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USE OF NON-SACCHAROMYCES *TORULASPORA DELBRUECKII* YEAST STRAINS IN WINEMAKING AND BREWING

ABSTRACT: Selected *Saccharomyces* yeast strains have been used for more than 150 years in brewing and for several decades in winemaking. They are necessary in brewing because of the boiling of the wort, which results in the death of all yeast cells, with the exception of some Belgian style beers (ex. Lambic), where the wort is left to be colonized by indigenous yeast and bacteria from the environment and ferment naturally. In winemaking their use is also pertinent because they provide regular and timely fermentations, inhibit the growth of indigenous spoilage microorganisms and contribute to the desired sensory characters.

Even though the use of selected *Saccharomyces* strains provides better quality assurance in winemaking in comparison to the unknown microbial consortia in the must, it has been debated for a long time now whether the use of selected industrial *Saccharomyces* strains results in wines with less sensory complexity and "terroir" character.

In previous decades, non-*Saccharomyces* yeasts were mainly considered as spoilage/problematic yeast, since they exhibited low fermentation ability and other negative traits. In the last decades experiments have shown that there are some non-*Saccharomyces* strains (*Candida, Pichia, Kluyveromyces, Torulaspora*, etc) which, even though they are not able to complete the fermentation they can still be used in sequential inoculation-fermentation with *Saccharomyces* to increase sensory complexity of the wines.

Through fermentation in a laboratory scale, we have observed that the overall effects of selected *Torulaspora delbrueckii* yeast strains, is highly positive, leading to products with pronounced sensory complexity and floral/fruity aroma in winemaking and brewing.

KEY WORDS: wine, beer, fermentation, yeast, *Saccharomyces cerevisiae*, *Torulaspora delbrueckii*

INTRODUCTION

Most of the non-*Saccharomyces* yeast strains are considered as spoilage yeast due to low ethanol tolerance, low fermentation ability and other negative

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sensory traits, but some strains have been isolated from a variety of species (*Candida, Pichia, Kluyveromyces, Torulaspora*, etc.) that even though they are not able to complete the fermentation, they can still be used in sequential inoculation-fermentation with *Saccharomyces* to increase sensory complexity of the wines (J o l y et al., 2003a,b; 2006, C i a n i et al*.,* 2010). N i s s e n et al. (2003) found that early death of *Torulaspora delbrueckii* during mixed fermentations with *S. cerevisiae* was not due to the presence of ethanol or any other toxic compound but cell–cell contact-mediated mechanism. These non-*Saccharomyces* strains have been commercialized and at least 3 *T. delbrueckii s*trains are now available to the winemaking industry. *T. delbrueckii* strains have also been used traditionally in the production of German style wheat beers (Hefeweizen) for their banana, bubblegum and clove-like flavors. During wine fermentation, *T. delbrueckii* yeast strains produce noticeably higher concentration of higher alcohols, esters, terpenes and phenolic aldehydes as well as other molecules like 2-phenylethanol, linalool, methylvanillin (F a g a n et al., 1981; H e r r a i z et al*.,* 1990; L e m a et al*.,* 1996; K i n g et al*.,* 2000; P l a t a et al., 2003; R e y n a l et al., 2011), which impart a distinct floral and fruity aroma and add to the sensory complexity giving a "wild/natural" fermentation effect. *T. delbrueckii* strains, when compared to *S. cerevisiae* strains, generally exhibit osmotolerance (A l v e s – A r a u j o et al., 2007; B e l y et al*.,* 2008), higher demand for nitrogen and oxygen (V i s s e r et al., 1991; M a u r i c i o et al., 1998; H o l m H a n s e n et al., 2001; H a n l et al., 2005), lower production of volatile acidity, acetaldehyde and acetoin (especially in high gravity fermentations) and depending on the strain, low/medium glycerol, succinic acid, polysaccharides production, volatile thiols like 3-sulfanylhexan-1-ol and other compounds (B e l y et al., 2008; R e y n a l et al., 2001; J o l y et al., 2003a,b; 2006; R e n a u l t et al., 2009; C i a n i et al., 2010; Z o t t et al., 2011).

MATERIALS AND METHODS

Yeast strains: One strain of *T. delbrueckii* (Td28), two strains of *S. cerevisiae*, (Sc12 and Sc31) isolated from fermenting musts in Greece and 3 commercial *T. delbrueckii* strains, Level 2® (Lallemand), Zymaflore® Alpha (Laffort), Viniflora® Prelude™ (Hansen), as well as a commercial brewing yeast WB-06 by Fermentis, were used in this experiment.

