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Effect of Different Strains of *Saccharomyces cerevisiae* on Production of Volatiles in Napa Gamay Wine and Petite Sirah Wine

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Napa Gamay grapes were fermented with four different strains of the yeast *Saccharomyces cerevisiae* (VL1, MI16, Fermirouge, and RA17). Petite Sirah grapes were fermented with seven different strains of the same yeast (BM45, Fermirouge, RA17, NI, CX3079, A350, and A796). Volatile compounds formed in the wines were analyzed by gas chromatography/mass spectrometry. Volatile compounds found in both wines were alcohols, esters, and acids, as well as some miscellaneous compounds. Isoamyl alcohol was the compound found in the highest relative amount with all four yeast strains in the Napa Gamay wines, followed by 2-phenyl ethanol, monoethyl succinate, and hexanoic acid. The relative amounts of isoamyl alcohol ranged from 30.84% (VL1) to 43.28% (RA17). Major volatile compounds found in Petite Sirah wines were isoamyl alcohol, 2-phenyl ethanol, 2-hydroxy ethyl propanoate, monoethyl succinate, and octanoic acid. The several esters, including 2-hydroxyethyl propanoate, may contribute to the fruity flavor of Petite Sirah wines. Overall, the *S. cerevisiae* yeast strains used to ferment Napa Gamay grapes and Petite Sirah grapes produced the same major components, with certain variations in formation levels.

KEYWORDS: Flavor chemicals; *Saccharomyces cerevisiae*; wine

INTRODUCTION

The use of yeast to produce alcoholic beverages has been known for centuries. The choice of yeast for the formation of desirable flavor components is of particular importance in the production of wine. The preferable flavors of wine depend on a balance of volatile constituents. The principal odor and flavor constituents responsible for the characteristics of specific wines include acids, alcohols, esters, carbonyls, acetals, amines, and sulfur-containing compounds (*1*, *2*).

The formation of volatile compounds during alcoholic fermentation depends on the particular yeast species (*3*) and the particular strain of the species (*4*). Although a number of flavor components are found in the original grape (*5*), most compounds found in wine are formed during yeast fermentation (*6*). These products determine the final aroma and flavor of the wine. There is a positive correlation between the yeast used and the production of volatile chemicals, including acids, alcohols, and esters, in fermenting must (*7*).

In modern wine making, *Saccharomyces cerevisiae* is commonly used for alcoholic fermentation (*8*). There have been some reports that various *S. cerevisiae* strains produced different volatile profiles in wines (*9*, *10*). The wine industry is greatly interested in wine yeast strains that produce unique flavor (*11*). It is important to know potential differences in volatile production by various strains of yeast in order to select the best strain to produce a wine of interest.

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The objective of this study was to investigate the effect of different strains of *S. cerevisiae* on volatile flavor compounds—principally alcohols, esters, and acids— produced during fermentation of wine made from the same pool of Napa Gamay grape must and Petite Sirah grape must. Strains of *S. cerevisiae* were used because they have been widely used by the wine makers in California.

MATERIALS AND METHODS

Chemicals. Tetradecane (99%), anhydrous sodium sulfate, dichloromethane (99%), and methanol (99%) were purchased from Sigma Chemical Company (St. Louis, MO). Authentic volatile chemicals were obtained from Takata Koryo Co., Ltd. (Osaka, Japan) as a gift.

***S. cerevisia* Yeast Strains.** Yeast strains BM45, Fermirouge, RA17, NI, CX3079, A350, and A796 were purchased from Christian Hanson (Fresno, CA), Gist Brocades (Fresno, CA), and Scott Labs. (Petaluma, CA).

