Oenological attributes of the yeast *Hanseniaspora vineae* and its application for white and red winemaking

V. Martin[1], L. Fariña[1], K. Medina[1], E. Boido[1], E. Dellacassa[1], A. Mas[2], and F. Carrau[1,a]

[1] Universidad de la República, Facultad de Quimica, Área Enología y Biotecnología de Fermentaciones, Montevideo, Uruguay

[2] Biochemistry and Biotechnology Department, Faculty of Oneology, University Rovira i Virgili, Tarragona, Spain

Abstract. Flavour and some compounds associated with wine colour are known to be yeast strain-dependent. These metabolites are important for the sensory quality of wines, studies searching for increase aroma and color are a key area today in winemaking. The aim of this work was to study the oenological potential of the two main strains of *Hanseniaspora vineae,* native to Uruguay to better understand their successful application at winery level. It is known that these strains contribute with extracellular proteases and β-glucosidase enzyme activities that might increase cell lysis and flavor depending in grape varieties. Application and nutrient management of the process of these strains in production of white wines (Chardonnay, Macabeo and Petit Manseng) and red wine Tannat are discussed. Wines were evaluated to determine the volatile compounds composition and their effect compared to conventional processes. Low production of short and medium chain fatty acids and ethyl esters, and high production of acetate esters and isoprenoids are found compared to *S. cerevisiae* strains. The most outstanding characteristic of the species *H. vineae* was the production of benzenoids, phenylpropanoids and acetate esters. This behavior was reflected in the sensory evaluation, where all the fermentations performed with *H. vineae* were considered superior compared to *Saccharomyces cerevisiae* wine strains.

1. Introduction

Wine markets continue growing in brands quantity, and the challenge of product style differentiation is always more competitive and difficult to attract consumers attention. It is considered that regional consumption will be the main alternative for the many new brands that appear in the market. This is also in agreement with an opposite situation that is happening in the distribution channels, as every year there is a reduction in distribution companies in the main importer countries, such as the USA, UK and Germany. The use of non-conventional yeasts is a strategy to create unique wine profiles within an extensive market, where region identity is the challenge [1].

Returning to low input winemaking strategies to develop particular characteristics of the "terroir" that might differentiate their wines, is one of the ways that was followed by some winemakers in the last 20 years [2,3]. New sites and soil selection, minimal handling of the grapes and fertilizers addition, decrease irrigation, old vines recovery, are some of the topics that are being discussed in wine quality definition in the last two decades searching for complexity [4]. One of the key components of the terroir concept are the native yeast associated with the mature grapes of a particular region. In this presentation we showed our experience with a native grape yeasts of the apiculate group, *Hanseniaspora vineae* [5]. Since 2009 we have used this species for commercial wine production, with many interesting results firstly in white wines [6,7], and now for red Tannat. Two strains genomes that were applied of this species were sequenced so as

to understand metabolic differences with the conventional *S. cerevisiae* strains [8]. Although there is an increase interest in non- *Saccharomyces* yeast application, there are still very limited commercial strains available for the winemaker [9,10]. We discuss here how we have applied small quantity production of liquid ferments that will improve non-conventional yeasts availability in particular regions, and a protocol for successful vinifications of these strains.

2. Materials and methods

2.1. Yeast strains

The commercial wine yeast strain used was *S. cerevisiae* ALG 804 (DSM, Denmark). The apiculate NS strain used was isolated from Tannat wine fermentation, *H. vineae* T02/5AF.

H. vineae was prepared by Lage y Cia in liquid sterile bags of 3 liters for inoculation at the winery to obtain an initial cell concentration of 5×10^5 cells/ml in triplicate 400 liters bins for Tannat and 225 L barrels for white grapes Petit Manseng wines. Final population inoculated was checked by microscope counting and by plating in WLN medium where green dark colonies can be clearly associated with *Hanseniaspora*.

