Journal of Microbiological Methods 165 (2019) 105699

Contents lists available at ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

Review

Characterisation of hybrid yeasts for the production of varietal Sauvignon blanc wine – A review

Rodney Sebastian Hart[a,b,*,1], Neil Paul Jolly[a], Bongani Kaiser Ndimba[a,b]

[a] Agricultural Research Council (ARC) Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch 7599, South Africa
[b] Department of Biotechnology, National Agricultural Proteomics Research, Services Unit (NAPRSU), University of the Western Cape, Private Bag X17, Bellville 7535, South Africa

ARTICLE INFO

Keywords:
CHEF
Hybrid yeast
MALDI-TOF MS
Wine organoleptic quality
Thiols
Enzymes

ABSTRACT

The wine yeast *Saccharomyces cerevisiae* forms an integral part of wine production by converting relatively 'neutral' flavoured grape must into varietal aromatic wines. Additionally, non-*Saccharomyces* strains can be used with *S. cerevisiae* for the production of wines with more complexity. Yeast strains, to varying extents, produce and/or mediate the release of a whole range of key metabolites, which in turn contribute to enhanced aroma and flavour of the final wine, especially Sauvignon blanc. These metabolites viz. thiols are dependent on yeast-expressed enzymes during fermentation. Inoculation with an appropriate yeast will, therefore, lead to more commercial wine sales due to resultant wines with sought-after aroma and flavour. Likewise, inoculation with the incorrect yeast will have a negative effect on sales. It is also important to have quality control measures in place to ensure that the inoculated yeast strain quickly dominate, and is present throughout the fermentation process. Traditionally, the laborious contour clamped homogeneous electric field (CHEF) DNA karyotyping technique was shown to be reliable in this regard, however cutting-edge matrix-assisted laser desorption/ionisation-time of flight mass spectrometry (MALDI-TOF MS) biotyping is proving to be a faster alternative. As both methods have advantages and disadvantages, they should be used in complementary as opposed to competitively. Standard chemical and descriptive sensory analyses of wine also serve as evaluation and/or characterisation tools of yeast starter cultures. Additionally, metabolomic and proteomic profiling using gas chromatography (GC) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) of final wines was shown to be instrumental yeast evaluation tools. Therefore, this review highlights the importance and practicality of a more inclusive approach to evaluate and characterise novel yeast strains used for winemaking by deploying traditional and modern chemical and organoleptic evaluation techniques of wines in conjunction with cutting-edge omics approaches towards enhancing white wine varietal aroma.

1. Introduction

The production of varietal wines with enhanced aromatic characteristics from relatively 'neutral' flavoured grape must (juice) is relatively easily achieved by deploying the wine yeast *Saccharomyces cerevisiae* (Swiegers et al., 2009; King et al., 2011; Erten et al., 2006). Subsequently, final wine aroma and flavour can be attributed to the yeast starter culture's metabolism during alcoholic fermentation (Nedović et al., 2015). *Saccharomyces cerevisiae* is, therefore, important for the production of varietal aromatic white wines from grapes of non-aromatic cultivars such as Sauvignon blanc (Lambrechts and Pretorius, 2000; Cadière et al., 2012). Wine yeasts vary in their ability to develop the full aroma potential of especially Sauvignon blanc, due to differences in their ability to release bound volatile aromatic metabolites, an important contributor to the tropical fruit and floral aromas expected in these wines (Pinu et al., 2012; Van Wyngaard, 2013; Von Mollendorf, 2013). However, the presence of naturally occurring *S. cerevisiae* on wine grapes is negligible compared to other ubiquitous non-*Saccharomyces* genera *e.g. Cryptococcus* spp., *Rhodotorula* spp. *Sporobolomyces* spp., *Candida* spp., *Hanseniaspora* spp., *Metschnikowia* spp., *Pichia* spp., *Zygosaccharomyces* spp., and *Torulaspora* spp. (Cray et al., 2013; Jolly et al., 2014). This observation, therefore, highlights the vigour of *S. cerevisiae* to be the dominant yeast strain at the end of spontaneous fermentation and so be responsible for the organoleptic quality and/or profile of the final wine (Álvarez-Pérez et al., 2014). Furthermore, the organoleptic profile of wines produced by means of

* Corresponding author at: ARC Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch 7599, South Africa.
E-mail address: hartr@arc.agric.za (R.S. Hart).
[1] Present address: ARC Infruitec-Nietvoorbij, Old Klapmuts Road, Stellenbosch, South Africa.

https://doi.org/10.1016/j.mimet.2019.105699
Received 3 July 2019; Received in revised form 15 August 2019; Accepted 21 August 2019
Available online 22 August 2019

spontaneous fermentation varies between fermentations. For this reason, commercial *S. cerevisiae* starter cultures are used to ensure consistent results.

The aforementioned non-*Saccharomyces* spp. nonetheless are still being explored for the production of wine with more complexity (Fleet, 2003; Ocón et al., 2010). However, the use of non-*Saccharomyces* commercial yeast starter cultures still requires co-fermentation with an *S. cerevisiae* strain to complete fermentation, as most non-*Saccharomyces* strains are not able to completely ferment grape must due to lower alcohol tolerance (Wang et al., 2016). As mentioned above, spontaneous fermentations then again have shortcomings as resultant wine chemical and sensory profiles are unpredictable, and in some instances less preferred than wines produced solely with S. cerevisiae strains (Navarrete-Bolaños, 2012; Velázquez et al., 2015). Furthermore, spontaneous fermentations also open the doors for spoilage organisms (e.g. Brettanomyces) to proliferate, resulting in wines with undesirable metabolites (e.g. 4-ethylphenol, acetic acid, etc.). Van Breda et al. (2013) did, however, report that some non-*Saccharomyces* spp. produced wine with similar chemical and sensory quality compared to wines produced with *S. cerevisiae* strains renowned for producing wine with good organoleptic properties. Subsequently, non-*Saccharomyces* (*viz. Torulaspora delbrueckii*) yeast starter cultures became commercially available and proved useful to enhance wine complexity.

The use of a non-*Saccharomyces* starter culture, namely *T. delbrueckii* was shown to be advantageous as it resulted in wines with lower volatile acidity (VA) levels, and thereby enhance final wine organoleptic quality (Jolly et al., 2014). This yeast was also shown to produce wine with enhanced varietal aromas as a single inoculum or in conjunction with *S. cerevisiae* (Van Breda et al., 2013; Belda et al., 2015; Renault et al., 2016). Therefore, *T. delbrueckii* is also a good candidate to be utilised in a breeding program to develop new yeast strains for the production of aromatic white wines with lower VA, a yeast criterion reported by Hart et al. (2016). Likewise, an *S. cerevisiae/T. delbrueckii* inter-genus hybrid was also reported to have a positive effect on wine flavour due to its metabolic activity (Santos et al., 2008). Indications, therefore, are that *T. delbrueckii* in conjunction with *S. cerevisiae* has a role to play in the production of varietal aromatic white wines with low VA. As *S. cerevisiae* intra-genus and *T. delbrueckii* inter-genus hybrids bred through classical mating/breeding (Fig. 1) for the commercial production of aromatic wines were previously identified as an industry priority, the ARC Infruitec-Nietvoorbij microbiology-group initiated a new hybrid breeding program that saw the development of *S. cerevisiae/T. delbrueckii* inter-genus hybrids.

Yeast strains were also reported to produce a whole range of metabolites during fermentation, referred to as its metabolome, which includes monosaccharide sugars, organic acids, fatty acids, amino acids, volatile thiols (imparts tropical fruit aromas and flavour), esters (imparts fruity aroma and flavour), and higher alcohols which contribute to the organoleptic characteristics of the final wine (Varela, 2016). Wine can, therefore, be seen as the 'metabolic footprint' of the yeast strain that carried out the fermentation (Chambers et al., 2009). Wine yeast, however can also produce undesirable metabolites (*e.g.* acetic acid and/or VA) which impart unpleasant vinegar-like off-flavours (Vilela-Moura et al., 2010; Luo et al., 2013) which will affect wine organoleptic negatively.

It is noteworthy that yeast derived and mediated metabolites (aroma and flavour enhancing compounds) are dependent on enzymes expressed by yeasts during fermentation (Holt et al., 2011). As a result, different yeast strains will produce wines with different sensory properties, since expressed proteins will influence metabolite release responsible for final wine organoleptic quality (Moreno-García et al., 2015). Moreover, different yeast strains were also observed to express proteins differentially even though they were cultivated in the same growth medium (Abrahams et al., 2017), hence the association between wine yeast expressed and/or released proteins and metabolites present at the end of fermentation, and their effect on Sauvignon blanc wine chemical and sensory profiles will be discussed. Therefore, the objective of this review is to encourage a paradigm shift by emphasising the importance and practicality of a more inclusive approach to breed, characterise and evaluate novel yeast strains by deploying traditional and modern chemical and organoleptic evaluation techniques of white wines in conjunction with cutting-edge metabolomic and proteomic analytical approaches towards enhancing white wine varietal aroma.

2. Wine yeast evaluation and/or characterisation

The use of commercial wine yeast starter cultures, *S. cerevisiae*, in particular, has revolutionised earlier methods of winemaking by allowing winemakers to consistently produce wines with comparable organoleptic properties (Mateo et al., 2001; Meersman et al., 2015; Pretorius, 2016). The wine yeast *S. cerevisiae,* whether natural isolates or hybrids has unsurpassed fermentation ability when compared to yeasts from other genera due to *viz*. higher alcohol tolerance. Yeast starter cultures commercially available undergo a thorough evaluation that involves fermentation trials, classical and molecular characterisation prior to commercialisation and/or release (García et al., 2012; Bonciani et al., 2016). Currently, enologists and/or wine technologists have a large variety of molecular tools at their disposal for the rapid characterisation of food-related microbes viz. wine yeast strains (Ivey and Phister, 2011).

Wine organoleptic quality varies based on the yeast starter culture used to conduct the fermentation as reported (Usbeck et al., 2014). Furthermore, some wine microorganisms are implicated in spoilage of final wine, resulting in financial loss (Loureiro and Malfeito-Ferreira, 2003; Bartowsky, 2009). Differentiation of yeast strains during winemaking, therefore, is of utmost importance as final wine organoleptic quality is dependent on the yeast starter culture (Sharma et al., 2012). Inoculation with the incorrect starter culture can result in wine with undesirable chemical parameters and sensory flavours, which in turn will have undesirable financial implications. Similarly, inoculation with a starter culture resulting in wines with sought-after aroma and flavour is always lucrative, since millions of litres of wines are annually produced and sold on a commercial scale in South Africa (SAWIS, 2015). South Africa produced a staggering 10.5. and 10.8 million hectolitres of wine during the 2016 and 2017 vintages, respectively (International Organisation of Vine and Wine (OIV), 2018). International wine production during the 2017 vintage was estimated to be 250 million hectolitres, with South Africa being the eight highest contributor behind Italy, France, Spain, United States of America, Australia, Argentina, and China. Some of these wines are also exported. It is also noteworthy, that yeast manufacturers benefit from increased commercial sales of yeast starter cultures, especially those exhibiting characteristics to produce sought-after varietal aromatic wines (M. Fundira, Personal communication, 2015). Therefore, a reliable and fast differentiation method is a useful tool to ensure the correct identification of microorganisms during the production of varietal wines (Chovanová et al., 2011; Panda et al., 2015).

Moreover, chemical and sensory analyses of final ferments (wine) are routinely conducted and fundamentally serve as a means of evaluating and characterising experimental yeast strains used for wine production (Ezeama and Ebia, 2015; Hart et al., 2016). Coupled to this are metabolomic and proteomic characterisation, as yeast-expressed proteins during fermentation are responsible for the release and mediation of sought-after and undesirable metabolites, which in turn have an effect on wine organoleptic quality as, previously reported (Moreno-García et al., 2015). Previously mentioned yeast evaluation and/or characterisation tools will further be discussed.

