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Use of non-*Saccharomyces* yeasts and oenological tannin in red winemaking: Influence on colour, aroma and sensorial properties of young wines

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ABSTRACT

Today, many non-*Saccharomyces* strains have been verified can be positive for the development of wine anthocyanin and aroma in different fermentation scenarios. Moreover, oenological tannins are widely used in wine industry to improve the colour profile and aroma complexity. The aim of this work is to analyze the fermentation characters of non-*Saccharomyces* strains and investigate the effects of pre-fermentative addition of oenological tannins on the wine components as well as sensory properties. For this purpose, five selected non-*Saccharomyces* strains and grape seed tannin were used to carry out the different fermentation trials. As a result, the grape seed tannin were less likely to influence growth kinetics of non-*Saccharomyces* strains. *Schizosaccharomyces pombe* has been proved can be effective to reduce the malic acid content while increase the level of vinylphenolic pyranoanthocyanin, which is positive for wine colour stability. Pre-fermentative use of oenological tannin was verified could be beneficial for the wines fermented with non-*Saccharomyces* regarding the improvement of wine colour, anthocyanin composition and the complexity of volatile compounds. Nevertheless, sensory analysis showed that oenological tannin could be less effective to modify the aroma impression of non-*Saccharomyces* wines.

1. Introduction

Wine fermentation is a complex biochemical process in which yeasts play an important role during their transformation of sugar into ethanol, carbon dioxide and hundreds of other secondary products (Ciani et al., 2010). Some studies have been carried out to determine the impact of yeasts on wine composition, sensory properties and final flavours (Lencioni et al., 2007; Benito et al., 2014, 2016; Ciani and Comitini, 2011). At present, it is known that the ecology of fermentation process is more complex than previous investigations, and some non-*Saccharomyces* yeasts are also playing relevant roles in the metabolic impact, anthocyanin composition and aroma complexity of the final products (Pretorius, 2000). Recently, there has been a growing demand of new and improved wine yeasts that adapted to different fermentation scenarios (Jolly et al., 2006). In order to improve chemical composition and sensory properties of red wine, the combined use of non-*Saccharomyces* and *Saccharomyces* has been proposed as a tool to take advantage of spontaneous fermentation as well as avoid the risk of stuck fermentations (Fleet, 2008).

Traditionally, many non-*Saccharomyces* strains were considered as a kind of spoilage yeast in terms of the production of undesirable metabolites that result in negative sensory impacts on wine quality (Batt and Tortorello, 2014). Moreover, most of the non-*Saccharomyces* wine-related species showed limited fermentation aptitudes, such as low fermentation kinetics and low sulfur dioxide (SO_2) resistance (Ciani and Comitini, 2011). However, in mixed fermentations, some negative oenological characters of non-*Saccharomyces* may not be expressed or could be modified by *Saccharomyces cerevisiae* (*S. cerevisiae*) (Bely et al., 2008). Many experimental evidences have highlighted the positive role of non-*Saccharomyces* in

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the analytical composition of wine (Comitini et al., 2011). For instance, *Schizosaccharomyces* not only can improve the fermentation behaviour of yeast starter cultures and aroma complexity but also produce a high amount of pyruvic acid which contributes to the formation of vinylphenolic pyranoanthocyanins (Benito et al., 2016; Morata et al., 2007); *Lachancea thermotolerans* (*L. thermotolerans*) can produce more L-lactic acid while limit the concentration of malic acid. It has been specially used to modify the wine acidity (Kapsopoulou et al., 2007); *Torulaspora delbrueckii* (*T. delbrueckii*) can produce high amount of higher alcohols and terpenes (Chen and Liu, 2015). Nevertheless, some researches indicate that the yeast growth kinetics could be reduced by addition of grape tannins. It is due to yeast cell wall interact with tannins, hence, the material exchange with must could be inhibited (Mekoue et al., 2015).

Nowadays, oenological tannins have been commonly used into different fermentation scenarios in order to modify aroma complexity, prevent colour loss and enhance well-rounded taste (Chen et al., 2016a,b). Particularly, the pre-fermentative addition of grape seed tannin which contains large amount of oligomeric proanthocyanidin (a set of bioflavonoid complexes that can perform the high antioxidant activity while lower astringency) has been widely reported as a practical tool to improve wine colour stability as well as aroma complexity (Chen et al., 2016a,b). During alcoholic fermentation (AF), tannin treatment could influence the development of pyruvic acid and acetaldehyde, which can directly link with malvidin-3-*O*-glucoside to form high stable pyranoanthocyanins by cycloaddition, such as vitisin A (Vit A) and vitisin B (Vit B). These pyranoanthocyanins are strongly resistant to SO_2 bleaching effect and can protect the wine from oxidation (Morata et al., 2007). With pre-fermentative addition of oenological tannin, the effects of non-*Saccharomyces* on wine components and sensory properties can be regarded as a valuable research since it has been scarcely ever reported.

Above all, the aim of this work is to analyze the interactions between selected non-*Saccharomyces* strains and oenological tannins during AF, meanwhile, the effects of oenological tannin on wine colour, aroma and sensory properties were also studied.

2. Methods and materials

2.1. Microorganism

Non-*Saccharomyces* strains, *Metschnikowia pulcherrima* (*M. pulcherrima*), *L. thermotolerans*, *T. delbrueckii* as well as two wild *Schizosaccharomyces pombe* (*S. pombe*) strains, 938 and V1 were all selected from the Institute of Industrial Fermentation (IFI, CSIC, Madrid) (Benito et al., 2014). *S. cerevisiae* strain (7VA) (Laboratorio de Tecnología de Alimentos, E.T.S.I. Agronomos, Madrid), which possess high capability to produce pyruvic acid and acetaldehyde (Morata et al., 2006), was carried out in this study as a comparison and supplimentary yeast of sequential fermentation. The yeast suspensions were cultivated at 25 °C for 48 h, until the initial inoculation scale was controlled at 10^6 CFU/mL (Benito et al., 2015a,b,c).

2.2. Micro-vinifications

All fermentations were undertaken by using the juice of Merlot grapes (*Vitis vinifera*) grown at Socuéllamos, Ciudad Real in Castilla la Mancha, Spain. The juice was extracted with classic flash thermovinification from fresh must in order to release more colour and tannins. The process heated grapes to a high temperature (about 82 °C) in few seconds, then immediately pumped the fruit into a vacuum chamber for depressurization. Sugars were amended up to 213 g/L, final pH was 3.2, lactic and acetic acids were less than 0.1 g/L. To facilitate the fermentations, nutrients were added at the level of 0.4 g/L (Nutrient-Vit, Lallemand, Montreal, PQ, Canada). Oenological tannin, Tanicol Vintage (40 g/hL) derived from grape seeds, purchased from Agrovin S.A., Ciudad Real, Spain. Six sets of fermentations were carried out with five strains of non-Saccharomyces and one strain of *S. cerevisiae*. Oenological tannin was added at the beginning of each set of fermentation. To make a comparison, each control was designed to be without exogenous tannin in different fermentations. Every treatment was done in triplicate.

In all treatments, *M. pulcherrima*, *L. thermotolerans* and *T. delbrueckii* were carried out with sequential fermentation, which was inoculated with *S. cerevisiae* (7VA) at the fourth fermentation day. It is required since *S. cerevisiae* must be used as a binding partner to supply these non-*Saccharomyces* stains accomplish AF (Benito et al., 2015a,b,c). *S. pombe* strains, 938 and V1 are capable enough to independently conduct the AF (Benito et al., 2014). Each treatment was fermented in a 150 mL microvessel capped with sulfuric acid (Panreac, Barcelona) filled Müller valve (Alamo, Madrid) to avoid microbial contamination and release carbon dioxide (CO_2). All the fermentation trials were accomplished at 25 °C until no weight loss was detected (12 days). After AF, all wines were centrifuged (5000 rpm, 10 min) and then transferred into 125 mL aseptic brown glass bottles, well-sealed and placed at 4 °C for the following analysis.

2.3. Analysis of physical-chemical parameters

Basic fermentation parameters, such as ethanol, total acidity, pH, volatile acid, L-malic acid, L-lactic acid, glucose, fructose, acetic acid were all detected with a Y15 enzymatic autoanalyzer (Biosystems S.A., Barcelona, Spain). These analyses were performed with the appropriate enzymatic reaction kits, which were purchased from Biosystems enterprise. Prior to detections, the Y15 equipment was calibrated with the external standards, which were technically supported by Biosystems enterprise (www.biosystems.com).

