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Research Article

Some sensory and chemical properties of mulberry wine fermented with mixed yeast cultures

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Abstract

This study reports the use of mixed yeast cultures in mulberry juice fermentation. The trials were conducted in the winery of Agricultural Technology Research Institute, Rajamangala University of Technology Lanna, Thailand. The purpose was to examine the quality of mulberry wines fermented with mixed yeast cultures. Four inoculation protocols of *Saccharomyces cerevisiae*V1, *S. cerevisiae*E1, coinoculation of *Torulaspora delbruekii* and *S. cerevisiae*V1 and sequential inoculation of *S. cerevisiae*V1and *S. cerevisiae*G1 were applied in mulberry winemaking. The respective concentrations of ethanol, anthocyanins and phenolic compounds in the products ranged from 13.6 to 14.0% (v/v), 208 to 299 mg/L and 2099 to 2269 mg/L. The 20 point evaluation scale of The American Wine Society was applied in sensory analysis of mulberry wines using 15 trained panelists. The results indicated that different inoculation protocols influenced the mulberry wine sensory quality only on the overall impression of character. The study demonstrated the applicability of mixed yeast cultures to mulberry winemaking. This is an example of the benefits of using multistarter cultures in fruit winemaking processes for the fruit wine industry in Thailand.

Keywords: fruit wine, non-*Saccharomyces*, sensory evaluation, *Morus* sp., Thailand.

Introduction

Extensive studies have been conducted on the production of wine, particularly grape wine, using mixed yeast culture fermentation [1, 2, 3, 4]. There is increasing evidence that metabolites of non-*Saccharomyces* yeasts contribute positively to the quality of complex wines. On the other hand, it should be noted that metabolites of certain non-*Saccharomyces* yeasts might be detrimental. The use of a mixed yeast culture could have some unexpected effects on the sensory characteristics of wines, therefore its fermentation should be approached with care. Using oligo cultures in winemaking, either as *Saccharomyces* species [5, 6, 7] or as a combination with *Saccharomyces* and non-*Saccharomyces* strains [8, 9], increases the complexity of the aroma when compared to a single strain. It is therefore understandable that aroma compounds produced by non-*Saccharomyces* yeasts have been studied for many strains [10, 11]. No study has examined the impact of mixed culture on mulberry wine. The aim of this work was to evaluate the quality of mulberry wine fermented with mixed yeast cultures.

Methods

Yeast strains and media

Yeast strainswere derived from the collection of the Section of Microbiology and Biochemistry, Geisenheim Research Center, Germany. Yeasts were grown at 25^{0}C on YEPD medium; glucose, 20 g/l; yeast extract, 10 g/l; peptone, 20 g/l; and agar, 15 g/l.

Mulberry juice

Mulberry juice was obtained from mulberry harvested in Lampang (2011). The mulberry juice was adjusted to get an initial sugar of 20^{0}Brix and acidity was 0.3% as measured by hand refractometer and titration method, respectively.

Fermentation conditions

Duplicate experiments were carried out in 750 ml sterile bottles (650 ml mulberry juice per bottle). The juice was inoculated with 24 h pre-culture to obtain an initial level of 1x10^6 cells/ml. Inoculation protocols shows in Table 1. The fermentation trials were performed at 20^{0}C and terminated after 14 days.

Table 1. Inoculation protocols for mulberry wine fermentation.

Treatment	Code	1st inoculation	2nd inoculation
1	V1	*S. cerevisiae*V1	-
2	E1	*S. cerevisiae*E1	-
3	T+V1	*T. delbruekii* and *S. cerevisiae*V1	-
4	V1/G1	*S. cerevisiae*V1	*S. cerevisiae*G1

Chemical analyses

Total soluble solid was determined byrefractometer (Atago Model N-1a, Japan). pH was measured by a pH meter (Model C831, Consourt, Belgium). Total acidity was determined by titrimetric method. Ethanol concentration was measured by ebulliometer. Anthocyanin content was investigated by spectrophotometric method (Australian Wine Research Institute, 2012). Modified method of Zoecklein, *et al* [12] was used to determine phenolic content as gallic acid.

Sensory analysis

A group of 15 trained panelists (10female, 5male) took part in this study. All the panelists were experienced in fruit wine sensory program. Selection of panelists was on the basis of interest and availability.

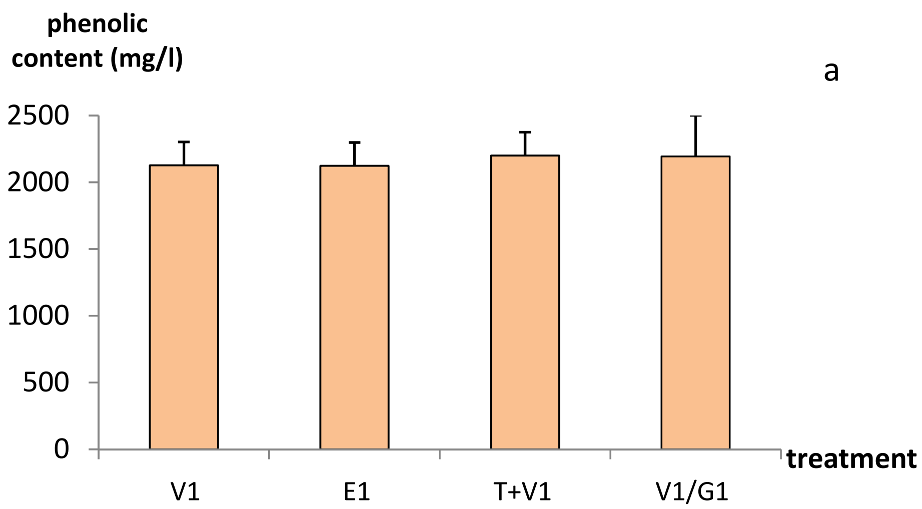
Two sets of the mulberry wine samples were assessed during the study. The tests were conducted from 10:00 am to 11:00 am. In each set, 4 samples were evaluated. The effect of order of presentation, the samples were served as per the method of Meilgaard *et al*. [13]. The panelists were presented with samples of 100 ml, at room temperature (25-30^{0}C). The wine glasses were randomly coded with three-digit numbers. They were asked to rate the appearance, aroma, taste, aftertaste and overall acceptance using the 20 point evaluation scale of The American Wine Society. The panelists were required to wash their mouths thoroughly with water between samples.