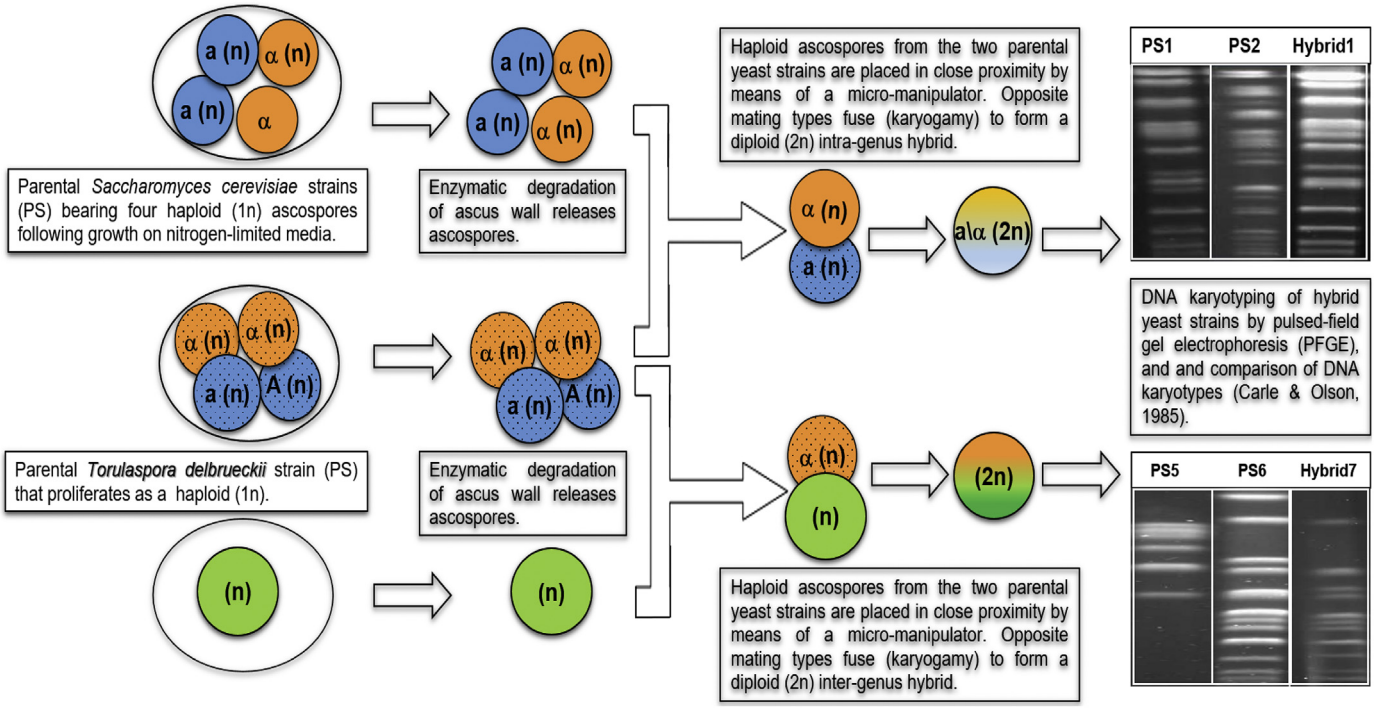


Fig. 1. Schematic depiction of inter-genus (inter-genus) hybrid yeast breeding between *Saccharomyces cerevisiae* and *Torulospora delbrueckii* by deploying the protoplast fusion technique (Hart, 2018, PhD dissertation).

2.1. Molecular characterisation

2.1.1. Contour-clamped homogeneous electric field (CHEF) DNA karyotyping

Pulsed-field gel electrophoresis (PFGE), a popular molecular-based tool to characterise wine yeast has been used for decades for the evaluation of experimental yeast strains (Van der Westhuizen and Pretorius, 1992). Contour-clamped homogeneous electric field (CHEF) DNA karyotyping, a variation of PFGE is a reliable technique to distinguish between yeast strains based on chromosomal DNA karyotypes (Hoff, 2012). The CHEF system comprises electrodes that are hexagonally arranged (Carle and Olson, 1985; Parizad et al., 2016). Subsequently, DNA stationed in an agarose gel is separated by sequentially alternating the electric field between electrodes based on pre-programmed pulse times (Fig. 2). Subsequently, smaller and larger DNA is efficiently separated as the latter requires more time to re-orientate and migrate every time the electric field is alternated, whilst the reverse applies to the former.

Other gel electrophoresis-based yeast differentiation tools that involves alteration of the electric field during separation of DNA include orthogonal field alternation gel electrophoresis (OFAGE), field inversion gel electrophoresis (FIGE), and transverse alternating field electrophoresis (TAFE), programmable autonomously controlled gel electrophoresis (PACE), pulsed homogeneous orthogonal field gel electrophoresis (PHOFE) and rotating gel electrophoresis (REG) (De Jonge et al., 1986). However, CHEF DNA karyotyping was successfully deployed to differentiate closely related yeasts to ensure that the correct yeast strain is used to inoculate and complete the fermentation (Hage and Houseley, 2013). Subsequently, a positive association between final wine organoleptic quality and yeast starter culture/s could be established. It is noteworthy that CHEF DNA karyotyping is a lengthy process, which requires up to 8 days to yield the identity of a pure culture (Van Breda et al., 2013).

2.1.2. Polymerase chain reaction (PCR) based characterisation

Polymerase chain reaction (PCR) amplification of targeted genes is a faster molecular characterisation tool compared to CHEF DNA karyotyping (Hoff, 2012; Colabella and Libkind, 2016). However, PCR of *e.g.* internal transcribed spacers (ITS) was repeatedly shown to have a limitation in differentiating closely related strains as rDNA amplicons every so often have similar sizes that can't be resolved by gel electrophoresis (Hierro et al., 2004). A relatively old technique *i.e.* restriction fragment length polymorphism (RFLP) of PCR amplification products is a fast method to determine yeast population dynamics during wine fermentation (Chial, 2008). Nevertheless, this approach cannot differentiate between closely related strains, especially in the absence of sequence data. Even though cloning can be used to bypass this limitation, it is not recommended due to the fact that it can be a lengthy process, as genotypes or species mixtures with two or more restriction patterns within the same profile first need to be purified and cloned into an appropriate plasmid vector prior to isolation and sequencing of plasmid DNA. Sequencing of PCR amplicons can be done relatively fast, however, this exercise can be time-consuming due to sequencing facilities running thousands of samples a day (Vaudano and Garcia-Moruno, 2008).

Variations of the PCR technique *i.e.*, random amplified polymorphic DNA (RAPDs), amplified fragment length polymorphism (AFLPs) and simple sequence repeats (SSRs) are frequently used to study population dynamics (Chial, 2008). A major advantage of RAPDs is that it does not require knowledge of the target genome sequence. However, the low-specificity PCR conditions used for RAPDs does not yield reproducible results. The AFLP approach contrariwise yields reproducible results, due to the stringent PCR annealing temperatures. A major drawback of AFLP is that it requires high molecular weight purified DNA. The use of SSRs is more advantageous in terms differentiating different yeast strains, however, this approach requires familiarity with the target genome sequence in order to design the primers for PCR (Bhattramakki et al., 2000). Overall, the accuracy of differentiating between strains using PCR-based approaches is dependent on the primer pair used (Hansen et al., 1998; Klindworth et al., 2013; Hulin and Wheals, 2014). Primers were also reported to be biased towards genomic DNA with matching sequences, especially with higher primer annealing

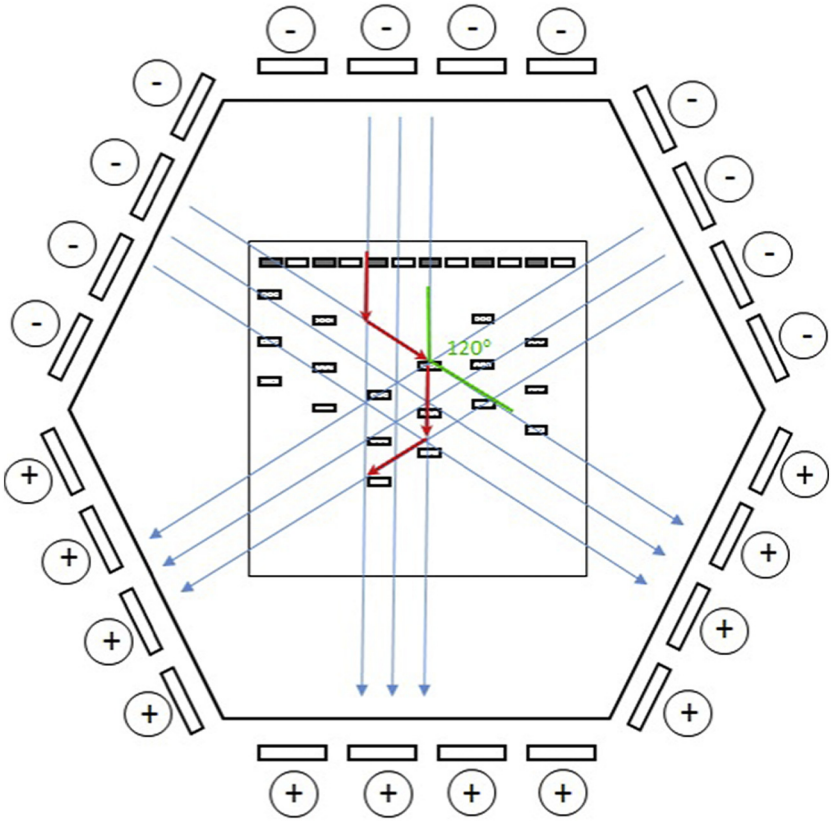


Fig. 2. Schematic depiction of principle behind pulsed-field gel electrophoresis (PFGE)/contour clamped homogeneous electric field (CHEF) used to separate large chromosomal DNA bands by alternating the direction of the electric field at an angle of 120° which is regulated by programmed pulse times (Hart, 2018, PhD dissertation).

temperatures which increases binding specificity (Sipos et al., 2007; Naqib et al., 2019). Moreover, PCR at times cannot show sufficient polymorphism to enable differentiation of closely related *S. cerevisiae* strains. It is noteworthy that, CHEF DNA karyotyping does not have these limitations, and the time required from PCR primer design, DNA purification, PCR amplification, cloning, and sequencing will be similar if not more to that required for CHEF DNA karyotyping.

2.1.3. Whole genome sequencing of wine yeast

Whole Genome Sequencing is routinely used to conduct in-depth comparisons between genetically related yeast species (Chen et al., 2016). It was also applied to successfully sequence whole genomes of numerous *Saccharomyces sensu stricto* species *viz. S. cerevisiae* and *S. bayanus* var. *uvarum* (Borneman and Pretorius, 2015; Naseeb et al., 2018). It is noteworthy that, whole-genome sequencing studies on industrial strains used haploid representations of diploids, due to genomic complexity in terms of genotypic heterogeneity between strains and heterozygosity within strains. It can, therefore, be tentatively said that whole genome sequencing is not an everyday approach to rapidly identify wine yeast starter cultures during fermentation. As CHEF DNA karyotypes are not affected by small genetic insertions and deletions, it still remains a reliable technique to distinguish between yeast strains (Van der Westhuizen and Pretorius, 1992; Van Breda et al., 2013; Hart et al., 2016). Nonetheless, genome sequencing provides a comprehensive understanding of the genomic make-up of industrial yeast strains that is useful to study population dynamics.

2.1.4. Genotyping by sequencing (GBS)

A simpler alternative to Whole Genome Sequencing *i.e.* genotyping by sequencing (GBS) shares the same advantage as RAPDs, due to the fact that it does not require knowledge of the target genome sequence (Elshire et al., 2011). However, as with Whole Genome Sequencing GBS more useful to study population dynamics, as opposed to rapid identification of wine yeast starter cultures (Drozdova et al., 2016). This approach can be time-consuming due to local sequencing facilities running numerous of samples a day as mentioned above and, therefore, not as advantageous as *e.g.* CHEF DNA karyotyping and MALDI Biotyping for relatively fast identification of yeast starter cultures.

2.1.5. Multi-locus sequence typing (MLST)

Another PCR-based technique *i.e.* multilocus sequence typing (MLST) analysis was previously reported to differentiate between wine yeast strains (Muñoz et al., 2009). As MLST also involves RFLP of PCR amplicons of selected genes, it can be used as a fast method to determine yeast population dynamics (Ivey and Phister, 2011). However, MLST is less discriminatory than SSRs mentioned above, as target genes used for wine yeast typing and resultant genotypes for the same strain will vary (Jubany et al., 2008). Therefore, the use of MLST to differentiate between closely related yeast strains, especially hybrids bred from mutual parental strains is not recommended. Nonetheless, MLST is a reliable tool to construct phylogenetic trees of wine yeasts.