Wine Preparation. Napa Gamay grapes and Petite Sirah grapes were harvested from the Sonoma Valley in September 1995. Grapes were crushed, and the must was fermented in 50-gal plastic containers fitted with a carbon dioxide vent through a water trap. Each batch of must was blended to 22 degree brix sugar measured with a Leica refractometer (Fisher Scientific, Pittsburgh, PA), and the titratable acidity ranged from 8.5 to 10 g/L. The sulfur dioxide concentration was adjusted to 50 ppm using a 10% stock solution of potassium metabisulfite (Glen Ellen Winery, Modesto, CA). Four 50-gal batches of must were each fermented with one of four types of *S. cerevisiae* strains (VL1, MI16, Fermirouge, and RA17) for Napa Gamay grapes and with one of seven types of *S. cerevisiae* strains (A350, A796, BM45, CY3079, Fermirouge, NI, and RA17) for Petite Sirah grapes. The amount of yeast added

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Table 1. *Saccharomyces cerevisiae* Yeast Strains Used To Ferment Napa Gamay Grapes and Amount of Sulfur Dioxide, pH, and Titratable Acidity of Resulting Wines

yeast strains	sulfur dioxide (mg/L)	pH	titratable acidity (g/L)
Napa Gamay			
VL1	39.95	3.50	7.58
MI16	36.65	3.70	7.30
Fermirouge	28.45	3.20	6.30
RA17	43.7	3.60	7.60
Petite Sirah			
A350	33.28	3.56	7.50
A796	26.88	3.62	7.50
BM45	39.68	3.61	7.80
CY3079	24.32	3.61	6.60
Fermirouge	39.68	3.56	7.50
NI	35.84	3.70	7.20
RA17	28.16	3.66	6.30

(22.7 g/50 gal) was that recommended by the winemaker at the Glen Ellen Winery (Modesto, CA).

Fermentation was allowed to proceed at 16 °C in a controlled temperature cooler for two weeks until the sugar concentration was reduced to <20 mg/100 mL. The sugar was monitored using a glucose oxidase test strip (Chemstrip, Boehringer Mannheim Corp., Indianapolis, IN). The fermented must was racked two times at intervals of two weeks. The must was then filtered through a cheesecloth into 750-mL wine bottles that were subsequently corked and stored in an 18 °C storage chamber until October 1998 at the Glen Ellen Winery. The wines were transferred to the Environmental Toxicology Department at the University of California, Davis, and stored at an ambient room temperature of 22 °C for 1−3 months. At that time, the wines were extracted and analyzed for flavor compounds. The three years aging period was used because it was recommended by the Glen Ellen Winery.

Sulfur Dioxide, pH, and Titratable Acidity. Free sulfur dioxide was quantitated using the Ripper titrametric method (*12*). A 50-mL wine or must sample was transferred to a 250-mL Erlenmeyer flask. A 5-mL portion of starch indicator solution (1% soluble starch) and a 5.0-mL sulfuric acid/water (1/3, v/v) solution were added to the flask. The free sulfurous acid generated was titrated with a 0.02 N iodine solution (Fisher Scientific, Pittsburgh, PA). The end point was a blue color that persisted for 30 s.

The pH was measured using a Corning 240 pH meter (Corning, NY). The soluble solids measured as sugar were determined using a Leica Brix meter no. 7531L; range at 0−50 (Fisher Scientific, Pittsburgh, PA). The sugar was measured using a Chemstrip glucose oxidase test strip that measures sugars between 20 and 800 mg/100 mL.

Titratable acidity was determined by titrating a 0.1 N sodium hydroxide against a 5.0-mL wine sample, with phenolphthalein (Fisher Scientific, Pittsburgh, PA) as an indicator.

Isolation of Volatile Flavor Compounds in Wine. A glass column (250 mm × 11 mm i.d.) packed with 5 mL of Porapak Q resin (50−80 mesh, Alltech Associates, Inc., Deerfield, IL) was conditioned with 2 column volumes each of dichloromethane, methanol, and water. A 200-mL sample of wine was placed in the column connected to a 200-mL reservoir. The eluted wine was discarded. The column was further eluted with 50 mL dichloromethane, and the eluate was collected in a separatory funnel. A second volume of 10 mL dichloromethane was added and eluted. A third 5-mL volume of dichloromethane was used to extract any remaining sample.