Statistical analysis

A completely randomized design and a randomized complete block design were used for this study. Analysis of variance (ANOVA) was used to compare mean differences of the samples. Mean separation was carried out using Duncan's Multiple Range Test (DMRT) for objectively measured data. Statistical significance was assigned as $p < 0.05$.

Results and Discussion

Four inoculation protocols of *Saccharomyces cerevisiae*V1, *S. cerevisiae*E1, co-inoculation of *Torulaspora delbruekii* and *S. cerevisiae*V1 and sequential inoculation of *S. cerevisiae*V1 and *S. cerevisiae*G1 were applied in mulberry winemaking. Ethanol content of the finished wine ranged from 13.6 to 14.0% (v/v). Investigation of concentrations of phenolic compounds and anthocyanins in the mulberry wines ranged from 13.6 to 14.0% (v/v), 208 to 299 mg/L and 2099 to 2269 mg/L, respectively (Figure 1). Both concentrations of phenolic compounds and anthocyanins in mulberry wines fermented with different inoculation protocols were not significantly different ($p>0.05$). Theresults indicated that the concentrations of phenolic compounds and anthocyanins were not influenced by the four inoculation protocols applied in this study.



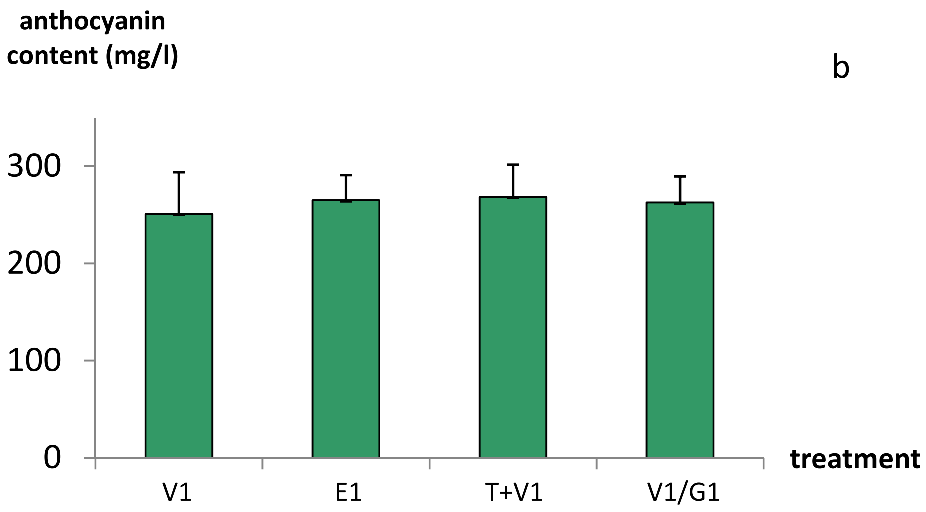


Figure 1. Phenolic (a) and anthocyanin (b) contents in mulberry winesfermented with different yeast inoculation protocols.

The 20 point evaluation scale of The American Wine Society was applied in sensory analysis of wines. Sensory scores of appearance, aroma, taste and aftertaste of mulberry wines were not significantly different (p>0.05). The results indicated that different inoculation protocols influenced the mulberry wine sensory quality on the character of overall impression (p<0.05). Variability of metabolite production of yeasts in mixed cultures fermentation could affect this organoleptic quality of the mulberry wines [14, 15, 16]. The study demonstrated the applicability of mixed yeast cultures to mulberry winemaking. This is an example of the benefits of using multi-starter cultures in fruit winemaking processes for the fruit wine industry in Thailand.

Table 2. Mean scores for sensory attributes of mulberry wines fermented with different yeast inoculation protocols.

Treatment	Appearance ^{ns}	Aroma ^{ns}	Taste ^{ns}	Aftertaste ^{ns}	Overall *
V1	2.47±0.54	4.09±0.95	4.02±0.94	2.17±0.83	1.26±0.62^{a}
E1	2.34±0.54	3.70±1.16	3.61±0.92	1.91±0.64	0.99±0.55^{b}
T+V1	2.35±0.53	3.82±1.10	3.82±0.90	2.08±0.55	1.24±0.41^{a}
V1/G1	2.43±0.55	3.94±0.91	4.01±0.97	2.12±0.69	1.31±0.49^{a}

Standard deviations are given in brackets.
Values followed by common letters within column are not significantly different (P > 0.05).

Conclusion

Application of mixed yeast cultures affected organoleptic properties only in overall appreciation of mulberry wines. Further studies on the practical use of mixed cultures are required to obtain a more fundamental picture of how and why this technology can be applied to improve and develop the sensory quality of fruit wine in general.

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