2.2. Classical characterisation

2.2.1. Morphological characterisation

Morphological characterisation of new yeasts isolate involves *viz.* sub-culturing on appropriate growth media to obtain a pure culture, followed culturing on different growth media to investigate macroscopic and microscopic characterisation, etc. This approach is still practical and was also shown to be a relatively cheaper alternative compared to molecular characterisation *e.g.* CHEF DNA karyotyping (Van Breda et al., 2013). However, morphological characterisation has limitations, as it can be laborious. Moreover, some of these techniques are also susceptible to misidentification, and can only differentiate between genera (Ciardo et al., 2006).

2.2.2. Biochemical characterisation

A commonly used biochemical characterisation approach, namely

ID 32 C AUX system (BioMérieux, South Africa) in conjunction with apiweb™ identification software was previously shown to differentiate yeasts on a genus level (Van Breda et al., 2013). However, the ID 32 C approach is known for misidentification of some yeast strains used for wine production, hence it is advised that the technique be used in conjunction with other complementary yeast differentiation tools.

2.3. Unconventional characterisation

2.3.1. Classical capillary gas chromatography (GC)

Cellular fatty acids analyses (CFAA) by means of coupled capillary gas chromatography-mass spectrometry (GC-MS) proved to be a reliable tool to differentiate between closely related yeast strains (Augustyn and Kock, 1989). However, the physiological conditions are to be completely rigid for successful differentiation. As a result, the cellular fatty acid profile of any given yeast will vary, which essentially means that a single strain will have different profiles in different fermentation matrixes. Therefore, CHEF DNA karyotyping still remains a practical and reliable characterisation method as this level of rigidity is not required.

2.3.2. Protein fingerprinting

Protein fingerprinting based on yeast cellular proteins following sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), is a relatively fast method to differentiate yeast isolates to a genus level (Dowhanick et al., 1990). As the protein fingerprint of a specific isolate was reported to correlate with gene expression of that particular isolate, it can be envisioned that the establishment of a database of commercially available yeast strains similar to a DNA fingerprinting database will be useful to rapidly classify an unknown isolate to genus-level or even study population dynamics during a fermentation. Mitterdorfer et al. (2002) reported that various *Saccharomyces* isolates had identical protein fingerprints following SDS-PAGE, however native polyacrylamide gel electrophoresis was successfully used to group isolates. This approach can, therefore, be advantageous compared to molecular methods, as results can be obtained within a shorter period. However, as matrices affect yeast protein expression, protein fingerprints for the same strain might show variations, resulting in poor reproducibility. Nonetheless, two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) was reported to differentiate S. cerevisiae strains (Mitterdorfer et al., 2002).

2.3.3. Matrix-assisted laser desorption/ionisation-time of flight mass spectrometry (MALDI-TOF/TOF MS) biotyping

Matrix-assisted laser desorption/ionisation-time of flight mass spectrometry biotyping is a relatively new technique for wine yeast characterisation, and was shown to be a relatively fast, accurate and dependable method to differentiate between closely related microorganisms (Moothoo-Padayachie et al., 2013; Deak et al., 2015; Hart et al., 2016). Biotyping involves the extraction of ribosomal protein and subsequent generation of peptide mass spectral fingerprints using tandem mass spectrometry (Fig. 3). Peptide spectra are automatically processed with the software package MALDI Biotyper Real-Time Classification (Bruker Daltonics, Bremen, Germany). The software performs smoothing and baseline correction using Savitsky-Golay, Multipolygon algorithms. Identification of unknown microorganisms is achieved by comparing their mass spectra to a database containing more than 4100 reference microorganisms (Rizzato et al., 2016). Moreover, Gekenidis et al. (2014) and Emami et al. (2015) reported that ribosomal protein mass spectra are not influenced by the microbial growth medium and growth phase. This is advantageous, since anecdotal evidence showed that cultivation of a single yeast strain on complete, *e.g.* yeast extract, peptone, and dextrose (YPD), and selective, *e.g.* yeast extract, malt extract, dextrose, and peptic digests of animal tissue (YM) media resulted in differential protein expression (C. Abrahams, Personal communication, 2016). Therefore, a single yeast strain isolated from different matrices, *e.g.* wine, beer or synthetic medium, etc., will be accurately identified. Moreover, yeasts are known to display different phenotypes when subjected to different physiological conditions, a difficulty that is easily circumvented by deploying MALDI-TOF MS biotyping (Gekenidis et al., 2014). Another advantage is that the database includes both the anamorphic and teleomorphic species name (Turvey et al., 2016; Du Plessis et al., 2017). Despite the faster yeast identification obtainable with MALDI-TOF MS biotyping, this method still remains relatively expensive, especially if the unknown isolate is not in the database (Croxatto et al., 2012; Panda et al., 2015). Singhal et al. (2015) also highlighted that identification of the unknown isolate is highly unlikely if the database does not contain a peptide mass spectral fingerprint of the relevant strain. Nevertheless, MALDI-TOF-MS biotyping provides fast and reliable differentiation of microorganisms, more so in circumstances where molecular differentiation of different isolates is impractical due to identical internal transcribed spacer (ITS) regions (Korabecná et al., 2003; Emami et al., 2015).

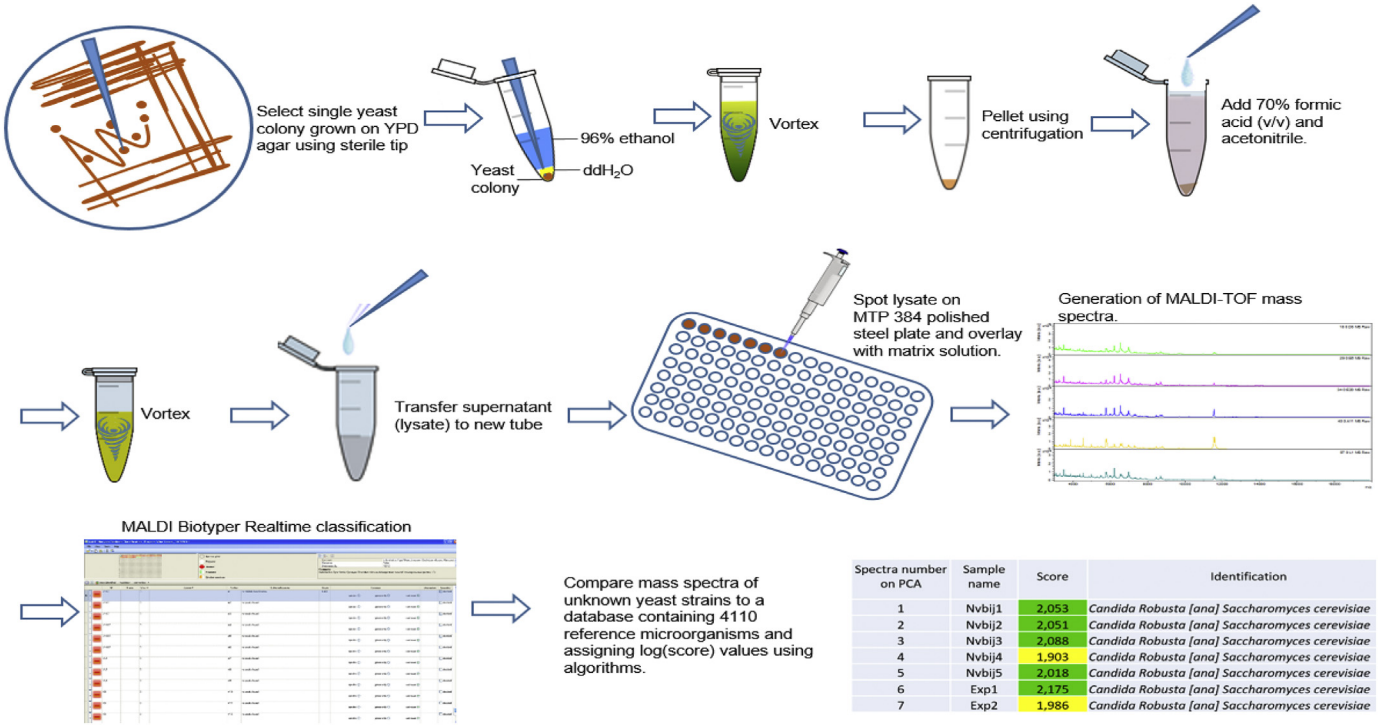
2.4. Characterisation based on fermentation kinetics

2.4.1. Fermentation potential

Evaluating the fermentation potential of promising experimental yeast strains is critical before commencing commercial trials and subsequent commercialisation of the yeast (Gonzalez et al., 2007; Pérez-Torrado et al., 2015). However, determining the fermentation potential of yeast strains through a series of lab- ($\leq$ 1 L), small- ($\leq$ 20 L), pilot- ($\leq$ 100 L) and semi-commercial ($\geq$ 1000 L) scale vinification (wine-making) trials has drawbacks, and can easily take up to 10 years to identify an experimental yeast strain worthy for commercialisation purposes (Hart and Jolly, 2008). The process starts with the identification of a new criterion for yeast development by industry, whereafter trials are initiated using a large number of experimental and commercial reference yeast strains (Hart and Jolly, 2008; Heymann et al., 2013; Hart et al., 2016). Promising experimental yeast strains will be re-evaluated during subsequent trials and/or vintages to establish that results obtained are repeatable. Subsequently, trails will be scaled-up to *e.g.* semi-commercial scale in conjunction with a commercial cellar/s once observations during re-evaluations are promising. This approach in all probability ensures the development and/or identification of the most promising yeast strains with the ability to produce good quality wines irrespective of vintages, since different vintages are known to result in wines with different organoleptic profiles (Von Mollendorf, 2013; Canonico et al., 2015; Padilla et al., 2016). Establishment of the fermentation potential of a yeast strain involves various criteria (*e.g.* determination of fermentation rate, measurement of wine chemical and sensory parameters, metabolite levels) which will be briefly discussed.

2.5. Fermentation rate

A classical method to investigate the fermentation rate following inoculation with an experimental yeast strain involves the labour-intensive weighing of the fermentation vessels to monitor CO_2-weight loss (Fairbairn, 2012; Parcunev et al., 2012). However, technological advances allow for monitoring of fermentation rate and wine parameters by deploying state of the art bioreactors (fermenters) in conjunction with computer software (Sonego et al., 2016). This approach is advantageous since data are rapidly generated and physical weighing circumvented. However, installation and operation of bioreactors are expensive, making this approach expensive especially in developing countries where budget constraints apply (Hashimura et al., 2012; University world news [UWN], 2013). Therefore, traditional monitoring of CO_2-weight loss still remains a viable and/or cheaper option.



Spectra number on PCA	Sample name	Score	Identification
1	Nvbij1	2,053	*Candida Robusta [ana] Saccharomyces cerevisiae*
2	Nvbij2	2,051	*Candida Robusta [ana] Saccharomyces cerevisiae*
3	Nvbij3	2,088	*Candida Robusta [ana] Saccharomyces cerevisiae*
4	Nvbij4	1,903	*Candida Robusta [ana] Saccharomyces cerevisiae*
5	Nvbij5	2,018	*Candida Robusta [ana] Saccharomyces cerevisiae*
6	Exp1	2,175	*Candida Robusta [ana] Saccharomyces cerevisiae*
7	Exp2	1,986	*Candida Robusta [ana] Saccharomyces cerevisiae*

Fig. 3. Schematic depiction of steps involved during matrix-assisted laser desorption/ionisation-time of flight mass spectrometry (MALDI-TOF/TOF MS) biotyping (Hart, 2018, PhD dissertation).