The water layer was discarded. The dichloromethane layer was transferred to a Erlenmeyer flask and dried over anhydrous sodium sulfate. After removal of anhydrous sodium sulfate, the sample solution was concentrated with a rotary evaporator and then further concentrated to a final volume of the sample of 1.2 mL with a purified nitrogen stream in a 5-mL glass vial with a Teflon liner. The sample was stored at −5 °C until analysis for volatiles.

Determination of Volatile Compounds in Wine. The GC Kovats retention index *I* (*13*) and the MS fragmentation pattern of each component were compared to those of the authentic compound to identify the volatiles in the samples. A Hewlett-Packard 5890 gas chromatograph equipped with a 30 m × 0.25 mm i.d. DB-Wax bonded-phase fused-silica capillary column (J&W Scientific, Folsom, CA) and a flame-ionization detector (FID) was used for routine quantitative analysis. The split ratio was 1:18. The peaks from chromatography were integrated with a Spectra Physics 4290 integrator (San Jose, CA). The injector and detector temperatures were maintained at 250 °C. The oven temperature was held at 50 °C for 8 min and then programmed at 3 °C/min to 180 °C for 35 min. The linear helium gas flow rate was 30 cm/s.

A HP 5890A Series II GC interfaced to a HP 5972 mass-selective detector was used for mass spectral identification of the GC components at MS ionization voltage of 70 eV. Column and oven conditions were as stated above.

Table 2. Volatile Compounds Found in Napa Gamay Wine Fermented with Four Different *Saccharomyces cerevisiae* Strains

compd	relative GC peak area%			
	VL1	MI16	FR[a]	RA17
Alcohols				
1-propanol	0.02	0.12	0.04	0.04
isobutanol	0.27	0.34	0.48	0.38
1-butanol	0.04	0.02	0.02	0.02
isoamyl alcohol	30.84	36.99	37.25	43.28
1-hexanol	1.14	1.22	1.61	1.48
(*E*)-3-hexen-1-ol	0.03	0.30	0.03	0.03
3-ethoxy-1-propanol	0.03	0.12	0.02	0.03
cyclohexanol	0.23	0.21	0.35	0.37
2,3-butanediol (D, L)	1.36	2.74	1.91	1.15
1-octanol	0.02	0.01	0.03	0.02
2,3-butanediol (*meso*)	0.26	0.57	0.36	0.23
isoheptanol	0.07	0.07	0.07	0.05
benzyl alcohol	0.04	0.06	0.05	0.04
2-phenylethanol	11.13	16.34	15.66	16.88
1-(4-hydroxyphenyl)ethanol	–[b]	–	0.57	0.60
Esters				
ethyl butanoate	0.07	0.09	0.10	0.14
isoamyl acetate	0.09	0.15	0.17	0.19
propyl butanoate	–	0.02	–	–
ethyl hexanoate	0.44	0.40	0.60	0.60
butyl butanoate	–	0.04	–	–
ethyl octanoate	0.52	0.49	0.75	0.72
ethyl 2-hydroxypentanoate	0.59	0.79	0.88	1.16
ethyl 3-hydroxybutanoate	0.08	0.08	0.07	0.07
ethyl decanoate	0.18	0.11	0.09	0.17
diethyl succinate	5.65	8.50	6.98	5.33
hydroxyl diethyl succinate	2.39	2.10	2.13	2.06
tartaric acid, diethyl ester	0.09	0.07	0.08	0.08
dipropyl succinate	–	0.05	–	–
monoethyl succinate	15.17	15.34	12.37	10.16
Acids				
acetic acid	0.31	0.48	0.41	0.20
propionic acid	0.01	0.03	0.03	0.01
isobutanoic acid	0.08	0.09	0.20	0.10
butanoic acid	0.13	0.14	0.14	0.13
hexanoic acid	3.59	2.97	4.24	3.50
octanoic acid	3.88	2.68	4.07	3.35
decanoic acid	0.83	0.8	0.66	0.51
hexadecanoic acid	0.69	0.04	–	–
Miscellaneous Compounds				
thiazolidine	0.06	0.10	0.38	0.22
N,*N*-dimethylformamide	–	0.13	0.34	0.25
naphthalene	0.28	0.07	0.05	0.06
propylene glycol	0.06	0.08	0.11	0.11

[a] Fermirouge. [b] GC area <0.01% or not detected.

