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Influence of Sensory Attributes and Volatile Compounds in Kallmet Wine Produced with Sequential fermentation

MAMICA RUCI[1]*, RENATA KONGOLI[2], MASSIMO IORIZZO[3], BRUNO TRIALA[3], ARBJOLA ABDYL[2], JULIAN KARAULLI[1], FATBARDHA LAMÇE[1], ONEJDA KYÇYK[1]

[1]Faculty of Biotechnology and Food, Food Research Center, Agricultural University of Tirana, Albania

[2] Faculty of Biotechnology and Food, Department of Agro-food Technology, Agricultural University of Tirana, Albania

[3] Department of Agriculture, Environmental and Food Sciences, University of Molise, 86100 Campobasso, Italy

*Corresponding author e-mail:mruci@ubt.edu.al

Abstract

Kallmet (Vitis vinifera L.) is an indigenous red grape variety widely cultivated in northern of Albania, and increasingly recognized for its potential to produce wines with distinctive aromatic and sensory characteristics. Despite its cultural and economic importance, systematic scientific studies on the sensory properties of Kallmet wines remain limited. This study aimed to provide an in-depth evaluation of the influence of sensory attributes and volatile compound in Kallmet red wine produced using sequential fermentation strategies. Specifically, the research focused on sequential fermentation conducted with Metschnikowia pulcherrima (M.pulcherrima) followed by Saccharomyces cerevisiae (S. cerevisiae), in order to assess the impact of non-Saccharomyces yeast involvement on the wine aroma, flavor, and overall quality.

Three experimental trials were carried out in laboratory scale using sequential inoculation. The first trial was inoculated with M.pulcherrima at the early stage of fermentation and after 48 hour was added the S.cerevisiae yeast to finish the alcoholic fermentation. To the second trial the S.cerevisiae was inoculated after 72 hour, and the third trial was inoculated only with S.cerevisiae used as control. At the end of fermentation process, the wines were analyzed for chemical and volatile compounds and a trained sensory panel assessed key attributes including aroma intensity, flavor complexity, balance, body, and overall acceptability.

The results revealed that sequential fermentation with M.pulcherrima and S.cerevisiae resulted in Kallmet wines with a rich and diverse volatile profile, with fruity and floral esters being dominant contributors to their aroma. Sensory analysis indicated high scores for overall quality and highlighted strong correlations between specific volatile compounds and sensory descriptors. These findings provide new insights into the chemical and sensory properties of Kallmet wines, contributing to a better understanding of their uniqueness and potential for market differentiation in the Albanian and international wine sectors.

Keywords: Kallmet red wine, volatile compounds, sensory evaluation, Albania

1. Introduction

Albanian is a Mediterranean country with ideal climate conditions, rich soil, and high levels of sunshine favoring the growth of unique grape varieties. The Kallmet grape (Vitis vinifera L.) is an indigenous Albanian variety that historically has been growing in the regions of Malësi e Madhe and Shkodra. Kallmet has been planted on hilly and sloping soils with sandy structure. Kallmet grapes has recognized for its potential to produce red wines with high-quality and with Geographical Indication (GI) status in Albania (Caso, A, 2022). The sensory attributes of the red wines are shaped by various factors, including grape variety, fermentation techniques, and environmental conditions (Ariola Morina, 2014). In the recent years more attention was paid to the influences of the selected non-Saccharomyces yeast strains in winemaking. These strains can produce secondary metabolites during the fermentation process, which influence on wine aroma and sensory profile. Due to their sensitivity to ethanol concentration and SO_2, these non-Saccharomyces