The commercial strain, *S. cerevisiae* ALG 804 was hydrated as instructed by the manufacturer and subsequent to microscope counting, the appropriate dilution of the rehydrated wine yeast was inoculated (at time 0 or 6 days after inoculation with strain H. vineae T02/5AF in sequential co-inoculations) to obtain an initial cell concentration of 1×10^6 cells/ml.

---

[a] e-mail: `fcarrau@fq.edu.uy`

Table 1. Analytical parameters of Tannat wines produced with *H. vineae* compared to a conventional commercial yeast *Saccharomyces cerevisiae* ALG 804 (*Sacch*). Data showed are before and after malolactic Fermentation MLF, and during barrel and bottle aging. ND not determined.

	Before MLF March 2013		After MLF (May 2013)		barrel aging Oct. 1st		Aug 1st 2014 and Bottled		Jul–15	
	Sacch	*H. vineae*	*Sacch*	*H. vineae*	*Sacch*	*H. vineae*	*Sacch*	*H. vineae*	*Sacch*	*H. vineae*
Free SO2 mg/L	ND	ND	11	12.4	17	22	22	25	18	21
Total SO2 mg/L	7	9.8	26.52	23.2	51	45	66	53	60	48
Volatile Ac. g/L in Sulf	0.255	0.27	0.5	0.48	0.54	0.4	0.6	0.49	0.59	0.44
Total Ac. g/L in sulf	4.65	5.1	4.1	4.3	4.3	4.3	4.2	4.1	4.2	4
pH	3.5	3.4	3.81	3.65	ND	ND	3.67	3.64	3.72	3.67
Sugars g/L	1.9	2.4	1.9	2.3	ND	ND	1.25	1.4	ND	ND
Alcohol % Vol.	13.2	13	13.2	13	ND	ND	ND	ND	ND	ND
OD 420nm	9.415	9.44	7.86	7.5	7.04	6.51	6.8	6.3	7	5.9
OD 520nm	22.075	21.81	15.4	15.17	12.73	11.78	10.7	9.7	9.3	7.9
OD 620nm	4.035	3.985	3.53	3.12	2.96	2.56	3	2.5	3	2.1
CI	35.55	35.25	26.79	25.79	22.73	20.85	20.5	18.5	19.3	15.9
Total Anthocyanin mg/L	1710	1563.5	1061	931	ND	ND	577	628	386	351
Polyphenol Index	88	88	84.8	81.7	80	76.2	75	74	69	67

2.2. Fermentation conditions

The grape of Petit Manseng must contained 178 mg N/L yeast assimilable nitrogen (YAN), 248 g/L total sugars, 5.5 g/L of total acidity was measured as sulphuric acid at pH 3.3. Grapes were crushed, cooled by refrigeration to 12 °C and pressed with the addition of 3 g/Hl of SO_2, allowed to settle for 12 h and then transferred to three 225 l French oak barrels for each yeast treatment. Red grapes of Tannat were crushed, and no SO_2 was added, so immediately were inoculated with the corresponding yeast treatments. Tannat grape contained 148 mgN/L of YAN, 235 g/L of total sugars, 5.8 g/L of total acidity and pH 3.3.

Inoculation of the must was done immediately except for the spontaneous trials. Treatments were as follows: coferm-*H. vineae* (initially inoculated with *H. vineae* and then inoculated six days later with strain ALG 804), Commercial (inoculation with strain ALG 804 at time 0) and Spontaneous (without inoculation). Twenty-four hours after inoculation, 0.3 mg/L thiamine was added to all barrels. A supplementation of 100 mg/L with diammonium phosphate (DAP) and an extra 0.3 mg/L of thiamine and 1 g/L of yeast extract were added when ALG 804 was added at day 6 to the *H. vineae* treatment. Fermentation activity was measured by juice density every day, together with temperature and room temperature was maintained at 20 °C. Procedures for wine chemical analysis and YAN levels by the formaldehyde method were described previously [11]. Samples for analysis were taken once a day, for cell growth measurement, using an improved Neubauer chamber and the numbers of dead cells were counted by the methylene blue-staining technique. Upon completion of alcohol fermentation, chemical and sensory analysis was done. Subsequently, malolactic fermentation (MLF) was done using *Oenococcus oeni* VP41 (Lallemand, Montreal). All barrels were maintained at 18–22 °C and malic and lactic acid production during MLF was analysed by thin-layer chromatography [12]. After MLF completion 50 mg/L SO_2 was added followed by 2 months barrel ageing before bottling and final sensory analysis. All samples for analysis were filter sterilised (0.45 µm membrane) and the free SO_2 content was then adjusted to 35 mg/L, before and after MLF.