RESULTS AND DISCUSSION

At least three different Vinifera (European) grape varieties are allowed to use the term “Gamay” on their label in the United

Table 3. Volatile Compounds Found in Petit Sirah Wine Fermented with Seven Different *Saccharomyces cerevisiae* Strains

	GC peak area %						
compd	BM45	Fermirouge	RA17	NI	CX3079	A350	A796
			Alcohols				
1-propanol	–[a]	0.44	–	0.41	–	0.64	0.42
isobutanol	0.87	–	2.19	1.91	1.57	1.23	2.48
isoamyl alcohol	35.29	38.98	40.87	38.87	36.53	37.68	32.73
3-ethoxy-1-propanol	–	–	–	–	–	0.26	–
2-methyl-3-buten-2-ol	0.45	0.46	–	–	–	–	–
1,3-butanediol	–	–	–	3.30	–	–	–
2,3-butanediol (D,L)	2.42	2.64	2.50	-	5.21	2.30	3.18
2,3-butanediol (*meso*)	0.55	0.67	–	0.82	1.09	0.50	1.60
2-phenylethanol	16.03	12.98	12.48	7.78	8.58	10.12	13.29
			Esters				
isoamyl acetate	0.74	1.08	1.97	–	0.82	0.32	3.29
ethyl hexanoate	0.66	0.86	–	–	–	0.17	0.43
2-hydroxyethylpropanoate	7.48	7.82	6.15	5.91	2.39	4.68	4.54
ethyl octanoate	1.05	1.33	–	–	–	–	0.81
ethyl decanoate	0.31	0.41	–	–	–	–	–
diethyl succinate	2.99	2.42	3.42	5.40	6.82	4.72	0.73
linalyl propanoate	0.55	0.46	–	–	–	–	–
2-phenylethyl acetate	–	–	–	–	–	–	3.01
3-hexenyl butanoate	1.28	1.02	–	0.18	ÑD	–	–
hydroxydiethyl succinate	–	–	1.17	-	-	0.25	–
monoethyl succinate	14.64	13.09	12.50	17.09	19.86	27.31	16.59
			Acids				
acetic acid	0.86	1.21	1.82	2.26	2.32	1.52	3.04
isoamyl acid	–	–	–	–	–	0.23	–
hexanoic acid	3.82	3.19	2.24	0.99	1.23	1.05	2.16
octanoic acid	5.46	4.64	2.71	1.06	1.31	0.82	4.61
decanoic acid	1.66	1.17	0.72	–	–	0.16	2.48
			Miscellaneous Compounds				
3-hydroxy-2-butanone	–	–	–	1.23	1.01	0.97	–
1-methoxy-2-methyl propane	–	–	1.51	–	–	–	–
γ-buryrolacton	–	–	-	0.61	–	–	–
thiazole	–	–	1.31	0.94	0.75	0.72	–
1*H*–i-ole-3-ethanol	–	–	–	–	2.43	0.25	–

[a] –, GC peak area <0.01% or not detected.

States. The Gamay Noir is responsible for the Beaujolais wine of France. The Gamay Beaujolais is a clone of Pinot Noir, the famous grape of Burgundy. Napa Gamay is grown in France under the name Valdiguie. In the United States, these varieties have all been treated in a similar way to produce generally light-to-medium-bodied fruity red wines, often utilizing the technique called carbonic maceration and frequently with little or no oak aging.