*Corresponding author: Mamica Ruci; E-mail: mruci@ubt.edu.al
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yeast couldn't drive alone the fermentation process for this reasons they can be involved in sequential fermentation with Saccharomyces yeast(Torres-Díaz et al., 2024) The sequential fermentation process, characterized by the application of diverse yeast strain such as Metchnikowia pulcherrima at different stages of fermentation process, following by Saccharomyces cerevisiae for completed the process. Involving these non-Saccharomyces yeast in the winemaking process can significantly influence on the wine quality parameters and impacting the sensory profiles by increasing the presence of diverse volatile compounds such as esters, terpenes, phenols, and alcohols (Muñoz-Redondo et al., 2021) (Ruci et al., 2025). For this research work, a selected non-saccharomyces yeast strain Metschnikowia pulcherrima (AS3C1) has been employed during early fermentation stage in sequential fermentation by commercial yeast Saccharomyces F15. The Metschnikowia pulcherrima (AS3C1) has been characterized by (Triala et al., 2024) and has been chosen for oenological and enzymatic activity like β-glucosidase, which contribute to the release of varietal aromas related to grape by breaking the bound monoterpenes . This work is the first research using the sequential fermentation strategies for evaluated the effects of this non-Saccharomyces yeast species on the sensory and volatile compounds of red wines obtained by the Kallmet grape variety. By investigating the implications of sequential fermentation on the sensory and volatile profile of Kallmet wine, this research aims to provide insights into the influence of the sensory attributes and volatile compounds, ultimately contributing to the preservation and promotion of this unique Albanian heritage variety.

2. Material and Methods

2.1 Yeast Strains and Growth conditions

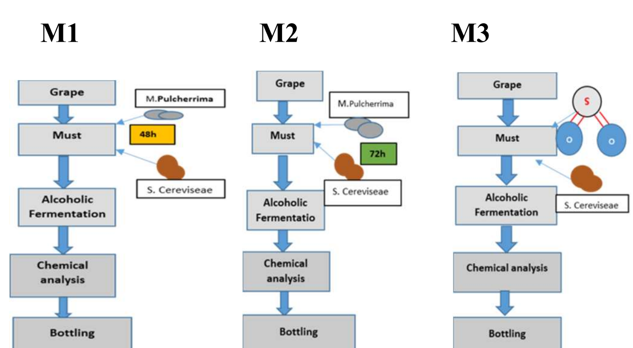
The yeast strain of M. pulcherrima AS3C1 belongs to the culture collection of the Di.A.A.A (Department of Agricultural, Environmental and Food Sciences, University of Molise, Campobasso, Italy) was used. This strain was previously isolated from Italian must grape and characterized from (Triala et al., 2024a). For the fermentation trials the S. cerevisiae commercial strain of S. cerevisiae Zymaflore F15 (LAFFORT Co., Bordeaux, France), was used as reference yeast.

The S. cerevisiae Zymaflore F15 was rehydrated according to the manufacturer's instructions. The strain of M. pulcherrima AS3C1 was cultured in Yeast Pepton Dextrose (YPD) broth at 280C under aerobic condition for 48 hours. After, the cultures were centrifuged at 5000 rpm for 10 minutes at 4 0C, the cell pellets were washed twice with sterile physiological solution (0.9% NaCl) before used, and inoculated in Kallmet grape juice to concentration of 7.0 log CFU/ml.

2.2 Fermentation trials (Winemaking process)

For the laboratory trials were used the Kallmet red grapes (Vitis vinifera cv.), grown in north of Albanian and transported to the laboratory of Research Food Center, Faculty of Biotechnology and Food, Agriculture University of Tirana. The grapes were destemmed and crushed without any addition of chemical preservatives. The must was used for three fermentations trials. Trial (M1) sequential inoculation was performed initially inoculated with M. pulcherrima AS3C1 and after 48 h, with S. cerevisiae F15. Trial (M2) the S. cerevisiae F15 was added after 72 hours, while the trial (M3) was inoculated only with commercial yeast of S. cerevisiae F15. The fermentation process as carried out at temperature 23 °C (± 2 °C). At the end of alcoholic fermentation, skins were pressed, and the wines obtained were subject to chemical analysis. The experiments schemes of fermentation trials with sequential inoculation are present at the figure 1.

Figure 1. The experiments schemes of fermentation trials with sequential inoculation



2.3 Volatile analysis

The determination of major volatile compounds (VOCs) was carried out using a gas chromatography (GC) according to International Organization of Vine and Wine (OIV) (Compendium of International Methods of Wine and Must Analysis, OIV, n.d.). The instrument, (Thermoquest Mod. 8000, Rodano, Milan, Italy), used was equipped with a fused capillary column ZB-Wax (30 m × 0.32 mm i.d., 0.50 µm film thickness, Phenomenex, Torrance, CA, USA) and a flame ionization detector. Briefly, after the addition of an internal standard (Butan-2-ol; 0.1 mg/mL in water), 1 µL of the sample was injected directly in split mode (1:50); injection port at 250 °C.; The oven temperature was increased from 40 °C (held for 5 min) to 240 °C at a rate of 7 °C/min.; Helium was used as the carrier gas at a flow rate of 60 kPa. All analyses were carried out in duplicate and reagents were obtained from Sigma-Aldrich.

