection threshold of geraniol and linalool is around four times lower than that of α-terpineol and nerol. It is easily detected, as its perception threshold is very low. Flavoured and semi-flavoured varieties, especially Muscat Ottonel and Târdea, contain the highest concentration of linanool (Târdea, 2007; Lengyel 2014). Methyl and ethyl vanilla, providing a pleasant vanilla flavour, are characteristic of wines obtained using selected yeasts (Terrier et al. 1998; Etaino et al., 2008; Itu, 2008).

2. MATERIALS AND METHODS

Must made of Muscat Ottonel grapes from Gârbova, Sebeș-Apold Vineyard, harvested in 2016, fermented using microvinification with 12 selected yeast strains, isolated in the Microbiology Laboratory from indigenous strains, coded as follows: S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S11, S12. The 12 samples underwent alcoholic fermentation under identical maceration, temperature and time conditions (12 hours, 18°C, 15 days). We quantified free volatile terpene flavours (FVT) and bound precursor terpene flavours (BPT) from the resulting wines, using the spectrophotometric method described by Târdea, 2007, using a CECIL 1021 UV-VIS spectrophotometer. The concentration of FVT and BPT was calculated with the formula: FVT/BPT(µg/L)=a*bc, where:

a=concentration of linalool read on the calibration curve (µg/L)
b=volume of distillate used (mL)
c=volume of wine used for distillation (mL)
d=volume of distillate used for spectrophotometric measurements.

3. RESULTS AND DISCUSSIONS

Wines resulting from the fermentation of must made of Muscat Ottonel grapes using the 12 yeast strains contain significant amounts of free volatile terpene flavours (FVT) and bound precursor terpene flavours (BPT). As shown in Figure 1, we recorded values between a minimum of 376.99 µg/L when using the strain S11, and a maximum of 943.97 µg/L free volatile terpene flavours when using the strain S6. The most valuable strains led to FVT accumulations of around 800 µg/L. Thus, strain S1 led to an accumulation of 832.23 µg/L, strain S7 to 823.44 µg/L, and strain S4 to 813.87 µg/L.
values were recorded when using strains S9, S10, S12, which led to amounts of 521.22 µg/L, 513.97 µg/L, respectively 621.11 µg/L. Important amounts of FVT were also reached when using strains S2–769.42 µg/L, S3–697.56 µg/L, S8–781.24 µg/L or S5–756.02 µg/L.

Figure 1. Evolution of the concentration of free volatile terpenic flavours (FVT) under the action of the 12 selected yeast strains (S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S11, S12).

Figure 2. Evolution of the concentration bound precursor terpene flavours (BPT). under the action of the 12 selected yeast strains (S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S11, S12).

The amount of bound precursor terpene flavours (BPT) reached a minimum of 445.44 µg/L when using strain S11, and a maximum of 1099.17 µg/L when using strain S6 (Figure 2).

Important values were also brought about by strains S7, S8, and S4, values of bound precursor terpene flavours (BPT) reaching 1011.12 µg/L, 978.45 µg/L, respectively 912.09 µg/L.

Moderate values were recorded when using strains S1, S2, S3, S9, and S10, the amounts determined reaching 888.89 µg/L, 812.11 µg/L, 782.65 µg/L, 715.55 µg/L, respectively 777.09 µg/L.

The results obtained were validated by the statistical model of regression Anova.

### SUMMARY OUTPUT

**Regression Statistics**

|                     |        |
|---------------------|--------|
| Multiple R          | 0.829395 |
| R Square            | 0.687897 |
| Adjusted R Square   | 0.653218 |
| Standard Error      | 98,33048 |
| Observations        | 11     |

**ANOVA**

|                     | df | SS    | MS    | F     | Significance |
|---------------------|----|-------|-------|-------|--------------|
| Regression          | 1  | 191797.8 | 191797.8 | 19,8366 | 0.001591     |
| Residual            | 9  | 87019.95 | 9668,883 |       |              |
| Total               | 10 | 278817.7 |       |       |              |
### Regression Analysis

|                | Coefficients | Standard Error | t Stat | P-value | Lower 95% | Upper 95% |
|----------------|--------------|----------------|--------|---------|-----------|-----------|
| Intercept      | 142,5125     | 127,0216       | 1.121955 | 0.290921 | -144,83   | 429,8554  |
| 888,89         | 0.691325     | 0.15522        | 4.45383 | 0.001591 | 0.340192  | 1.042457  |

### Residual Output

| Observation | Predicted 832,23 | Residuals | Standard Residuals | Percentile 832,23 |
|-------------|------------------|-----------|--------------------|-------------------|
| 1           | 703,9442         | 65,47575  | 0.701893           | 4,545455          |
| 2           | 683,5778         | 13,98218  | 0.149888           | 13,63636          |
| 3           | 773,0629         | 40,80711  | 0.437448           | 22,72727          |
| 4           | 627,2763         | 128,7437  | 1.380119           | 31,81818          |
| 5           | 902,3959         | 41,57408  | 0.44567            | 40,90909          |
| 6           | 841,5248         | -18,0848  | -0.19387           | 50                |
| 7           | 818,9392         | -37,6992  | -0.40413           | 59,09091          |
| 8           | 637,1899         | -115,97   | -1.24319           | 68,18182          |
| 9           | 679,7341         | -165,764  | -1.77697           | 77,27273          |
| 10          | 450,4562         | -73,4662  | -0.78755           | 86,36364          |
| 11          | 500,7086         | 120,4014  | 1.29069            | 95,45455          |

**Figure 3.** Regression rate of evolution of the concentration of free volatile terpenic flavours (FVT) and bound precursor terpene flavours (BPT) under the action of the 12 selected strains of yeast (S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S11, S12)

**Figure 4.** The prediction of the concentration of free volatile terpenic flavours (FVT) and bound precursor terpene flavours (BPT) under the action of the 12 selected strains of yeast (S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S11, S12)
The statistical interpretation of the obtained results confirms that: Among the leaky free volatile terpene flavours (FVT) and bound precursor terpene flavours (BPT) there is a strong link. 68% of the results obtained depend on the strain of yeast used. The test F is 19.8366 and the significance F is 0.001591 (value less than 0.05), the regression model is valid and can be used to analyze the dependence between the two variables.

4. CONCLUSIONS

Using selected yeast strains results in a higher accumulation of terpene compounds, both free or precursor. The highest potential strains were S6, S7, and S8, followed by S1 and S4. The lowest potential strains were S11 and S12, with values even half lower than the most valuable.

We also noticed that, out of 12 strains, only three are highly recommendable for the fermentation process, four are moderately recommendable, while the others can be used as strains whose properties can be improved through biotechnological methods.

Wine yeast contributes to the accumulation of aromatic compounds in wine, especially volatile and bound terpenes.

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