Torulaspora delbrueckii Yeast Strains for Small-scale Chenin blanc and Pinotage Vinifications

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Nine *Torulaspora delbrueckii* yeast strains, a commercial *T. delbrueckii* strain and a commercial *Saccharomyces cerevisiae* yeast strain were used in the production of small-scale Chenin blanc and Pinotage vinifications. The fermentations were carried out at 15°C and 24°C respectively. Four *T. delbrueckii* yeasts were used as single inoculants, while the remainder were inoculated sequentially. The commercial *S. cerevisiae* yeast strains were added at zero, 24 and 48 hours after the *T. delbrueckii* strain. The wines were evaluated chemically and sensorially and the data was analysed statistically. The results for the white wine vinification trial showed that two *T. delbrueckii* treatments could produce novel wines, either on their own or as a component of co-inoculated fermentations. These compared well with, and even exceeded, the quality of wine produced by the *S. cerevisiae* reference treatment regarding chemical composition and overall sensory quality. One *T. delbrueckii* strain showed its robustness by being re-isolated from the yeast lees at the end of fermentation. The red wine vinifications were less conclusive, and no distinctive *T. delbrueckii* "fingerprint" was observed in the chemical and sensory data, neither was a pattern observed regarding the different inoculation times.

INTRODUCTION

Modern wine production relies on selected pure yeast strains with desired qualities as starter cultures (Lema *et al*., 1996; Egli *et al*., 1998; Pretorius, 2000). This gives the winemaker better control over the fermentation process, and a predictable outcome (Degré, 1993; Henick-Kling *et al.*, 1998). The alternative method, of spontaneous fermentation, in which the fermentation is driven by the yeast naturally present in grape must, carries a higher risk of an undesirable outcome. The yeasts responsible for spontaneous fermentations come either directly from the grapes, or are transferred from the processing equipment (Pretorius, 2000). Despite the high risks and unpredictability associated with spontaneous fermentations, many winemakers prefer this method. This is partially due to the desirable effects on wine flavour, complexity and distinct vintage variability caused by the many non-*Saccharomyces* yeasts present on the grapes and in the crushed grape must (Lambrechts & Pretorius, 2000; Romano *et al*., 2003; Jolly *et al*., 2014; Padilla *et al*., 2016). As much as 90% to 100% of the total population of indigenous yeasts are non-*Saccharomyces* yeasts. These yeasts are capable of initiating alcoholic fermentation (Fleet & Heard, 1993), and the predominant genera can include *Hanseniaspora* (*Kloeckera*)*, Dekkera* (*Brettanomyces*), *Cryptoccocus, Metschnikowia* (*Candida*)*, Debaryomyces, Lachancea* (*Kluyveromyces*)*, Pichia* (*Candida*)*, Rhodotorula, Saccharomycodes, Schizosaccharomyces, Torulaspora* (*Candida*)*, Lindnera* (*Williopsis*) and *Zygosaccharomyces* (Erten & Campbell, 2001; Holm Hansen *et al*., 2001; Van Keulen *et al*., 2003; Deák, 2007; Ciani & Comitini, 2011; Kurtzman *et al*., 2011; Jolly *et al*., 2014; Alonso *et al*., 2015; Renault *et al*., 2016; Benito *et al*., 2017; Domizio *et al*., 2017).

In contrast to earlier studies, which showed that non-*Saccharomyces* yeasts only dominated during the early stages of wine fermentation, with *S. cerevisiae* completing the fermentation (Amerine & Kunkee, 1969), more recent studies have shown that non-*Saccharomyces* yeasts survive at significant levels and for longer periods, thereby influencing the chemical and sensory profiles of the wine (Moreno *et al*., 1991; Ciani & Maccarelli, 1998; Granchi *et al*., 1998; Farías *et al*., 2003; Fleet, 2003; Combina *et al*., 2005; Ciani & Comitini, 2011; Jolly *et al*., 2014). It has

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further been shown that some non-*Saccharomyces* species greatly improve the quality and sensorial properties of wine (Loureiro & Malfeito-Ferreira, 2003; Hornsey, 2007; Jolly *et al*., 2003a; 2003b; Benito *et al*., 2016; Padilla *et al*., 2016; Renault *et al*., 2016; Benito *et al*., 2017).

Metabolic, chemical and sensory profile studies of non-*Saccharomyces* yeast fermentations have shown that *T. delbrueckii* species in particular make a positive contribution toward the flavour of alcoholic beverages (Ciani & Picciotti, 1995; Ciani & Maccarelli, 1998; Jolly *et al*., 2003a; Van Breda *et al*., 2013; Belda *et al*., 2015; Loira *et al*., 2015; Renault *et al*., 2015; Chen & Liu, 2016; Renault *et al*., 2016; Ngqumba *et al*., 2017). This was further demonstrated in a study conducted by Hernández-Orte *et al*. (2008), in which wines produced with *T. delbrueckii* had high concentrations of aliphatic lactones and ethyl dihydrocinnamate, imparting sensory notes such as "dried fruit" and "coconut" to the wine. Compounds such as linalool, β-phenylethanol and β-phenylethyl acetate, which impart "rose" notes, were also detected. Studies conducted by Bely *et al*. (2008) showed that *T. delbrueckii* had high fermentation purity by producing very low volatile acidity (acetic acid and ethyl acetate) and acetaldehyde levels. In addition, this species also produced low quantities of undesirable compounds such as acetoin.

In recent years, selected non-*Saccharomyces* wine yeasts have been commercially dried, or have been included as part of mixed active dried starter cultures with *S. cerevisiae* (Comitini *et al*., 2011; Jolly *et al*., 2014; Azzolini *et al*., 2015). This gives the winemaker the beneficial effects of spontaneous fermentation without the accompanying risks (Romano *et al*., 2003; Ciani *et al*., 2006).

The aim of this study was to investigate the potential of nine *T. delbrueckii* yeast isolates used singly or co-inoculated with *S. cerevisiae* for the production of small-scale white and red vinifications to produce wine with lower alcohol concentrations, while at the same time maintaining or improving the wine quality.