2.4. Analysis of phenolic and colour parameters

An Agilent 8453 UV-Visible spectrophotometer (Santa Clara, CA, USA) was used for the detection of phenolic and colour parameters. Beforehand, samples were analysed by full wavelength range (200–1100 nm) with 1 mm quartz cuvette. Absorbance at 420 nm, 520 nm, and 620 nm was measured and then colour intensity was calculated as the sum of absorbance at the three wavelengths, while hue was calculated as the ratio between the absorbance at 420 nm and 520 nm. Total polyphenols index, total tannin content and total anthocyanin content were carried out based on the methods of Ribereau-Gayon et al. (2006). Four important phenolic parameters were also analysed for each trial. Gelatine index is related to the percentage of tannins which are able to combine with protein and mainly used to detect the level of tannin astringency in wine (Oberholster et al., 2013); HCL index indicates the percentage of polymerized tannins combine with wine polysaccharides and salts; Ethanol index is the percentage of tannins which can combine with wine polysaccharides; Vanillin index expressed with mg/L of catechin reflects the flavonoids react with vanillin to form red pigments (Ribereau-Gayon et al., 2006). In addition, CIELAB scales, L^*, a^*, b^* are the colour scales based on the Opponent-Colour Theory that assumes the receptors in the human eye perceive colour as the following pairs of opposites (Gil-Muñoz et al., 1997). L^* scale: Light vs. dark where a low value (0–50) indicates dark and a high value (51–100) indicates light. a^* scale: Red vs. green where a

positive value indicates red and a negative value indicates green. b* scale: Yellow vs. blue where a positive value indicates yellow and a negative value indicates blue (Gil-Muñoz et al., 1997; Schlesier et al., 2009).

On the other hand, anthocyanin composition of each treatment was analysed by the method of Boulton (2001). Prior to determinations, wine samples were centrifuged 5000 rpm, 10 min at 4 °C. Equations are as follows:

$$\text{Total anthocyanin (mg/L)} = 18.9 \times A^{*}_{520\text{-H}} \quad (1)$$

$$\text{Coloured anthocyanin (mg/L)} = 18.9 \times (A^{*}_{520}\text{-}A^{*}_{520\text{-S}}) \quad (2)$$

$$\text{Polymeric anthocyanin (mg/L)} = 18.9 \times (5/3)A^{*}_{520\text{-S}} \quad (3)$$

$$\text{Monomeric anthocyanin (mg/L)} = 18.9 \times [A^{*}_{520\text{-H}}\text{-}(5/3)A^{*}_{520\text{-S}}] \quad (4)$$

*Explanations of relative indexes on above; $A^{*}_{520} = A_{520} \times 10$ (wine was directly detected at wavelength 520 nm with 1 mm cuvette); $A^{*}_{520\text{-S}} = A_{520\text{-S}} \times 1.05 \times 10$ (100 μL 6% sodium metabisulphite was added into 2 mL wine sample, vortex blending and then wait 1 min, detection was conducted under 520 nm wavelength with 1 mm cuvette in terms of $A_{520\text{-S}}$); $A^{*}_{520\text{-H}} = A_{520\text{-H}} \times 100$ (100 μL wine sample was added into 10 mL 1M HCL, vortex blending and then wait 4 h at 25 °C. The detection was conducted under 520 nm wavelength with 1 cm cuvette for in terms of $A_{520\text{-H}}$).

2.5. Anthocyanin analysis by HPLC/ESI-MS

Nineteen anthocyanins and derived pigments were determined by using an Agilent Technologies (Palo Alto, CA, USA) series 1100 HPLC equipped with a diode array detector and a quadrupole mass spectrometer with an electrospray interface. Gradients of solvent A (water/formic acid, 95:5, v/v) and B (methanol/formic acid, 95:5, v/v) were used in a reverse-phase Kinetex C18 column (Phenomenex, Torrance, CA, USA) (100 × 4.6 mm; particle size 2.6 μm) as follows: 20–50% B linear (0.8 mL/min) from 0 to 27 min, 50% B from 27 to 28 min, 20–50% B linear (0.8 mL/min) from 28 to 29 min, and re-equilibration of the column from 29 to 30 min. Detection was performed by scanning in the 500–600 nm range. Quantification was performed by comparison against an external standard at 525 nm and expressed as a function of the concentration of M3G (Extrasynthèse, Genay, France). The different anthocyanins were identified by their retention times with reference to the majority anthocyanin M3G, and by comparing the UV–Visible and mass spectra with data in the literature (Morata et al., 2012, 2007, 2016). The electrospray ionization variables were: drying gas (N_2) flow rate 10 mL/min; temperature 350 °C; nebulizer pressure 380 Pa (55 psi); and capillary voltage 4000 V. Mass spectrometry was performed in positive mode scanning from m/z 100 to m/z 1500, using a fragmentary voltage of 150 V from 0 to 23 min. One hundred microliters sample of previously filtered (0.45 μm membrane filters made of cellulose methylic esters (Tecknokroma, Barcelona)) wines were injected into the HPLC apparatus. The detection limit was 0.1 mg/L. Each wine sample was done in triplicate.

2.6. Analysis of volatile compounds

Seventeen volatile compounds were determined at the end of AF by Agilent Technologies 6850 gas chromatograph with a flame ionization detector (Hewlett-Packard, Palo Alto, CA, USA). The apparatus was calibrated with 4-methyl-2-pentanol as an internal standard at 50 mg/L. GC quality standard reagents were purchased from Fluka, Sigma-Aldrich Corp., Switzerland. The system was prepared with a DB-624 column (60 m × 250 μm × 1.40 μm), injector temperature was 250 °C and the detector temperature 300 °C. The column temperature was 40 °C for the first 5 min, rising linearly by 10 °C/min until reaching 250 °C and then maintained for 5 min. Hydrogen was used as the carrier gas, which was provided by a hydrogen generator (LNI Schmidlin SA, Geneva, Switzerland). The flow rate was 22.1 L/min, injection split ratio was 1:10, and detection limit was 0.1 mg/L. One hundred microliters of internal standard (50 mg/L) was added to 1 mL test samples and filtered through syringe membrane filters (0.45 μm pore size) (Teknokroma, Barcelona, Spain). They were then placed in 1.5 ml glass vials sealed with a PTFE/silicon septum. One microliter of this filtrate was injected into the GC apparatus. Each wine sample was done in triplicate.

2.7. Sensory analysis

Sensory analysis was conducted with the method organized by professional panellists from the Polytechnic University of Madrid. The experimental wines were evaluated with blind tasting by experienced wine tasters (6 females and 4 males) who were invited from the Chemistry and Food Technology Department (UPM, Madrid, Spain). No specific training was carried out before tasting sessions. Previous to the tasting session, the studied parameters were established by consensus. Twelve blended wines were respectively made from the repetition mixture of each treatment, were evaluated in a randomized order. The wines were presented in tasting glasses identified with numbers from one to twelve in an air-conditioned (24 °C) tasting room. Twenty millilitres of each wine was served at 14 °C for once taste and more litre was available to be provided according to the panellist's need. The panellists were asked to rate the wines on seventeen attributes according to an unstructured scale 0 (absent) to 5 (very intense), to rate the intensity of the parameters. Additionally, the panellists were asked to name descriptors as free comments for each wine sample.

2.8. Statistics

All the statistical analysis of experimental data were carried out with SPSS 19.0 software (IBM SPSS Inc., Chicago). For each fermentation trial, group differences were identified with ANOVA analysis followed by Duncan's multiple range test at $p < 0.05$. Cluster analysis (CA) with Ward's method and Squared Euclidean Distance was used for variances analysis. Meanwhile, Principal component analysis (PCA) with the method of varimax rotation was carried out to highlight the main contributors of different observations.

3. Results and discussion

3.1. Yeast population kinetics

The inoculation methods and yeasts developments during AF had been investigated. Based on previous experiences and relative literatures, sequential inoculation is needed for low fermentative non-*Saccharomyces* (*M. pulcherrima*, *L. thermotolerans* and *T. delbrueckii*) which are not able to conduct whole fermentation. Early disappearances were observed as a result of low alcohol resistance (8–9% v/v) as well as antimicrobial peptides, which was released by *S. cerevisiae* metabolism (Benito et al., 2015a,b,c). Previous study mentioned the microbe activities can be inhibited by tannins, which have bonding effects with the proteins on yeast cell wall (Ribereau-Gayon et al., 2006). However, it was observed the 40 g/hL oenological tannin did not affect yeasts development in each fermentation. *S. pombe* strains, 938 and V1, were verified have a high fermentative ability (Benito et al., 2016), which is similar to

S. cerevisae 7VA. The highest yeast population was recorded at the 5th fermentation day. As expected, *S. pombe* and *S. cerevisiae* independently accomplished the whole fermentation process. Accordingly, the influence of low amount of oenological tannin (40 g/hL) on non-*Saccharomyces* yeasts growth kinetics is not significant.