Free Sulfur Dioxide, pH, and Titratable Acidity. Table 1 presents the quantities of free sulfur dioxide, pH, and titratable acidity in the fermented wines. The values are the average of two duplicate experiments. All values were within the ranges acceptable to the wine industry. No significant differences in the levels of these items among the wines were observed.

Volatiles in Napa Gamay Wine Fermented with Four Different *S. cerevisiae* strains. The recovery method used for volatile compounds was the one previously reported (*14*). The use of Porapak Q as a trapping agent and dichloromethane as a solvent achieved satisfactory recovery of wine volatiles. For example, the major wine volatiles, such as 2-phenylethanol, diethyl succinate, and octanoic acid, could be nearly 100% recovered using this method (*14*).

Table 2 shows the volatile compounds found in the Napa Gamay grapes fermented with different *S. cerevisiae* yeast strains, along with their relative GC peak area. The compound produced in the highest relative amounts by all four yeast strains was isoamyl alcohol, followed by 2-phenylethanol and monoethyl succinate. The relative amounts of isoamyl alcohol ranged from 30.84% (VL1) to 43.28% (RA17). Isoamyl alcohol possesses a peculiar winey-brandy-like flavor in proper dilution (*15*). The strain RA17 may be useful in enhancing a brandy-like taste in wine, because it produced the greatest relative amount of isoamyl alcohol. 2-Phenyl ethanol had the second greatest relative amount detected among the alcohols found in the wines, in relative amount, ranging from 11.13% (VL1) to 16.88% (RA17). 2-Phenyl ethanol possesses a rose-honey-like odor and has been used widely for manufacturing perfumes (*15*). 2,3-Butanediol was detected in appreciable relative amounts. The strain MI16 produced the greatest relative amount of 2,3-butanediol (2.74%) among the four yeast strains. Alcohols can be produced directly from fermented sugar or through catabolism of amino acids (*16*, *17*). MI16 also produced the greatest relative amount of diethyl succinate (8.50%) and monoethyl succinate (15.34%) among the four yeast strains.

Among the 14 esters found in Napa Gamay wine volatiles, monoethyl succinate was detected in the greatest relative amounts, ranging from 10.16% (RA17) to 15.34% (MI16). Diethyl succinate was detected in the second greatest relative amounts, ranging from 5.33 (RA17) to 8.50% (MI16). Succinic acid is one of the major acids found in wine. It contributes a pleasant acidic taste. Many esters of succinic acids may be present in wines. In addition to the above two esters, diethyl hydroxy succinate and dipropyl succinate were found in the present study. These succinic acid esters have been used in various flavor compositions, such as imitation butter, rum, brandy, grape, and raspberry (*15*). The other esters found in the present study—including ethyl butanoate, isoamyl acetate, and ethyl hexanoate—give fruity flavors to wines.

Hexanoic acid (2.97−4.24%) and octanoic acid (2.68−4.07%) were the acids produced in the highest relative amounts. Fermirouge produced the greatest relative amount of these two acids among the four yeast strains. The titratable acid value of Fermirouge wine showed it to be the most acidic among these four wines. This may be due to the formation of these two acids in high quantities.

The VL1 yeast has traditionally been used where high green and vegetable aromas are in need of reduction. This yeast is used for late fermented wines when the winemaker desires to bring out a hint of green flavor notes. In the present study, the VL1 yeast produced a wine with the lowest percentage (1.14%) of hexanol of the four wines.

The RA17 yeast produced a wine with 1.48% hexanol, and the Fermirouge yeast, 1.61% hexanol. The RA17 yeast strain, which produced the most sulfur dioxide in the present study (**Table 1**), is used by most winemakers only in red wines. Control of hydrogen sulfide formation during the fermentation process may be the most critical factor in the usage of RA17.

