The Ecology of Red Wine Fermentation

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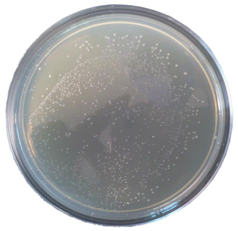
Introduction

The general trend of modern winemaking currently tends to favor higher alcohol levels and hence the requirement of higher must sugar concentrations (1). High sugar concentrations can lead to increased levels of residual L-malic acid and non-reducing sugars which may serve as substrates for spoilage microbes (2). The sugar concentration in grape juice selectively influences yeast growth during alcoholic fermentation (3). Low concentrations of sugars may support the growth of lactic acid bacteria during malolactic fermentation. However, the impact of sugar concentration on the ecology of wine fermentations has not been examined in any scientific depth. In this study, microbe population changes and morphological differences were studied at different fermentation stages in Cabernet Sauvignon wines with must sugar levels of 20, 24, and 28 Brix. In addition, chemical components were measured to analyze the effect of sugar concentration during wine fermentation.

Materials and Methods

Cabernet Sauvignon grapes from Cold Creek Vineyard, Mattawa, WA, Columbia Valley AVA were harvested at 20 Brix. A 20 Brix must served as control, and two 20 Brix musts were adjusted to 24 and 28 Brix with the addition of sugar. Fermentations were sampled at 7,10, 25, and 40 days. Sulfur dioxide was added (50mg/L) during filling of the fermenters. Fermaid K and Diammonium phosphate were added to bring yeast assimilable nitrogen (YAN) to 225mg/L. Yeast (Lalvin EC-1118, Lallemand, Montreal, Canada) were added at a rate of 250mg/L 4 hours after crush. Malolactic bacteria (Lalvin VP41, Lallemand, Montreal, Canada) were added at a rate of 10mg/L 48 hours after yeast addition. All fermentations were carried out in triplicate in 200 L temperature controlled fermenters (28 ± 2 °C) with automatic pump over (6 times/daily). Sugar consumption was measured with an automated Brix reader (Cypress Semiconductor, San Jose, CA, USA) at the research and teaching winery in the Ste. Michelle Wine Estates WSU Wine Science Center, Richland, WA. Wine samples were measured enzymatically with the Y15 Analyzer by BioSystems (Admeo, CA). Yeast samples were serially diluted in YPD media and 100 µl samples were plated onto YPD agar plates at dilutions of: 1:100 and 1:1,000. Plates were incubated at 30°C for 3-5 days. Bacteria samples were serially diluted 1:10, 1:100 in MRS media and 100 µl samples were plated onto MRS agar (pH=5.6) plates containing 100 mg/l cycloheximide. Plates were incubated at 30°C for 10 days. Colony forming units (CFU) were counted for each Brix sample.

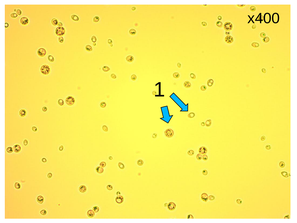
Bacteria and Yeast Colony Morphology



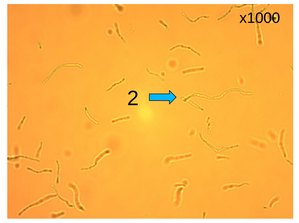
Bacteria agar plate with 4.24E+05 CFU, from 40 day 20 Brix sample. Bacteria colonies appeared small (<0.5 mm) to medium (0.5-1mm), circular to irregularly shaped, flat, and transparent, with a shiny surface.

Yeast agar plate with 8.08E+06 CFU, from 7 day 28 Brix sample. Most yeast colonies appeared large (1-2.0 mm), circular, and convex, with a opaque dull surface. Small (<0.5mm) and medium sized colonies were also present.

Bright-Field Microscope Cell Morphology



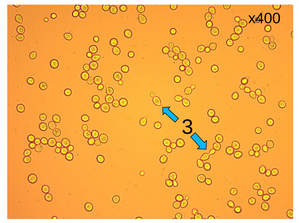
1. Round and ovoid yeast cells. (7 day 20 Brix sample)



2. Bacteria in a streptococci arrangement. (40 day 20 Brix sample)

A master plate by streaking colonies from the direct plating.