2.3. Sensory analysis

Duplicate sensory analysis was performed on fifteen samples, comprising three wines from each of the white wine treatments and two red Tannat wines. The three treatments as shown, are coded as follows, Spontaneous, Sacch and *H. vineae*, this last one refers to sequential co-fermentation. Sensory aroma description was made by a panel of eight established winemakers. Wines were presented in individual testing booths with normalised red lighting, to eliminate the colour perception of the white wine samples in flavour descriptions. Samples of 60 ml were served at 18 ± 1 °C in 250 ml, clear, tulip shaped wine glasses (ISO 3591, 1977) covered with a watch glass, and were identified with three digit random codes. Two samples were evaluated for aroma characteristics in each session. Panelists were required to rate secondary and tertiary tier terms using a 10-point intensity scale [13].

2.4. GC and GC–MS analysis

Aroma volatile compounds extraction of aroma compounds was done by adsorption and subsequent elution and separation from an Isolute (IST Ltd., Mid Glamorgan, U.K.) ENV+ cartridge packed with 1 g of highly, cross-linked, styrene-divinyl benzene (SDVB) polymer. Sample preparation and GC analysis was described previously [14].

Wine aroma components were identified by comparison of their Linear Retention Indices, with pure standards or data reported in the literature. Comparisons were also made with MS fragmentation patterns obtained with those in databases. GC-FID and GC–MS methods with an internal standard (1-heptanol) were used for quantitative purposes.

2.5. Statistical analysis

ANOVA of chemical and volatile compound analysis was done for the different fermentation treatments. ANOVA for sensory descriptors was done for different treatment and panel assessors' effect. Mean rating and Least Significant Differences (LSD) for each treatment were calculated from each analysis of variance with Statistica 7.1.

3. Results and discussion

In Table 1 we showed the basic wine quality parameters of Tannat red wines produced as an average of three processes with two yeast treatments for the Cerro Chapeu region. As it is shown wines obtained with *H. vineae* treatment give similar results in terms of final alcohol, volatile

Table 2. Aroma compounds produced by *H. vineae* and *S. cerevisiae* of Tannat grapes of two different regions. Results are the average of triplicates and SD. *, ** indicate significance at $p < 0.05$, $p < 0.01$ between the mean.