2.6. Characterisation based on wine organoleptic quality

2.6.1. Chemical analyses

Monitoring of chemical parameters (*e.g.* sugar utilisation, alcohol production, progression of pH, free amino nitrogen (FAN), yeast assimilable nitrogen (YAN), total sulphur (SO_2) etc.) of ferments constitutes chemical characterisation of the wine yeast strain used to conduct the alcoholic fermentation (Heymann et al., 2013; Cramer et al., 2014). Traditionally, analyses of fermenting grape must and/or wine chemical parameters (*e.g.* residual sugar) involved wet chemistry (*e.g.* Rebelein titrations) (Jolly et al., 2008; Hoon, 2015). Wet chemistry techniques are advantageous because it does not require expensive state-of-the-art equipment (*e.g.* density meter, alcoholyzer, OenoFoss™ Fourier transform infrared spectrophotometer (King and Heymann, 2014; Friedel et al., 2016). However, the key disadvantages of deploying wet-chemistry techniques are that they have variances as the perception of critical colour changes during titrations, for instance, differs between technicians. This will result in inconsistencies for a specific parameter measured. Nonetheless, insufficient calibrations and servicing of the aforementioned modern equipment can produce inaccurate results. Therefore, one or the other analytical chemistry technique has advantages and disadvantages, hence respective techniques should be used in a complementary manner.

2.6.2. Sensory analyses

Sensory evaluation of fermented must (wine) by a panel of trained judges also constitutes sensory characterisation of the wine yeast strain used to conduct the alcoholic fermentation (Molina et al., 2009; Campo et al., 2010). Arroyo et al. (2009), and Bhattacharyya and Bandhopadhyay (2010) reported that the human olfactory system has a wider range for detecting aroma and flavour compounds compared to sophisticated analytical gas chromatographic equipment. Perception of wine aroma and flavour involves smelling and tasting of wines, which enables multiple nerve fibres (filaments) within the olfactory system referred to as the olfactory bulb (receptor) cells that detect aroma compounds (*e.g.* esters, thiols). Thereafter, in conjunction with various regions within the brain an association is made with preconceived aromas and/or flavours (*e.g.* banana, passionfruit) as schematically depicted (Fig. 4) (Swiegers et al., 2005; Shepherd, 2006; Tham et al., 2009; Bushdid et al., 2014). Various approaches are deployed to conduct experimental wine sensory analyses *e.g.* descriptive sensory analyses using an intensity scale, unipolar numerical scale, multi-wine preference sorting. Descriptive sensory evaluation involves visual (colour), aroma (nose) and flavour (taste) characterisation of wine based on the perceived intensity of aroma and flavour descriptors (*e.g.* fruitiness, herbaceous, berry, vegetative, citrus, acidity, mouthfeel). Sensory evaluation using a unipolar numerical scale is conducted in a similar manner, whilst multi-wine preference sorting, on the other hand, is a discriminative analysis which involves sorting of wines in two groups based on liking and disliking. This is a useful approach to rapidly determine whether treatments (*e.g.* yeast inoculum) differ or are similar (Weightman et al., 2016). Favoured wines can subsequently be subjected to descriptive sensory evaluation in order to generate data that can be statistically analysed.

2.7. Omics-based characterisation

2.7.1. Metabolomic analyses

Wine contains various metabolites (*e.g.* monosaccharide sugars, organic acids, fatty acids, amino acids, volatile thiols, esters, higher alcohols), some of which are yeast-derived referred to as the yeast's exo-metabolome (Hart et al., 2016; Varela, 2016). A bottled wine with a desirable organoleptic profile can mostly be attributed to the yeast starter culture produced and/or mediated metabolites that contribute to *viz.* tree and tropical fruit aroma of white wines, especially Sauvignon blanc wines (Rollero et al., 2016; Hart et al., 2017a). Yeast produced and/or mediated metabolites can either be sought-after (contributes to tropical fruit, floral, vegetative fresh aromas and flavour) or undesirable (contributes to vinegar-like, bruised-apple, rotten egg off-flavours). Sensorial detectable levels of undesirable metabolites are known to lower wine chemical and sensory quality and, therefore, consumer preference. Consequently, financial loss will be incurred by wine

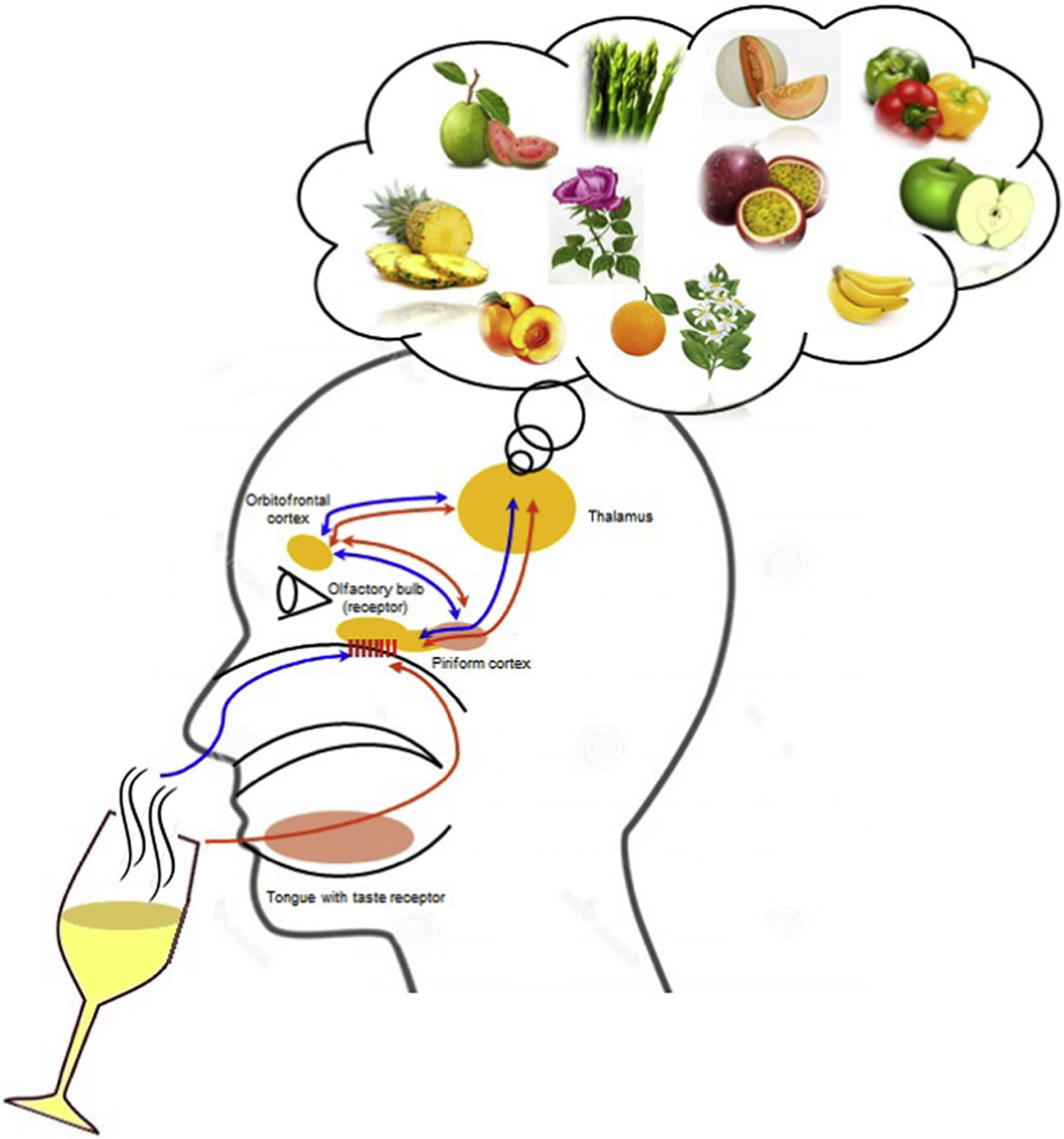


Fig. 4. Schematic depiction of the human olfactory system and the process of olfaction that allows for the perception of wine aroma and flavour. The olfactory bulb (receptor) consisting of multiple fibers transfer regular ortho-nasal (blue arrow) and retro-nasal (orange arrow) smell to the brain for interpretation and/or identification (Hart, 2018, PhD dissertation). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

producers, firstly due to lower wine sales and secondly by deploying expensive reverse osmosis to remove excessive VA from wine (Vilela et al., 2013). Worst case scenario would be to discard the entire affected tank, which will also result in financial loss due to production costs involved during viticultural procedures in the vineyard and wine-making processes in the cellar up to the point where excessive VA was detected. Furthermore, commercial yeast manufacturers will also incur financial losses if and when a commercial yeast strain is implicated in excessive VA formation. Preventative measures involve the use of wine yeast strains that are known to be low VA producers. Said strains will also inhibit the growth of acetic acid bacteria (AAB) that have the ability to produce acetic from glucose and ethanol. It is, therefore, of paramount importance to utilise appropriate analytical tools for metabolite identification in order to characterise yeast starter cultures (Sirén et al., 2019).

Various gas chromatographic (GC) based techniques (*e.g.* gas chromatography coupled to a flame ionisation detector [GC-FID], gas chromatography-mass spectrometry [GC-MS], solid-phase extraction [SPE] coupled with GC-MS/MS analysis, and ultra-pressure liquid chromatography-tandem mass spectrometry [UPLC-MS/MS]) are routinely deployed to measure those metabolite levels in final wines following fermentation (Schueuermann et al., 2016). This process, in essence, constitutes metabolomic characterisation of the yeast starter culture used to produce the wine (Sirén et al., 2019).

Aggio et al. (2014) and Savolainen et al. (2016) reported that GC-MS based methods are advantageous since it allows for the simultaneous identification and quantification of various volatile thiols in Sauvignon blanc wines. Gas chromatography coupled with mass spectrometry also complements descriptive analyses, since correlations between detection thresholds and sensory perception thresholds can easily be established (Schmidtke et al., 2013). Canuti et al. (2009) also reported that capillary GC-MS in conjunction with solid-phase microextraction (SPME) allows for fast and reliable characterisation of metabolites in wines. Another advantage of GC-MS based metabolite analyses is that it can distinguish between wines produced from different cultivars (*e.g.* Chardonnay, Sauvignon blanc, Chenin blanc) as well as wine produced from the same cultivar grown in different terroirs (Sagratini et al., 2012).

2.7.2. Proteomic evaluation

Wine yeast expressed proteins are instrumental in the production of varietal aromatic wines, as yeast derived and/or mediated aroma compounds (metabolites) are reliant on said proteins which play a key role in metabolic pathways of yeast cells (Moreno-García et al., 2015; Hart et al., 2017a, 2017b). It was also reported that yeast strains differentially express proteins even though a standard medium or grape juice was used for fermentation (Mostert, 2013). Additionally, proteins that are always associated with a certain physiological condition (*e.g.* high aroma compounds), can be used as biomarkers for said condition (Parker and Borchers, 2014). The advantage of using a protein biomarker above a gene biomarker is that an organism's genome is relatively constant, irrespective of the physiological condition, whilst protein expression varies based on physiological condition (Gómez-Pastor et al., 2010). Transcriptomics is also a viable characterisation tool, however, mRNA which is directly transcribed from an expressed gene is less stable compared to proteins and are degraded more rapidly than proteins (Parker, 2014). Furthermore, mRNA was shown to be translated into more than one protein, each with a different function following post-translational modification (Gibson et al., 2017). It is, therefore, evident that proteomic analyses of wine yeast can be used as a practical characterisation tool to differentiate fermenting yeast strains (Hansen et al., 2006; Salvado et al., 2008; Mostert, 2013).