2.4 Sensory analysis

The evaluations of sensory attributes of the wines were conducted by a trained panel. The sensory descriptors Results of the analysis of volatile compounds in obtained wines are present in Table 1. For the identified compounds are found data from the literature on the odor descriptors. In our study, were evaluate the concentration of higher alcohols and terpens which are the main contribute to the aroma profile of wine. Higher alcohols are volatile compounds produced from yeasts during the fermentation and values below 300mg/L provide fruity and floral notes, while above this thresholds can give wines pungent and unpleasant aromas (Carpena et al., 2020). The sequential fermentation with M pulcherrima has been show to an increase in the concentration of the higher alcohols (Kręgiel et al., 2022) (Seguinot et al., 2020). Our results are show that the concentration of the phenylethyl alcohol and isobutanol were higher to those Trial carried out with sequential inoculation of M pulcherrima and S. cerevisiae (Trial M1 and Trial M2). The highest concentration was in Trial M2, followed by Trial M1 and Trial M3. Phenylethyl alcohol,derived from phenylalanine, is associated with rose, floral

were according the ONAV methodology (Gomis-Bellmunt et al., 2024). The panelist were evaluated the following attributes: overall judgment, spiciness, herbal notes, acidity, astringency, softness, sweet cherry, red fruits, retro-nasal spiciness, retro-nasal red fruits, and color by intensity (0 = absent to 9 = very intense).

2.5 Statistical Analysis

The SPSS software was used for statistical analysis of the data obtained from the experiments. The data are present as mean ± standard deviation (SD) and analyzed using ANOVA. The data represent three replicates for each trial fermentation (n=3) and were considered significant if p-values ≤ 0.05. The significant differences were determined using Duncan test.

3. Results and Discussion

3.1 Volatile Aroma Compounds

aroma enhancing the overall sensory quality of the red wines. Isobutanol is a higher alcohol produced during fermentation by decarboxylation of amino acids such as valine. It has been reported that the concentration of Isobutanol in sequential inoculation with M. pulcherrima increased significantly compare to fermentation with S. cerevisiae alone (Seguinot et al., 2020). Also, other studies showed that the co-inoculation with M pulcherrima and S. cerevisiae resulted in an increased of concentration of isoamyl alcohol which have a strong sensory impact due to its low perception thresholds (30 µg/L) (Arias-Pérez et al., 2021). Enzymatic activities of M pulcherrima, particulary β-glucosidase and α-arabinofuranosidase, play a central role in transforming bound terpenes into their volatile forms that enhance the aroma of wine (Triala et al., 2024b). In our results, the wines with sequential inoculation Trials M1 and M2 shown higher concentration of monoterpenes like linalool, geraniol and nerol compared to control Trial M3.

Higher alcohols (mg/L)	*M1*	*M2*	*M3*	*Odor descriptor*

2,3-Butanediol	118.4 ±0.06a	119.6 ±0.06a	120.9 ±0.06a	Butter, creamy
Phenylethyl alcohol	82.5 ±0.06b	93.9 ±1.4a	38.3 ±1.6c	Honey, spice, rose, lilac, floral
Isobutanol	134.5 ±0.2b	169.4 ±0.5a	97.3 ±0.8c	Balsamic, solvent whiskey
Isoamyl alcohol	104.5 ±0.5b	119.7 ±0.06a	60.0 ±0.6c	Whiskey, malt, burnt
Methionol	4.2 ±0.06 a	3.9 ±0.06 a	2.9 ±0.06 a	Alcohol, Pungent
Hexanol	4.4 ±0.2 a	5.1 ±0.2 a	4.2 ±0.4 a	Ethereal, fruity, alcoholic, sweet, herbaceous
Butanol	66.2 ±0.4 a	66.6 ±0.5 a	65.4 ±0.06 a	Medicine, fruit
Pentanol	24.7 ±0.06 a	24.4 ±0.06 a	23.5 ±0.06 a	Balsamic
Octanol	0.49 ±0.02 a	0.51 ±0.02 a	0.48 ±0.01 a	Chemical, metal, bunt
Terpenes (µg/L)	M1	M2	M3	Odor descriptor
Linalool	94.0 ±0.2b	116.0 ±0.2a	15.1 ±0.06c	Citrus, floral, woody, green, blueberry
Geraniol	83.6 ±0.6b	90.9 ±0.2a	15.9 ±0.2c	Floral, rose, fruity, lemongrass, citrus
Nerol	94.9 ±0.1b	105.5 ±0.3a	15.1 ±0.2c	Rose-like aromas