Isolation and conservation media were: YEPD agar consisting of 10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose, 20 g/L agar-agar; Lysine agar consisting of 11.7 g/L yeast carbon base, 0.9 g/L L-Lysine, 20 g/L agar-agar; YM agar consisting of 3g/L malt extract, 5g/L peptone, 10 g/L glucose, 20 g/L agar-agar. The media were supplemented with 0.1 g/L chloramphenicol. Sterilization occurred at 121 ºC for 15 minutes.

Fermentation media for wine: for inoculums and fermentations, a synthetic must simulating the grape must composition (N a g a t a n i et al., 1968; S t r e h a i a n o et al., 1984) was used with the following composition: 1 g/L

yeast extract, 2 g/L $(NH_4)_2SO_4$, 0.4 g/L $MgSO_4.7H_2O$, 5 g/L KH_2PO_4, 50 g/L glucose (for inoculums) and 195 g/L glucose (for fermentations), 3 g/L tartaric acid, 3 g/L L-malic acid, 0.2 g/L citric acid, 2 g/L, pH was adjusted at 3.5 with 1N KOH. For sensory analysis a natural wine produced from fermented grape must of the Greek *Vitis vinifera* var. *Assyrtiko* was used, with the following composition: 204 g/L sugars, total acidity 6.1 g/L expressed as tartaric acid, yeast-assimilable nitrogen 243 mg/L, pH 3.24 and total sulfur dioxide of 35 mg/L. Inoculum $3*10^6$ cells/L, with viability over 96%.

Fermentation medium for brewing: the medium was reconstituted from malt extracts made from wheat and barley malts (dry unhopped extract "Spraymalt Wheat" and liquid "Connoisseurs Range" hopped extract for "Wheat Beer" from Muntons plc) with bottled chlorine-free water to an original gravity of 1.044, and fermentation was followed through weight loss. The inoculum ratio was 7g dry yeast/10 L, with viability over 96%.

Sterilization occurred at 121 ºC for 15 minutes. Media components were purchased as following: yeast extract from bioMerieux, Yeast carbon base, peptone and L-Lysine from Difco, and all other from Sigma-Aldrich.

Analyses for sugars, organic acids, ethanol and glycerol were performed with an ELITE LaChrom HPLC system comprised of a VWR HITATCH L-2130 pump, VWR HITATCH L-2200 autosampler fitted with 20 μL sample loop, and VWR HITATCH L-2455 Diode Array detector and RI detector. Peaks data were collected with Agilent EZChrom Elite Client/Server Enterprise Data System. The column was an Aminex HPX-87X from Biorad, the mobile phase was H_2SO_4 0,05N at 0.4 mL/min, with a column temperature of 40 ºC. Samples were treated for protein removal by mixing 8 parts of sample with 1 part $Ba(OH)_2$ 0.3N and 1 part 5 % $ZnSO_4$ solutions, left for 10 min at room temperature, centrifuged and sterile filtrated through 0.45 mm cellulose acetate filters (Sartorius).

Volatile substances were measured using 8500 Perkin Elmer Gas Chromatographer, with a Head Space Perkin Elmer 8500μ, with a Shimadzu integrator C-R3A using a silica SGE 25 AQ3/BP 20, 25m x0.33 mm column with 0.5 μm film thickness (T a t a r i d i s et al., 1998; T a t a r i d i s, 2001).

Yeast cell number was determined using a Thoma type haemocytometer and yeast cell viability using the methylene blue method by L a n g e et al., (1993). Yeast biomass was measured by dry weight and correlated with optical density measures (O.D.) at 620 nm.

Sensory analysis was conducted with a panel of 10 expert enologists and brewers. All experiments were conducted in triplicate with 1.8 L for synthetic must, 5L for grape must, and 3.5L for wort. Samples were taken and analyzed at regular intervals.

Statistical analysis for the percentage of error, standard error, standard deviation, variation coefficient and curve fitting (smoothing by spline functions) was conducted using Microsoft Excel (N e u i l l y and C e t a m a, 1998; R e i n c h, 1967).

RESULTS AND DISCUSSION

In synthetic must fermentation at 20 ºC (Figures 1, 2 and 3) there was a clear difference between the fermentation kinetics of *S. cerevisiae* strains Sc12, Sc31 and *T. delbrueckii* Td28. Sc12 was a rapid fermenting strain, Sc31 was a slow fermenting strain. Td28 was even slower fermenting strain than expected for *T. delbrueckii* strain, but it was able to complete the fermentation leaving no sugars, despite the popular belief that due to low alcohol tolerance *T. delbrueckii* strains are not capable of doing so. No lag phase was observed for the three strains.