The Fermirouge yeast is used in wines to bring out the green and fruity aromas, which could explain the higher quantity of the hexanol and its ester produced by this yeast. The Fermirouge yeast strain is a cold-tolerant, floral yeast that is used in making white wines. Cryotolerant yeast strains produce higher relative amounts of glycerol, succinic acid, and malic acid, and lower relative amounts of acetic acid and ethanol (*18*−*20*). The formation of higher alcohols is influenced by the must used in the fermentation as well as the yeast strain (*21*).

The MI16 yeast strain is a proprietary, all-around yeast used by wine makers. This yeast produced the highest relative amount of 2,3-butanediol and butanoic acid esters and the lowest relative amount of ethyl esters of octanoic acid. Overall, the wine produced by fermentation of Napa Gamay grapes was neither too floral nor too heavy with sulfur compounds or fermented (acidic) flavors.

Volatile Components Found in Petite Sirah Wine Fermented with Seven Different Yeast Strains. The Petite Sirah grape is a very dark-skinned red grape that produces wines with an inky color and heavily tannic structure. The wine tends to be robust, rustic, and simple, often with black pepper in the aroma and taste. This grape was once confused in California with the true Syrah/Shiraz, which is the most important red grape from the Northern Rhone region of France. They are descended from the Duriff Rhone grape of France. For most of their history, Petite Sirah grapes were used only to blend into other wines. They gained a lot of attention in the 1970s because of the general popularity of red wines, and because of their full, tannic taste. Typical flavors include plum, raspberry, blackberry, and black pepper.

Table 3 shows the major volatile components found in Petite Sirah grapes fermented with seven different *Saccharomyces* yeast strains. The major volatile compounds produced were isoamyl alcohol, 2-phenyl ethanol, 2-hydroxy ethyl propanoate, monoethyl succinate, and octanoic acid. The major compounds found in Petite Sirah wine were the same as those found in Gamay wine except for 2-hydroxyethyl propanoate. 2-Hydroxyethyl propanoate, which possesses a fruity flavor, was found in rather high levels ranging from 2.39 (CX3079) to 7.82% (Fermirouge). It was not found in Gamay wine. Isoamyl alcohol composed over one-third of the total volatiles. Its relative amount ranged from 32.73 (A796) to 40.87% (RA17). It is interesting that RA17 also produced the greatest relative amount (43.28%) of isoamyl alcohol in the Napa Gamay wine. The ester found in the greatest relative amounts among those identified in Petite Sirah wine was monoethyl succinate. Its relative amounts ranged from 12.50% (RA17) to 27.31% (A350).

The differences in the relative amounts of 2-phenylethanol found in the seven wines was large: BM45 (16.03%) produced approximately twice as much as NI (7.78%) did. The presence of many ethyl esters might be indicative of fruity−berry flavor notes that BM45 yeast contributes to the final flavor of the Petite Sirah wine (*15*).

CX3079, which is an excellent yeast for fermentation of Chardonnay grape must, produced the greatest relative amount of 2,3-butanediol (D, L) (5.21%) among the seven yeast strains, whereas NI did not produce any detectable levels. A796 produced the greatest relative amount of isoamyl acetate (3.29%), which has a sweet−fruity, banana-like flavor (*15*).

The information presented here is to be a steppingstone toward enhancing the ability to assay flavor compounds as our research methods and our understanding of wine flavors grows.

LITERATURE CITED

(1) Van Stratten, S., Ed. Lists of volatile compounds in food. Rappt. R 4030. Central Institute for Nutrition and Food Research, Zeist 1977, Netherlands.

(2) Ribereau-Gayon, P. Wine flavor. In *Flavor in Foods and Beverages*; Charalambous, G., Inglett, G., Eds.; Academic Press: London, 1978.

(3) Rankine, B. C. Formation of higher alcohols by wine yeasts and relationships to taste thresholds. *J. Sci. Food Agric.* **1967**, *18*, 583−589.