Table 1. Data of the Volatile compounds in wines obtained from sequential inoculation strategies:

Trial M1 (M. pulcherrima AS3C1 + S. cerevisiae F15 after 48 h), Trial M2 (M. pulcherrima AS3C1 + S. cerevisiae F15 after 72 h), and Trial M3 (S. cerevisiae F15). Values above the perception threshold levels are highlighted in bold. Different letters (a-b) within a row indicate significant differences ($p < 0.05$).

3.2 Sensory evaluation of wines

The sensory analyses of wines are shown graphically in Figure 2. The wines are assessed for the sensory attributes like color, acidity, astringency, softness, red fruits, sweet cherry and overall judgment. The wine obtained in Trial M2 shown the highest overall scores in a most of the evaluated attributes. In the color of the wines, anthocyanins are the primary contributors but during the maturation and aging, these compounds lead to the formation of more complex pigments that resulting in color changes. The perceived color intensity and the acidity from the panelist were similar across all Trials wines with no significantly differences. Trial M2 was received the highest ratings for red fruit aroma and astringency compare to Trial M1 and M3. The judgments attributed to the perception of sweet cherry and herbaceous notes did not show significant differences between trials.In our results of sensory evaluation, for the softness, higher scores were obtained in the wines carried out with sequential inoculation (M1 and M2) than in the wine only with S. cerevisiae (M3). This difference may be due to the amount of glycerol produced by M pulcherrima. Trial

M2 also obtained the highest scores for Ro-red fruits and Ro-spicy, followed by Trial M1. While the Trial M3 scored lowest in these attributes.

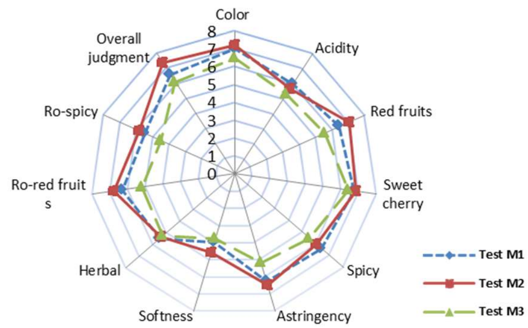


Figure 2. Evaluation of sensory profiles of wines: Trial M1 (M. pulcherrima AS3C1 + S. cerevisiae F15 after 48 h), Trial M2 (M. pulcherrima AS3C1 + S. cerevisiae F15 after 72 h), and M3 (S. cerevisiae F15)

4. Conclusions

Metschnikowia pulcherrima is primarily proposed as a biocontrol agent in winemaking due to the antimicrobial activity against some unwanted bacteria and yeast present in the grape must. In particular, the M. pulcherrima AS3C1 strain exhibits specific enzymatic activities, such as, β-glucosidase, which could enhance the release of grape-derived volatile compounds and improve the aromatic profile of the final wine. This study demonstrates that the sequential inoculation of M. pulcherrima AS3C1 strain with S.cerevisiae provide a sustainable and innovative approach to enhance the aromatic complexity and authenticity of wines produced from indigenous varieties such as Kallmet. Furthermore, it aligns with the growing global trend toward environmental friendly and low-intervention winemaking practices. Future work should further investigate the phenolic fraction and profiling of more volatile compounds in order to understand in more detail the interaction of metabolic pathways during fermentation process using M. pulcherrima in sequential inoculation with S.cerevisiae.

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