MATERIALS AND METHODS

Yeast strains

Nine *T. delbrueckii* strains (654, M2/1, 301, 704, 206, M2/15, M2/19, M2/27 and M2B/27) from the ARC Infruitec-Nietvoorbij culture collection, one isolate (TdH) from a commercial *T. delbrueckii* yeast blend (Viniflora® Harmony. nsac, CHR Hansen, Denmark) and one *S. cerevisiae* reference strain (VIN 13, Anchor Bio-Technologies, South Africa) were selected for the production of white and red wines. The *T. delbrueckii* strain selection was based on data from a laboratory-scale study (Van Breda *et al*., 2013). All yeasts were maintained on yeast peptone dextrose agar (YPD agar, Biolab, Merck South Africa) slants and stored at 4°C until required.

Yeast inoculum propagation

A three-phase propagation procedure was used to grow the yeast inocula: 10 mL of YPD broth (Biolab, Merck, South Africa) at 30°C for 24 hours; 100 mL of YPD broth incubated at 30°C for eight hours on an orbital shaker (RO20, Gerhardt); and 900 mL of YPD broth inoculated with the preceding 110 mL culture at room temperature (± 20°C) overnight on a Gyrotory® shaker (G10, New Brunswick Scientific).

Small-scale white and red vinifications

A white grape variety, Chenin blanc (22.15°B, 7 g/L total acidity (TA), pH 3.42), and a red grape variety, Pinotage (25.1°B, 6.3 g/L TA, pH 3.38), were used for small-scale vinifications. All fermentations were performed in duplicate.

A standardised white wine production method was followed, according to which the Chenin blanc grapes were crushed, the juice and skins were pressed at 1 Bar and a sedimentation enzyme (0.50 g/L, Ultrazym, Novozymes, Switzerland) and SO_2 (50 mg/L) were added. The clarified Chenin blanc must was dispensed into stainless steel containers (18 L per 20 L container) fitted with fermentation caps. Seventeen fermentation treatments were initiated, comprising single yeast inoculations and co-inoculations. Three *T. delbrueckii* yeast isolates (strains 654, 301 and M2/1) and the commercial *T. delbrueckii* yeast strain (TdH) were inoculated individually. Four *T. delbrueckii* yeast isolates (strains 206, 704, M2/15 and M2/27) were co-inoculated with the *S. cerevisiae* reference strain. For the co-inoculated fermentations, *T. delbrueckii* was inoculated at zero hours, followed by inoculation with the *S. cerevisiae* reference strain at zero hours, 24 and 48 hours respectively. A reference sample inoculated with *S. cerevisiae* only was also included. Di-ammonium phosphate (0.50 g/L) was added as a source of nitrogen. The fermentations were conducted at 15°C and monitored by measuring CO_2 weight loss. The fermentations were allowed to continue until the wines were dry.

The red wine production method was also standardised as described by Minnaar *et al*. (2015). Four *T. delbrueckii* yeast isolates (strains 654, 206, 301 and M2/1) were inoculated individually. The remaining three *T. delbrueckii* yeast isolates (strains 704, M2/19 and M2B/27) and the commercial *T. delbrueckii* yeast strain (TdH) were inoculated in combination with the *S. cerevisiae* reference strain. The timing strategy for the co-inoculated fermentation was the same as that for Chenin blanc, and a *S. cerevisiae* reference fermentation was also included. Residual sugar analyses were performed on all wines to confirm the end of fermentation. After bottling, the wines were stored at 15°C until required for sensory evaluation and chemical analyses.

Isolation and identification of yeast from white wine lees

After racking, lees samples were taken from the stainless steel fermentation containers of the single-inoculant Chenin blanc wines and preserved cryogenically in glycerol at -80°C. These lees samples were inoculated (5 µL) into D-mannitol broth (4 g mannitol, 1.34 g yeast nitrogen base, Merck, South Africa) and incubated at room temperature (± 20°C) for five to seven days. Once sufficient growth was observed, 10 µL of the D-mannitol broth was streaked out onto lysine medium (Biolab, Merck, South Africa) and incubated at room temperature (± 20°C) for five days. Single colonies were then further purified by streaking onto YPD agar (Biolab, Merck, South Africa) plates, followed by incubation at 30°C for three days.

The BioMeriéux identification system (ID32C,

BioMeriéux, South Africa) with manual reading was used to identify the colonies according to the manufacturer's instructions. The identities supplied by the apiweb™ identification software were used as a presumptive identification. The identification was confirmed by CHEF gel electrophoresis, as described in Van Breda *et al*. (2013).

Chemical analyses and sensory evaluation

The wines were analysed for alcohol, volatile acidity (VA) (Koelenhof Wynlaboratorium, South Africa), glycerol (Winescan, Institute for Wine Biotechnology, Stellenbosch University), reducing sugar (Rebelein method) and SO_2 (Ripper method). The wet chemistry methods were according to the methods prescribed by the South African Wine Laboratories Association (Anonymous, 2002). Five months after bottling, two different panels of 13 expert wine tasters sensorially evaluated all the wines. The wines were presented to the tasters based on a randomised block design, and descriptive sensory analysis was performed by scoring each sample on a 10 cm unstructured line scale. The descriptors used were: "fruity and fermentation aroma", "guava aroma", "body" and "general quality" for Chenin blanc, and "berry/cherry/plum aroma", "body" and "general quality" for Pinotage.

Statistical analysis

The experimental design was a completely randomised design. Data (chemical and sensory) were subjected to analysis of variance (ANOVA) using PROC GLM of the SAS® software (Version 9.2; SAS Institute Inc., Cary, USA). A Shapiro–Wilk test was used to verify the normality of the standardised residuals of the variables (Shapiro & Wilk, 1965). Fisher's least significant difference was calculated at the 5% level to compare treatment (yeast) means (Ott & Longnecker, 2001). A probability level of 5% was considered significant for all significance tests. Principal component analysis (PCA), using the correlation matrix, was performed on the sensory and chemical data separately by XLSTAT (version 2015.1.03.15485, Addinsoft, New York, USA) to examine the relationships among and between the variables and observations.

