Bioprotection as a tool to produce natural wine: Impact on physicochemical and sensory analysis

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Abstract. With an increasing concern of the food security, more and more winemakers choose bioprotection as an alternative of SO_2 in winemaking process as a practice of natural wine producing. In order to insight a way to produce reliable natural wine, three wines were produced in vintage 2021, in the Republic Moldova. This study provides two comparisons of physicochemical property and sensory analysis: (1) Comparison between commercial yeast and wild yeast, two wines followed a conventional fermentation technique, but different yeasts were used: *Saccharomyces cerevisiae* (wine A), wild yeast (wine); (2) Comparison between bioprotection and SO_2 usage: Wine A and Wine C (which were inoculated two non-*Saccharomyces*: *Torulaspora delbrueckii* and *Metschnikowia pulcherrima* before alcohol fermentation). As a result of this comparative study, it is found that the commercial yeast is more capable of converting sugar in the alcoholic fermentation, but after malolatic fermentation (MLF) the alcohol levels of each are almost same. From physicochemical poit of view, the bioprotectors obviously modified the volatile acidity, total polyphenol index (TPI), phenolic, anthoyanin and ethyl acetate. From sensory perspective, the smell intensity of bioprotection wine is higher and with more fruity aroma.

1 Introduction

SO_2 is an antioxidant which is able to avoid developing oxygen aroma, and its capacity of antimicrobial restrains unwanted bacteria cultivating. Based on those advantages, SO_2 has become an important protection tool of road usage in conventional winemaking process. However, SO_2 may cause allergy and other health problems. It's a trend to reduce the usage of SO_2 in winemaking. A contrastive study of red wine with and without SO_2 added was carried out in Bordeaux, which shows a larger number of ``non-added'' wines with defects [1]. In order to improve the quality of $SO_2$ ``non-added'' wine, non-saccharomyces yeasts were used for bioprotection in recent year, results from which has shows that bioprotection has a significant impact on the aromatic profile of wine [2].

Traditional winemaking processes massively rely on the ambient yeast especially Saccharomyces *cerevisiae* on the grape surface [3]. It plays a principal role in wine fermentation, producing various chemical compositions, makes the wine show different ``terrior'' [4]. Nowadays, with a growing concern about food safety, winemakers are trying to return to tradition, consequently ``natural wine'' gets more and more popular. Although there is not an unambiguous definition of natural wine, it is considered a type of wine with low input.

This research aims at the approach to producing "natural wine", two objects were studied:

How does the wild yeast from local grapes work.

Compare the bioprotection wine and the SO_2 protection wine from physicochemical and sensory aspects.

2 Materials and methods

2.1 Experimental procedure

This research was carried out with Rara Neagra grapes from Olanesti, PGI Stefan Voda, and Moldova. Grapes were harvested from an organic vineyard at the moment when they reached the optimal must weight. For each type of wine, 7000 kg grapes were used, 80% of which crushed and destemmed while 20% were whole bunches grapes. The grapes were fermented together. For wine A, *Saccharomyces cerevisiae* authorized for organic wine were inoculated, meanwhile wine B inoculated the wild yeast from itself, they are both protected by SO_2 (PMS 400 g). For wine C, the same *Saccharomyces cerevisiae* inoculated with *Torulaspora delbrueckii* and *metschnikowia pulcherrima* for bioprotection. The density measured twice a day after each punching down. Each type of wine with duplication ($n = 2$).

2.2 Physicochemical properties analysis

The FTIR spectroscopy method was used for general physico-chemical analysis: alcohol and pH. Capillary electrophoresis was used to detect and quantify the organic acid. The composition of volatile substances was

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determined by gas chromatography, the polyphenolic pole was determined with the help of UV spectrophotometry, and the component of anthocyanins was established by the HPLC method refer to Ribereau-Gayon. The total fenolice was measured by method Folin-Ciocaleteu.

Color Intensity (CI) was calculated by summery of absorbance value of 420 nm, 520 nm and 620 nm. The method refers to what Glories described. The A420/520 represent hue which is determined ratio between value at 420 nm and 520 nm.

In addition, the CIELAB analysis followed what Ayala mentioned.

2.3 Sensory analysis

The sensory analysis was carried out in the specialized tasting room of the National Center for checking the quality of wine products. The samples were served together at a temperature of 20 degrees in individual booths and in ISO-INAO glasses into which about 75 ml of wine was poured. Each of the analyzed samples was coded with a three-digit code.

The samples were evaluated by ten panelists (women 4/men 6, average age 37.4) from the professional wine industry who have worked for at least 5 years in different spheres of the value chain of the wine industry: winegrowers, oenologists, marketers and traders, logistics, catering, and hospitality (wine tourism).