Td28 cells were significantly smaller than *S. cerevisiae* cells, however the total dry biomass was higher for Td28 when compared to Sc31, but lower when compared to Sc12 (Table 1).

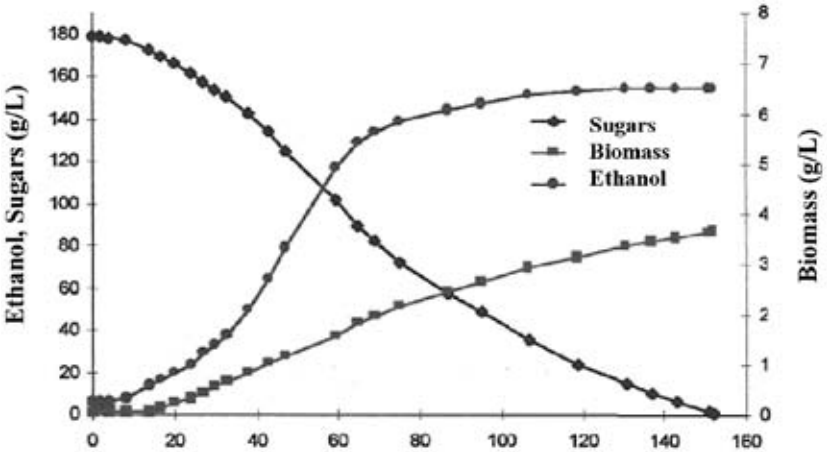

Fig. 1. – Fermentation kinetics of Sc12 at 20 ºC.

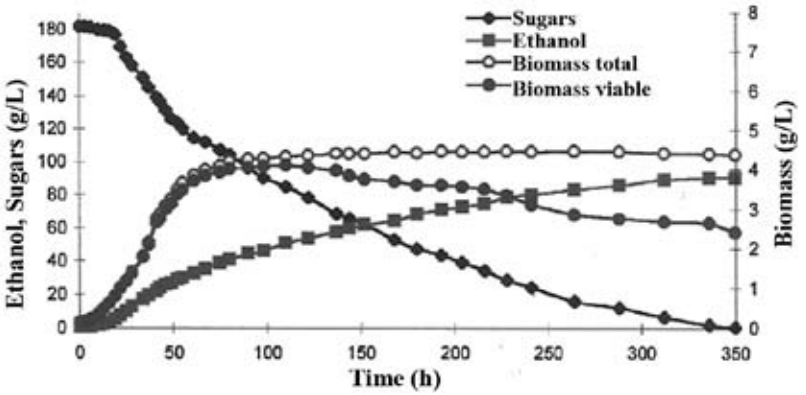

Fig. 2. – Fermentation kinetics of Sc31 at 20 ºC.

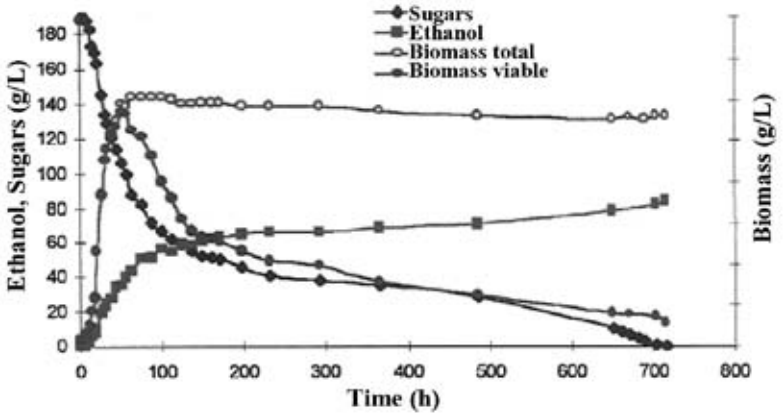

Fig. 3. – Fermentation kinetics of Td28 at 20 ºC.

