

Yeasts on Arkansas White Riesling grapes and in musts

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Wine makers wanting to know about natural fermentations (i.e., those not intentionally inoculated with yeast starter cultures) are interested in the kinds of yeasts present naturally on wine grapes at harvest and which yeasts gain predominance during must fermentation. This information about Arkansas grapes and naturally-fermented musts is not available. Therefore, the objective of this study was to determine the major yeasts present on Arkansas-grown White Riesling (*Vitis vinifera*) grapes and in fermenting model musts.

Randomly selected clusters of grapes were harvested from three different locations (i.e., three replications) within an experimental vineyard at Fayetteville. Sound berries were removed from the clusters and blended for 2 minutes in sterile blender jars. The slurry (must) was divided into four 225-mL portions and dispensed into sterile 250-mL Erlenmeyer flasks equipped with air locks. Potassium metabisulfite was added as a single dose to must samples to give total SO₂ concentrations of 0, 25, 50, or 100 mg/L. The set of flasks, containing each of the four sulfite treatments, was incubated at 13°C and sampled at 0, 5, 10, and 20 days.

Table 1. Enumeration of yeasts and bacteria in White Riesling model musts at 13°C.

Sampling Time (Days)	SO ₂ Level (mg/L)	Log (CFU/mL) ^{1,2,3}				
		PDA	YM + P	LM	CSA	PCA
0	0	5.9 _a	5.8 _a	5.9 _a	5.0 _a	6.0 _a
	25	5.2 _a	5.3 _a	5.2 _a	4.4 _a	5.1 _a
	50	5.5 _a	5.4 _a	5.2 _a	4.7 _a	5.4 _a
	100	5.3 _a	5.3 _a	5.3 _a	5.0 _a	5.3 _a
5	0	6.9 _b	7.0 _b	6.7 _b	4.9 _b	6.7 _b
	25	7.6 _b	7.5 _b	7.5 _b	5.0 _b	7.4 _b
	50	7.1 _b	7.2 _b	7.2 _b	5.0 _b	7.4 _b
	100	7.0 _b	7.0 _b	7.0 _b	5.0 _b	7.0 _b
10	0	7.6 _b	7.7 _b	7.6 _b	5.2 _b	7.7 _b
	25	8.0 _b	8.0 _b	8.0 _b	6.2 _b	7.9 _b
	50	7.9 _b	8.0 _b	8.1 _b	4.1 _b	8.0 _b
	100	7.7 _b	7.5 _b	7.9 _b	5.5 _b	7.6 _b
20	0	7.7 _b	7.8 _b	7.8 _b	7.8 _b	7.8 _b
	25	7.9 _b	7.8 _b	7.8 _b	6.8 _b	7.9 _b
	50	7.4 _b	7.6 _b	7.7 _b	7.6 _b	7.6 _b
	100	7.2 _b	7.5 _b	7.5 _b	7.5 _b	7.5 _b

¹Values are means of six determinations (three experiments with duplicate determinations/replications).
²Means in vertical columns within each sampling time followed by the same superscript (a or b) are not significantly different at p<0.05 by the Least Significant Difference (LSD) test.
³Means in horizontal rows within each sulfite treatment followed by the same subscript (x, y, or z) are not significantly different at p<0.05 by the Least Significant Difference (LSD) test.

A 10-mL sample was removed from each flask and dispensed into 90 mL of sterile deionized water at each sampling time. Serial dilutions were prepared and pour-plated in duplicate using Plate Count Agar (PCA), Potato Dextrose Agar acidified with sterile 10% tartaric acid to pH 3.5 (PDA), Yeast Malt Agar containing 0.125% sodium propionate (YM + P), Lysine Medium (LM), and Czapek Solution Agar (CSA) for enumerating aerobic bacteria, total yeasts, yeasts capable of utilizing L-lysine as a sole nitrogen source, and yeasts capable of utilizing nitrate as a sole nitrogen source, respectively. Plates were incubated at 25°C for 5-7 days before counting colonies.

Microbial counts (log Colony Forming Units/cm²) on berry surfaces were: aerobic bacteria, 3.5-4.9 (X = 4.5); total yeasts, 3.5-4.9 (X = 4.5); yeasts capable of utilizing L-lysine as a sole nitrogen source, 3.8-4.6 (x = 4.3); and yeasts capable of utilizing nitrate as a sole nitrogen source, 3.8-4.1 (X = 4.0).

Plate counts of yeasts and bacteria (log CFU/mL) in musts are summarized in Table 1. A two log cycle increase in counts occurred during the first 10 days for all the organisms tested. At day 0, sulfite addition (regardless of level) caused significant decreases in counts of total

yeasts (PDA or YM + P), lysine-positive yeasts (LM), and bacteria (PCA). Generally, counts of lysine-positive yeasts (LM) and nitrate-positive yeasts (CSA) were not significantly different from total yeast counts (PDA or YM + P) at any sampling time or sulfite level. Most of the must yeasts enumerated on LM or CSA were of the wild-type and possessed the ability to utilize L-lysine or nitrate as a sole nitrogen source, properties lacking in commercially-used wine starter cultures of *Saccharomyces* (Kreger-van Rij, 1984).

Forty yeast isolates, ten each from the following treatments, were selected for further identification: (i) 0 days, 0 mg/L SO₂; (ii) 0 days, 100 mg/L SO₂; (iii) 20 days, 0 mg/L SO₂; and (iv) 20 days, 100 mg/L SO₂. These isolates were identified to be 10 species of 8 genera (Table 2). Surfaces of intact grapes had the same yeasts as those shown for 0 day, 0 mg/L SO₂.

The ten different species of yeasts isolated and identified from the musts in this study were similar to those reported for grapes, juices, musts, and wines in other wine grape regions of the world.

Kreger-van Rij, N.J.W. (Ed.). 1984. "The Yeasts: A Taxonomic Study," 3rd ed. North-Holland Publishing Co., Amsterdam, The Netherlands.

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Table 2. Yeast isolates identified¹ from White Riesling model musts.

Sample Code	Yeasts Recovered	Number of Isolates
0 Day;	<i>Cryptococcus albidus</i>	1
0 mg/L SO ₂	<i>Hanseniaspora guilliermondii</i>	6
	<i>Pichia membranaefaciens</i>	1
	<i>Trichosporon cutaneum</i>	2
0 Day;	<i>Candida diversa</i>	1
100 mg/L SO ₂	<i>Hanseniaspora guilliermondii</i>	1
	<i>Hanseniaspora uvarum</i>	2
	<i>Metschnikowia pulcherrima</i>	1
	<i>Pichia membranaefaciens</i>	1
	<i>Saccharomycopsis vini</i>	1
	<i>Trichosporon cutaneum</i>	3
20 Days;	<i>Hanseniaspora guilliermondii</i>	1
0 mg/L SO ₂	<i>Pichia membranaefaciens</i>	1
	<i>Saccharomyces cerevisiae</i>	6
	<i>Saccharomyces kluyveri</i>	2
20 Days;	<i>Hanseniaspora uvarum</i>	4
100 mg/L SO ₂	<i>Metschnikowia pulcherrima</i>	2
	<i>Pichia membranaefaciens</i>	2
	<i>Saccharomyces cerevisiae</i>	2

¹According to Kreger-van Rij, 1984.