The sheet below was applied to the sensory analysis:

Name		Vintage				
Date		Code				
View						
Color Indensity	0	1	2	3	4	5
Tone	Brown	Orange	Caramel	Red	Purple	
Smell						
Smell Indenity	0	1	2	3	4	5
Smell Cleaness	Yes		No			
Fruity	0	1	2	3	4	5
Vegetal	0	1	2	3	4	5
Smell Complexity	0	1	2	3	4	5
Taste						
Taste Cleaness	Yes		No			
Structure	0	1	2	3	4	5
Body	0	1	2	3	4	5
Tannins	dry		prominent			velvet
Balance	0	1	2	3	4	5
Persistance	0	1	2	3	4	5

Figure 1. Sensory analysis sheet.

The sheet contains the usual sensory descriptors that must be developed according to an intensity scale from 0 (no sensation) to 5 (very strong sensation). Besides these descriptors, there are 2 positions that contain a binary answer and refer to the purity of aroma and taste (absence or presence of a defect). If present, the taster was asked to identify this defect. Also the ladder of appreciation of the quality of the tannins in the taste to limit to three levels: dry, pronounced and supple.

2.4 Statistical analysis

Statistical analysis was performed by R.4.04 for IOS. ANOVA (one-way analysis of variance) and Tukey test were used to compare differences between samples, when $p < 0.05$, it regarded as significant.

3 Results and Discussion

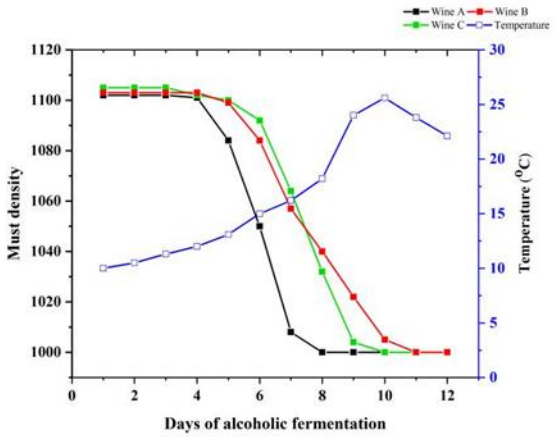

Figure 2. The dynamic of alcoholic fermentation. End of alcoholic fermentation analysis: Wine A with 14.3±0.28, Wine B 13.1±0.14, Wine C 14.8±0.

As Figure 1 shows, both the musts density of Wine A and Wine C (inoculated with commercial yeast) falling faster than Wine B, and the end of alcoholic fermentation analysis showed Wine B with a lower alcohol level. But after MLF, three wines didn't show significant difference, refer to Table 1.

In the general analysis results, the pH among three wines has a significant difference, where wine B is larger than others. What more interesting is that Wine C with a significantly larger value in volatile acidity.

The volatile acidity is considered with an important contribution for aroma, when it was more than 1.2 g/L, the unpleasant vinegar aroma will appear [5]. The volatile acidity of Wine C (0.45 g/L) was still at a normal level.

As the Table 1 shows, the phenolic and anthocyanins have significant difference between bioprotection wine and others. It is widely accepted that they contribute to the quality of wine, especially astringency, bitterness and color. There are lots of factors that affect the color of wine: grape variety, winemaking practice, the strain of yeast and lactic acid bacteria. *Lb.plantarum* and *O.oeni* modify the wine color, astringency and bitterness[6]. But significant differences in taste and color haven't been detected by panelist.

Table 1. The physico-chemical analysis results.

	Wine A	Wine B	Wine C
Alcohol (% v/v)	14.44±0.01a	14.46±0.10a	14.49±0.04a
pH	3.61±0.01b	3.68±0.01a	3.63±0.01b
Total Acidity (g/L)	4.80±0.00a	4.85±0.07a	4.65±0.07a
Volatile Acidity (g/L)	0.21±0.01b	0.21±0.04b	0.45±0.01a
Tartaric Acid (g/L)	1.59±0.15a	1.36±0.02a	1.51±0.06a
Lactic Acid (g/L)	1.52±0.23a	1.43±0.04a	1.65±0.01a
TPI	34.30±0.15c	39.27±1.20b	46.98±0.06a
Total phenolic substance (mg/L)	899.95±132.02b	944.97±44.22b	1312.35±15.34a
Total Anthocyannins (mg/L)	191.65±14.64b	189.6±11.60b	492.00±3.39a
Acetic Aldehyde	4.35±1.20a	4.30±12.97a	9.80±0.14a
Ethyl acetate	60.55±0.35c	70.25±0.35b	89.50±0.71a
Glycerol	5.40±0.14a	5.70±1.27a	6.80±0.14a
2,3-butanediol	164.15±11.95a	172.05±43.06a	223.30±18.10a

All concentrations are listed with average value and standard deviation. Different letters are significantly different between the samples ($p < 0.05$).