RESULTS AND DISCUSSION

In comparison to *S. cerevisiae*, the deliberate use of non-*Saccharomyces* yeast in wine production is in its relative infancy. While an estimated 150 to 200 commercial *S. cerevisiae* yeasts are available to the wine industries worldwide, there are only a limited number of commercial *T. delbrueckii* strains (Jolly *et al*., 2014). These strains are all recommended for use as co-inoculants, usually with a specific *S. cerevisiae* strain. Although the number of commercial *T. delbrueckii* strains will probably never reach that of *S. cerevisiae*, there is undoubtedly scope for strains with improved oenological characteristics (Velázquez *et al*., 2015). The selection of yeasts used for this study was based on their individual performance and fermentation characteristics during a laboratory-scale investigation (Van Breda *et al*., 2013). It was found that considerable variation occurred in the fermentation characteristics of the *T. delbrueckii* strains investigated. While most were unable to completely utilise all the grape sugar, some had the potential to complete the fermentation as a single inoculant under the conditions tested. The length of the fermentations ranged from 14 to 32 days (Van Breda *et al*., 2013), which, for the faster fermenting yeasts, is comparable to other published work, where fermentations were found to take between 14 and 24 days (Belda *et al*., 2015; Loira *et al*., 2015). Consequently, the choice of strains for this investigation included weaker fermenters for co-inoculation, and the more vigorous fermenters for single inoculations.

The choice of the internationally lesser known grape varieties, Chenin blanc and Pinotage, for the investigation was made due to these two varieties being of economic and traditional importance to the South African wine industry. Chenin blanc is the most widely planted cultivar and is used to make a variety of wine styles and products. Pinotage, a hybrid between Pinot noir and Cinsaut, was bred in South Africa and is therefore considered a true South African variety. Both varieties deliver wines of varying quality, so an enhancement of wine quality is often sought.

Small-scale vinification: Chenin blanc

From the fermentation curves of the small-scale vinifications, it can be seen that all the co-inoculated fermentations (Groups 1 and 2) fermented faster (14 days) that the single *T. delbrueckii* inoculant fermentations (Group 3) (32 days) (Fig. 1). The duration of these co-inoculated fermentations is similar to that in other studies, which took between 12 and 24 days, but the single-inoculant group took much longer (24 vs. 32 days) (Azzolini *et al*., 2015; Belda *et al*., 2015). The zero-hour co-inoculations fermented at the same rate (slope of logarithmic growth phase) as the *S. cerevisiae* reference yeast. The 24-hour co-inoculations fermented for an intermediate length of time, and the 48-hour fermentations were the slowest. The single-inoculated fermentations also had a notably longer lag phase than the co-inoculated fermentations. This was expected, as it is known that the *T. delbrueckii* yeasts are slower fermenters and take longer than *S. cerevisiae* to acclimatise to the conditions of the grape must (Bely *et al*., 2008; Van Breda *et al*., 2013). Longer lag phases have also been linked to a positive association with the fruitiness and complexity of wine (Albertin *et al*., 2017). Three of the *T. delbrueckii* single fermentations (strains 301, 654 and M2/1) appeared to ferment at a similar rate and finished within 32 days, only slightly longer than the commercial *T. delbrueckii* yeast (TdH).

All the wines fermented to dryness (sugar ≤ 5 g/L), in accordance with South African legislation, with one exception that was very close to dryness (5.45 g/L) (Table 1). The low sugars in the co-inoculated wines can be ascribed to the *S. cerevisiae* yeast completing the fermentation. The *T. delbrueckii* single-inoculant wines had, as was expected, the highest residual sugar, ranging from 2.60 to 5.45 g/L, indicating that the *T. delbrueckii* single-inoculant strategy could lead to complete fermentations, as also shown in the laboratory-scale trials using a sterile must (Van Breda *et al*., 2013). However, as the trials in this study were conducted in a non-sterile must, a possible background *S. cerevisiae* yeast population could have played a role. The alcohol levels of the various treatments were very similar, and a one-way ANOVA

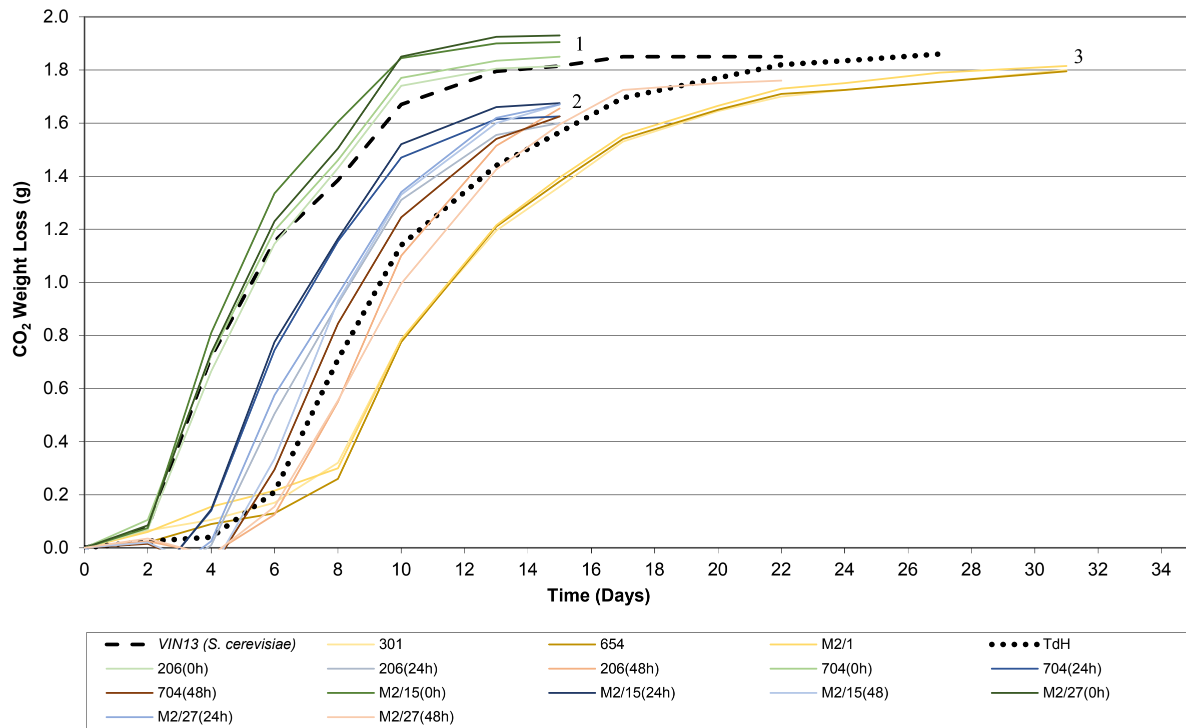

FIGURE 1

Average fermentation curves of duplicate small-scale Chenin blanc wines fermented at 15°C. The co-inoculated fermentations are indicated in the legend by the time of *Saccharomyces cerevisiae*, strain VIN 13 inoculation (Group 1 = 0h [zero hours]; Group 2 = 24h [24 hours after start]; Group 3 = 48h [48 hours after start]).