Correlation between optical density (O.D.) at 620 nm and dry weight was calculated for each strain, from exponentially growing cells, after appropriate dilutions:

Sc12	dry biomass g/L=2.0045*(O.D.)+ 0.1962	(R^2=0.9883)
Sc31	dry biomass g/L=1.9739*(O.D.)+0.0942	(R^2=0.9928)
Td28	dry biomass g/L=1.4678*(O.D.)+0.1511	(R^2=0.9948)

Maximum total cell population was $118*10^6$ cells/mL for Sc12, $110*10^6$ cells/mL for Sc31, and $277*10^6$ cells/mL for Td28. At the end of the fermentation, dry biomass yield was higher for Sc12; however, at maximum biomass Td28 exhibited higher yield than the *S. cerevisiae* strains. Further experiment (data not shown) revealed that oxygen additions in the first stages of fermentation can improve *T. delbrueckii* growth, biomass yield and survival rate/viability. Ethanol production, final sugar concentration and final pH were similar for all strains, without significant differences. Ethanol yields were similar for the three strains, with *S. cerevisiae* strains having slightly higher values. Ethanol productivity, as well as sugar consumption rate, for Sc12 was significantly higher than those of Sc31 and Td28. Biomass productivity was higher for Td28, lower for Sc12 and even lower for Sc31. Maximum specific growth rate was higher for Td28 and lower for Sc12 and Sc31. Cell viability for Sc12 was over 96% throughout the fermentation, for Sc31 after the growth phase (maximum biomass) viability declined gradually to 60% at the end of the fermentation, and for Td28 the loss of viability was rapid, down to 10% at the end of the fermentation.

Tab. 1. – Kinetic characteristics (mean values) of fermentations at 20 ºC.

	Yeast strains		
	Sc12	Sc31	Td28
Initial sugars (g/L)	179±8.95	182.2±8.23	189.2±8.79
Final sugars (g/L)	1±0.45	0.25±0.49	0
Initial ethanol (g/L)	1±0.07	0.36±0.09	0.22±0.03
Final ethanol (g/L)	87.23±4.32	90.04±5.3	89.43±4.6
Ethanol (% vol)	11.05±0.32	11.41±0.45	11.33±0.51
Initial biomass (g/L)	0.25±0.013	0.14±0.017	0.17±0.02
Initial biomass (g/L)	6.51±0,035	4.38±0.041	5.69±0.09
Maximum population (cell/mL)	$118*10^6$±6.3%	$110*10^6$±6.5%	$277*10^6$±7.3%
Initial pH	3.5±0.05	3.43±0.05	3.45±0.05
Final pH	3±0.05	3.16±0.05	3.03±0.05
Biomass yield (g/g)	0.035 (end)	0.023 (end)	0.029 (end)
Biomass yield (g/g) at max biomass	0.048 (at 87 h)	0.052 (at 80 h)	0.058 (at 63 h)
Ethanol yield (g/g)	0.484	0.492	0.4715
Max specific growth rate (h^{-1})	0.065	0.1048	0.1435
Sugar consumption rate (g/L/h)	1.171	0.520	0.264
Ethanol productivity (g/L/h)	0.567	0.256	0.124
Biomass productivity (g/L/h)	0.067 (at 63 h)	0.051 (at 80 h)	0.093 (at 63 h)
Fermentation time (h)	152±6.3%	350±8.4%	717±9%

Means of triplicate fermentations ±SD or % of error

Glycerol production was higher for Sc31, followed by Sc12, with Td28 having lower concentration (Table 2). Volatile acidity (acetic acid) was slightly higher for Td28 than Sc31 and Sc12, just as the lactic acid concentration. However, Td28 had much higher (almost twice as much) production of succinic acid than the two *S. cerevisiae* strains (Table 2).

Acetaldehyde production was low for all strains, with Sc31 having less than the other two. Propanol-1 production was higher for Td28, isobutanol production was the same for Td28 and Sc12 and 25% higher for Sc31. Ethyl acetate concentrations for the three strains had no significant differences, and the sum of amyl alcohols was lower for Td28. However, the concentration of 2-phenyl ethanol with its distinctive rose-like aroma was significantly higher for Td28 than Sc31 (almost half) and Sc12 (almost a third) (Table 2). As *T. delbrueckii* strains are used in consecutive fermentation in winemaking, followed by inoculation by *S. cerevisiae,* in order to achieve fast fermentation completion, the fermentation with Td28 was also analyzed during the mid fermentation point in order to see which metabolite concentrations would be found. According to the data shown below, in the middle of the fermentation by Td28, acetic acid and ethyl acetate production was low, but organic acids and other metabolite production was high, even higher than that of the *S. cerevisiae* at the end of their fermentations (Table 2).

Tab. 2. – Fermentation products (mean values) at 20 ºC.