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As the secondly important compound of volatile acidity of wine, ethyl acetate affected by the yeast strain selected, this makes different results from wine A and wine B. Another important reason for variation amount of ethyl acetate is aeration, the more aeration protection is, the smaller the value. Previous research pointed out that a concentration of ethyl acetate less than 70mg/L contributes positive aroma [7]. But from sensory analysis results, aroma defect was not detected by panelists.

Table 2. The sensory analysis results.

	Wine A	Wine B	Wine C
Color Intensity	2.48±0.03a	2.50±0.71a	2.81±0.12a
Tone	3.77±0.32a	3.67±0.04a	4.10±0.28a
Smell Intensity	2.15±0.08b	2.54±0.37ab	3.34±0.22a
Smell Fruit	2.19±0.12b	2.16±0.48b	3.76±0.05a
Smell Vegetal	1.57±0.10a	1.76±0.08a	1.87±0.33a
Smell	2.30±0.56a	2.40±0.57a	2.49±0.30a
Structure	2.46±0.37a	2.63±0.24a	2.90±0.01a
Body	2.42±0.31a	2.63±0.51a	2.95±0.06a
Tannin	2.05±0.06a	1.81±0.12a	2.20±0.28a
Balance	3.17±0.66a	2.38±0.03a	2.96±0.33a
Persistence	2.84±0.48a	2.57±0.04a	2.90±0.01a

The sensory analysis evaluations are listed with average value and standard deviation. Different letters are significantly different between the samples ($p < 0.05$).

As another important influence factor to aroma, the phenolic substance also enjoys a significant difference between Wine A and Wine C. From Table 2, the smell intensity difference was detected by panelist, especially the fruity aroma.

Non-*Saccharomyces* enhancing complexity and fruity characters has been provided by many researches. The Impact from *Metschinikowia pulcherrima* as a bioprotection hasn't found on wine quality. But *Torulaspora delbrueckii* is always linked with "fruitness", which has a good consistence with sensory analysis results in this research [8].

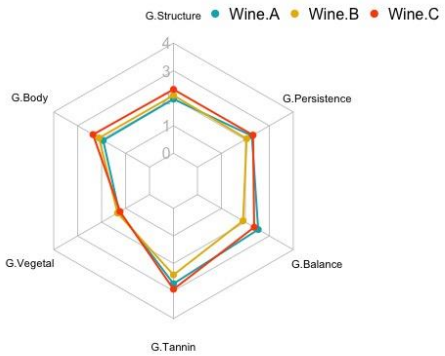

Figure 3. Radar graph of gustative characteristics.

The sensory analysis didn't show significant difference of gustative characteristics, but from the radar graph, a little variation can be observed, especially from the perspective of body and structure.

Table 3. The color study.

	Wine A	Wine B	Wine C
L	75.15±0.21b	73.70±0.57b	80.55±0.49a
a	20.49±0.09a	21.55±0.55a	16.60±0.85b
b	9.55±0.14a	9.62±0.16a	6.10±0.04b
C	22.60±0.03a	23.60±0.44a	17.54±0.60b
H	25.00±0.42a	24.05±0.89a	20.90±0.18b
Color intensity	4.86±0.05a	4.64±0.60a	3.60±0.08a
A420/520	0.96±0.01a	0.98±0.01ab	1.03±0.03b

The color study results are listed with average value and standard deviation. Different letters are significantly different between the samples ($p < 0.05$)

In contrast with sensory analysis, the results of CIELAB and A420/A520 show that bioprotection wine is significantly different from conventional wine. As mentioned previously, MLF modifies wine color, bioprotection wine permits simultaneous MLF and alcoholic fermentation, as a result that even it has an obviously different color from conventional wine is not remarkable. Considering the complexity of microorganism activities, samples of must from different stages of winemaking process were took for microbiology study in future. From what Table 3 shows, wine C is significantly different from A and B on Lightness (L) and Hue angle (H), the H from 0-60 represents red, and H > 60 means yellow. The CIELAB result is the same as sensory analysis that bioprotection wine is of darker red hue. The evaluation from the panelists didn't show significant difference, however, the hue value measured by A420/520 shows that significant difference exists only between Wine A and Wine C.

4 Conclusion

This research is based on a real production scale of vinification, chemical-fizico and sensory analysis that were done with three types of wine. Firstly, in terms of conversion the commercial yeast worked better than wild yeast in the alcoholic fermentation, but after MLF, they get similar alcohol level. From other perspectives, except pH, ethyl acetate and TPI, the qualities of wine made from commercial yeast and wild yeast are almost the same. Secondly, from the comparison between bioprotection wine and conventional wine:

The former has obviously higher concentration of volatile acidity, TPI, phenolic, anthocyanin and ethyl acetate. And it also has more fruit aroma and higher smell intensity.

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