	Tannat Chapeu				Tannat Melilla				
Alcohols	H vineae + Sc Ch	SD	Sc Ch	SD	H vineae + Sc B	SD	Sc B	SD	
1-propanol	40290	480	38970	2380	68600	980	28100	3570	**
2-methyl-1-propanol	113280	3460	65340	1400	47180	380	42060	240	**
1-butanol	129	6	140	0.3	251	10	283	10	
3-methyl-1-butanol	370520	2430	263210	33350	269180	3640	259340	1310	*
1-hexanol	966	59	920	6	1585	13	1478	15	
2-ethyl-1-hexanol	245	3	22	1	18	1	15	1	
3-ethoxi-1-propanol	151	10	435	5	407	6	1113	6	**
benzyl alcohol	80	14	94	4	203	5	165	5	*
2-phenethyl alcohol	35076	279	41685	2113	23844	80	23298	120	
methanol	59510	3540	54290	2090	100310	3040	84020	80	
methionol	919	159	977	238	330	14	401	10	
Ethyl Esters	Hv + Sc C		Sc C		Hv + Sc B		Sc B		
ethyl isobutirate	50	3	33	0.03	22	2	37	1	
ethyl butirate	85	4	72	1	157	4	159	**5**	
ethyl hexanoic	175	16	161	2	195	6	239	9	
ethyl octanoic	170	10	132	3	125	3	158	12	
ethyl decanoic	45	3	33	1	43	1	46	1	
ethyl lactate	18610	321	17079	324	10211	118	10450	**120**	
ethyl pyruvate	nc	nc	4	1	24	1	40	1	
diethyl succinate	3649**	680	1245	131	616	17	662	16	
ethyl succinate	48837	2876	59456	1431	25660	380	24605	200	
2-hidroxi-glutarato de dietilo	392	25	388	1	375	12	375	12	
Acetate Esters	Hv + Sc C		Sc C		Hv + Sc B		Sc B		
2-phenylethyl acetate	134	25	133	21	433	5	193	6	**
isobutyl acetate	60	0	40	0	65	1	74	1	
isoamyl acetate	788	56	553	52	2050	45	2281	43	
ethyl acetate	35810	2280	32610	7630	57440	1000	32930	360	**
1.3-propanediol diacetate	465	78	970	16	467	2	772	**20**	**
Acids	Hv + Sc C		Sc C		Hv + Sc B		Sc B		
butanoic acid	214.13	26.10	232.10	3	354	6	494	7	*
hexanoic acid	664.89	97.35	755.32	47	840	3	956	12	*
octanoic acid	845.06	143.38	950.54	20	988	21	1089	21	*
decanoic acid	119.85	16.64	81.42	7	174	13	148	10	*
isobutanoic acid	1660.65	220.26	1017.56	14	954	7	1464	8	
isovaleric acid	619.35	13.56	979.22	50	548	7	637	**9**	
Lactones	Hv + Sc C	C	Sc C		Hv + Sc B		Sc B		
γ-butyrolactone	761	47	1019	112	328	3	441	3	
pantolactone	66	7	80	1	47	2	94	4	
Others	Hv + Sc C	C	Sc C		Hv + Sc B		Sc B		
2,3-Butanodione	152	3	224	12	206	4	250	10	
2,3-Pentanodione	67	5	65	3	170	3	168	15	
3-hydroxi-2-butanone	102	12	222	52	98	5	183	14	
acetaldehyde	5250	810	3330	1380	8600	600	2370	310	**
guaiacol	102	16	116	8	220	25	88	**6**	**
4-vinyl-guaiacol	119	12	225	41	133	3	103	10	
2,6-dimethoxy phenol	379	16	592	16	867	51	438	15	
methyl vanillylether	123	11	166	6	158	2	156	**12**	
4-(4-hydroxy-3-methoxy-phenyl)butan-2-ol	11	0	76	65	17	1	18	1	
zingerone	3	0	4	0	12	0	10	0	
ethyl-b-(4-hydroxy-3-methoxy-phenyl-propionate	7	0	10	1	28	0	19	1	
3-oxo-α-ionol	29	2	30	0	51	0..20	37	3	*
vomifoliol	38	9	58	5	55	1	43	2	*

Table 3. Aroma compounds produced by the 3 treatments of Petit Manseng barrel fermented. Results are the average of triplicates and SD. *, ** indicate significance at $p < 0.05$, $p < 0.01$ between the mean.