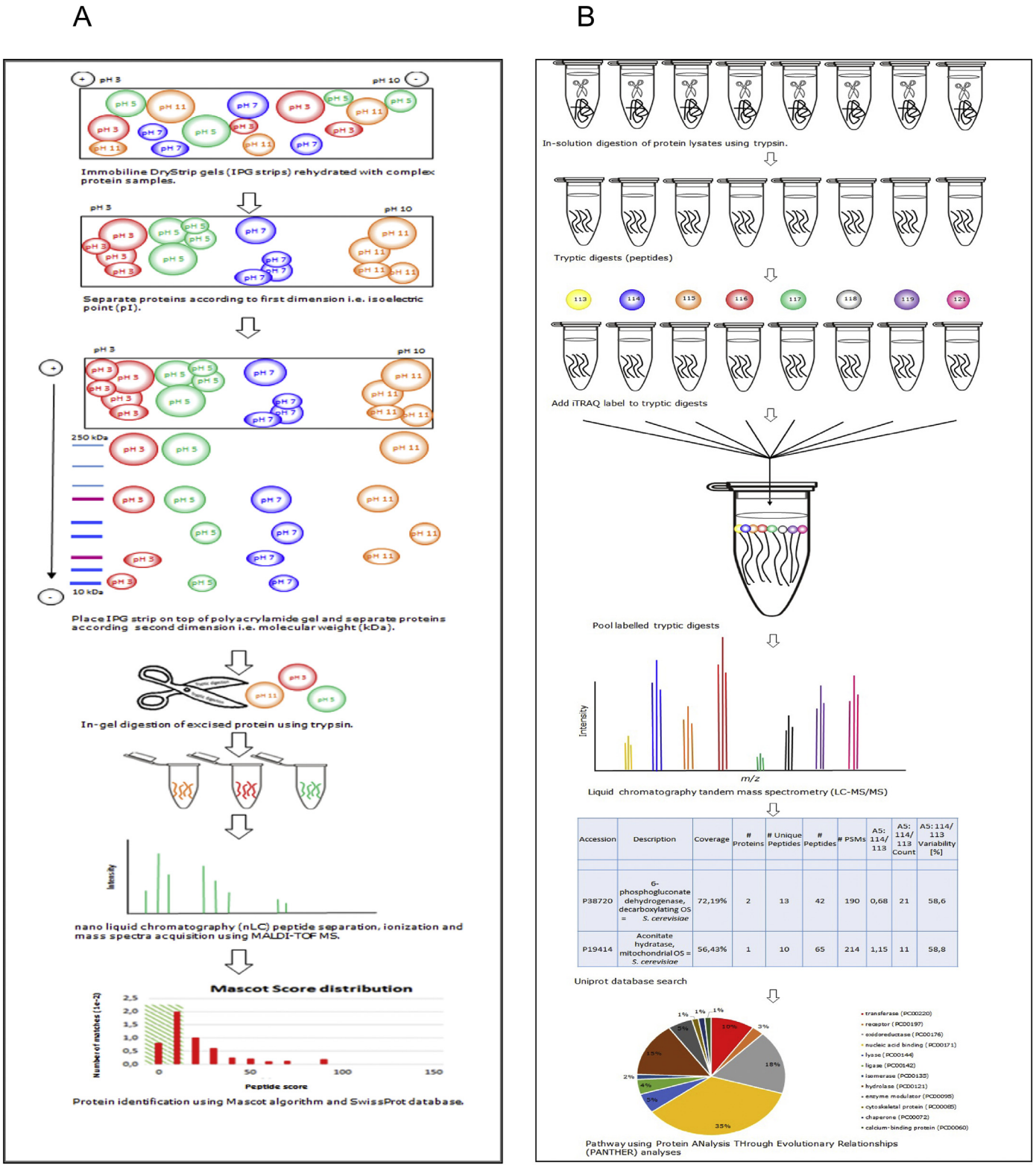
Proteomic characterisation involves qualitative and quantitative analyses of protein extracts (Gillet et al., 2012). Protein quality, on the other hand, can be determined using basic gel-based approaches in conjunction with innovative liquid chromatography-tandem mass spectrometry (LC-MS/MS) techniques (Milac et al., 2012; Nowakowski et al., 2014). The most basic gel-based proteomic analyses involve one-dimensional (1D) SDS-PAGE, whereby extracted proteins are resolved based on their molecular weight (MW) (Kolkman et al., 2006). However, the disadvantage of 1D-PAGE is that proteins with a similar molecular weight cannot be separated and are visualised as one protein band. This predicament is easily circumvented by means of two-dimensional (2D) PAGE, whereby proteins are separated in two steps based on two independent properties (Ndimba et al., 2010). The first step entails the separation of proteins according to their isoelectric points (pH where the protein has a neutral net charge) followed by the second step which is a basic SDS-PAGE. Individual proteins normally manifest as different spots on polyacrylamide gels following a staining technique using *e.g.* Coomassie blue, Silver staining (Westermeier, 2006). It is improbable that two or more expressed proteins from the same organism will have identical molecular weight and isoelectric points (Shevchenko et al., 1996a, 1996b). Differentially expressed proteins are then excised and characterised by means of peptide mass fingerprinting (PMF) using MALDI-TOF/MS (Sutton et al., 1995; Shevchenko et al., 1996a, 1996b; Gevaert et al., 1997; Jimenez et al., 2001; Zhou et al., 2012). The major drawback of gel-based proteomics is that protein identification is limited to a specific molecular weight- and pH-range. Nonetheless, 2D-PAGE is a useful tool to identify and characterise single proteins associated with certain wine chemical (lower VA) and sensory profiles (enhanced tropical fruit aroma), and these proteins can serve as biomarkers for experimental yeast starter cultures during winemaking trials.

Protein quantitation can be conducted by subjecting protein lysate to a rapid and simple spectrophotometric analyses (*e.g.* Bradford Coomassie G-250 assay) (Ngara et al., 2012). A major drawback of protein quantitation using a spectroscopic based assay is that complex protein lysates might have different adsorption properties compared to bovine serum albumin (BSA) protein used to construct the standard curve. As a result, various proteins will bind differently to the Coomassie G-250 compared to BSA, therefore giving incorrect measurements when extrapolated on a BSA-based standard curve (Zaia et al., 2005).

Cutting-edge liquid chromatography-mass spectrometry (LC-MS) based isobaric peptide tags for relative and absolute quantification (iTRAQ), where unknown protein peptides are tagged with reporter molecules that allows for quantification is a practical alternative (Kim et al., 2012; Nie et al., 2013). A key advantage of iTRAQ is that complex protein samples originating from different yeast strains, can be analysed simultaneously (Latosinska et al., 2015). Therefore, protein expression of an experimental yeast strain can simultaneously be compared to various commercial yeast strains, respectively. Furthermore, unlike gel-based proteomics, iTRAQ protein identification is not limited to a given molecular weight (MW)- and pH-range, thereby allowing for quantification and identification of vast numbers of proteins during a single analysis. This approach does, however require expensive state-of-the-art equipment (*e.g.* Linear trap quadrupole [LTQ] Orbitrap Velos MS) that is not always readily available, especially in developing countries. Label-free MS, contrariwise to iTRAQ-labelling is also a practical alternative for identifying complex protein samples originating from different sources simultaneously, but also requires above-mentioned LTQ Orbitrap Velos MS (Li et al., 2012; Latosinska et al., 2015). Label-free approaches though are significantly cheaper, since the use of expensive iTRAQ labels is not required (M. Vlok, Personal communication, 2016). Quantitative and qualitative proteomic analysis procedures discussed are graphically illustrated (Fig. 5).

2.8. Conclusions and future remarks

The wine industry is an important contributor to the South African economy as wine forms an integral part of exports from the agricultural sector. Furthermore, anecdotal evidence suggests that many of the yeasts commercially available are inadequate to fulfil to the changing requirements of not only South African winemakers, but international winemakers as well. Therefore, South African oenologists are continually striving to increase wine quality in an ever increasing competitive market, especially in light of varietal aromatic new world wines, lower in VA becoming increasingly popular. However, yeasts strains with sought-after characteristics can be generated by deploying genetic modification (GM). However, the use of genetically modified organisms (GMO) for wine production is illegal to date (Hart, 2018). Furthermore, the Cape Winemakers Guild (CWG) and South African Wine Industry Council (SAWIC) is also largely against the use of GMO in wine production (CWG, 2015), as both emphasised the dependence of the SA wine industry on the highly sensitive European market which are largely against GM food products. Nonetheless, improvements in yeast starter cultures used for the wine production is of utmost importance, as it is directly linked to wines with improved organoleptic quality. Failure to improve yeast strains as industry criteria are changing will result in financial loss due to lower wine and yeast sales. In future, climate change with its impact on viticulture and possibly grape physiology will create new fermentation challenges for the winemaker. As a result, continuous yeast breeding by means of classical mating, as well as characterisation of novel hybrids by deploying traditional and modern chemical and organoleptic evaluation techniques of white wines in conjunction with cutting-edge metabolomic and proteomic analytical



Accession	Description	Coverage	# Proteins	# Unique Peptides	# Peptides	# PSMs	A5: 114/113	A5: 114/113 Count	A5: 114/113 Variability [%]
P38720	6-phosphogluconate dehydrogenase, decarboxylating OS = *S. cerevisiae*	72,19%	2	13	42	190	0,68	21	58,6
P19414	Aconitate hydratase, mitochondrial OS = *S. cerevisiae*	56,43%	1	10	65	214	1,15	11	58,8

Fig. 5. Schematic depiction of qualitative and quantitative proteomic analysis tools. A) Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE), followed by peptide mass fingerprinting (PMF) using matrix-assisted laser desorption ionization-time of flight coupled mass spectrometry (MALDI-TOF/MS) to identify differentially expressed proteins; and B) quantification and identification of proteins using liquid chromatography-mass spectrometry (LC-MS) based isobaric peptide tags for relative and absolute quantification (iTRAQ) (Hart, 2018, PhD dissertation).

approaches towards enhancing white wine varietal aroma is important and relevant in order for the SA industry to remain globally competitive.

Acknowledgements

The authors wish to thank the Agricultural Research Council (ARC) for the infrastructure.

Funding

The authors wish to thank the Agricultural Research Council (ARC), South Africa (Parliamentary Grant) and the National Research Foundation (NRF), South Africa (Research and Technology Fund [RTF] grant UID 98693) for financial support. The opinions and conclusions expressed in this publication are those of the authors, and the National Research Foundation accepts no liability in this regard.

Declaration of competing interest

All authors declares no conflict of interest.

References

Abrahams, C.D., Ndiko, N.N., Hart, R.S., 2017. Molecular and proteomic characterisation of wine and wild yeast strains used for vinification, presented at The South African Association for Food Science & Technology (SAAFoST) 22nd Biennial congress, Century City Convention Centre, Cape Town, South Africa, 03–06 September 2017.

Aggio, R.B., Mayor, A., Reade, S., Probert, C.S., Ruggiero, K., 2014. Identifying and quantifying metabolites by scoring peaks of GC-MS data. BMC Bioinformatics. 15, 374. https://doi.org/10.1186/s12859-014-0374-2.

Álvarez-Pérez, J.M., Álvarez-Rodríguez, M.L., Campo, E., Sáenz de Miera, L.E., Ferreira, V., Hernández-Orte, P., Garzón-Jimeno, E., Coque, J.J.R., 2014. Selection of *Saccharomyces cerevisiae* strains applied to the production of Prieto Picudo Rosé wines with a different aromatic profile. S. Afr. J. Enol. Vitic. 35, 242–256. https://doi.org/10.21548/35-2-1013.

Arroyo, T., Lozano, J., Cabellos, J.M., Gil-Diaz, M., Santos, J.P., Horrillo, C., 2009. Evaluation of wine aromatic compounds by a sensory human panel and an electronic nose. J. Agric. Food Chem. 57, 11543–11549. https://doi.org/10.1021/jf902109y.

Augustyn, O.P.H., Kock, L.F., 1989. Differentiation of yeast species, by cellular fatty acid analysis. 1. Application of an adapted technique to differentiate between strains of *Saccharomyces cerevisiae*. J. Microbiol. Methods 10, 9–23.

Bartowsky, E.J., 2009. Bacterial spoilage of wine and approaches to minimize it. Lett. Appl. Microbiol. 48, 149–156. https://doi.org/10.1111/j.1472-765X.2008.02505.x.

Belda, I., Navascués, E., Marquina, D., Santos, A., Calderon, F., Benito, S., 2015. Dynamic analysis of physiological properties of *Torulaspora delbrueckii* in wine fermentations and its incidence on wine quality. Appl. Microbiol. Biotechnol. 99, 1911–1922. https://doi.org/10.1007/s00253-014-6197-2.

Bhattacharyya, N., Bandhopadhyay, R., 2010. Electronic nose and electronic tongue. In: Nondestructive Evaluation of Food Quality. Springer, Berlin, Heidelberg, pp. 73–100.

Bhattramakki, D., Dong, J., Chhabra, A.K., Hart, G.E., 2000. An integrated SSR and RFLP linkage map of *Sorghum bicolor* (L.) Moench. Genome 43, 988–1002.

Bonciani, T., Solieri, L., De Vero, L., Giudici, P., 2016. Improved wine yeasts by direct mating and selection under stressful fermentative conditions. Eur. Food Res. Technol. 242, 899–910. https://doi.org/10.1007/s00217-015-2596-6.

Borneman, A.R., Pretorius, I.S., 2015. Genomic insights into the *Saccharomyces sensu stricto* complex. Genetics 199, 281–291.