3.2. Analysis of physical-chemical parameters

The physical-chemical parameters of different fermentation scenarios are shown in Table 1. *S. pombe* V1 trial showed the highest amount of ethanol production in all fermentations. The high pH value could be related to the degradation of organic acids. Comparing with the *S. cerevisiae* (7VA), *S. pombe* (V1 and 938) trials showed lower level of total acidity and no L-malic acid was detected as it is usually expected. *T. delbrueckii* trials showed low level of acetic acid, which is an opposite result to the wines fermented with *S. pombe* 938. Moreover, the total acidity of *M. pulcherrima* trials was significantly increased 24% by oenological tannin. It is probably due to grape seed tannin, which can stimulate some non-*Saccharomyces* strains, such as *M. pulcherrima*, to produce higher amount of L-malic acid (Bonciani et al., 2016). A similar effect was observed in *L. thermotolerans* trials, the amount of malic acid was increased 13% with addition of oenological tannin. At the end of AF, it was observed that *L. thermotolerans* produced a certain amount of lactic acid which could offset or increase the wines acidity (Benito et al., 2015a,b,c; Lu et al., 2016). On the other hand, no L-malic acid was detected from V1 and 938 trials. It indicates *S. pombe* V1 is promising to be used for acids reduction in cold regions where the wine contains too much acidity. However, in all fermentation trials, 938 trial with grape seed tannin produced the highest level of acetic acid that could affect the wine quality. In addition, residual glucose and fructose in 938 trials were reduced by tannin addition, 42% and 35% decreased respectively. However, tannin addition was non-significant to influence the consumption of glucose/fructose in other fermentations.

3.3. Analysis of phenolic & colour parameters

According to the phenolic results in Table 2, tannin addition was less effective to influence polyphenols accumulation in *L. thermotolerans* and *T. delbrueckii* trials. With centrifugation, it was confirmed that the total tannin in each fermentation was irrelevant to addition of oenological tannin. Meanwhile, oenological tannin did not affect the development of total anthocyanin in non-*Saccharomyces* trials. On the contrary, total anthocyanin of *S. cerevisiae* trials were significantly increased by the addition of 40 g/hL tannin. Gelatine index highlights the capacity of tannins to react with wine proteins. It can be used for the evaluation of tannin astringency level. With tannin addition, *M. pulcherrima* and *S. cerevisiae* 7VA trials showed similar result of gelatine index, which were increased 63% and 83% respectively. In other words, after being treated with oenological tannin, the *M. pulcherrima* and *S. cerevisiae* wines could be more astringent during AF. Ethanol index of *S. pombe* and *S. cerevisiae* trials indicates there were high percentage of tannin combined with wine polysaccharides in these fermentations. Moreover, in *M. pulcherrima* and *L. thermotolerans* trials, the ethanol index can be significantly increased by tannin addition. HCL index indicates polymerization level of tannins (Ribereau-Gayon et al., 2006). Obviously, *S. pombe* trials expressed a higher polymerization level than other yeasts trials. Particularly, *S. pombe* 938 trials showed the highest value of HCL index. Nevertheless, vanillin index indicates the low-molecular weight flavonoids react with vanillin to form red colour pigments (Sarni-Manchado et al., 2006). As a result, the fermentations with

Table 1
Physical-chemical parameters of wine samples fermented with different yeast strains, the influence of oenological tannin was investigated.

Parameters	*M. pulcherrima*		*L. thermotolerans*		*T. delbrueckii*		*S. pombe V1*		*S. pombe 938*		*S. cerevisiae 7VA*	
		+ TV40		+ TV40		+ TV40		+ TV40		+ TV40		+ TV40
Ethanol (g/L)	12.6 ± 0.00^{abc}	12.5 ± 0.06^{bcd}	12.6 ± 0.06^{ab}	12.6 ± 0.06^{ab}	12.6 ± 0.09^{abc}	12.6 ± 0.00^{abc}	12.7 ± 0.00^{a}	12.7 ± 0.00^{a}	12.5 ± 0.00^{cd}	12.5 ± 0.06^{bcd}	12.5 ± 0.06^{d}	12.5 ± 0.12^{d}
Total acidity (g/L)	5.2 ± 0.10^{e}	6.1 ± 0.25^{ab}	6.4 ± 0.10^{a}	6.1 ± 0.67^{ab}	5.3 ± 0.06^{de}	5.3 ± 0.00^{de}	3.9 ± 0.21^{g}	3.8 ± 0.12^{g}	4.3 ± 0.17^{f}	4.4 ± 0.17^{f}	5.6 ± 0.12^{cd}	5.9 ± 0.06^{bc}
pH	3.6 ± 0.01^{c}	3.6 ± 0.02^{d}	3.4 ± 0.03^{f}	3.4 ± 0.02^{f}	3.6 ± 0.02^{c}	3.7 ± 0.03^{b}	3.7 ± 0.03^{a}	3.8 ± 0.02^{a}	3.8 ± 0.01^{a}	3.8 ± 0.02^{a}	3.5 ± 0.01^{e}	3.5 ± 0.01^{e}
L-Malic acid (g/L)	1.7 ± 0.15^{bc}	2.1 ± 0.07^{a}	1.6 ± 0.17^{c}	1.8 ± 0.04^{b}	1.6 ± 0.08^{c}	1.6 ± 0.15^{c}	0.0 ± 0.00^{d}	0.0 ± 0.00^{d}	0.0 ± 0.00^{d}	0.0 ± 0.00^{d}	2.0 ± 0.11^{a}	2.1 ± 0.06^{a}
L-Lactic acid (g/L)	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	1.70 ± 0.20^{a}	1.70 ± 0.13^{a}	0.01 ± 0.02^{b}	0.04 ± 0.07^{b}	0.00 ± 0.01^{b}	0.00 ± 0.00^{b}	0.04 ± 0.03^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.02 ± 0.03^{b}
Glucose (g/L)	1.2 ± 0.15^{b}	1.1 ± 0.11^{b}	1.2 ± 0.15^{b}	1.2 ± 0.06^{b}	1.1 ± 0.00^{b}	1.3 ± 0.12^{b}	1.3 ± 0.15^{b}	1.0 ± 0.15^{b}	1.9 ± 0.55^{a}	1.1 ± 0.20^{b}	1.3 ± 0.17^{b}	1.1 ± 0.26^{b}
Fructose (g/L)	1.0 ± 0.15^{cde}	0.7 ± 0.10^{de}	0.4 ± 0.20^{e}	0.5 ± 0.17^{de}	1.3 ± 0.10^{cde}	1.3 ± 0.06^{bcd}	1.6 ± 0.45^{bc}	1.6 ± 0.25^{bc}	3.4 ± 1.26^{a}	2.2 ± 0.80^{b}	1.0 ± 0.15^{cde}	0.8 ± 0.06^{cde}
Acetic acid (g/L)	0.17 ± 0.05^{c}	0.16 ± 0.06^{c}	0.16 ± 0.05^{c}	0.16 ± 0.05^{c}	0.06 ± 0.02^{d}	0.12 ± 0.03^{cd}	0.17 ± 0.02^{c}	0.17 ± 0.01^{c}	0.73 ± 0.06^{b}	0.83 ± 0.04^{a}	0.19 ± 0.03^{c}	0.18 ± 0.02^{c}

Different letters show significant difference at $p < 0.05$.
TV40: Tanicol Vintage 40 g/hL

Table 2
Phenolic & Colour parameters of wine samples fermented with different yeast strains, the influence of oenological tannin was investigated.