Concentration	Yeast strains			
	Sc12	Sc31	Td28	Td28 mid fermentation
Glycerol (g/L)	5.49±0.33	5.98±0.41	6.03±0.09	4.87±0.07
Acetic acid (g/L)	0.15±0.01	0.21±0.02	0.16±0.01	0.08±0.01
Lactic acid (g/L)	0.13±0.01	0.08±0.01	0.2±0.02	0.05±0.01
Succinic acid (g/L)	0.34±0.02	0.44±0.02	0.7±0.02	0.405±0.02
Acetaldehyde (mg/L)	9±0.71	5.3±0.84	10±0.89	7±0.63
Propanol-1 (mg/L)	32.5±4.01	35.4±3.72	55±4.15	76±3.89
Isobutanol (mg/L)	31±3.53	41.4±3.72	31±4.23	22.2±3.51
Ethyle acetate (mg/L)	45±4.32	43.5±3.91	41.5±4.00	29.2±3.67
Sum of Amyl alcohols (mg/L)	82.6±5.06	100±0.01±5.21	63±4.31	82.5±6.34
Phenylethanol* (mg/L)	3.6±0.27	5.9±0.8	9±0.04	not analyzed

* determined by direct injection
Means of triplicate fermentations ±SD

With regard to the sensory analysis of wines produced from *Assyrtiko* grapes, ten experienced enologists were asked to assess comparatively the wines produced with the three strains, using a scale from 1 (worst) to 10 (best) for 16 attributes in 4 major groups: Sight (color, viscosity, brilliance, depth), Nose (aroma, faults, variety, intensity), Palate (complexity, concentration, fruit, length), Finish (aftertaste, balance, tannin / phenolics, acid). As it is shown below (Figure 4) the wine produced with Td28 scored significantly higher averages for aroma, variety, intensity, complexity, fruit and acid. The panel concluded that the Td28 wine was more crisp and fresh, with higher flower/fruit aromas (Figure 4). *Assyrtiko* grape musts were also fermented by consecutive fermentation with each commercial *T. delbrueckii*, followed by *S. cerevisiae*, according to the manufacturer's recommendations, with results similar to Td28.

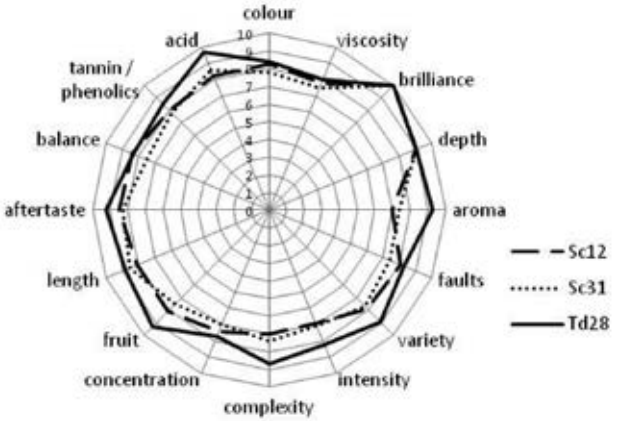

Fig. 4. – Average sensory notes for the wine produced by three strains Sc12, Sc31, Td28.

In brewing, almost all yeast strains that are used belong either to *S. cerevisiae* species (in case of ales-top fermenting yeast) or *S.pastorianus* (ex *uvarum*, ex *carlsbergenesis*) species (in case of pils/lagers-bottom fermenting yeast). There are some exceptions, the case of Lambics, were a consortium of indigenous yeast and bacteria from the air populate/contaminate the wort and are left to ferment naturally (or inoculated with commercial mixed cultures). Recently, some brewers have started using non-*Saccharomyces* (like Brettanomuces/ Dekkera strains), in order to obtain specific sensory characteristics. Even though *T. delbrueckii* is frequently mentioned on several web pages on the internet as a typical yeast used in the production of Bavarian style "weiss" (wheat) beers, we have not been able to find scientific references on their use in beer. The only references that could be found on them are related to them as spoilage yeast. Thus, we have undertaken the task of conducting some preliminary experiments on their use in brewing "wheat" style beers. Wheat beers are produced from wort that has been obtained using barley malts and a percentage of wheat, malted or unmalted.

Brewing was conducted at 20 ºC with wort reconstituted from liquid and dry malt extract specific for this beer type. Fermentation of 3.5 L batches with either Td28 *T. delbrueckii* strain or commercial WB-06 *S. cerevisiae* strain revealed that Td28 was able to ferment maltose (A l v e s – A r a u j o et al., 2007), but at a rate of 30% slower than with the WB-06 strain. Final gravity was high for both strains (Table 3). Maturation was conducted after the primary fermentation in capped beer bottles with the same yeast, at room temperature (25 ºC) for 7 days, followed by 14 days at 10 ºC.