(4) Webb, A. D.; Kepner, R. E.; Muggiora, L. Sherry aroma. IV. Some volatile components of flor sherry of Spanish origin. Neutral substances. *Am. J. Enol. Vitic.* **1966**, *18*, 252−256.

(5) Schreier, P.; Drawert, F.; Junder, A. Identification of volatile constituents from grapes. *J. Agric. Food Chem.* **1976**, *24*, 331−336.

(6) Margalith, P.; Schwartz, Y. Flavor and microorganisms. In *Advances in Applied Microbiology*; Perlmand., Ed.; Academic Press: New York, 1970; Vol. 13, p. 35.

(7) Nakajima, T.; Yamagata, K. Changes of yeast population and volatile components in fermenting must and aged wine. *Nippon Shokuhin Kogyo Gakkaishi* **1983**, *30*, 509−515.

(8) Esteve-Zarzoso, B.; Manzanares, P.; Ramond, D.; Querol, A. The role of non-*Saccharomyces* yeasts in industrial winemaking. *Internat. Microbiol.* **1998**, *1*, 143−148.

(9) Cabrera, M. J.; Moreno, J.; Ortega, J. M.; Medina, M. Formation of ethanol higher alcohols, esters, and terpenes by five yeast strains from Pedro Ximenez grapes in various degrees of ripeness. *Am. J. Enol. Vitic.* **1988**, *39*, 283−287.

(10) Alvarez, M. A.; Garcia, E. Componentes aromáticos en la fermentación alcohólica del Jerez. *Microbiol. Espanol* **1984**, *37*, 37−45.

(11) Pretorius, I. S. Tailoring wine yeast for the new millennium: Novel approaches to the ancient art of winemaking. *Yeast* **2000**, *15*, 675−729.

(12) Ough, C. S.; Amerine, M. A. *Methods of Analyses of Musts and Wines*, 2nd ed.; Wiley and Sons: New York, 1988; pp 198−235.

(13) Kovats, E. Gas Chromatographic Characterization of Organic Substances in the Retention Index System. *Adv. Chromatogr.* **1965**, *1*, 229−247.

(14) Wada, K.; Shibamoto, T. Isolation and identification of volatile compounds from a wine using solid-phase extraction, gas chromatography, and gas chromatography/mass spectrometry. *J. Agric. Food Chem.* **1997**, *45*, 4362−4366.

(15) Arctander, S. *Perfume and Flavor Chemicals*; Published by the author, Montclair, NJ, 1969.
(16) Ingraham, J. L.; Guymon, J. F.; Crowell, E. A. The pathway of formation of *n*-butyl and *n*-amyl alcohols by mutant strains of *Saccharomyces cerevisiae*. *Arch. Biochem. Biophy.* **1961**, *95*, 169−174.
(17) Giudici, P.; Romano, P.; Zambonelli, C. A biometric study of higher alcohol production in *Saccharomyces cerevisiae*. *Can. J. Microbiol.* **1990**, *36*, 61−64.
(18) Castellari, L.; Pachioli, G.; Zambonelli, C.; Tini, V.; Grazi, L. Isolation and initial characterization of cryotolerant *Saccharomyces* strains. *Ital. J. Food Sci.* **1992**, *3*, 179−186.
(19) Castellari, L.; Ferruzzi, M.; Magrini, A.; Giudici, P.; Passarelli, P.; Zambonelli, C. Unbalanced wine fermentation by cryotolerant vs noncryotolerant *Saccharomyces* strains. *Vitis* **1994**, *33*, 49−52.
(20) Giudici, P.; Zambonelli, C.; Passarelli, P.; Castellari, L. Improvement of wine composition with cryotolerant *Saccharomyces* strains. *Am. J. Enol. Vitic.* **1995**, *46*, 143−147.
(21) Bertolini, L.; Zambonelli, C.; Giudici, P.; Castellari, L. Higher alcohol production by cryotolerant *Saccharomyces* strains. *Am. J. Enol. Vitic.* **1996**, *47*, 343−345.

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