Bushdid, C., Magnasco, M.O., Vosshall, L.B., Keller, A., 2014. Humans can discriminate more than 1 trillion olfactory stimuli. Science 343, 1370–1372. https://doi.org/10.1126/science.1249168.

Cadière, A., Aguera, E., Caillé, S., Ortiz-Julien, A., Dequin, S., 2012. Pilot-scale evaluation the enological traits of a novel, aromatic wine yeast strain obtained by adaptive evolution. Food Microbiol. 32, 332–337. https://doi.org/10.1016/j.fm.2012.07.010.

Campo, E., Ballester, J., Langlois, J., Dacremont, C., Valentin, D., 2010. Comparison of conventional descriptive analysis and a citation frequency-based descriptive method for odor profiling: an application to Burgundy Pinot noir wines. Food Qual. Prefer. 21, 44–55.

Canonico, L., Comitini, F., Ciani, M., 2015. Influence of vintage and selected starter on *Torulaspora delbrueckii/Saccharomyces cerevisiae* sequential fermentation. Eur. Food Res. Technol. 241, 827–833. https://doi.org/10.1007/s00217-015-2507-x.

Canuti, V., Conversano, M., Calzi, M.L., Heymann, H., Matthews, M.A., Ebeler, S.E., 2009. Headspace solid-phase microextraction-gas chromatography-mass spectrometry for profiling free volatile compounds in Cabernet Sauvignon grapes and wines. J. Chromatogr. A 1216, 3012–3022.

Cape Winemakers Guild (CWG), 2015. South African Wine Industry. [Online]. http://wosa.co.za/sa/sustainable_gmyeast_statement.php accessed on 30 Jul 2019.

Carle, G.F., Olson, M.V., 1985. An electrophoretic karyotype for yeast. Proc. Natl. Acad. Sci. U. S. A. 82, 3756–3760.

Chambers, P.J., Borneman, A.R., Schmidt, S.A., Hack, J.C., Varela, C., Mercurio, M., Curtin, C.D., Cozzolino, D., Ugliano, M., Herderich, M.J., Pretorius, I.S., 2009. The dawn of a new paradigm for wine yeast strain development. Aust. NZ. Wine Indus. J. 24, 16–18.

Chen, P., Dong, J., Yin, H., Bao, X., Chen, L., He, Y., Chen, R., Wan, X., Zhao, Y., Hou, X., 2016. Genome comparison and evolutionary analysis of different industrial lager yeasts (*Saccharomyces pastorianus*). J. Inst. Brew. 122, 42–47.

Chial, H., 2008. DNA fingerprinting using amplified fragment length polymorphisms (AFLP): no genome sequence required. Nat. Educ. 1, 176.

Chovanová, K., Kraková, L., Zenišová, K., Turcovská, V., Brežná, B., Kuchta, T., Pangallo, D., 2011. Selection and identification of autochthonous yeasts in Slovakian wine samples using a rapid and reliable three-step approach. Lett. Appl. Microbiol. 53, 231–237. https://doi.org/10.1111/j.1472-765X.2011.03097.x.

Ciardo, D.E., Schär, G., Böttger, E.C., Altwegg, M., Bosshard, P.P., 2006. Internal transcribed spacer sequencing versus biochemical profiling for identification of medically important yeasts. J. Clin. Microbiol. 44, 77–84. https://doi.org/10.1128/JCM.44.1.77-84.2006.

Colabella, F., Libkind, D., 2016. PCR-based method for the rapid identification of astaxanthin-accumulating yeasts (*Phaffia* spp.). Rev. Argent. Microbiol. 48, 15–20. https://doi.org/10.1016/j.ram.2015.10.006.

Cramer, G.R., Ghan, R., Schlauch, K.A., Tillett, R.L., Heymann, H., Ferrarini, A., Delledonne, M., Zenoni, S., Fasoli, M., Pezzotti, M., 2014. Transcriptomic analysis of the late stages of grapevine (*Vitis vinifera* cv. Cabernet Sauvignon) berry ripening reveals significant induction of ethylene signaling and flavor pathways in the skin. BMC Plant Biol. 14, 370. https://doi.org/10.1186/s12870-014-0370-8.

Cray, J.A., Bell, A.N.W., Bhaganna, P., Mswaka, A.Y., Timson, D.J., Hallsworth, J.E., 2013. The biology of habitat dominance; can microbes behave as weeds? Microb. Biotechnol. 6, 453–492. https://doi.org/10.1111/1751-7915.12027.

Croxatto, A., Prod'hom, G., Greub, G., 2012. Applications of MALDI-TOF mass spectrometry in clinical diagnostic microbiology. FEMS Microbiol. Rev. 36, 380–407. https://doi.org/10.1111/j.1574-6976.2011.00298.x.

De Jonge, P., de Jongh, F.C., Meijers, R., Steensma, H.Y., Scheffers, W.A., 1986. Orthogonal-field-alternation gel electrophoresis banding patterns of DNA from yeasts. Yeast 2, 193–204. https://doi.org/10.1002/yea.320020307.

Deak, E., Charlton, C.L., Bobenchik, A.M., Miller, S.A., Pollett, S., McHardy, I.H., Wu, M.T., Garner, O.B., 2015. Comparison of the Vitek MS and Bruker Microflex LT MALDI-TOF MS platforms for routine identification of commonly isolated bacteria and yeast in the clinical microbiology laboratory. Diagn. Microbiol. Infect. Dis. 81, 27–33. https://doi.org/10.1016/j.diagmicrobio.2014.09.018.

Dowhanick, T., Sobczak, J., Russell, I., Stewart, G., 1990. The rapid identification by protein fingerprinting of yeast and bacterial brewery contaminants. J. Am. Soc. Brew. Chem. 48, 75–79.

Drozdova, P.B., Tarasov, O.V., Matveenko, A.G., Radchenko, E.A., Sopova, J.V., Polev, D.E., Inge-Vechtomov, S.G., Dobrynin, P.V., 2016. Genome sequencing and comparative analysis of *Saccharomyces cerevisiae* strains of the Peterhof genetic collection. PLoS One 11, e0154722. https://doi.org/10.1371/journal.pone.0154722.

Du Plessis, H.W., Du Toit, M., Hoff, J.W., Hart, R.S., Ndimba, B.K., Jolly, N.P., 2017. Characterisation of non-*Saccharomyces* yeasts using different methodologies and evaluation of their compatibility with malolactic fermentation. S. Afr. J. Enol. Vitic. 38. https://doi.org/10.21548/38-1-819.

Elshire, R.J., Glaubitz, J.C., Sun, Q., Poland, J.A., Kawamoto, K., Buckler, E.S., Mitchell, S.E., 2011. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PLoS One 6, e19379.

Emami, K., Hack, E., Nelson, A., Brain, C.M., Lyne, F.M., Mesbahi, E., Day, J.G., Caldwell, G.S., 2015. Proteomic-based biotyping reveals hidden diversity within a microalgae culture collection: an example using *Dunaliella*. Sci. Rep. 12, 10036. https://doi.org/10.1038/srep10036.

Erten, H., Tanguler, H., Cabaroglu, T., Canbas, A., 2006. The influence of inoculum level on fermentation and flavour compounds of white wines made from cv. Emir. J. Inst. Brew. 112, 232–236.

Ezeama, C.F., Ebia, O.O., 2015. Yeast performance, chemical and sensory properties of wine produced from Chinese star apple (*Averrhoa carambola*). Am. J. Food Nutr. 5, 9–15. https://doi.org/10.5251/ajfn.2015.5.1.9.15.

Fairbairn, S., 2012. Stress, Fermentation Performance and Aroma Production by Yeast. MSc thesis. Stellenbosch University, Private Bag X1, 7602, Matieland (Stellenbosch), South Africa.

Fleet, G.H., 2003. Yeast interactions and wine flavour. Int. J. Food Microbiol. 86, 11–22.

Friedel, M., Sorrentino, V., Blank, M., Schüttler, A., 2016. Influence of berry diameter and colour on some determinants of wine composition of *Vitis vinifera* L. cv. Riesling. Aust. J. Grape Wine Res. 22, 215–225. https://doi.org/10.1111/ajgw.12210.

García, V., Rivera, J., Contreras, A., Ganga, M.A., Martínez, C., 2012. Development and characterization of hybrids from native wine yeasts. Braz. J. Microbiol. 43, 482–489. https://doi.org/10.1590/S1517-83822012000200008.

Gekenidis, M., Studer, P., Wüthrich, S., Brunisholz, R., Drissner, D., 2014. Beyond the matrix-assisted laser desorption ionization (MALDI) biotyping workflow: in search of microorganism-specific tryptic peptides enabling discrimination of subspecies. Appl. Environ. Microbiol. 80, 4234–4241. https://doi.org/10.1128/AEM.00740-14.

Gevaert, K., Demol, H., Puype, M., Broekaert, D., De Boeck, S., Houthaeve, T., Vandekerckhove, J., 1997. Peptides adsorbed on reverse-phase chromatographic beads as targets for femtomole sequencing by post-source decay matrix-assisted laser desorption ionization-reflectron time of flight mass spectrometry (MALDI-RETOF-MS). Electrophoresis 18, 2950–2960.

Gibson, B., Geertman, J.M.A., Hittinger, C.T., Krogerus, K., Libkind, D., Louis, E.J., Magalhães, F., Sampaio, J.P., 2017. New yeasts—new brews: modern approaches to brewing yeast design and development. FEMS Yeast Res. 17. https://doi.org/10.1093/femsyr/fox038.

Gillet, L.C., Navarro, P., Tate, S., Röst, H., Selevsek, N., Reiter, L., Bonner, R., Aebersold, R., 2012. Targeted data extraction of the MS/MS spectra generated by data-independent acquisition: a new concept for consistent and accurate proteome analysis. Mol. Cell. Proteomics 11https://doi.org/10.1074/mcp.O111.016717. O111-016717.

Gómez-Pastor, R., Pérez-Torrado, R., Cabiscol, E., Matallana, E., 2010. Transcriptomic and proteomic insights of the wine yeast biomass propagation process. FEMS Yeast Res. 10, 870–884. https://doi.org/10.1111/j.1567-1364.2010.00667.x.

Gonzalez, S., Gallo, L., Climent, M., Barrio, E., Querol, A., 2007. Enological characterization of natural hybrids from *Saccharomyces cerevisiae* and *S. kudriavzevii*. Int. J. Food Microbiol. 116, 11–18.

Hage, A.E., Houseley, J., 2013. Resolution of budding yeast chromosomes using pulsed-field gel electrophoresis. Methods Mol. Biol. 1054, 195–207. https://doi.org/10.1007/978-1-62703-565-1_13.

Hansen, M.C., Tolker-Nielsen, T., Givskov, M., Molin, S., 1998. Biased 16S rDNA PCR amplification caused by interference from DNA flanking the template region. FEMS Microbiol. Ecol. 26, 141–149.

Hansen, R., Pearson, S.Y., Brosnan, J.M., Meaden, P.G., Jamieson, D.J., 2006. Proteomic analysis of a distilling strain of *Saccharomyces cerevisiae* during industrial grain fermentation. Appl. Microbiol. Biotechnol. 72, 116–125. https://doi.org/10.1007/s00253-006-0508-1.

Hart, R.S., 2018. Proteomic and Metabolomic Characterisation of Novel Wine Yeasts: Towards the Evaluation and Improvements of Their Ability to Produce Aromatic Sauvignon Blanc Wines. PhD thesis. University of the Western Cape, Bellville, Cape Town, 7535, South Africa.

Hart, R.S., Jolly, N.P., 2008. New Wine Yeasts for South African Winemakers. Wineland November, 20-24.

Hart, R.S., Jolly, N.P., Mohamed, G., Booyse, M., Ndimba, B.K., 2016. Characterisation of *Saccharomyces cerevisiae* hybrid yeasts selected for low volatile acidity formation and the production of aromatic Sauvignon blanc wine. Afr. J. Biotechnol. 15, 2068–2081. https://doi.org/10.5897/AJB2016.15388.

Hart, R.S., Ndimba, B.K., Jolly, N.P., 2017a. Characterisation and evaluation of thiol-releasing and lower volatile acidity forming intra-genus and inter-genus hybrid yeast strains for Sauvignon blanc wine. Afr. J. Microbiol. Res. 11, 740–755.