Parameters	*M. pulcherrima*		*L. thermotolerans*		*T. delbrueckii*		*S. pombe V1*		*S. pombe 938*		*S. cerevisiae 7VA*	
		+ TV40		+ TV40		+ TV40		+ TV40		+ TV40		+ TV40
Phenolic indexes												
Total polyphenol index	98.77 ± 5.97^{d}	119.97 ± 5.54^{a}	98.70 ± 7.53^{d}	98.77 ± 7.26^{d}	100.63 ± 0.47^{cd}	108.60 ± 2.65^{bcd}	112.70 ± 3.87^{ab}	118.07 ± 5.58^{ab}	112.53 ± 5.33^{ab}	120.30 ± 11.35^{a}	109.77 ± 3.89^{abc}	118.73 ± 0.46^{ab}
Total tannin (g/L)	1.52 ± 0.18^{b}	1.88 ± 0.18^{ab}	2.06 ± 0.28^{ab}	2.09 ± 0.3^{a}	2.29 ± 0.03^{a}	2.04 ± 0.04^{ab}	1.78 ± 0.39^{ab}	1.87 ± 0.31^{ab}	2.01 ± 0.44^{a}	1.88 ± 0.16^{ab}	2.04 ± 0.45^{ab}	1.91 ± 0.02^{ab}
Total anthocyanin (mg/L)	420.9 ± 13.1^{ab}	426.3 ± 18.0^{a}	417.4 ± 19.7^{ab}	415.9 ± 7.5^{ab}	388.5 ± 17.7^{bc}	398.4 ± 8.9^{abc}	388.1 ± 5.8^{bc}	386.1 ± 8.10^{bc}	368.2 ± 14.1^{cd}	397.9 ± 39.1^{abc}	342.5 ± 31.0^{d}	384.5 ± 15.8^{bc}
Gelatine index	21.15 ± 0.49^{ef}	34.75 ± 5.36^{bcde}	39.08 ± 8.90^{abcd}	44.24 ± 10.73^{abc}	47.33 ± 8.43^{ab}	34.74 ± 6.41^{bcde}	38.48 ± 7.86^{abcd}	32.68 ± 8.44^{cde}	52.13 ± 6.20^{a}	28.06 ± 6.59^{def}	16.95 ± 2.37^{f}	31.10 ± 9.86^{cde}
Ethanol index	4.01 ± 0.16^{f}	17.37 ± 11.72^{def}	9.16 ± 4.16^{ef}	25.82 ± 12.18^{cde}	12.80 ± 7.74^{def}	10.06 ± 5.92^{ef}	48.47 ± 10.83^{b}	44.09 ± 6.45^{b}	27.26 ± 11.63^{cd}	40.26 ± 10.18^{bc}	65.17 ± 11.52^{a}	65.91 ± 8.41^{a}
HCL index	32.03 ± 2.36^{cd}	30.76 ± 4.58^{cd}	31.60 ± 1.88^{cd}	26.52 ± 4.87^{d}	33.17 ± 1.29^{c}	29.80 ± 2.54^{cd}	41.47 ± 2.06^{b}	41.59 ± 1.50^{b}	50.50 ± 8.06^{a}	47.87 ± 0.42^{a}	31.90 ± 1.30^{cd}	32.49 ± 1.53^{cd}
Vanillin index (Catechin g/L)	0.92 ± 0.02^{de}	0.95 ± 0.04^{bcd}	0.89 ± 0.01^{e}	0.93 ± 0.02^{cde}	0.89 ± 0.03^{de}	0.93 ± 0.02^{cde}	1.01 ± 0.05^{ab}	1.06 ± 0.05^{ab}	0.99 ± 0.06^{bc}	1.00 ± 0.03^{ab}	0.89 ± 0.01^{e}	1.01 ± 0.02^{ab}
Colour indexes												
Colour intensity	14.5 ± 0.6^{d}	19.0 ± 0.7^{b}	15.2 ± 0.2^{d}	16.7 ± 0.3^{c}	15.7 ± 0.5^{cd}	18.3 ± 0.3^{b}	21.9 ± 0.8^{a}	21.7 ± 1.2^{a}	21.5 ± 0.5^{a}	22.1 ± 2.0^{a}	18.2 ± 0.3^{b}	19.1 ± 0.7^{b}
Hue	0.63 ± 0.00^{a}	0.64 ± 0.01^{a}	0.54 ± 0.01^{f}	0.56 ± 0.01^{e}	0.59 ± 0.00^{bc}	0.64 ± 0.00^{a}	0.60 ± 0.01^{b}	0.63 ± 0.02^{a}	0.57 ± 0.00^{de}	0.59 ±0.01^{bc}	0.60 ± 0.01^{b}	0.58 ± 0.01^{cd}
L*	61.60 ± 1.25^{a}	53.94 ± 1.13^{c}	60.06 ± 0.40^{ab}	57.75 ± 0.56^{b}	59.01 ± 0.86^{b}	55.00 ± 0.63^{c}	48.08 ± 1.48^{d}	48.45 ± 1.80^{d}	49.28 ± 0.91^{d}	48.54 ± 3.37^{d}	55.15 ± 0.43^{c}	53.81 ± 0.97^{c}
a*	41.88 ± 0.69^{g}	48.74 ± 1.01^{e}	48.55 ± 0.73^{e}	49.07 ± 0.77^{cde}	45.62 ± 0.75^{f}	46.30 ± 0.69^{f}	50.12 ± 0.18^{bcd}	48.29 ± 1.37^{f}	51.99 ± 0.16^{a}	50.34 ± 0.52^{bc}	48.86 ± 0.63^{de}	51.32 ± 0.53^{ab}
b*	−0.32 ± 0.08^{de}	2.25 ± 0.34^{ab}	−0.98 ± 0.12^{f}	0.40 ± 0.12^{d}	−0.45 ± 0.13^{ef}	2.03 ± 0.19^{b}	1.26 ± 0.37^{c}	1.23 ± 0.58^{c}	2.52 ± 0.35^{ab}	2.73 ± 0.31^{ab}	2.29 ± 0.36^{ab}	1.95 ± 0.70^{b}

Different letters show significant difference at $p < 0.05$.
TV40: Tanicol Vintage 40 g/hL

S. pombe produced more low-molecular weight flavonoids and showed darker colour than other yeasts trials.

On the other hand, colour intensity and hue are commonly used for wine colour evaluation (Benito et al., 2015a,b,c; Mylona et al., 2016). Based on the colour indexes in Table 2, *S. pombe* trials showed higher value of colour intensity than other fermentations. Oenological tannin significantly improved colour intensity of *M. pulcherrima*, *L. thermotolerans* and *T. delbrueckii* trials. Moreover, oenological tannin can be positive to the development of hue for all non-*Saccharomyces* trials, while the hue of *S. cerevisiae* trials were slightly reduced by tannin treatment. CIELAB scales indicate specific colour elements. *S. pombe* trials possess darker and redder colour than others according to the results of L* and a*. Moreover, tannin treatment significantly reduced the L* value of *M. pulcherrima*, *L. thermotolerans*, *T. delbrueckii*, 938 and 7VA. It indicates these fermentations could be developed into darker colour. It is probably due to the monomeric pigments were introduced into the fermentations by addition of exogenous tannin or the effect of copigmentation during AF (Chen et al., 2016a,b), which has been widely reported that the tannin-anthocyanins combination can effectively improve wine colour stability (Liu et al., 2013; Morata et al., 2012). Regarding *S. pombe* and *S. cerevisiae* trials, tannin addition was less effective to influence b* value. However, all the negative b* value become positive after being treated with grape seed tannin. In other words, the original colour of young red wine can be slightly turned to yellow from blue by addition of oenological tannin. This phenomenon could be related to tannin combined the anthocyanin to form pyranoanthocyanins, which are characterized with orange colour (Morata et al., 2016; Rentzsch et al., 2007).

3.4. Analysis of anthocyanins

Fig. 1 illustrated the anthocyanins composition of different fermentations. *S. cerevisiae* 7VA developed the lowest amount of total anthocyanins (Fig. 1A). Sequential fermentations, *M. pulcherrima*, *L. thermotolerans* and *T. delbrueckii* produced a lower amount of coloured anthocyanin than that in the *S. pombe* and *S. cerevisiae* trials, which also showed similar content of coloured anthocyanin. Moreover, tannin addition increased the amount of coloured anthocyanins in each fermentation. On the other hand, *S. pombe* trials (938 and V1) showed lower content of monomeric anthocyanins but higher content of polymeric anthocyanins than other non-*Saccharomyces* (Fig. 1B). It indicates the red wines fermented with *S. pombe* could have higher colour stability. In addition, it was observed that the pre-fermentative use of oenological tannin can increase the content of monomeric anthocyanins for different fermentations. However, in *S. pombe* and *M. pulcherrima* trials, the use of oenological tannin could result in a negative effect on the development of polymeric anthocyanins.

Table 3 showed the contents of anthocyanins in the different yeasts trials. Significantly, vitisin type pigments produced by *S. pombe* were higher than that from other yeast strains during AF. This property of *S. pombe* was previously reported by A. Morata (Morata et al., 2012). Furthermore, oenological tannin positively contributed to the development of monomeric anthocyanins and pyranoanthocyanins in some non-*Saccharomyces* trials (*M. pulcherrima*, *L. thermotolerans* and *T. delbrueckii*) as well as the fermentation of *S. cerevisiae* 7VA. However, tannin addition could affect the development of monomeric anthocyanins and pyranoanthocyanins in *S. pombe* trials. For instance, C3G in V1 trials was significantly reduced 65%, which is the largest drop of anthocyanin content in *S. pombe* trials. Meanwhile, after being treated with oenological tannin, the amount of highly stable pyranoanthocyanins, Vitisin A (Vit A), Vitisin B (Vit B) and Vit A-Ac