|	Petit Manseng Melilla						
Alcohols	Spontaneous	SD	Sacch	SD	H. vineae	SD	
1-propanol	181	43.9	220	13.5	188	26.1	
2-methyl-1-propanol	3412	42.0	4507	10.8	2649	16.1	**
1-butanol	360	42.8	376	13.9	395	15.5	
3-methyl-1-butanol	71093	28.7	67586	13.2	65990	9.2	
3-hydroxy-2-butanone	235	56.6	145	47.6	119	16.8	**
1-hexanol	785	21.0	831	8.0	795	7.2	
3-hexen-1-ol	29	19.8	33	9.9	32	5.0	
2-propanol	20	14.4	16	25.7	20	11.8	
2-ethyl-1-hexanol	48	13.2	37	21.9	52	39.6	
2-furanmethanol	24	21.7	35	14.6	34	42.0	
benzyl alcohol / benzenemethanol	49	8.8	44	22.2	40	23.5	
benzeneethanol / Phenethyl alcohol	14757	11.3	12810	15.9	12135	15.3	
tyrosol	4546	16.2	4738	22.3	2747	21.5	**
3-methylthio-1-propanol	340	16.9	324	14.0	300	30.8	
Esters							
ethyl isobutirate	84	43.6	108	13.9	109	22.0	**
acetate 3-methyl-1-butanol	1248	33.9	987	34.9	953	20.2	
hexanoic acid ethyl ester	248	13.1	245	16.2	283	9.4	
hexyl ester acetic acid	84	28.7	54	63.1	52	25.4	
2-hydroxy-propanoic acid	23660	18.2	20447	30.2	24202	5.7	
ethyl ester octanoic acid	528	14.1	549	4.9	625	7.7	
ethyl ester 3-hydroxybutanoic acid	125	18.7	139	8.4	93	12.0	
ethyl ester decanoic acid	228	11.8	222	12.8	247	12.3	
diethyl succinate	3222	22.1	3239	16.4	2099	24.0	**