Hart, R.S., Ndimba, B.K., Jolly, N.P., 2017b. Characterisation of thiol-releasing and lower volatile acidity forming intra-genus hybrid yeast strains for Sauvignon blanc wine. S. Afr. J. Enol. Vitic. 38 (2), 144–155. https://doi.org/10.21548/38-2-1322.

Hashimura, Y., Giroux, D., Lee, B., 2012. Designing the ideal bioreactor with single-use technology. Bioprocess Int. 10 (5).

Heymann, H., LiCalzi, M., Conversano, M.R., Bauer, A., Skogerson, K., Matthews, M., 2013. Effects of extended grape ripening with or without must and wine alcohol manipulations on Cabernet Sauvignon wine sensory characteristics. S. Afr. J. Enol. Vitic. 34, 86–99. https://doi.org/10.21548/34-1-1084.

Hierro, N., González, A., Mas, A., Guillamón, J.M., 2004. New PCR-based methods for yeast identification. J. Appl. Microbiol. 97, 792–801. https://doi.org/10.1111/j.1365-2672.2004.02369.x.

Hoff, J.W., 2012. Molecular Typing of Wine Yeasts: Evaluation of Typing Techniques and Establishment of a Database. MSc thesis. Stellenbosch University, Private Bag X1, 7602, Matieland (Stellenbosch), South Africa.

Holt, S., Cordente, A.G., Williams, S.J., Capone, D.L., Jitjaroen, W., Menz, I.R., Curtin, C., Anderson, P.A., 2011. Engineering *Saccharomyces cerevisiae* to release 3-mercaptohexan-1-ol during fermentation through overexpression of an *S. cerevisiae* gene, STR3, for improvement of wine aroma. Appl. Environ. Microbiol. 77, 3626–3632. https://doi.org/10.1128/AEM.03009-10.

Hoon, A., 2015. The Evaluation of Fourier Transform Infrared (FT-IR) Spectroscopy and Multivariate Data Analysis Techniques for Quality Control at an Industrial Cellar. MSc thesis. Stellenbosch University, Private Bag X1, 7602, Matieland (Stellenbosch), South Africa.

Hulin, M., Wheals, A., 2014. Rapid identification of *Zygosaccharomyces* with genus-specific primers. Int. J. Food Microbiol. 173, 9–13. https://doi.org/10.1016/j.ijfoodmicro.2013.12.009.

International Organisation of Vine and Wine (OIV), 2018. OIV Statistical Report on World Vitiviniculture. [accessed on 15 August 2019]. http://www.oiv.int/public/medias/6371/oiv-statistical-report-on-world-vitiviniculture-2018.pdf.

Ivey, M.L., Phister, T.G., 2011. Detection and identification of microorganisms in wine: a review of molecular techniques. J. Ind. Microbiol. Biotechnol. 38, 1619–1634. https://doi.org/10.1007/s10295-011-1020-x.

Jimenez, C.R., Huang, L., Qiu, Y., Burlingame, A.L., 2001. In-gel digestion of proteins for MALDI-MS fingerprint mapping. Curr. Protoc. Protein Sci. https://doi.org/10.1002/0471140864.ps1604s14. Chapter 16, Unit 16.4.

Jolly, N.P., O'Kennedy, K., Reid, G., 2008. Fructose Utilisation by Anchor Wed Wine Yeast Strains. Online. http://wineland.archive.shapeshift.co.za/archive/index.php?option=comzine&view=article&id=154:fructose-utilisation-by-anchor-red-wine-yeast-strains accessed on 09 May 2019.

Jolly, N.P., Varela, C., Pretorius, I.S., 2014. Not your ordinary yeast: non-*Saccharomyces* yeasts in wine production uncovered. FEMS Yeast Res. 14, 215–237. https://doi.org/10.1111/1567-1364.12111.

Jubany, S., Tomasco, I., Ponce de León, I., Medina, K., Carrau, F., Arrambide, N., ... Gaggero, C., 2008. Toward a global database for the molecular typing of *Saccharomyces cerevisiae* strains. FEMS Yeast Res. 8, 472–484.

Kim, P.D., Patel, B.B., Yeung, A.T., 2012. Isobaric labeling and data normalization without requiring protein quantitation. J. Biomol. Tech. 23, 11–23. https://doi.org/10.7171/jbt.12-2301-002.

King, E.S., Francis, I.L., Swiegers, J.H., Curtin, C., 2011. Yeast strain-derived sensory differences retained in Sauvignon blanc wines after extended bottle storage Am. J. Enol. Vitic. 62, 366–370.

King, E.S., Heymann, H., 2014. The effect of reduced alcohol on the sensory profiles and consumer preferences of white wine. J. Sens. Stud. 29, 33–42. https://doi.org/10.1111/joss.12079.

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., Glöckner, F.O., 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res. 41, e1. https://doi.org/10.1093/nar/gks808.

Kolkman, A., Daran-Lapujade, P., Fullaondo, A., Olsthoorn, M.M., Pronk, J.T., Slijper, M., Heck, A.J., 2006. Proteome analysis of yeast response to various nutrient limitations. Mol. Syst. Biol. 2, 0026.

Korabecná, M., Liska, V., Fajfrlík, K., 2003. Primers ITS1, ITS2 and ITS4 detect the intraspecies variability in the internal transcribed spacers and 5.8S rRNA gene region in clinical isolates of fungi. Folia Microbiol. (Praha) 48, 233–238.

Lambrechts, M.G., Pretorius, I.S., 2000. Yeast and its importance to wine aroma – a review. S. Afr. J. Enol. Vitic. 21, 97–129.

Latosinska, A., Vougas, K., Makridakis, M., Klein, J., Mullen, W., Abbas, M., Stravodimos, K., Katafigiotis, I., Merseburger, A.S., Zoidakis, J., Mischak, H., Vlahou, A., Jankowski, V., 2015. Comparative analysis of label-free and 8-Plex iTRAQ approach for quantitative tissue proteomic analysis. PLoS One 10, e0137048. https://doi.org/10.1371/journal.pone.0137048.

Li, Z., Adams, R.M., Chourey, K., Hurst, G.B., Hettich, R.L., Pan, C., 2012. Systematic comparison of label-free, metabolic labeling, and isobaric chemical labeling for quantitative proteomics on LTQ Orbitrap Velos. J. Proteome Res. 11, 1582–1590. https://doi.org/10.1021/pr200748h.

Loureiro, V., Malfeito-Ferreira, M., 2003. Spoilage yeasts in the wine industry. Int. J. Food Microbiol. 86, 23–50.

Luo, Z., Walkey, C.J., Madilao, L.L., Measday, V., Van Vuuren, H.J.J., 2013. Functional improvement of *Saccharomyces cerevisiae* to reduce volatile acidity in wine. FEMS Yeast Res. 13, 485–494. https://doi.org/10.1111/1567-1364.12053.

Mateo, J., Jiménez, M., Pastor, A., Huerta, T., 2001. Yeast starter cultures affecting wine fermentation and volatiles. Food Res. Int. 34, 307–314.

Meersman, E., Steenselsa, J., Paulusa, T., Struyfa, N., Saelsa, V., Mathawanc, M., Koffic, J., Vranckenc, G., Verstrepena, K.J., 2015. Breeding strategy to generate robust yeast starter cultures for cocoa pulp fermentations. Appl. Environ. Microbiol. 81, 6166–6176. https://doi.org/10.1128/AEM.00133-15.

Milac, T.I., Randolph, T.W., Wang, P., 2012. Analyzing LC-MS/MS data by spectral count and ion abundance: two case studies. Stat. Interface 5, 75–87. https://doi.org/10.4310/SII. 2012.v5. n1. a7.

Mitterdorfer, G., Mayer, H.K., Kneifel, W., Viernstein, H., 2002. Protein fingerprinting of *Saccharomyces* isolates with therapeutic relevance using one-and two-dimensional electrophoresis. Proteomics. 2, 1532–1538.

Molina, A.M., Guadalupe, V., Varela, C., Swiegers, J.H., Pretorius, I.S., Agosin, E., 2009. Differential synthesis of fermentative aroma compounds of two related commercial wine yeast strains. Food Chem. 117, 189–195.

Moothoo-Padayachie, A., Kandappa, H.R., Krishna, S.B.N., Maier, T., Govender, P., 2013. Biotyping *Saccharomyces cerevisiae* strains using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Eur. Food Res. Technol. 236, 351–364.

Moreno-García, J., García-Martínez, T., Millán, M.C., Mauricio, J.C., Moreno, J., 2015. Proteins involved in wine aroma compounds metabolism by a *Saccharomyces cerevisiae* flor-velum yeast strain grown in two conditions. Food Microbiol. 51, 1–9.

Mostert, T.T., 2013. Investigating the Secretome of Non-*Saccharomyces* Yeast in Model Wine. MSc thesis. Stellenbosch University, Private Bag X1, 7602, Matieland (Stellenbosch), South Africa.

Muñoz, R., Gómez, A., Robles, V., Rodríguez, P., Cebollero, E., Tabera, L., Carrascosa, A.V., Gonzalez, R., 2009. Multilocus sequence typing of oenological *Saccharomyces cerevisiae* strains. Food Microbiol. 26, 841–846.

Naqib, A., Jeon, T., Kunstman, K., Wang, W., Shen, Y., Sweeney, D., Hyde, M., Green, S.J., 2019. PCR effects of melting temperature adjustment of individual primers in degenerate primer pools. PeerJ 7, e6570.

Naseeb, S., Alsammar, H., Burgis, T., Donaldson, I., Knyazev, N., Knight, C., Delneri, D., 2018. Whole genome sequencing, de novo assembly and phenotypic profiling for the new budding yeast species *Saccharomyces jurei*. G3 (Bethesda) 8, 2967–2977.

Navarrete-Bolaños, J.L., 2012. Improving traditional fermented beverages: how to evolve from spontaneous to directed fermentation. Eng. Life Sci. 12, 410–418. https://doi.org/10.1002/elsc.201100128.

Ndimba, B.K., Thomas, L.A., Ngara, R., 2010. Sorghum 2-dimensional proteome profiles and analysis of Hsp70 expression under salinity stress. Kasetsart J. (Nat. Sci.) 44, 768–775.

Nedović, V., Gibson, B., Mantzouridou, T.F., Bugarski, B., Djordjević, V., Kalušević, A., Paraskevopoulou, A., Sandell, M., Šmogrovičová, D., Yilmaztekin, M., 2015. Aroma formation by immobilized yeast cells in fermentation processes. Yeast 32, 173–216.

Ngara, R., Ndimba, R., Borch-Jensen, J., Jensen, O.N., Ndimba, B., 2012. Identification and profiling of salinity stress-responsive proteins in *Sorghum bicolor* seedlings. J. Proteome 75, 4139–4150.

Nie, S., Lo, A., Zhu, J., Wu, J., Ruffin, M.T., Lubman, D.M., 2013. An isobaric protein-level labeling strategy for serum glycoprotein quantification analysis by LC-MS/MS. Anal. Chem. 85, 5353–5357.

Nowakowski, A.B., Wobig, W.J., Petering, D.H., 2014. Native SDS-PAGE: high resolution electrophoretic separation of proteins with retention of native properties including bound metal ions. Metallomics 6, 1068–1078. https://doi.org/10.1039/c4mt00033a.

Ocón, E., Gutiérrez, A.R., Garijo, P., Tenorio, C., López, I., López, R., Santamaría, P., 2010. Quantitative and qualitative analysis of non-*Saccharomyces* yeasts in spontaneous alcoholic fermentations. Eur. Food Res. Technol. 230, 885–891. https://doi.org/10.1007/s00217-010-1233-7.