TABLE 1

Chemical profiles of Chenin blanc wines produced in small-scale fermentations at 15°C[1]

	Chemical analyses					
Yeast strains[2]	**Residual sugar (g/L)**	**Alcohol (%, v/v)**	**Glycerol (g/L)**	**Total SO_2 (mg/L)**	**Volatile acidity (g/L)**	**Total acidity (g/L)**
VIN 13 (*S. cerevisiae* reference)	1.00 ± 1.41	13.31 ± 0.04	5.54 ± 0.21	114.05 ± 1.63	0.25 ± 0.01	5.35 ± 0.06
Harmony (commercial reference)	2.60 ± 2.55	12.99 ± 0.40	6.67 ± 0.21	145.80 ± 6.08	0.35 ± 0.06	5.73 ± 0.13
301	5.45 ± 0.07	13.04 ± 0.00	6.93 ± 0.17	121.05 ± 10.68	0.37 ± 0.00	5.61 ± 0.02
654	4.85 ± 0.21	12.78 ± 0.39	6.41 ± 0.69	167.20 ± 6.08	0.35 ± 0.04	5.70 ± 0.20
M2/1	4.85 ± 0.78	13.07 ± 0.03	6.85 ± 0.29	156.25 ± 20.86	0.36 ± 0.01	5.59 ± 0.02
206 (0 h)	1.60 ± 0.00	13.28 ± 0.01	5.56 ± 0.22	137.85 ± 5.44	0.26 ± 0.03	5.27 ± 0.02
206 (24 h)	0.75 ± 0.07	13.24 ± 0.02	6.18 ± 0.09	105.40 ± 4.81	0.27 ± 0.01	5.42 ± 0.03
206 (48 h)	2.39 ± 0.42	13.16 ± 0.02	6.28 ± 0.06	131.71 ± 2.05	0.31 ± 0.03	5.49 ± 0.13
704 (0 h)	0.75 ± 0.21	13.24 ± 0.01	5.33 ± 0.07	128.35 ± 1.34	0.28 ± 0.01	5.27 ± 0.01
704 (24 h)	0.95 ± 0.07	13.28 ± 0.04	5.69 ± 0.06	112.45 ± 1.91	0.27 ± 0.01	5.35 ± 0.00
704 (48 h)	0.90 ± 0.00	13.23 ± 0.03	5.94 ± 0.63	120.85 ± 3.61	0.29 ± 0.04	5.42 ± 0.14
M2/15 (0 h)	1.05 ± 0.07	13.15 ± 0.06	5.57 ± 0.35	124.35 ± 14.92	0.29 ± 0.03	5.26 ± 0.02
M2/15 (24 h)	0.95 ± 0.92	13.15 ± 0.01	5.71 ± 0.09	127.75 ± 5.02	0.28 ± 0.01	5.36 ± 0.01
M2/15 (48 h)	1.00 ± 0.00	13.13 ± 0.06	6.67 ± 0.53	117.60 ± 4.53	0.37 ± 0.01	5.75 ± 0.16
M2/27 (0 h)	1.05 ± 0.07	13.22 ± 0.04	5.37 ± 0.09	113.75 ± 3.32	0.28 ± 0.01	5.27 ± 0.01
M2/27 (24 h)	1.20 ± 0.28	13.20 ± 0.04	6.01 ± 0.21	120.45 ± 4.45	0.27 ± 0.03	5.44 ± 0.05
M2/27 (48 h)	0.20 ± 0.28	13.30 ± 0.04	5.82 ± 0.04	109.95 ± 3.18	0.27 ± 0.01	5.51 ± 0.01

[1] Means ± standard deviation (n = 2)

[2] Yeast strains used and time of co-inoculation with *S. cerevisiae*

performed on the alcohol data showed that the differences were not significant (results not shown). The alcohol levels were only lower where less sugar was consumed, showing that these *T. delbrueckii* isolates are not suitable as a means for lowering the alcohol content of the wine. This is in contrast to other published works where, *T. delbrueckii* vinifications produced between 0.14% and 0.47% lower alcohol levels than reference *S. cerevisiae* fermentations (Azzolini *et al*., 2015; Belda *et al*., 2015; Renault *et al*., 2015).

Glycerol produced by the single-inoculant fermentations was found to be slightly higher than in the co-inoculated wines and the *S. cerevisiae* reference fermentation, which is similar to what has been observed in other trials (Jolly *et al*., 2003a; Belda *et al*., 2015). The exception was the treatment of M2/15 (48 h), which was in the same range as the single inoculants (Table 1). Higher glycerol production has previously been shown for non-*Saccharomyces* yeasts (García *et al*., 2010; Romani *et al*., 2010; Loira, *et al*., 2015) and can contribute to improved mouthfeel, sweetness and complexity in wines (Ciani & Maccarelli, 1998; Loira *et al*., 2015).

Total SO_2 levels for the single-inoculant fermentations were higher than those of the co-inoculated fermentations and the *S. cerevisiae* reference fermentation (Table 1). This trait has previously been shown for single *T. delbrueckii* fermentations (Jolly *et al*., 2003a). High SO_2 is not desirable, as this can negatively affect wine quality and inhibit subsequent malolactic fermentation by sensitive lactic acid bacteria (Lerm *et al*., 2010). Although the SO_2 levels were higher for the single-inoculant wines, all except one fell well within the legal limits for South African wine standards (< 160 mg/L) (South African Liquor Products Act 60 of 1989) (Anonymous, 1989).

