Foods 2021

MDPI

Proceedings

Sensory profile of cv. Savvatiano (*Vitis vinifera* L.) wines fermented with the *Metschnikowia pulcherrima* and *Saccharomyces cerevisiae* yeasts in individual and mixed fermentation †

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† Presented at The 2nd International Electronic Conference on Foods.

Abstract: The objective of this study was to explore the use of non-*Saccharomyces* yeasts, individually or in mixed culture with *Saccharomyces cerevisiae* for the vinification of must originating from the native white wine grape cultivar Savvatiano *(Vitis vinifera* L.). Savvatiano is the most planted grape cultivar in Greece, cultivated predominantly in Central Greece. Grapes were harvested during October 2020, were pressed, and the must after cold settlement was inoculated with a. *Saccharomyces cerevisiae*, b. *Metschnikowia pulcherrima* and c. mixed culture, in sequential inoculation (*M. pulcherrima*, followed by *S. cerevisiae* after 7 days). The progress of fermentations was monitored and the finished wines were analyzed for main wine parameters, as well as sensory attributes by a panel of experts. The results of this study provide useful data in order to further explore the effect of mixed cultures use on fermentation of musts originating from native grape varieties with low aromatic intensity.

Keywords: wine; yeasts; mixed-fermentation; *Saccharomyces cerevisiae*; non-*Saccharomyces; Metschnikowia pulcherrima*; sensory evaluation; chemical analysis

Citation: Bouloumpasi, E.; Petraina, A.; Karampatea, A. Sensory profile of cv. Savvatiano (*Vitis vinifera* L.) wines fermented with the *Metschnikowia pulcherrima* and *Saccharomyces cerevisiae* yeasts in individual and mixed fermentation. **2021**, *68*, x. https://doi.org/10.3390/xxxxx

Published: 15 October 2021

1. Introduction

The potential enological use of non-*Saccharomyces* yeasts has gained interest during recent years, due to their natural presence in musts, to their ability to produce secondary compounds impacting the sensory characteristics of wines, as well as to their possible use for production of low alcohol wines [1-3]. The role of various non-*Saccharomyces* yeasts in natural must fermentation has been investigated in order to assess the effect of using specific strains individually or in combinations in wine aroma (quality and complexity), as well as in alcohol content [4-5]. It is known that alcoholic fermentation of must is initiated by apiculate yeasts, followed by *S. cerevisiae*, which eventually replaces them, carries on the utilization of sugars and finishes the fermentation [6]. *Metschnikowia* is one of the genera isolated along with others i.e., *Torulaspora, Candida*, at various stages of alcoholic fermentation [7].

Vitis vinifera L. cv. Savvatiano is the most planted grape cultivar in Greece, cultivated predominantly in Central Greece, and consists the main grape used for the production of flavored wine Retsina. It produces wines with a low aromatic character but well balanced in terms of structure and taste when cultivated in high altitude [8].

The main purpose of this study was to explore the use of non-*Saccharomyces* yeast species *Metschnikowia pulcherrima*, individually or in mixed culture with *Saccharomyces*

cerevisiae for the vinification of must originating from the native white grape cultivar Savvatiano, which is characterized by low aromatic intensity.

2. Materials and Methods

2.1. Fermentations

Grapes of *Vitis vinifera* cv. Savvatiano were harvested during October 2020, in Askri village, Viotia area, Central Greece (latitude, 38° 31', longitude 23° 11', altitude 382m). The microclimate of the area ensures proper maturation at low temperatures. The soil composition is sandy and loamy, while the vineyard is planted with an average slope of 6% and presents good drainage. Healthy ripe grapes were collected at the industrial maturity (sugars content 229 g/L, pH 3.35 and total acidity 4.8 g/L tartaric acid) were destemmed, and pressed. The freshly extracted must was homogenized, sulfur dioxide 60 ppm, pectolytic enzymes 4 gr/hl and PVPP 100 mg/L were added, followed by cold settlement for 24 h at 4°C. Clear must (100 NTU) was transferred to glass containers (5 L each) for batch fermentation. All fermentations were conducted in triplicate at controlled temperature (14 °C), with commercial yeast strains *Saccharomyces cerevisiae* (Excellence® FTH) (Lamothe – Abiet, France), and *Metschnikowia pulcherrima* (Excellence® B-Nature®) (Lamothe – Abiet, France). Each container was inoculated with a starter culture as follows: W1: *Saccharomyces cerevisiae,* 1g/hl, W2: *Metschnikowia pulcherrima,* 1g/hl, and W3: *M. pulcherrima*, 1g/hl, followed by *S. cerevisiae,* 1 g/hl, after 7 days (mixed culture, in sequential inoculation) [9]. The second day was added in each container 0.3 g/L tartaric acid and 0.2 g/L nutrients Vitaferment® (Lamothe – Abiet, France) containing Ammonium sulphate and thiamine hydrochloride.