Padilla, B., Gil, J.V., Manzanares, P., 2016. Past and future of non-*Saccharomyces* yeasts: from spoilage microorganisms to biotechnological tools for improving wine aroma

complexity. Front. Microbiol. 7, 411. https://doi.org/10.3389/fmicb.2016.00411.
Panda, A., Ghosh, A.K., Mirdha, B.R., Xess, I., Paul, S., Samantaray, J.C., Srinivasan, A., Khalil, S., Rastogi, N., Dabas, Y., 2015. MALDI-TOF mass spectrometry for rapid identification of clinical fungal isolates based on ribosomal protein biomarkers. J. Microbiol. Methods 109, 93–105.
Parcunev, I., Naydenova, V., Kostov, G., Yanakiev, Y., Popova, Z., Kaneva, M., Ignatov, I., 2012. Modelling of alcoholic fermentation in brewing-some practical approaches. In: Troitzsch, K.G., Möhring, M., Lotzmann, U. (Eds.), Proceedings 26th European Conference on Modelling and Simulation, pp. 434–440 ISBN: 978-0-9564944-4-3.
Parizad, E.G., Parizad, E.G., Valizadeh, A., 2016. The application of pulsed-field gel electrophoresis n clinical studies. J. Clin. Diagn. Res. 10, 1–4.
Parker, R., 2014. RNA degradation in *Saccharomyces cerevisae*. Genetics 191, 671–702. https://doi.org/10.1534/genetics.111.137265.
Parker, C.E., Borchers, C.H., 2014. Mass spectrometry based biomarker discovery, verification, and validation—quality assurance and control of protein biomarker assays. Mol. Oncol. 8, 840–858.
Pérez-Torrado, R., Querol, A., Guillamón, J.M., 2015. Genetic improvement of non-GMO wine yeasts: strategies, advantages and safety. Trends Food Sci. Technol. 45, 1–11.
Pinu, F.R., Jouanneau, S., Nicolau, L., Gardner, R.C., Villas-Boas, S.G., 2012. Concentrations of the volatile thiol 3-mercaptohexanol in Sauvignon blanc wines: no correlation with juice precursors. Am. J. Enol. Vitic. 63, 407–412.
Pretorius, I.S., 2016. Conducting wine symphonics with the aid of yeast genomics. Beverages 2, 36–63. https://doi.org/10.3390/beverages2040036.
Renault, P., Coulon, J., Moine, V., Thibon, C., Bely, M., 2016. Enhanced 3-sulfanylhexan-1-ol production in sequential mixed fermentation with *Torulaspora delbrueckii*/*Saccharomyces cerevisiae* reveals a situation of synergistic Interaction between two industrial strains. Front. Microbiol. 7, 293. https://doi.org/10.3389/fmicb.2016.00293.
Rizzato, C., Lombardi, L., Zoppo, M., Antonella, Lupetti, Tavanti, A., 2016. Pushing the limits of MALDI-TOF mass spectrometry: beyond fungal species identification. J. Fungi. 1, 367–383.
Rollero, S., Mouret, J., Sanchez, I., Camarasa, C., Ortiz-Julien, A., Sablayrolles, J., Dequin, S., 2016. Key role of lipid management in nitrogen and aroma metabolism in an evolved wine yeast strain. Microb. Cell Factories 15, 32–46.
Sagratini, G., Maggi, F., Caprioli, G., Cristalli, G., Ricciutelli, M., Torregiani, E., Vittori, S., 2012. Comparative study of aroma profile and phenolic content of Montepulciano monovarietal red wines from the Marches and Abruzzo regions of Italy using HS-SPME-GC-MS and HPLC-MS. Food Chem. 132, 1592–1599.
Salvado, Z., Chiva, R., Rodríguez-Vargas, S., Rández-Gil, F., Mas, A., Guillamo, J.M., 2008. Proteomic evolution of a wine yeast during the first hours of fermentation. FEMS Yeast Res. 8, 1137–1146.
Santos, J., Sousa, M.J., Cardoso, H., Inacio, J., Silva, S., Spencer-Martins, I., Leao, C., 2008. Ethanol tolerance of sugar transport, and the rectification of stuck wine fermentations. Microbiology 154, 422–430.
Savolainen, O.I., Sandberg, A., Ross, A.B., 2016. A simultaneous metabolic profiling and quantitative multimetabolite metabolomic method for human plasma using gas-chromatography tandem mass spectrometry. J. Proteome Res. 15, 259–265. https://doi.org/10.1021/acs.jproteome.5b00790.
Schmidtke, L.M., Blackman, J.W., Clark, A.C., Grant-Preece, P., 2013. Wine metabolomics: objective measures of sensory properties of semillon from GC-MS profiles. J. Agric. Food Chem. 61, 11957–11967. https://doi.org/10.1021/jf403504p.
Schueuermann, C., Khakimov, B., Engelsen, S.B., Bremer, P., Silcock, P., 2016. GC-MS metabolite profiling of extreme Southern Pinot noir wines: effects of vintage, barrel maturation, and fermentation dominate over vineyard site and clone selection. J. Agric. Food Chem. 64, 2342–2351. https://doi.org/10.1021/acs.jafc.5b05861.
Sharma, A.K., Pingh, P.N., Sawant, S.D., 2012. Evaluation of fermentation efficiency of yeast strains and their effect on quality of young wines. Indian J. Microbiol. 52, 495–499.
Shepherd, G., 2006. Smell images and the flavour system in the human brain. Nature 444, 316–321. https://doi.org/10.1038/nature05405.
Shevchenko, A., Wilm, M., Vorm, O., Mann, M., 1996a. Mass spectrometric sequencing of proteins from silver-stained polyacrylamide gels. Anal. Chem. 68, 850–858.
Shevchenko, A., Jensen, O.N., Podtelejnikov, A.V., Sagliocco, F., Wilm, M., Vorm, O., Mortensen, P., Shevchenko, A., Boucherie, H., Mann, M., 1996b. Linking genome and proteome by mass spectrometry: large-scale identification of yeast proteins from two dimensional gels. Proc. Natl. Acad. Sci. U. S. A. 93, 14440–14445.
Singhal, N., Kumar, M., Kanaujia, P.K., Virdi, J.S., 2015. MALDI-TOF mass spectrometry: an emerging technology for microbial identification and diagnosis. Front. Microbiol. 6, 791. https://doi.org/10.3389/fmicb.2015.00791.
Sipos, R., Székely, A.J., Palatinszky, M., Révész, S., Márialigeti, K., Nikolausz, M., 2007. Effect of primer mismatch, annealing temperature and PCR cycle number on 16S rRNA gene-targeting bacterial community analysis. FEMS Microbial. Ecol. 60, 341–350.
Sirén, K., Mak, S.S.T., Fischer, U., Hansen, L.H., Gilbert, M.T.P., 2019. Multi-omics and potential applications in wine production. Curr. Opin. Biotechnol. 56, 172–178.
Sonego, J.L.S., Lemos, D.A., Pinto, C.E.M., Cruz, A.J.G., Badino, A.C., 2016. Extractive fed-batch ethanol fermentation with CO_2 stripping in a bubble column bioreactor: experiment and modelling. Energy Fuel 30, 748–757. https://doi.org/10.1021/acs.energyfuels.5b02320.
South African Wine Industry Information and Systems (SAWIS), 2015. Online. http://www.sawis.co.za/info/download/Macro-economic_impact_study_-_Final_Report_Version_4_30Jan2015.pdf [accessed on 30 July 2019].
Sutton, C.W., Pemberton, K.S., Cottrell, J.S., Wheeler, C.H., Dunn, M.J., Pappin, D.J., 1995. Identification of myocardial proteins from two-dimensional gels by peptide mass fingerprinting. Electrophoresis. 16, 308–316.
Swiegers, J.H., Chambers, P.J., Pretorius, I.S., 2005. Olfaction and taste: human perception, physiology and genetics. Aust. J. Grape Wine Res. 11, 109–113. https://doi.org/10.1111/j.1755-0238.2005.tb00282.x.
Swiegers, J.H., Kievit, R.L., Siebert, T., Lattey, K.A., Bramley, B.R., Francis, I.L., King, E.S., Pretorius, I.S., 2009. The influence of yeast on the aroma of Sauvignon blanc wine. Food Microbiol. 26, 204–211. https://doi.org/10.1016/j.fm.2008.08.004.
Tham, W.P., Stevenson, R.J., Miller, L.A., 2009. The functional role of the mediodorsal thalamic nucleus in human olfaction. Brain Res. Rev. 62, 109–126. https://doi.org/10.1016/j.brainresrev.2009.09.007.
Turvey, M.E., Weiland, F., Meneses, J., Sterenberg, N., Hoffmann, P., 2016. Identification of beer spoilage microorganisms using the MALDI Biotyper platform. Appl. Microbiol. Biotechnol. 100, 2761–2773.
University world news, 2013. The Role of Research Universities in Developing Countries. Online. http://www.universityworldnews.com/article.php?story=20130811091502202 accessed on 09 May 2019.
Usbeck, J.C., Wilde, C., Bertrand, D., Behr, J., Vogel, R.F., 2014. Wine yeast typing by MALDI-TOF MS. Appl. Microbiol. Biotechnol. 98, 3737–3752. https://doi.org/10.1007/s00253-014-5586-x.
Van Breda, V.M., Jolly, N.P., van Wyk, J., 2013. Characterisation of commercial and natural *Torulaspora delbrueckii* wine yeast strains. Int. J. Food Microbiol. 163, 80–88.
Van der Westhuizen, T.J., Pretorius, I.S., 1992. The value of electrophoretic fingerprinting and karyotyping in wine yeast breeding programmes. Antonie Van Leeuwenhoek 61, 249–257.
Van Wyngaard, E., 2013. Volatiles Playing an Important Role in South African Sauvignon Blanc Wines. MSc thesis. Stellenbosch University, Private Bag X1, 7602, Matieland (Stellenbosch), South Africa.
Varela, C., 2016. The impact of non-*Saccharomyces* yeasts in the production of alcoholic beverages. Appl. Microbiol. Biotechnol. 100, 9861–9874.
Vaudano, E., Garcia-Moruno, E., 2008. Discrimination of *Saccharomyces cerevisiae* wine strains using microsatellite multiplex PCR and band pattern analysis. Food Microbiol. 25, 56–64.
Velázquez, R., Zamora, E., Álvarez, M.L., Hernández, L.M., Ramírez, M., 2015. Effects of new *Torulaspora delbrueckii* killer yeasts on the must fermentation kinetics and aroma compounds of white table wine. Front. Microbiol. 6, 1222. https://doi.org/10.3389/fmicb.2015.01222.
Vilela, A., Schuller, D., Mendes-Faia, A., Côrte-Real, M., 2013. Reduction of volatile acidity of acidic wines by immobilized *Saccharomyces cerevisiae* cells. Appl. Microbiol. Biotechnol. 97, 4991–5000. https://doi.org/10.1007/s00253-013-4719-y.
Vilela-Moura, A., Schuller, D., Mendes-Faia, A., Côrte-Real, M., 2010. Effects of acetic acid, ethanol, and SO_2 on the removal of volatile acidity from acidic wines by two *Saccharomyces cerevisiae* commercial strains. Appl. Microbiol. Biotechnol. 87, 1317–1326.
Von Mollendorf, A., 2013. The Impact of Wine Yeast Strains on the Aromatic Profiles of Sauvignon Blanc Wines Derived from Characterized Viticultural Treatments. MSc thesis. Stellenbosch University, Private Bag X1, 7602, Matieland (Stellenbosch), South Africa.
Wang, C., Mas, A., Esteve-Zarzoso, B., 2016. The interaction between *Saccharomyces cerevisiae* and non-*Saccharomyces* yeast during alcoholic fermentation is species and strain specific. Front. Microbiol. 7, 502. https://doi.org/10.3389/fmicb.2016.00502.
Weightman, C., Brand, J., Nieuwoudt, H., 2016. Sensory Evaluation of Wine (Part 1). Online. http://wineland.co.za/technical/sensory-evaluation-of-wine-part-1 accessed on 09 May 2019.
Westermeier, R., 2006. Sensitive, quantitative, and fast modifications for Coomassie Blue staining of polyacrylamide gels. Proteomics 6, 61–64. https://doi.org/10.1002/pmic.200690121.
Zaia, D.A.M., Marques, F.R., Zaia, C.T.B.V., 2005. Spectrophotometric determination of total proteins in blood plasma: a comparative study among dye-binding methods. Braz. Arch. Biol. Technol. 48, 385–388. https://doi.org/10.1590/S1516-89132005000300008.
Zhou, G., Yang, L.T., Li, Y.R., Zou, C.L., Huang, L.P., Qiu, L.H., Huang, X., Srivastava, M.K., 2012. Proteomic analysis of osmotic stress-responsive proteins in sugarcane leaves. Plant Mol. Biol. Report. 30, 349–359. https://doi.org/10.1007/s11105-011-0343-0.