The volatile acidity produced by the single-inoculant fermentations was similar to that of the wines produced by the co-inoculated fermentations and slightly higher than the *S. cerevisiae* reference fermentation (Table 1). However, all the values fell within the legal limit for South African wines (≤ 1.2 g/L) (Anonymous, 1989). The higher values were not expected, as it has been mostly reported that *T. delbrueckii* strains generally produce lower levels of volatile acidity (Lafon-Lafourcade, 1983; Bely *et al*., 2008; Renault *et al*. 2009; Azzolini *et at*., 2015). However, some reports show higher levels of acetic acid (major component of volatile acidity) for single-inoculant *T. delbrueckii* wines compared to reference *S. cerevisiae* wines (Belda *et al*., 2015).

The TA values for both single and co-inoculated fermentations (5.27 to 5.76 g/L) showed no notable differences. However, this varied from previously published work, in which TA levels for *T. delbrueckii*-only fermentations were lower than those of *S. cerevisiae* and mixed fermentations (Belda *et al*., 2015). However, it is generally accepted that yeasts do not affect tartaric acid levels (the most abundant acid in grapes).

The PCA of the sensory data (Fig. 2) showed that the co-inoculated treatments grouped together with the *S. cerevisiae* reference treatment. These were clearly separated from the single inoculation treatments, which appeared on the far right of the plot. The PCA analyses of the standard wine chemical data (Fig. 3) showed a similar grouping, confirming the different profiles obtained with the single-inoculation treatments compared to the co-inoculation and *S. cerevisiae* treatments.

The sensory descriptors “fruity and fermentation”, “guava” and “body” were most prominent in the single-inoculant small-scale wines produced from the *T. delbrueckii* yeast isolates (strains 654 and M2/1) (Fig. 2). The PCA results for the sensory evaluation were complemented by the ANOVA results (Table 2), which showed that the wine made from yeast strain 654 often scored significantly higher

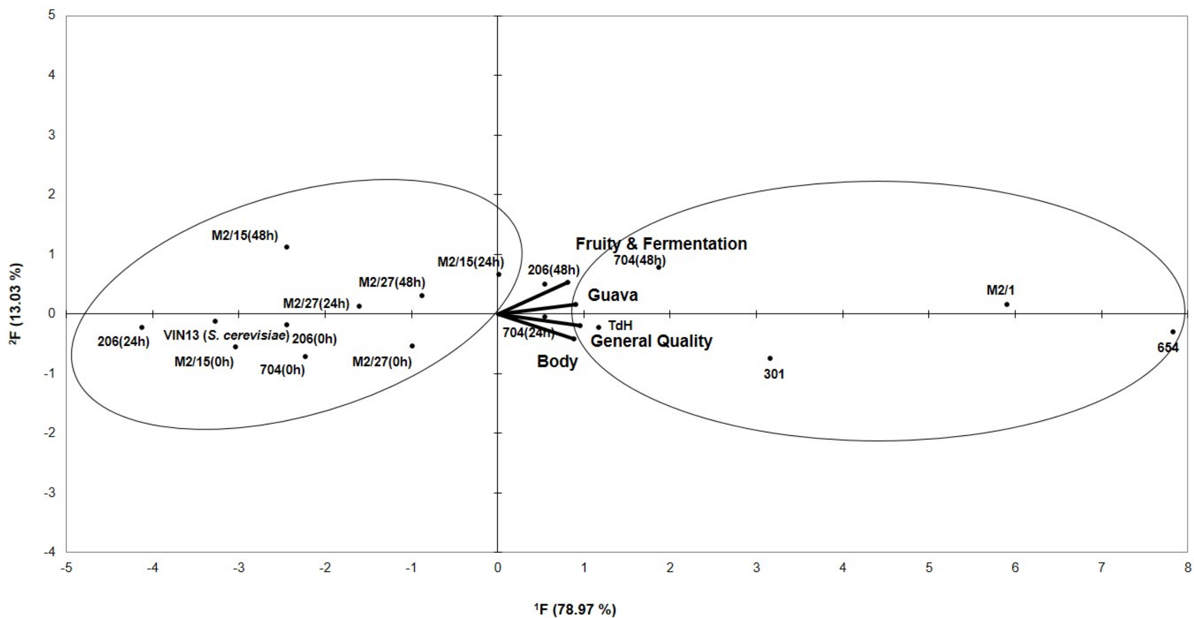

FIGURE 2

Principal component analysis of the average sensory analysis data (n = 2) for Chenin blanc wines.

[F1] First principal component; [F2] Second principal component

TABLE 2
Sensory analyses of Chenin blanc wines produced in small-scale fermentations with single *Torulaspora delbrueckii* inocula or co-inoculated with *Saccharomyces cerevisiae* at different times.

Yeast strain[1]	Fruity & fermentation	Guava	Body	General quality
VIN 13 (*S. cerevisiae* reference)	46cd[2]	33bc	44de	43c
Harmony (Commercial *T. delbrueckii*)	52abcd	35abc	51bcd	49bc
301	50abcd	38abc	53abc	54ab
654	57ab	43a	58a	58a
M2/1	58a	40ab	54ab	56a
206 (0 h)	47bcd	33bc	45de	45c
206 (24 h)	46cd	30c	43de	44c
206 (48 h)	54abcd	35abc	46d	49bc
704 (0 h)	45d	33bc	48bcd	45c
704 (24 h)	50abcd	37abc	48bcd	48bc
704 (48 h)	57ab	37abc	49bcd	47c
M2/15 (0 h)	46cd	31bc	47cd	44c
M2/15 (24 h)	55abc	34abc	46de	47c
M2/15 (48 h)	51abcd	36abc	39e	43c
M2/27 (0 h)	46cd	35abc	47cd	48bc
M2/27 (24 h)	48bcd	36abc	45de	44c
M2/27 (48 h)	49abcd	37abc	44de	46c

[1] Yeast strains used and time of co-inoculation with *S. cerevisiae*
[2] Values within columns followed by the same letter do not differ significantly ($p > 0.05$)

in terms of its "guava" aroma note, "body" (mouthfeel) and "general quality". Wines produced from the *T. delbrueckii* strain M2/1 treatment also had high scores and were often judged to be significantly better than the other wines in terms of the "fruity and fermentation" character and "general quality" (Table 2). These results correspond to the findings of previous studies, which found that non-*Saccharomyces* yeasts contributed to mouthfeel and improved the quality of wines (Ciani & Picciotti, 1995; Ciani & Maccarelli, 1998; Minnaar *et al*., 2015; Ngqumba *et al*., 2017). The results also support the findings of Albertin *et al*. (2017) on the already mentioned link between non-*Saccharomyces* yeasts, the duration of the *S. cerevisiae* lag phase and the wine's fruitiness and complexity. The reference *S. cerevisiae* wine had amongst the lowest sensory scores of all the wines (Table 2).