Tab. 3. – Final gravity and primary fermentation duration at 20 ºC.

	Yeast strains	
	WB-06	**Td28**
Initial gravity	1.044	
Final gravity	1.009	1.012
Primary fermentation (h)	157.2	204.4

Td28 also exhibited slightly less sedimentation (giving beers with more typical wheat haze appearance) in comparison to WB-06 which was also considered as a low sedimentation strain. Still, further experiments are necessary for validation. Sensory analysis performed by expert brewers found (on a scale 1 to 10) that WB-06 strain was not a very fast strain (compared to other yeasts recommended for wheat beers previously used in our laboratory), but exhibited a subtle estery character and phenol flavor which were typical of wheat beers and also mentioned by the manufacturer. Td28 showed higher estery notes than WB-06 with rose, bubblegum and banana aromas, but lower phenol flavors. Buttery notes (diacetyl) varied considerably between the bottles, and the differences between the two yeasts were also high and they could not be properly quantified. The overall average note of the brewer's panel was

higher for the Td28 strain, thus demonstrating a potential for brewing wheat beers (Figure 5).

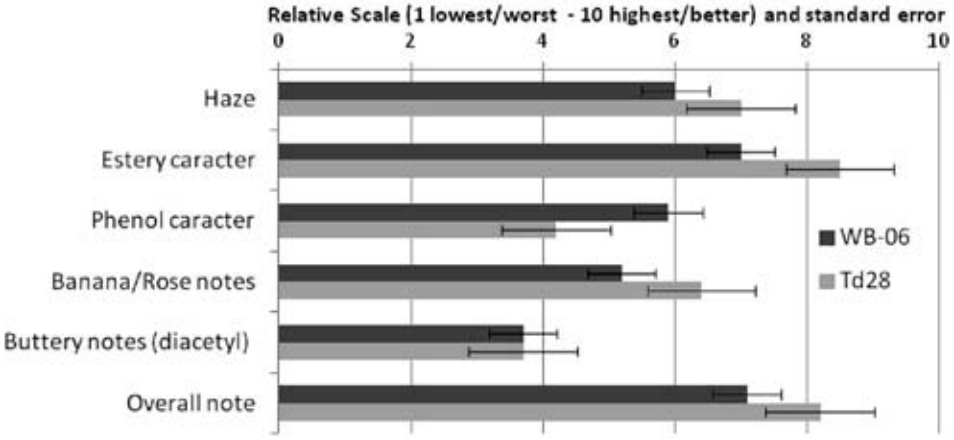

Fig. 5. – Comparison between wheat beers produced with WB-06 and Td28 strains at 20 ºC.

CONCLUSIONS

With respect to the wine production, results from laboratory scale fermentation showed that the *T. delbrueckii* Td28 strain was a slow fermenting strain. However, it has the ability to complete the process of wine fermentation twice or three times needed for *S. cerevisiae*. In addition, it is capable of higher production of organic acids, as well as 2-phenyl ethanol, acceptable production of acetic acid and glycerol. From a sensory point of view, the wines produced with Td28 retain high acidity and fresh character, while also having significantly higher sensory notes regarding the overall complexity and fresh flower and fruity aromas.

With regards to brewing, Td28 was able to consume maltose, which is the major sugar in wort, more slowly than the commercial *S. cerevisiae* strain WB-06. Td28 exhibited more pronounced ester character, complexity and intensity, but lower phenol character.

Although further experiments with more strains are necessary, the overall effects of selected *Torulaspora delbrueckii* yeast strains are highly positive, leading to pronounced sensory complexity and floral/fruity aroma in wine-making and brewing.

REFERENCES

A l v e s – A r a u j o, C., P a c h e c o, A., A l m e i d a, M.J., S p e n c e r – M a r t i n s, I., L e a o, C., S o u s a, M.J. (2007): Sugar utilization patterns and respiro-fermentative metabolism in the baker's yeast *Torulaspora delbrueckii*. Microbiol. 153:898–904.

B e l y, M., S t o e c k l e, P., M a s n e u f – P o m a r è d e, I., D u b o u r d i e u, D. (2008): Impact of mixed *Torulaspora delbrueckii–Saccharomyces cerevisiae* culture on high-sugar fermentation. Int. J. Food Microbiol. 122:312–320.

C i a n i, M., C o m i t i n i, F., M a n n a z z u, I., D o m i z i o, P. (2010): Controlled mixed culture fermentation: a new perspective on the use of non-*Saccharomyces* yeasts in wine-making. FEMS Yeast Res. 10:123–133.