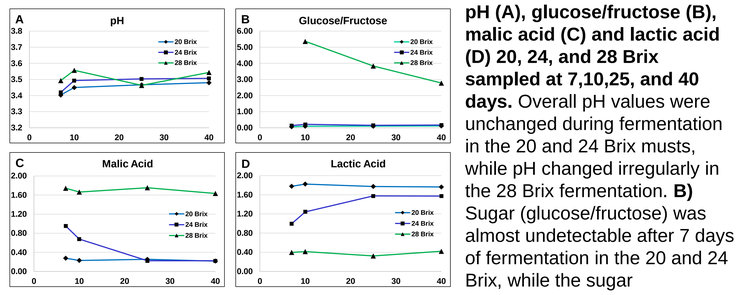
7 day 28 Brix sample



3. Budding yeast cells. (Isolated yeasts from master plate.)

Results

Chemical Characteristics of Fermented Grape Must.



pH (A), glucose/fructose (B), malic acid (C) and lactic acid (D) 20, 24, and 28 Brix sampled at 7,10,25, and 40 days. Overall pH values were unchanged during fermentation in the 20 and 24 Brix musts, while pH changed irregularly in the 28 Brix fermentation. **B)** Sugar (glucose/fructose) was almost undetectable after 7 days of fermentation in the 20 and 24 Brix, while the sugar concentration is still high after 40 days of fermentation. **C)** Malic acid concentrations were low in 20 Brix, high in 28 Brix, and intermediate in 24 Brix musts for the 7 day measurement; the malic acid concentrations were unchanged after 7 days in 20 Brix and 28 Brix fermentation, while a decrease was recorded in Brix 24 fermentation. **D)** The lactic acid concentrations were high in 20 Brix, low in 28 Brix, and intermediate in 24 Brix musts for the 7 day measurement; the lactic acid concentrations were similar after 7 days in 20 and 28 Brix fermentation, while it increased in the 24 Brix must.

Microbe Populations as Related to Sugar Concentrations

CFU/ml	20 Brix		24 Brix		28 Brix	
Days	Yeast	Bacteria	Yeast	Bacteria	Yeast	Bacteria
7	1.71E+06	1.58E+04	7.34E+06	4.30E+03	8.08E+06	1.53E+03
10	2.81E+05	1.99E+05	5.70E+05	7.33E+02	7.26E+05	3.60E+03
25	2.22E+03	1.60E+05		1.06E+04	6.22E+04	1.67E+02
40		2.09E+05		1.74E+04		2.67E+02

Yeast activity was still present in the 25 day 28 Brix sample at 6.22E+04 CFU, indicating a less vigorous fermentation.

Bacteria activity for the 28 Brix sample was the lowest of the 3 sugar concentrations, with the highest recorded CFU measurement of 3.6E+03.

Summary and Conclusion

In this study, the highest population densities of bacteria were observed with low concentrations of malic acid and high concentrations of lactic acid in the 20 Brix must, while reverse results were detected in the 28 Brix must. These results indicate that high populations of lactic acid bacteria stimulated malolactic fermentation during the early stages of fermentation (day 7) in low sugar grape must (20 Brix). In 24 Brix grape must, the MLF ended after 25 days of fermentation. High sugar grape must resulted in almost no MLF, which might be related to low populations of lactic acid bacteria. This research suggests that a lower sugar concentration may favor lactic acid bacteria growth during wine fermentation by resulting in a quicker and more complete MLF. Undoubtedly, the alcohol concentration of the wine also affects wine microbe growth since many lactic acid bacteria and non-*Saccharomyces* yeast species are impeded at high ethanol levels. The microbe colonies that were grown in agar plates exhibited morphological differences across the three different sugar concentrations. However, due to the variable nature of the morphology of microbe species, and the viable but non-culturable (VBNC) state of some microbes, a genomic profiling is needed to accurately identify the microorganisms in wine fermentations.

References

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Author Contribution

Designed the experiments: HP.
Performed the experiments: NR.
Generated and analyzed the Data: NR and HP.
Winemaking project design: JH.
Winemaking: RL and CM.