Small-scale vinification: Pinotage

The Pinotage fermentations were all completed within five days. Of note is that, in contrast to the Chenin blanc wines, all the *T. delbrueckii* single-inoculated treatments fermented dry (under 4 g/L residual sugar) (Table 3). However, the contribution by the *S. cerevisiae* natural population cannot be discounted (the red wine production process is more susceptible to contamination by resident *S. cerevisiae* cellar populations). *T. delbrueckii* contributed to a considerably higher total SO_2 in the white wines, while this was not evident in the red wines. The results of the analyses of the other routine wine parameters were very similar to the *S. cerevisiae* reference wine. The sensory results showed no notable differences in the wines, especially in "general quality" and "body" (Table 4), as was also observed in the white wine trial. The PCA of the sensory and chemical data (Figs 4 and 5) showed no trends regarding inoculation times or single vs. co-inoculations. This therefore reinforces the observation that *T. delbrueckii* may have been dominated by the growth of the natural *S. cerevisiae* background resident population.

Isolation and identification of yeast from wine lees

Wine lees samples of the single-inoculant treatments were inoculated into D-mannitol broth to enrich for, and isolate, *T. delbrueckii* yeasts, since *S. cerevisiae* is unable to utilise mannitol (Kurtzman, 2011; Vaughan-Martini & Martini, 2011). The use of lysine agar served as a further selective medium for non-*Saccharomyces* yeast, and colonies differing in appearance were observed, i.e. white and cream. The white colonies gave the desired identification of *Candida colliculosa* (anamorph of *T. delbrueckii*) with a 99.9% probability. However, *C. colliculosa* could only be isolated from the lees of the wine made from yeast strain 654, and not from the other single-inoculant wines.

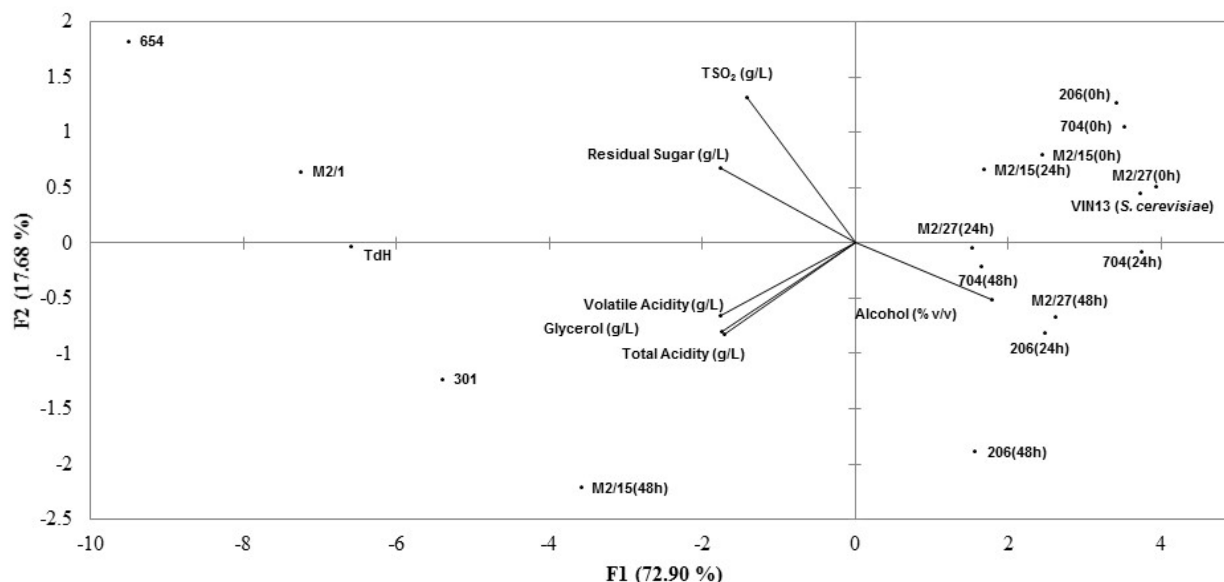

FIGURE 3
PCA bi-plot of chemical analyses (n = 2) of Chenin blanc wines, illustrating the yeasts that group together for specific compounds produced. [F1] First principal component; [F2] Second principal component

TABLE 3
Average data of chemical analyses of Pinotage wines produced in small-scale fermentations with different combinations of yeast at 24°C[1]