C i a n i, M., M a c c a r e l l i, F. (1998): Oenological properties of non-*Saccharomyces* yeasts associated with wine-making. World J. Microbiol. Biotechnol. 14:199–203.

F a g a n, G.L., K e p n e r, R.E., W e b b, A.D. (1981): Production of linalool, cis-nerolidol and trans-nerolidol, and trans, trans-farnesol by *Saccharomyces fermentati* growing as a film on simulated wine. Vitis 20:36-42.

H a n l, L., S o m m e r, P., A r n e b o r g, N. (2005): The effect of decreasing oxygen feed rates on growth and metabolism of *Torulaspora delbrueckii*. Appl. Microbiol. Biotechnol. 67(1):113-118.

H e r r a i z, T., R e g l e r o, G., H e r r a i z, M., M a r t i n – A l v a r e z, P.J., C a b e z u d o, M. D. (1990): The influence of the yeast and type of culture on the volatile composition of wines fermented without sulfur dioxide. Am. J. Enol. Vitic. 41:313–318.

H o l m H a n s e n, E., N i s s e n, P., S o m m e r, P., N i e l s e n, J. C., A r n e b o r g, N. (2001): The effect of oxygen on the survival of non-*Saccharomyces* yeasts during mixed culture fermentations of grape juice with *Saccharomyces cerevisiae*. J. Appl. Microbiol. 91:541-547.

J o l l y, N.P., A u g u s t y n, O.P.H., P r e t o r i u s, I.S. (2003a): The occurrence of non-*Saccharomyces* yeast species over three vintages in four vineyards and grape musts from four production regions of the Western Cape, South Africa. S. Afr. J. Enol. Vitic. 24:35-42.

J o l l y, N.P., A u g u s t y n, O.P.H., P r e t o r i u s, I.S. (2003b): The Effect of Non-*Saccharomyces Yeasts* on Fermentation and Wine Quality. S. Afr. J. Enol. Vitic. 24(2):55-62.

J o l l y, N.P., A u g u s t y n, O.P.H., P r e t o r i u s, I.S. (2006): The Role and Use of Non-*Saccharomyces* Yeasts in Wine Production. S. Afr. J. Enol. Vitic. 27(1):15-39.

K i n g, A. and D i c k i n s o n, J.R. (2000): Biotransformation of monoterpene alcohols by *Saccharomyces cerevisiae, Torulaspora delbrueckii* and *Kluyveromyces lactis.* Yeast 16: 499-506.

L a n g e, H., B a v o u z e t, J.M., T a i l a n d i e r, P., D e l o r m e, C. (1993): Systematic error and comparison of four methods for assessing viability of *Saccharomyces cerevisiae* suspension. Biotechnol. Techni. 7:223-228.

L e m a, C., G a r c i a – J a r e s, C., O r r i o l s, I., A n g u l o, L. (1996): Contribution of *Saccharomyces* and non-*Saccharomyces* populations to the production of some components of Albarino wine aroma. Am. J. Enol. Vitic. 47:206–216.

M a u r i c i o, J.C., M i l l a n, C., O r t e g a, J.M. (1998): Influence of oxygen on the biosynthesis of cellular fatty acids, sterols and phospholipids during alcoholic fermentation by

Saccharomyces cerevisiae and *Torulaspora delbrueckii*. W. J. Microbiol. Biotechnol. 14:405-410.

N a g a t a n i, M., S h o d a, M., A i d a, S. (1968): Kinetics of product inhibition in alcoholic fermentation. J. Ferm. Tech. 46:241-248.

N e u i l l y, M. and C e t a m a (1999): Modeling and estimation of measurement errors. Ed. Lavoisier Tec&Doc, Paris.

N i s s e n, P., N i e l s e n, D., A r n e b o r g, N. (2003): *Saccharomyces cerevisiae* cells at high concentrations cause early growth arrest of non-*Saccharomyces* yeasts in mixed cultures by a cell-cell contact-mediated mechanism. Yeast 20:331-341.

P l a t a, C., M i l l a n, C., M a u r i c i o, J.C., O r t e g a, J.M. (2003): Formation of ethyl acetate and isoamyl acetate by various species of wine yeasts. Food Microbiol. 20:217–224.

R a y n a l, C., W a r d r o p, F., P i l l e t, O., A m o s, J., L a n g u e t, P., D u m o n t, A., O r t i z J u l i e n, A. (2011): An innovative tool for winemakers sequential inoculation with a non-*Sacchoromyces* yeast and a *Saccharomyces cerevisiae* yeast Wine Vitic. J. 1:22-31.