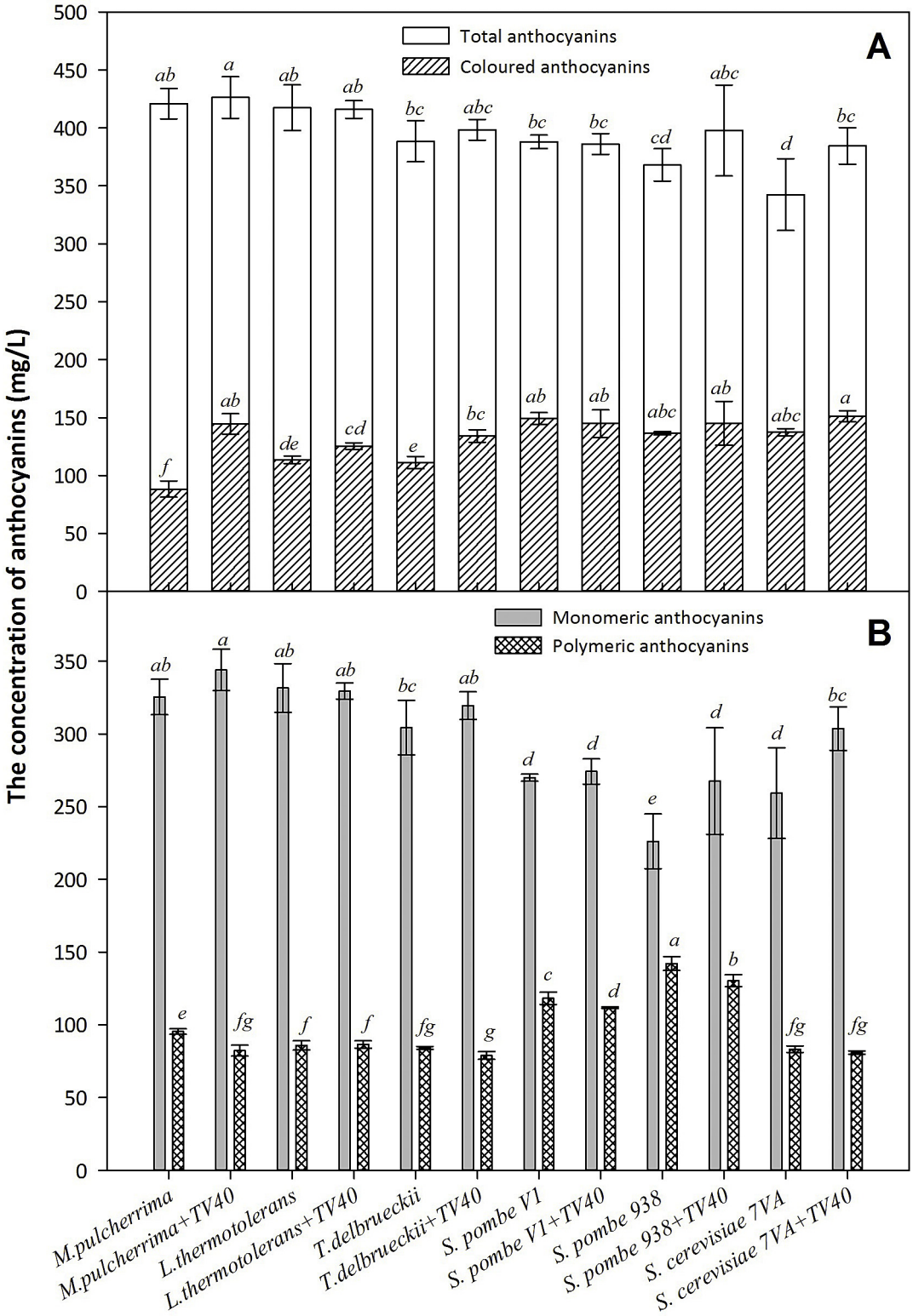

Fig. 1. Anthocyanin composition in different fermentation scenarios. (A) The content of total and coloured anthocyanin. (B) The content of polymerized anthocyanin and monomeric anthocyanin. Grape seed tannin, Tanicol Vintage 40 g/hL (TV40) was applied at the beginning of fermentation for each yeast strain. Different letters indicate the significant differences at $p < 0.05$.

were decreased to some extent. For example, Vit A + Vit B was reduced 12.3% and 12.4% in V1 and 938 trials respectively, while Vit A-Ac was respectively reduced 23.5% and 11.6% in V1 and 938 trials. The decreases probably result from oenological tannin affected the *S. pombe* metabolism to carry out material exchanges with must. Consequently, the production of pyruvic acid and acetaldehyde, which are the predecessor of Vit A and Vit B, were reduced during AF (Domizio et al., 2017). On the other hand, tannin addition could

Table 3
Anthocyanin composition of different fermentation scenarios, the influence of oenological tannin was investigated.