	Chemical analyses					
Yeast strain[2]	**Residual sugar (g/L)**	**Alcohol (%, v/v)**	**Glycerol (g/L)**	**Total SO_2 (mg/L)**	**Volatile acidity (g/L)**	**Total acidity (g/L)**
VIN 13 (*S. cerevisiae* reference)[3]	2.00 ± 0.49	15.10 ± 0.35	10.30 ± 0.25	79.00 ± 4.24	0.36 ± 0.04	5.64 ± 0.08
654	2.20 ± 0.13	15.10 ± 0.01	9.36 ± 0.08	76.00 ± 5.66	0.38 ± 0.01	5.57 ± 0.13
M2/1	5.00 ± 0.03	14.78 ± 0.16	8.87 ± 0.27	78.00 ± 1.41	0.38 ± 0.00	5.60 ± 0.01
206	6.40 ± 0.04	14.87 ± 0.01	9.40 ± 0.06	80.00 ± 8.49	0.38 ± 0.03	5.65 ± 0.06
301	4.00 ± 0.20	14.84 ± 0.02	9.00 ± 0.15	72.50 ± 10.61	0.40 ± 0.01	5.63 ± 0.01
TdH (0 h)	2.40 ± 0.45	15.22 ± 0.25	9.80 ± 0.10	86.50 ± 2.83	0.40 ± 0.02	5.61 ± 0.05
TdH (24 h)	4.60 ± 0.20	14.72 ± 0.01	9.60 ± 0.18	86.00 ± 7.07	0.36 ± 0.01	5.74 ± 0.06
TdH (48 h)	6.40 ± 0.28	14.84 ± 0.19	9.73 ± 0.06	84.00 ± 1.41	0.36 ± 0.04	5.76 ± 0.01
704 (0 h)	6.00 ± 0.10	14.81 ± 0.33	9.39 ± 0.31	63.00 ± 0.71	0.36 ± 0.01	5.59 ± 0.02
704 (24 h)	6.40 ± 0.04	15.22 ± 0.08	10.11 ± 0.11	67.00 ± 1.41	0.36 ± 0.01	5.64 ± 0.03
704 (48 h)	1.80 ± 0.40	14.90 ± 0.11	9.70 ± 0.25	71.00 ± 0.00	0.36 ± 0.02	5.71 ± 0.04
M2/19 (0 h)	4.80 ± 0.48	14.74 ± 0.04	9.48 ± 0.20	58.00 ± 19.80	0.37 ± 0.04	5.58 ± 0.06
M2/19 (24 h)	3.60 ± 0.78	14.45 ± 0.09	9.70 ± 0.05	61.00 ± 28.28	0.36 ± 0.03	5.75 ± 0.00
M2/19 (48 h)	5.00 ± 0.04	14.81 ± 0.16	9.96 ± 0.54	52.00 ± 24.04	0.32 ± 0.04	5.82 ± 0.02
M2B/27 (0 h)	4.80 ± 1.24	14.69 ± 0.01	9.76 ± 0.16	80.00[3]	0.41 ± 0.06	5.66 ± 0.01
M2B/27 (24 h)	3.40 ± 0.06	14.49 ± 0.06	9.80 ± 0.09	76.00 ± 4.24	0.36 ± 0.03	5.76 ± 0.06
M2B/27 (48 h)	4.80 ± 0.47	14.29 ± 0.19	9.75 ± 0.10	65.00 ± 8.49	0.36 ± 0.01	5.78 ± 0.11

[1] Means ± standard deviation (n = 2).
[2] Yeast strains used. Time of co-inoculation with *S. cerevisiae* indicated in brackets.
[3] Only one sample.

Six of the re-isolated *C. colliculosa* colonies were subjected to CHEF gel electrophoresis to compare them to the profile of the yeast initially inoculated at the start of the fermentation. The chromosomal banding patterns of three of the yeasts matched (lanes 6 to 8 in Fig. 6), confirming that the inoculated strain 654 was responsible for these fermentations. Three isolates appeared similar, but had an extra band (as indicated by the arrows in Fig. 6). This could possibly be due to the selected *T. delbrueckii* colonies being yeasts that were naturally present in the must. Despite the failure to isolate yeasts from the other single-inoculant treatments, the various PCA analyses of the chemical

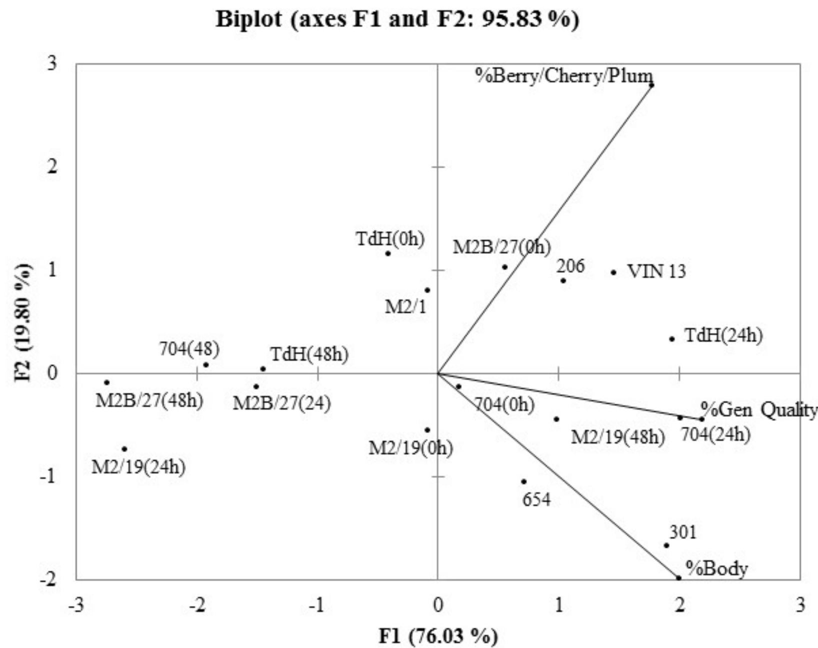

FIGURE 4
Principal component analysis of the average sensory analysis data (n = 2) for Pinotage wines.
F1 First principal component; F2 Second principal component

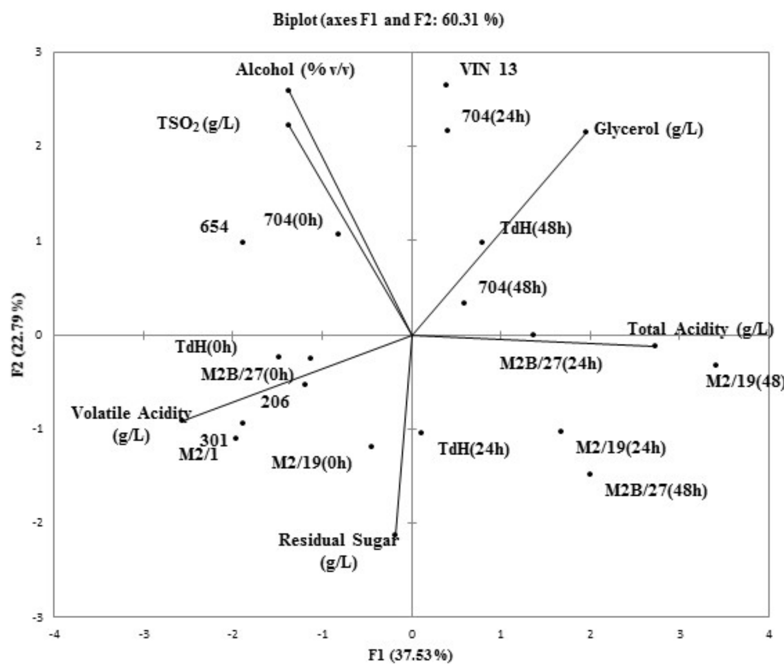

FIGURE 5
Principal component analysis of the average chemical analysis data (n = 2) for Pinotage wines.
F1 First principal component; F2 Second principal component

TABLE 4
Sensory analyses of Pinotage wines produced in small-scale fermentations with different combinations of yeast.