R e i n s c h, S. (1967): Numerische Matematik 10:177-183 (Ge).

R e n a u l t, P., M i o t – S e r t i e r, C., M a r u l l o, P., H e r n á n d e z – O r t e, P., L a g a r r i g u e, L., L o n v a u d – F u n e l, A., B e l y, M. (2009): Genetic characterization and phenotypic variability in *Torulaspora delbrueckii* species: Potential applications in the wine industry. Int. J. Food Microbiol. 134:201–210.

S t r e h a i a n o, P., U r i b e l a r r e a, J.L., M o t a, M., G o m a, G. (1985): Observation sur les mechanisms d'inhibition en fermentation alcoolique. Rev. Franc. D' Oenol. Cahier Scientifique 97 (Fr).

T a t a r i d i s, P.**,** T a i l l a n d i e r, P., S t r e h a i a n o, P., L a n a r i d i s, P. (1998): Αλκοολική ζύμωση από διάφορα είδη ζυμών προερχόμενα από οινοποιήσεις της Σαντορίνης και του Αμυνταίου: κινητική και χαρακτηρισμός. 2° Πανελλήνιο Συνέδριο Οινολογίας, Ένωση Ελλήνων Οινολόγων. 8-10 Μαΐου. Τόμος Α, ΕΚΕΦΕ ΔΗΜΟΚΡΙΤΟΣ, Αθήνα, 119-126, (Gr).

T a t a r i d i s, P. (2001): Etude des interaction entre micro-organisme du vin: du qualitative au quantitative. Doctoral dissertation. Institut National Polytechnique de Toulouse, Ecole Nationale Supérieure des Ingénieurs en Arts Chimiques Et Technologiques, Toulouse (Fr).

V i s s e r, W., S c h e f f e r s, W. A., B a t e n b u r g – v a n d e r V e g t e, W. H., v a n D i j k e n, J. P. (1991): Oxygen requirements of yeasts. Appl. Environ. Microbiol. 56(12):3785-92.

Z o t t, K., T h i b o n, C., B e l y, M., L o n v a u d – F u n e l, A., D u b o u r d i e u, D., M a s n e u f – P o m a r e d e, I. (2011): The grape must non-*Saccharomyces* microbial community: Impact on volatile thiol release. Int. J. Food Microbiol. 151:210–215.

КОРИШЋЕЊЕ NE-SACCHAROMYCES СОЈЕВА КВАСЦА *TORULASPORA DELBRUECKII* У ПРОИЗВОДЊИ ВИНА И СЛАДА

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Резиме

Одабрани сојеви квасца *Saccharomyces* се више од 150 година користе у производњи слада а у винарству неколико деценија. Ови сојеви су неопходни у производњи пива и слада због кувања сладовине, током које долази до уништавања свих ћелија квасаца са изузетком неких врста пива у белгијском стилу (нпр. Ламбиц) код којих се сладовина оставља да се колонизује нативним квасцима и бактеријама из околине и на тај начин природно ферментише. У производњи вина, примена *Saccharomyces* сојева је стална јер обезбеђује правилну и уредну ферментацију, спречава раст нативних (дивљих) микроорганизама изазивача кварења и доприноси жељеним сензорским карактеристикама вина.

Иако коришћење одабраних *Saccharomyces* сојева обезбеђује сигурније очување квалитета у производњи вина у односу на непознату, дивљу микрофлору у шири, већ дуже време се расправља о томе да ли коришћење индустријских *Saccharomyces* сојева има за последицу вина слабије сензорске комплексности и карактера који мање зависи од климатских услова, локалитета и земљишта (тероар).

У претходним деценијама, ne-Saccharomyces квасци су сматрани проблематичнима и изазивачима кварења јер су показивали слабију ферментациону способност и друге нежељене особине. Током последњих деценија, показано је да неки ne-Saccharomyces родови попут *Candida, Pichia, Kluyveromyces, Torulaspora*, иако не могу да комплетирају ферментацију, могу да се користе у поступку секвенционалне инокулације-ферментације заједно са *Saccharomyces* квасцем и доприносе сензорској комплексности вина.

Током ферментације у лабораторијским условима, уочили смо да је укупан ефекат одабраних квасних сојева *Torulaspora delbrueckii* веома задовољавајући и доприноси добијању вина и пива изражене сензорске комплексности и цветно/воћне ароме.

КЉУЧНЕ РЕЧИ: вино, пиво, ферментација, квасац, *Saccharomyces cerevisiae*, *Torulaspora delbrueckii*