Anthocyanins (mg/L)	*M. pulcherrima*		*L. thermotolerans*		*T. delbrueckii*		*S. pombe V1*		*S. pombe 938*		*S. cerevisiae 7VA*	
		+ TV40		+ TV40		+ TV40		+ TV40		+ TV40		+ TV40
D3G	11.95 ± 0.19^{e}	22.95 ± 0.90^{b}	21.29 ± 1.11^{b}	22.83 ± 0.84^{b}	14.60 ± 0.68^{de}	16.27 ± 0.15^{cd}	27.86 ± 2.28^{a}	23.29 ± 4.30^{b}	27.47 ± 1.17^{a}	26.50 ± 1.11^{a}	17.87 ± 0.93^{c}	23.77 ± 0.43^{b}
C3G	0.22 ± 0.05^{b}	0.60 ± 0.04^{b}	0.82 ± 0.17^{b}	1.10 ± 0.03^{b}	0.28 ± 0.05^{b}	0.47 ± 0.06^{b}	2.84 ± 0.98^{a}	0.98 ± 0.69^{b}	3.00 ± 0.49^{a}	2.49 ± 1.10^{a}	0.36 ± 0.01^{b}	0.59 ± 0.04^{b}
Pt3G	18.59 ± 0.78^{e}	26.37 ± 0.72^{a}	23.33 ± 0.52^{bc}	23.85 ± 0.90^{b}	21.47 ± 0.34^{d}	23.03 ± 0.44^{bcd}	24.64 ± 1.31^{b}	24.05 ± 1.38^{b}	23.22 ± 0.84^{bc}	23.79 ± 0.89^{b}	22.04 ± 0.55^{cd}	26.97 ± 1.35^{a}
Pn3G	4.58 ± 0.43^{e}	8.65 ± 0.40^{bc}	8.25 ± 0.73^{bc}	9.57 ± 0.08^{b}	5.82 ± 0.56^{de}	7.55 ± 0.37^{bcd}	14.04 ± 1.89^{a}	9.31 ± 3.00^{b}	14.48 ± 1.03^{a}	13.57 ± 2.00^{a}	6.44 ± 0.18^{cde}	8.99 ± 0.23^{b}
M3G	102.75 ± 1.70^{e}	125.47 ± 1.30^{a}	110.27 ± 2.14^{cd}	112.82 ± 1.39^{cd}	113.09 ± 0.80^{c}	119.43 ± 1.77^{b}	101.83 ± 3.63^{e}	107.61 ± 0.63^{d}	92.47 ± 4.32^{g}	96.77 ± 2.77^{f}	114.11 ± 3.36^{c}	128.95 ± 0.37^{a}
VitA + VitB	4.20 ± 0.61^{f}	6.09 ± 0.77^{cd}	5.11 ± 0.74^{def}	5.32 ± 0.81^{def}	4.64 ± 0.26^{ef}	4.63 ± 0.06^{ef}	6.82 ± 0.49^{c}	5.98 ± 0.43^{cd}	11.43 ± 0.67^{a}	10.01 ± 1.12^{b}	5.02 ± 0.34^{def}	5.65 ± 0.09^{de}
VitA-Ac	0.55 ± 0.01^{f}	0.75 ± 0.10^{ef}	0.68 ± 0.05^{ef}	0.67 ± 0.01^{ef}	0.67 ± 0.04^{ef}	0.58 ± 0.05^{ef}	1.36 ± 0.10^{c}	1.04 ± 0.13^{d}	2.67 ± 0.06^{a}	2.36 ± 0.31^{b}	0.78 ± 0.06^{e}	0.76 ± 1.10^{ef}
C3GAc	1.08 ± 0.02^{g}	1.23 ± 0.03^{ef}	1.40 ± 0.09^{c}	1.33 ± 0.06^{cd}	1.29 ± 0.03^{de}	1.28 ± 0.02^{de}	1.51 ± 0.05^{b}	1.56 ± 0.05^{b}	2.67 ± 0.06^{a}	2.36 ± 0.31^{b}	0.78 ± 0.06^{e}	0.76 ± 0.10^{ef}
Pt3GAc	4.14 ± 0.18^{f}	4.73 ± 0.11^{de}	4.90 ± 0.11^{cd}	4.74 ± 0.13^{de}	4.65 ± 0.18^{de}	5.00 ± 0.11^{bc}	5.21 ± 0.00^{b}	5.53 ± 0.05^{a}	5.02 ± 0.09^{bc}	5.03 ± 0.18^{bc}	4.63 ± 0.27^{e}	5.15 ± 0.06^{bc}
Pn3GAc	3.04 ± 0.18^{d}	3.80 ± 0.43^{ab}	3.55 ± 0.18^{abc}	3.34 ± 0.04^{cd}	3.29 ± 0.11^{cd}	3.17 ± 0.15^{cd}	3.39 ± 0.26^{bcd}	3.19 ± 0.13^{cd}	3.92 ± 0.30^{a}	3.78 ± 0.40^{ab}	3.22 ± 0.08^{cd}	3.52 ± 0.07^{abc}
M3GAc	24.77 ± 0.49^{ef}	29.92 ± 0.19^{b}	26.74 ± 0.62^{d}	27.46 ± 0.36^{d}	27.09 ± 0.26^{d}	28.68 ± 0.68^{c}	25.39 ± 0.71^{e}	26.84 ± 0.14^{d}	23.11 ± 0.76^{g}	24.00 ± 0.45^{f}	27.48 ± 0.77^{d}	31.04 ± 0.16^{a}
C3GCm	0.73 ± 0.02^{def}	0.84 ± 0.02^{ab}	0.88 ± 0.01^{a}	0.81 ± 0.01^{abc}	0.77 ± 0.03^{bcde}	0.79 ± 0.03^{bcd}	0.71 ± 0.01^{efg}	0.75 ± 0.02^{cdef}	0.64 ± 0.03^{g}	0.69 ± 0.03^{fg}	0.73 ± 0.04^{def}	0.87 ± 0.12^{a}
Pt3GCm	2.32 ± 0.05^{b}	2.35 ± 0.04^{b}	2.45 ± 0.06^{a}	2.47 ± 0.04^{a}	2.32 ± 0.01^{b}	2.34 ± 0.08^{b}	2.02 ± 0.03^{d}	2.22 ± 0.01^{c}	1.86 ± 0.09^{e}	1.94 ± 0.07^{e}	1.92 ± 0.05^{e}	2.32 ± 0.02^{b}
Pn3GCm	0.26 ± 0.01^{de}	0.26 ± 0.01^{cde}	0.33 ± 0.03^{b}	0.32 ± 0.02^{bc}	0.29 ± 0.02^{bcd}	0.28 ± 0.01^{bcde}	0.41 ± 0.04^{a}	0.43 ± 0.01^{a}	0.40 ± 0.05^{a}	0.41 ± 0.07^{a}	0.24 ± 0.02^{de}	0.23 ± 0.00^{e}
M3GCm	19.94 ± 0.47^{a}	19.34 ± 0.84^{ab}	19.75 ± 0.15^{a}	19.77 ± 0.43^{a}	18.64 ± 0.16^{bc}	18.41 ± 0.27^{c}	15.98 ± 0.31^{e}	17.37 ± 0.13^{d}	14.93 ± 0.39^{f}	15.71 ± 0.88^{ef}	15.55 ± 0.20^{ef}	18.52 ± 0.21^{c}
M3G4Vp	0.26 ± 0.02^{ab}	0.24 ± 0.02^{b}	0.24 ± 0.01^{b}	0.23 ± 0.01^{b}	0.24 ± 0.02^{b}	0.29 ± 0.01^{a}	0.00 ± 0.00^{e}	0.16 ± 0.05^{c}	0.09 ± 0.01^{d}	0.09 ± 0.01^{d}	0.26 ± 0.01^{ab}	0.25 ± 0.00^{b}
M3G4Vg	0.00 ± 0.00^{d}	0.03 ± 0.00^{b}	0.02 ± 0.00^{bcd}	0.00 ± 0.00^{d}	0.03 ± 0.01^{b}	0.05 ± 0.00^{a}	0.07 ± 0.03^{a}	0.01 ± 0.02^{cd}	0.02 ± 0.00^{bcd}	0.01 ± 0.00^{bcd}	0.03 ± 0.00^{b}	0.03 ± 0.00^{bc}
M3GAc4Vp	0.00 ± 0.00^{e}	0.07 ± 0.00^{ab}	0.08 ± 0.01^{a}	0.00 ± 0.00^{e}	0.06 ± 0.00^{cd}	0.06 ± 0.00^{cd}	0.00 ± 0.00^{e}	0.07 ± 0.00^{ab}	0.06 ± 0.01^{cd}	0.07 ± 0.01^{bc}	0.06 ± 0.00^{cd}	0.06 ± 0.00^{d}
M3GCm4Vp	0.00 ± 0.00^{e}	0.10 ± 0.01^{bc}	0.12 ± 0.01^{ab}	0.08 ± 0.02^{c}	0.11 ± 0.00^{ab}	0.12 ± 0.00^{a}	0.00 ± 0.00^{e}	0.08 ± 0.02^{c}	0.03 ± 0.00^{d}	0.03 ± 0.00^{d}	0.11 ± 0.02^{ab}	0.11 ± 0.00^{ab}

Different letters show significant difference at $p < 0.05$.
TV40: Tanicol Vintage 40 g/hL.
Abbreviations of each anthocyanin: D3G: delphinidin-3-*O*-glucoside, C3G: cyanidin-3-*O*-glucoside, Pt3G: petunidin-3-Oglucosid, Pn3G: peonidin-3-*O*-glucoside, M3G: malvidin-3-*O*-glucoside, Vit A + Vit B: Vitisin A + Vitisin B, Vit A-Ac: Vitisin A-(6″-acetylglucoside), C3GAc: cyanidin-3-*O*-(6″-acetylglucoside), Pt3GAc: petunidin-3-*O*-(6″-acetylglucoside), Pn3GAc: peonidin-3-*O*-(6″-acetylglucoside), M3GAc: malvidin-3-*O*-(6″-acetylglucoside), C3GCm: cyanidin-3-*O*-(6″-*p*-coumaroylglucoside), Pt3GCm: petunidin-3-*O*-(6″-*p*-coumaroylglucoside), Pn3GCm: peonidin-3-*O*-(6″-*p*-coumaroylglucoside), M3GCm: malvidin-3-*O*-(6″-*p*-coumaroylglucoside), M3G4Vp: Malvidin-3-glucoside-4-vinylphenol, M3G4Vg: Malvidin-3-4-vinylguaiacol, M3GAc4Vp: Malvidin-3-(6″-acetylglucoside)-4-vinyphenol, M3GCm4Vp: Malvidin-3-(6″-*p*-coumaroylglucoside)-4-vinylphenol.