acetato de etilo	17960	17.7	27820	22.1	42077	23.3	**
1,3-propanediol acetate	958	13.0	742	13.8	1065	12.9	**
ethyl hidroxy butyrate	4429	11.5	4041	19.6	3357	14.9	**
2-phenethyl acetate	281	18.0	259	25.2	229	22.1	
butanedioic acid , hydroxy diethyl ester	600	12.3	633	21.3	614	15.1	
ethyl succinate	58273	21.4	52686	11.5	32247	18.0	
Acids							
acetic acid	726	23.9	711	14.8	1211	13.3	**
propanoic acid	39	16.1	27	16.5	38	22.8	
isobutanoic acid	451	5.7	476	10.0	365	9.4	
butanoic acid	532	12.8	564	10.8	576	7.0	
isovaleric acid	348	9.6	363	9.6	336	11.5	
hexanoic acid	3786	19.5	3685	13.6	3629	13.6	
octanoic acid	7649	11.7	7373	16.5	6444	12.9	
decanoic acid	2578	18.2	2530	25.6	2421	19.0	
dodecanoic acid	613	26.7	415	10.2	418	48.1	
other							
g-butyrolactone	565	22.4	521	20.9	541	16.1	
Pantolactone	91	11.6	80	13.0	96	22.8	
Sum of aroma compounds							
Alcohols	76268	29.1	73929	12.6	70421	9.2	*
Esters	7131	3.6	6465	10.0	5855	8.8	*
Fatty acids	15196	10.7	14594	15.2	13525	12.6	*
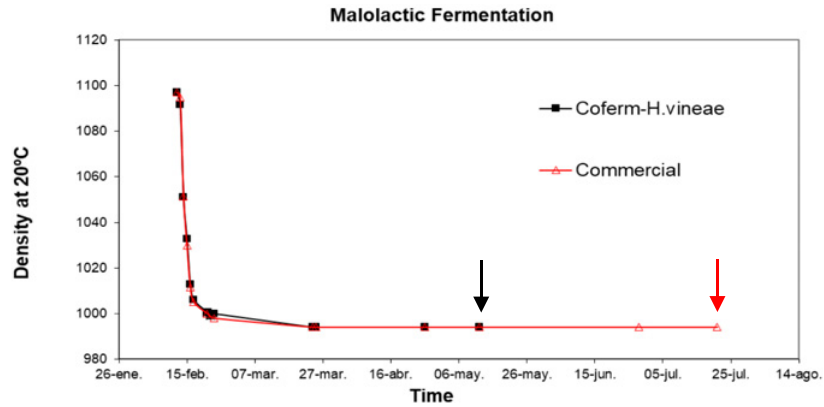
Figure 1. Malolactic fermentation is stimulated by *H. vineae* fermentations in Tannat. Similar results were obtained with barrel fermentation of Petit Manseng.
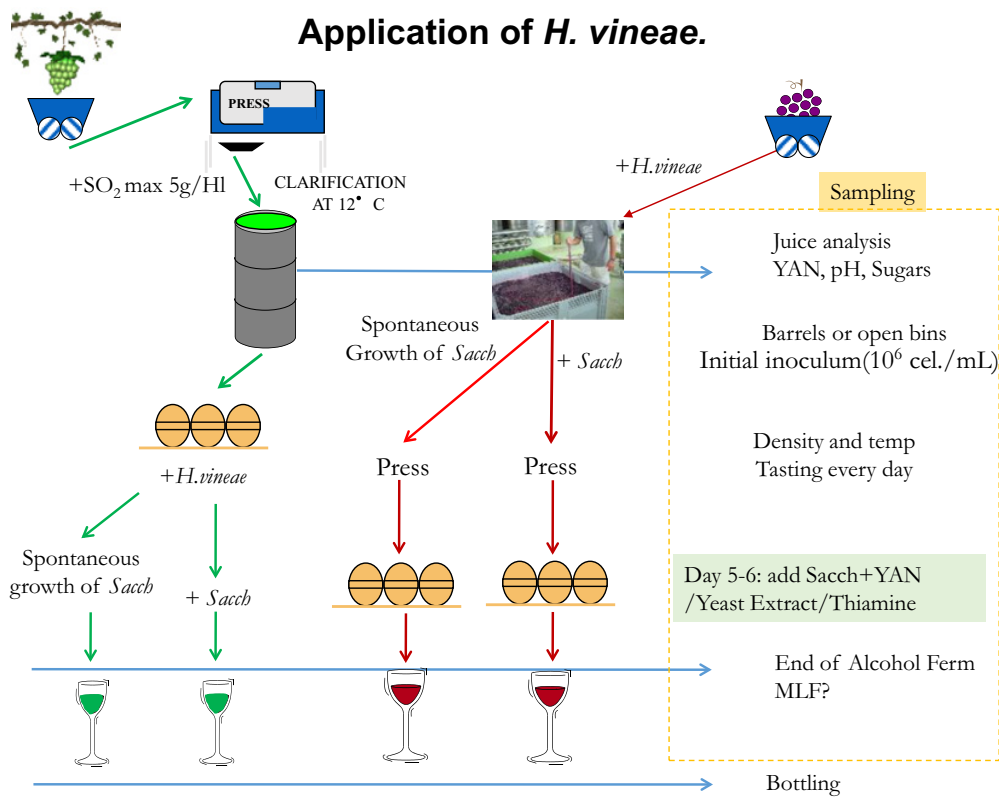