Yeast strain[1]	Berry/cherry/ plum aroma intensity	Body	General quality
VIN 13 (*S. cerevisiae* references)	61a[2]	54a	52ab
654	51abcd	56a	52ab
M2/1	56abc	50a	49ab
206	58ab	52a	53ab
301	51abcd	59a	56a
704 & VIN 13 (0 h)	53abcd	54a	49ab
704 & VIN 13 (24 h)	56abc	57a	56a
704 & VIN 13 (48 h)	48cd	47a	47ab
Td Harmony & VIN 13 (0 h)	56abc	48a	50ab
Td Harmony & VIN 13 (24 h)	58ab	55a	55a
Td Harmony & VIN 13 (48 h)	49bcd	48a	48ab
M2/19 & VIN 13 (0 h)	50abcd	53a	50ab
M2/19 & VIN 13 (24 h)	44d	49a	43b
M2/19 & VIN 13 (48 h)	54abcd	56a	52ab
M2B/27 & VIN 13 (0 h)	59ab	53a	49ab
M2B/27 & VIN 13 (24 h)	49bcd	49a	46ab
M2B/27 & VIN 13 (48 h)	46cd	48a	42b

[1] Yeast strains used and time of co-inoculation with *S. cerevisiae*
[2] Values within columns followed by the same letter do not differ significantly ($p < 0.05$)

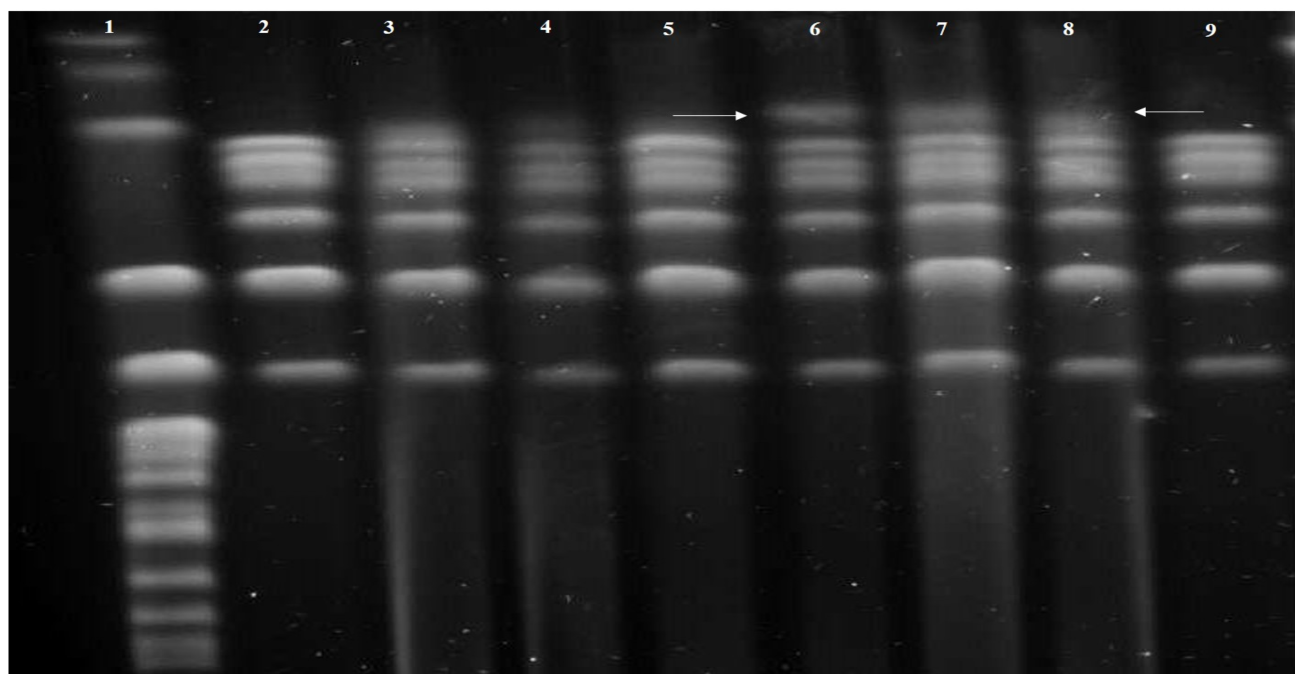

FIGURE 6
CHEF DNA profiles of the *Torulaspora delbrueckii* isolates in comparison to those isolated from the lees. Lanes 1: VIN 13 reference; Lanes 2 - 8: Seven isolates from lees 654; Lane 9: 654 mother culture (control). The extra bands are indicated by the white arrows.

and sensory data, as previously shown, indicate that the *T. delbrueckii* strains were actively involved in the Chenin blanc fermentations, and their "fingerprint" is evident. The same cannot be concluded for the Pinotage treatments, where no *T. delbrueckii* "fingerprint" is evident, and in all probability the treatments were dominated by the natural *S. cerevisiae* populations. *T. delbrueckii* inhibition by *S. cerevisiae* has been shown by other authors (Taillandier *et al*., 2014; Albergaria & Arneborg, 2016; Wang *et al*., 2016).

CONCLUSIONS

The various co-inoculation treatments all led to dry wines, and a *T. delbrueckii* chemical and sensory profile fingerprint was evident in the white wines. A similar fingerprint was not observed in the red wines. From the results obtained in this study, two out of the nine *T. delbrueckii* yeast strains (i.e. strains 654 and M2/1) appeared to show potential as single-inoculant yeasts in commercial white wine production. Strain 654 is the more robust of the two, as it could be re-isolated from the yeast lees after fermentation, confirming its activity to the end of the fermentation. However, the protocol used in the investigation was not successful in lowering the wine alcohol content significantly.

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