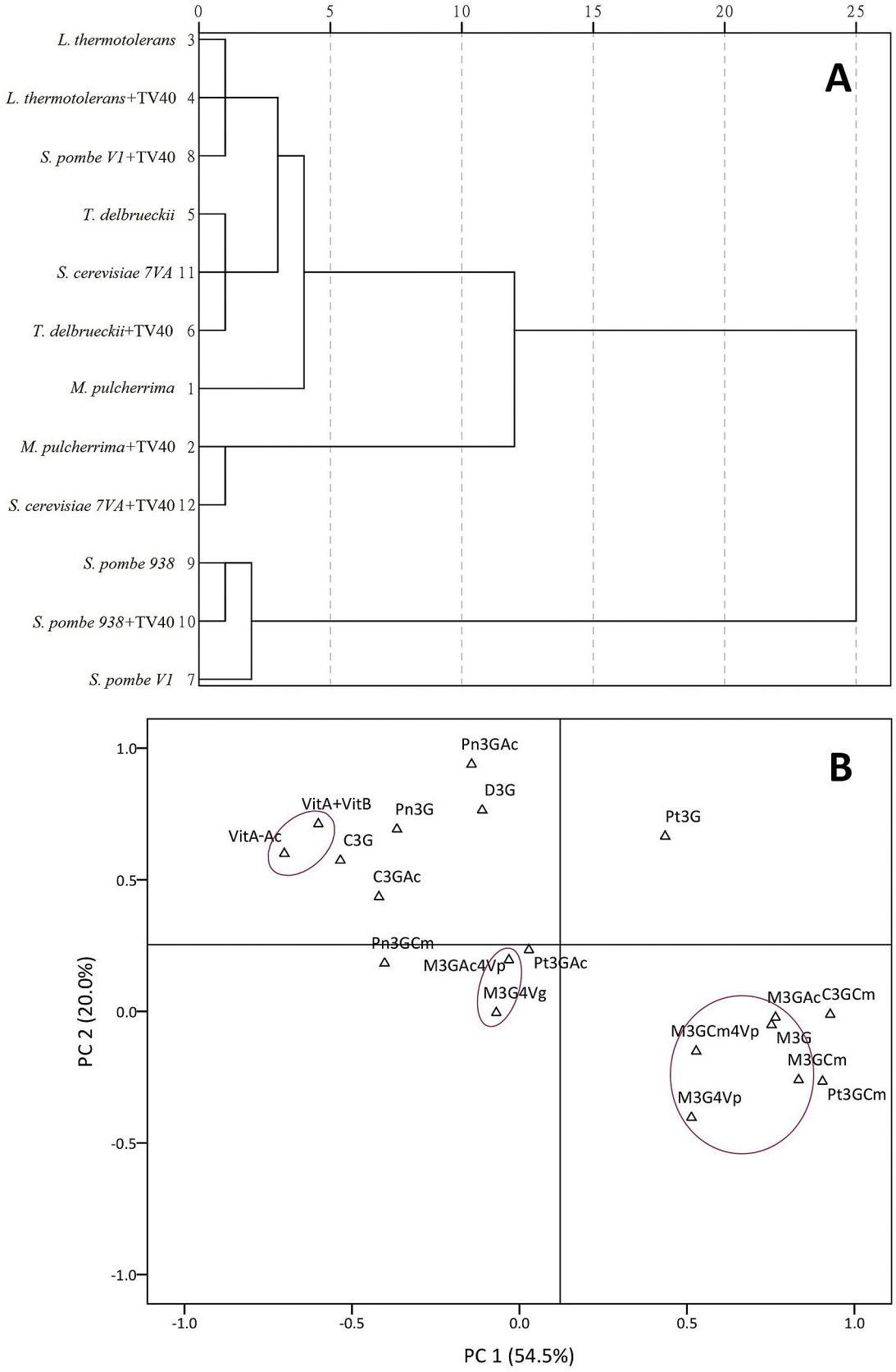

Fig. 2. The analysis of anthocyanin composition for variances and observations. (A) Cluster analysis of the fermentation variances. (B) Principal component analysis of the anthocyanins observations. Grape seed tannin, Tanicol Vintage 40 g/hL (TV40) was applied at the beginning of fermentation for each yeast strain.

be beneficial to the development of M3G and its derivatives, such as M3GAc and M3GCm, which were increased to different levels in all fermentations. Particularly, the fermentations involving 7 VA (sequential inoculations and 7VA single inoculation) possess higher level of M3G than *S. pombe* trials during AF. It's worth mentioning that wine aging could affect the evolution of pyranoanthocyanins which results in monomeric anthocyanins link with some yeast fermentative metabolites to form vinylphenolic pyranoanthocyanins and improve wine colour stability (Boulton, 2001). Refer to previous study, the use of oenological tannin can be a helpful tool for anthocyanin evolution and wine colour stability (Chen et al., 2016a,b).

In order to get more anthocyanins details, cluster analysis and principal component analysis were jointly carried out to evaluate the anthocyanin composition in all fermentations (Fig. 2). Two main groups of anthocyanins composition are classified at scale 15 (Fig. 2A), one part is involved in sequential fermentation and the other is related with *S. pombe*. Obviously, anthocyanin composition of *S. pombe* 938 is different from that in *S. cerevisiae* related trials. With tannin treatment, *S. pombe* V1 can be grouped with sequential fermentations at scale 15. It indicates tannin treatment could significantly influence anthocyanin composition of *S. pombe* V1 and makes the anthocyanins similar to the fermentations involving *S. cerevisiae*. On the contrary, no anthocyanin difference was found between *S. pombe 938* trials and the *S. pombe V1* trial without tannin addition. Additionally, at scale 5, tannin treatment is not significant to affect the anthocyanin composition of *L. thermotolerans* and *T. delbrueckii* trials, which were grouped as first category. Due to *S. cerevisiae* is dominant at the second half of sequential fermentation, *L. thermotolerans* and *T. delbrueckii* showed similar anthocyanin composition to *S. cerevisae 7VA*. However, *M. pulcherrima* and *S. cerevicae 7VA* can be affected by oenological tannin, which results in different anthocyanin composition from other non-*Saccharomyces* trials.

On the other hand, anthocyanin observations were mapped at PCA plot (Fig. 2B). Referring to the analysis of Table 3 and Fig. 2A, PC 1 (54.5%) reflects the anthocyanins related to tannin treatments, while PC 2 (20.0%) is corresponding with the anthocyanins in control (no exogenous tannin). As expected, vitisin type of pyranoanthocyanins (Vit A, Vit B and VitA-Ac) are close to PC 2, which is irrelevant with tannin treatment. Meanwhile, M3G and some pinotin type of its derivatives, such as M3GAc4Vp, M3G4Vg, M3GCm4Vp and M3G4Vp, are close to PC1 where oenological tannin is the dominant factor. It indicates the development of pinotin type of pyranoanthocyanins, which is positive to colour stability during AF, could be improved by pre-fermentative use of oenological tannin.

3.5. Analysis of volatile compounds

The volatile compounds of different fermentations were shown in Table 4. Accordingly, *S. pombe* produced the highest amount of acetaldehyde (threshold is 100–125 mg/L in red wine), which could be responsible for off-flavour. Meanwhile, oenological tannin was less effective to affect the development of acetaldehyde. Comparing with *S. pombe* and *S. cerevisiae* trials, sequential fermentations (*M. pulcherrima*, *L. thermotolerans* and *T. delbrueckii*) produced higher amount of ethyl acetate, which would give unpleasant glue odour to wine. Moreover, oenological tannin increased the amount of higher alcohols (isobutanol, 2-methyl-1-butanol, 3-methyl-1-butanol), which were less than 300 mg/L and could be positive to wine aroma complexity (Chen et al., 2016a,b). Sequential fermentations of *M. pulcherrima*, *L. thermotolerans*, *T. delbrueckii* and 7VA trials were considered to be more effective to produce higher alcohols than *S. pombe* trials. On the other hand, the development of

Table 4
Volatile compounds of different fermentation scenarios, the influence of oenological tannin was investigated.