Figure 2. Application protocol for vinification in white and red wine with *H. vineae* strains.

acidity and final SO_2. We found that the application of *H. vineae* facilitates the development of native yeast diversity during fermentation and also the malolactic fermentation MLF bacteria are stimulated as shown in Fig. 1. This result is in agreement with our previous work with white wine barrel fermented Chardonnay [6]. Similar results were obtained here also for white Petit Manseng barrel fermented. In Table 2, we present the data for Tannat vinificated in two different regions. Data are the average of triplicates, and from 47 compounds determined, 16 were significantly affected compared to the pure *S. cerevisiae* commercial strain utilized. Here we can found some consistent results with our white wine previous experiences with Chardonnay [6] and Macabeo [7]. Benzyl alcohol, 2-phenylethyl slvohol and ethyl acetate, guaiacol and an increase of norisoprenoids in

the Tannat of Melilla were detected. Sensory analysis of the Tannat treatments showed an increase of fruity and caramel descriptors in *H. vineae* compared to the most obvious oak and herbal flavors of the Sacch treatment. In Table 3, the flavor compounds analysis for Petit Manseng barrel fermented is shown and from 42 compounds determined 9 were significantly affected ($p < 0.01$) compared to the conventional yeast treatment. Although it was demonstrated previously that 2-phenylethyl acetate and benzenoids were the main synthetize compounds by *H. vineae* in Chardonnay and Macabeo [6,7]. Results for Petit Manseng showed a similar behavior for these compounds than with the conventional yeast treatment. Figure 2 shows the application protocol defined as successful to apply in red and white wine winery scale production. Sluggish fermentations are avoided by a

rational nutrient complementation of the co-inoculated strain at day 5 or 6 of the process. This is a simple operation that allowed the Sacch strain to obtained limited nutrients that were removed by *H. vineae* initial activity as it was demonstrated [15].

4. Conclusions

H. vineae showed oenological capacities for production of white and red wines, resulting in more complex sensory wines. Its moderate competitive capacity during vinification compared to Saccharomyces strains, help to understand the concept of "friendly yeast" that allowed to increase microbial diversity in the process. The nutrient management when sequential inoculation of *S. cerevisiae* is done should be the key activity to avoid a sluggish fermentation. It was shown that for Tannat wines the vinification process without addition of sulfites before fermentation and with sequential inoculation of *S. cerevisiae* after 6 days, resulted in wines of similar basic quality parameters than conventional vinification methods in terms of alcohol and volatile acidity. We presented an easy vinification protocol for making real wine experiments at the winery with or without commercial strains addition.

References

[1] F. Carrau, C. Gaggero, P.S. Aguilar, Trends Biotechnol. **33**, 148 (2015)
[2] F.M. Carrau, Agrociencia **9**, 387 (2005)
[3] D. Ramey, In *Proceedings Australian Wine Industry Technical Conference*, edited by C.S. Stockley, R.S. Johnstone and T.H. Lee, A. N. S (Adelaide, Australia, 1995), pp. 26–29
[4] S. Tempère, A. Marchal, J.C. Barbe, M. Bely, I. Masneuf-Pomarede, P. Marullo, W. Albertin, Appl. Microbiol. Biotechnol. **102**, 3995 (2018)
[5] V. Martin, M.J. Valera, K. Medina, E. Boido, F. Carrau, Fermentation **4.3**, 76 (2018)
[6] K. Medina, E. Boido, L. Fariña, O. Gioia, M.E. Gomez, M. Barquet, C. Gaggero, E. Dellacassa, F. Carrau, Food Chem. **141**, 2513 (2013)
[7] J. Lleixà, V. Martín, M. Portillo, C. del, F. Carrau, G. Beltran, A. Mas, Front. Microbiol. **7**, 338 (2016)
[8] F.M. Giorello, L. Berná, G. Greif, L. Camesasca, V. Salzman, K. Medina, C. Robello, C. Gaggero, P.S. Aguilar, F. Carrau, Genome Announc. **2** (2014)
[9] C. Varela, A.R. Borneman, Yeast, **34**, 111 (2017)
[10] N.P. Jolly, C. Varela, I.S. Pretorius, FEMS Yeast Res. **14**, 215 (2014)
[11] B.W. Zoecklein, K. Fugelsang, B.H. Gump, F.S. Nury, *Wine Analysis and Production* (Chapman & Hall, New York, 1995)
[12] E. Boido, E. Dellacassa, F. Carrau, P. Moyna, J. Planar Chromatogr. – Mod. TLC **12**, 269 (1999)
[13] A. Gámbaro, E. Boido, A. Zlotejablko, K. Medina, A. Lloret, E. Dellacassa, F. Carrau, Aust. J. Grape Wine Res. **7**, 27 (2001)
[14] E. Boido, A. Lloret, K. Medina, L. Farñia, F. Carrau, G. Versini, E. Dellacassa, J. Agric. Food Chem. **51**, 5408 (2003)
[15] K. Medina, E. Boido, E. Dellacassa, F. Carrau, Int. J. Food Microbiol. **157**, 245 (2012)