After the fermentation was concluded (25 days from inoculation, when the ethanol content remained constant) the wines were transferred, sulfur dioxide was added and stored in cellar conditions at low temperature (5 °C) for spontaneous decantation. After this natural stabilization process, all wines were bottled

2.2. Analyses

The progress of fermentations was monitored and the finished wines were analyzed for main wine parameters, as well as sensory attributes by a panel of experts.

Classical enological parameters: All wines were subjected to analysis occurred along two months. Classical enological parameters, i.e. ethanol content (% vol), residual sugars (g/L), pH value, total acidity (tartaric acid g/L), volatile acidity (acetic acid g/L), free SO_2 (mg/L), total SO_2 (mg/L), were determined according to the official methods of OIV [10].

Sensory analysis: The obtained wines were assessed for the attributes: appearance (limpidity and color), odor, fruity aroma, aroma quality, fruity flavor, astringency, taste, aftertaste and overall quality, through an expert tasting panel of 10 tasters, in tasting room kept at 20 °C. Wine samples were codified and served in certified tasting glasses of 200 mL filled with 30 mL of wine at 18 °C. All the samples were tested in one session and the nine wine attributes were evaluated by each taster according the following scale from 1 to 9: desirable (7–9), acceptable (4–6) and undesirable (1–3). The final punctuation was obtained as the mean of the 10 evaluations with their respective standard deviation. Each taster also provided an overall impression of the wines produced, taking into account olfactory and taste features, including any defects.

Statistical data processing was performed using IBM SPSS Statistics, v.25 statistical software (International Business Machines - IBM Corporation). Significant differences among results were determined using one-way ANOVA and Least Significant differences (LSD) test. Significance level was set at $p < 0.05$.

3. Results and discussion

3.1. Fermentations and chemical analyses

Three treatments were carried out to evaluate wine flavour profile in Savvatiano wines inoculated with either *S. cerevisiae (W1)*, *M. pulcherrima (W2)*, or *M. pulcherrima*/*S.*

cerevisiae (W3). Savvatiano grape cultivar was selected because of its lower aromatic intensity, which would allow the perception of fermentation aroma.

All treatments resulted at the same fermentation yield. The kinetics of fermentations are presented in Fig.1.

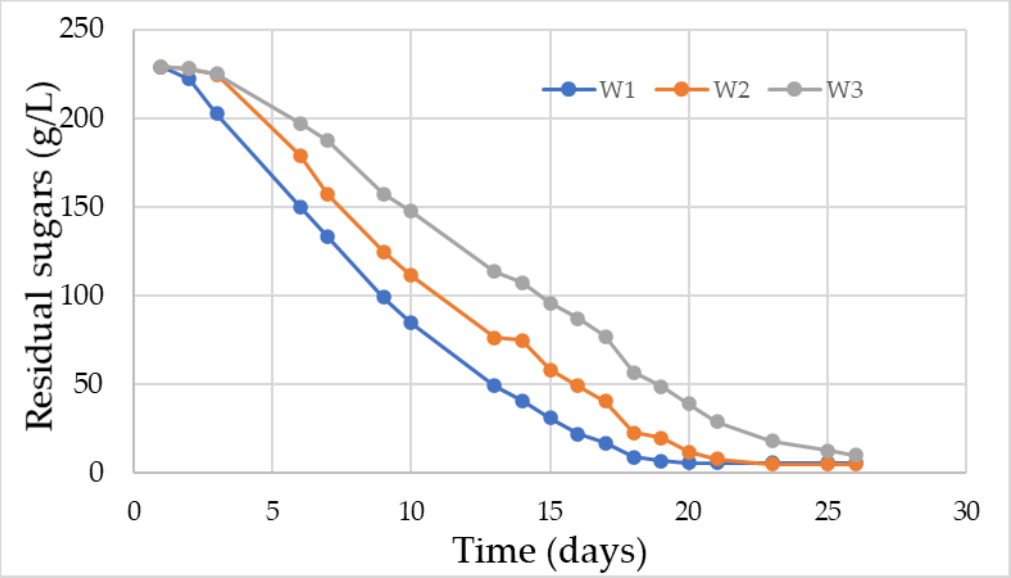

Figure 1. Sugar utilization profiles during fermentation. W1: Single-culture fermentation with *S. cerevisiae*; W2: single-culture fermentation with *M. pulcherrima*; W3: sequential fermentations with *M. pulcherrima* and *S. cerevisiae* (the latter inoculated after 7 days).

Fermentations inoculated with *M. pulcherrima* and *M. pulcherrima*/*S. cerevisiae* showed similar sugar consumption kinetics and completed alcoholic fermentation in 21 and 25 days, respectively (Fig. 1). Musts W2 and W3, which were inoculated with *M. pulherrima* showed a delay in the beginning of fermentation, which is accordance to Contreras et al.[11] that reported previously that M. pulcherrima's growth was inhibited by other yeasts naturally present in the must, such as *Hanseniaspora uvarum*, *Torulaspora delbrueckii* and/or *Pichia kluyveri*.

Must inoculated with *S. cerevisiae* showed faster sugar utilization kinetics and completed fermentation in 19 days. Fermentations were conducted at 14 ◦C. Low temperature fermentations, according to Torija et al. [12] start more slowly as there is a delay in reaching the maximal population but consume faster all the sugars. Fermentation inoculated sequentially with *M. pulcherrima*/*S. cerevisiae* exhibited the slowest sugar utilization kinetics, presenting a delay at the beginning and further delaying after inoculation with *S. cerevisiae* (Fig. 1).

The principal enological characteristics of the fermentation trials carried out in 5L containers with free *S. cerevisiae*, and/or *M. pulcherrima* cells are summarized in Table 1.

Treatments W1 and W2 resulted in similar ethanol and residual sugars concentrations, with *M. pulcherrima* (W2) having a slower evolution of fermentation by two days than *S. cerevisiae* (W1) but better utilization of sugars and higher alcohol content by 0.7% v/v. Wine W3 where *M. pulcherrima* started the fermentation, followed by inoculation with *S. cerevisiae* after 7 days had 2.2% v/v lower ethanol concentration (Table 1). Varela et al. [2,3] used *M. pulcherrima* in order to produce reduced-alcohol wines at laboratory scale and produced wine with 1% lower alcohol content [3], while Hranilovic et al. [9] produced wines with 0.6-1.2% (v/v) lower ethanol content by testing *M. pulcherrima* strains in sequential cultures with *S. cerevisiae*.

Although significantly different, there were minimal residual sugar concentration differences between treatments W1 and W2, where W3 had twice higher residual sugars. Compared to S. cerevisiae wine (W1), W2 had lower pH value and significantly lower

volatile acidity, whereas W1 and W3 exhibited similar values in both parameters. Total acidity was not significantly different among treatments.

Table 1. Enological parameters[1] of the Savvatiano wines fermented with *S. cerevisiae* (W1), with *M. pulcherrima* (W2), sequential fermentations with *M. pulcherrima* and *S. cerevisiae* (W3).

Wines	Ethanol (% vol)	Residual sugars (g/L)	pH	Total Acidity (tartaric acid g/L)	Volatile Acidity (acetic acid g/L)	SO_2_free (mg/L)	SO_2_total (mg/L)
W1	13.6±0.1^a	5.7±0.2^a	3.32±0.0	4.9±0.3^a	0.29±0.3^a	17±2^a	89±5^a
W2	13.7±0.1^a	5.1±0.2^b	3.26±0.1	5.0±0.2^a	0.22±0.2^b	8±2^b	48±5^b
W3	13.3±0.1^b	10.1±0.1^c	3.31±0.1	4.6±0.2^a	0.33±0.3^a	9±1^b	69±2^c

[1] Values are means ± standard deviation of three independent replicates.
Shared superscript letters (a, b, c) in the same column indicate no significant difference (LSD test, p= 0.05).

3.2. Sensory analysis

W2 found to be possess undesirable characteristics, whereas W1 and W3 exhibited higher scores for the attributes tested, and were found at least acceptable, with W3 being the most positively evaluated for all attributes by the testers (Fig. 2).

In sensory analysis (Fig. 2), overall perception was better for the wine produced by sequential fermentation (W3), than by single-culture fermentation with *S. cerevisiae* (W1) or *M. pulcherrima* (W2). All descriptors evaluated by the tasters were rated higher for W3, followed by W1. The aromatic quality and fruity aroma were also better in the case of sequential fermentation (W3), followed by W1. Hranilovic et al. [9] also obtained wines with increased acetate esters content, which are responsible for fruity aromas, from sequential fermentations of *M. pulcherrima* strains with *S. cerevisiae*. With regard to taste parameters, including taste, fruity flavor, astringency, as well as for aftertaste wine W3 had higher scores than W1 and W2. With regard to appearance, all wines exhibited similar scores.

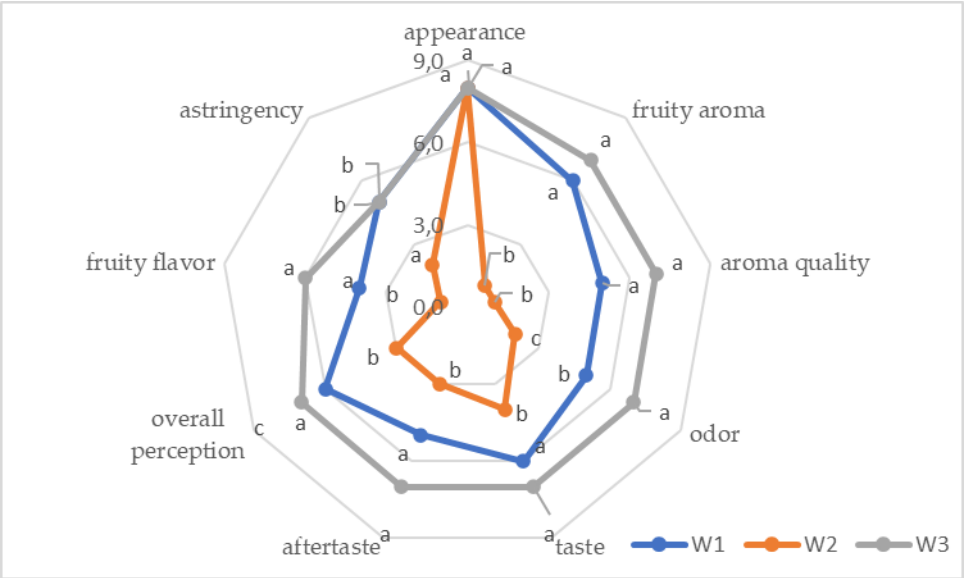

Figure 2. Spider web graph of taste panel results. Different letters in the same series indicate significant differences between means (p < 0.05). Scale used desirable (7–9), acceptable (4–6) and undesirable (1–3).

Moreover, significantly different sensory profiles were found among treatments (Table 2). This is in compliance with other researchers that noted the effect of non-*Saccharomyces* yeasts on wine sensory characteristics, for several grape varieties and with various non-*Saccharomyces* species [13]. Wines fermented with *S. cerevisiae* were characterized by low aromatic intensity and low flavor on the palate, while wines fermented with *M. pulcherrima* exhibited low scores in general acceptance. However, sequential inoculation with *M. pulcherrima*, followed by *S. cereviasiae* produced wine with rich mouthfeel, characterized by aromatic complexity, citrusy aromas, lasting palate aromas, which exhibited high scores in general acceptance.

Table 2. Sensory descriptive analysis.

Wines	Color	Aroma	Taste
W1	Light color, with green highlights	Aromas of pear, low aromatic intensity, slightly heavy aromas	Crisp in the mouth, soft, moderate structure, low flavor, balanced acidity/sugar ratio
W2	Light color, with green highlights	Lack of varietal aromas, closed nose, moldy, wet paper, low genuineness	Short aftertaste, lack of flavor, low acidity, slightly bitter and dry
W3	Bright yellow color	Quite complex and fresh nose with medium intensity	Rich mouthfeel (maybe because of reducing sugars), aromatic complexity, sour orange aromas, long lasting persistent flavor

4. Conclusions

This work shows that sequential inoculation with *M. pulcherrima* and *S. cerevisiae* produced wines with increased aroma complexity, high scores for desirable sensory attributes, and low scores for negative descriptors. In contrast, wines produced with single culture *M. pulcherrima* were characterized by unusual and negative sensory characteristics. It was, therefore, demonstrated the successful application of sequential inoculation with *M. pulcherrima* and *S. cerevisiae* for the production of pilot-scale Savvatiano wines with good quality sensory profile, which could help improve the quality of some wine types.

Funding: This research received no external funding.

Acknowledgments: Authors would like to thank The Muses Estate winery (Askri, Viotia, Greece) for the provision of grapes and materials used for experiments and also the sensory panelists.

Conflicts of Interest: The authors declare no conflict of interest.

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