Volatile compounds (mg/L)	*M. pulcherrima*		*L. thermotolerans*		*T. delbrueckii*		*S. pombe V1*		*S. pombe 938*		*S. cerevisiae 7VA*	
		+ TV40		+ TV40		+ TV40		+ TV40		+ TV40		+ TV40
Acetaldehyde	7.94 ± 0.96^{d}	7.97 ± 0.16^{d}	7.42 ± 0.43^{d}	9.62 ± 3.98^{cd}	8.39 ± 0.88^{d}	9.16 ± 0.42^{cd}	11.46 ± 0.58^{bc}	11.51 ± 1.56^{bc}	14.87 ± 2.46^{a}	14.20 ± 1.62^{ab}	10.11 ± 1.20^{cd}	7.42 ± 0.49^{d}
Methanol	92.00 ± 9.88^{c}	93.16 ± 3.36^{bc}	96.06 ± 5.44^{bc}	97.74 ± 3.80^{bc}	101.02 ± 2.63^{abc}	102.97 ± 1.08^{ab}	95.45 ± 6.10^{bc}	108.11 ± 6.07^{a}	96.78 ± 3.36^{bc}	94.33 ± 4.83^{bc}	98.29 ± 4.32^{bc}	97.99 ± 4.34^{bc}
1-Propanol	33.02 ± 3.92^{b}	23.57 ± 1.21^{cd}	47.71 ± 3.10^{a}	50.40 ± 2.32^{a}	48.38 ± 1.84^{a}	47.31 ± 0.33^{a}	14.90 ± 0.37^{f}	20.02 ± 3.37^{de}	17.33 ± 0.40^{ef}	17.70 ± 1.16^{ef}	27.01 ± 1.24^{c}	23.42 ± 0.82^{cd}
Diacetyl	9.40 ± 0.93^{a}	6.02 ± 1.04^{c}	9.38 ± 1.23^{a}	9.89 ± 0.73^{a}	7.40 ± 0.24^{b}	7.88 ± 0.67^{b}	2.11 ± 0.09^{e}	2.94 ± 0.45^{e}	2.19 ± 0.16^{e}	2.58 ± 0.48^{e}	4.03 ± 0.07^{d}	5.88 ± 0.94^{c}
Ethyl acetate	51.20 ± 12.78^{a}	50.37 ± 11.33^{a}	36.52 ± 1.85^{bcd}	40.08 ± 1.02^{bc}	41.93 ± 3.26^{ab}	43.89 ± 5.90^{ab}	16.16 ± 1.46^{g}	23.66 ± 1.06^{efg}	21.78 ± 2.41^{efg}	19.21 ± 3.69^{fg}	31.50 ± 1.53^{cde}	27.98 ± 1.46^{def}
Isobutanol	41.62 ± 10.59^{a}	22.03 ± 2.81^{c}	29.13 ± 2.25^{b}	32.52 ± 0.76^{b}	21.81 ± 0.90^{c}	22.38 ± 1.44^{c}	13.21 ± 1.76^{df}	22.64 ± 1.02^{c}	8.69 ± 0.43^{e}	14.13 ± 5.74^{e}	16.64 ± 0.86^{cd}	19.50 ± 0.62^{cd}
1-Butanol	9.82 ± 4.99	7.19 ± 2.69	10.36 ± 2.67	7.46 ± 0.91	10.35 ± 0.46	9.38 ± 3.40	7.75 ± 3.30	7.06 ± 2.09	10.96 ± 0.94	6.16 ± 1.62	8.95 ± 1.11	7.55 ± 1.28
Acetoin	9.15 ± 0.40^{bc}	8.62 ± 0.53^{c}	9.28 ± 0.40^{bc}	8.89 ± 0.34^{c}	8.83 ± 0.87^{c}	8.97 ± 0.12^{c}	15.20 ± 2.54^{a}	9.09 ± 0.24^{bc}	16.10 ± 2.29^{a}	11.98 ± 4.21^{b}	8.62 ± 0.19^{c}	8.15 ± 0.27^{c}
2-Methyl-1-Butanol	116.77 ± 6.53^{cd}	149.09 ± 12.05^{a}	109.25 ± 9.65^{d}	118.54 ± 2.11^{cd}	106.36 ± 7.83^{d}	127.28 ± 5.61^{bc}	34.85 ± 4.72^{f}	70.75 ± 4.75^{e}	34.37 ± 1.15^{f}	45.32 ± 4.88^{f}	132.12 ± 8.40^{b}	155.89 ± 6.84^{a}
3-Methyl-1-Butanol	23.37 ± 13.77^{bc}	38.72 ± 1.77^{a}	22.74 ± 1.28^{bc}	23.15 ± 1.43^{bc}	20.53 ± 0.55^{bcd}	19.38 ± 2.35^{bcd}	17.57 ± 2.88^{cd}	26.08 ± 1.64^{b}	14.49 ± 0.35^{d}	17.55 ± 1.31^{cd}	35.48 ± 3.09^{a}	38.74 ± 1.50^{a}
Ethyl lactate	7.37 ± 1.19^{f}	13.84 ± 0.98^{cde}	43.50 ± 8.21^{b}	59.59 ± 3.95^{a}	17.78 ± 2.61^{c}	16.25 ± 1.49^{cd}	9.87 ± 0.52^{ef}	10.01 ± 0.56^{ef}	9.97 ± 1.36^{ef}	8.60 ± 1.37^{ef}	11.76 ± 1.24^{def}	10.06 ± 2.05^{ef}
2,3-Butanediol	164.16 ± 37.31^{d}	188.61 ± 11.08^{cd}	140.62 ± 52.10^{d}	157.94 ± 3.12^{d}	179.46 ± 24.56^{d}	179.96 ± 4.37^{d}	274.22 ± 20.75^{b}	336.47 ± 32.54^{a}	244.57 ± 18.12^{bc}	254.37 ± 13.64^{b}	168.99 ± 77.69^{d}	174.81 ± 19.07^{d}
Isoamyl acetate	8.02 ± 3.35^{a}	8.08 ± 1.51^{a}	2.80 ± 0.65^{cde}	4.23 ± 0.86^{bcd}	5.15 ± 1.99^{abc}	6.73 ± 1.92^{ab}	0.00 ± 0.00^{e}	4.74 ± 1.30^{bc}	0.00 ± 0.00^{e}	1.83 ± 1.59^{de}	6.61 ± 0.92^{ab}	6.14 ± 1.33^{ab}
Hexanol	4.00 ± 0.09^{a}	3.72 ± 0.02^{c}	3.75 ± 0.05^{bc}	3.75 ± 0.02^{bc}	3.79 ± 0.14^{bc}	3.84 ± 0.08^{abc}	3.97 ± 0.17^{a}	3.90 ± 0.05^{abc}	3.91 ± 0.11^{abc}	3.74 ± 0.07^{bc}	3.74 ± 0.07^{bc}	3.93 ± 0.06^{ab}
2-Phenylethanol	27.82 ± 1.80^{cd}	52.04 ± 8.15^{ab}	25.31 ± 4.56^{cd}	33.22 ± 13.53^{bcd}	21.24 ± 1.67^{cd}	30.89 ± 9.99^{cd}	16.81 ± 0.43^{d}	12.24 ± 3.50^{d}	29.39 ± 0.84^{cd}	17.50 ± 13.48^{d}	41.16 ± 27.25^{abc}	59.61 ± 10.44^{a}
2-Phenylethyl acetate	5.42 ± 0.40^{f}	7.11 ± 1.16^{bc}	5.84 ± 0.60^{cd}	7.12 ± 0.38^{bc}	7.10 ± 1.23^{bc}	6.88 ± 0.63^{bc}	7.31 ± 0.18^{ab}	7.43 ± 0.15^{ab}	8.51 ± 0.64^{a}	7.41 ± 0.15^{ab}	6.60 ± 0.40^{bcd}	6.30 ± 1.08^{bcd}

Different letters show significant difference at $p < 0.05$.
TV40: Tanicol Vintage 40 g/hL

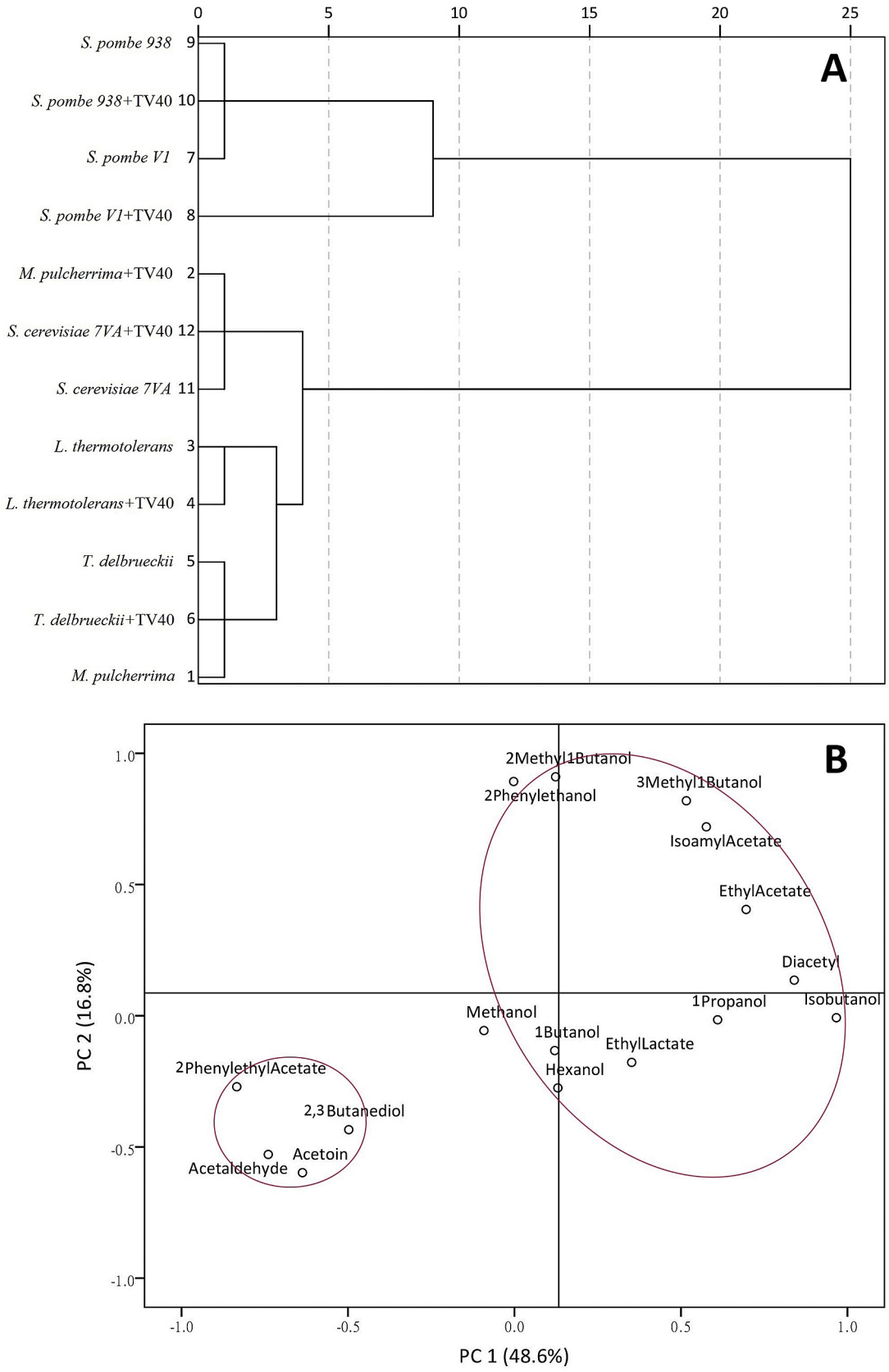

Fig. 3. Variances and observations analysis of volatile compounds. (A) Cluster analysis of the fermentation variances. (B) Principal component analysis of the volatile compounds observations. Grape seed tannin, Tanicol Vintage 40 g/hL (TV40) was applied at the beginning of fermentation for each yeast strain.

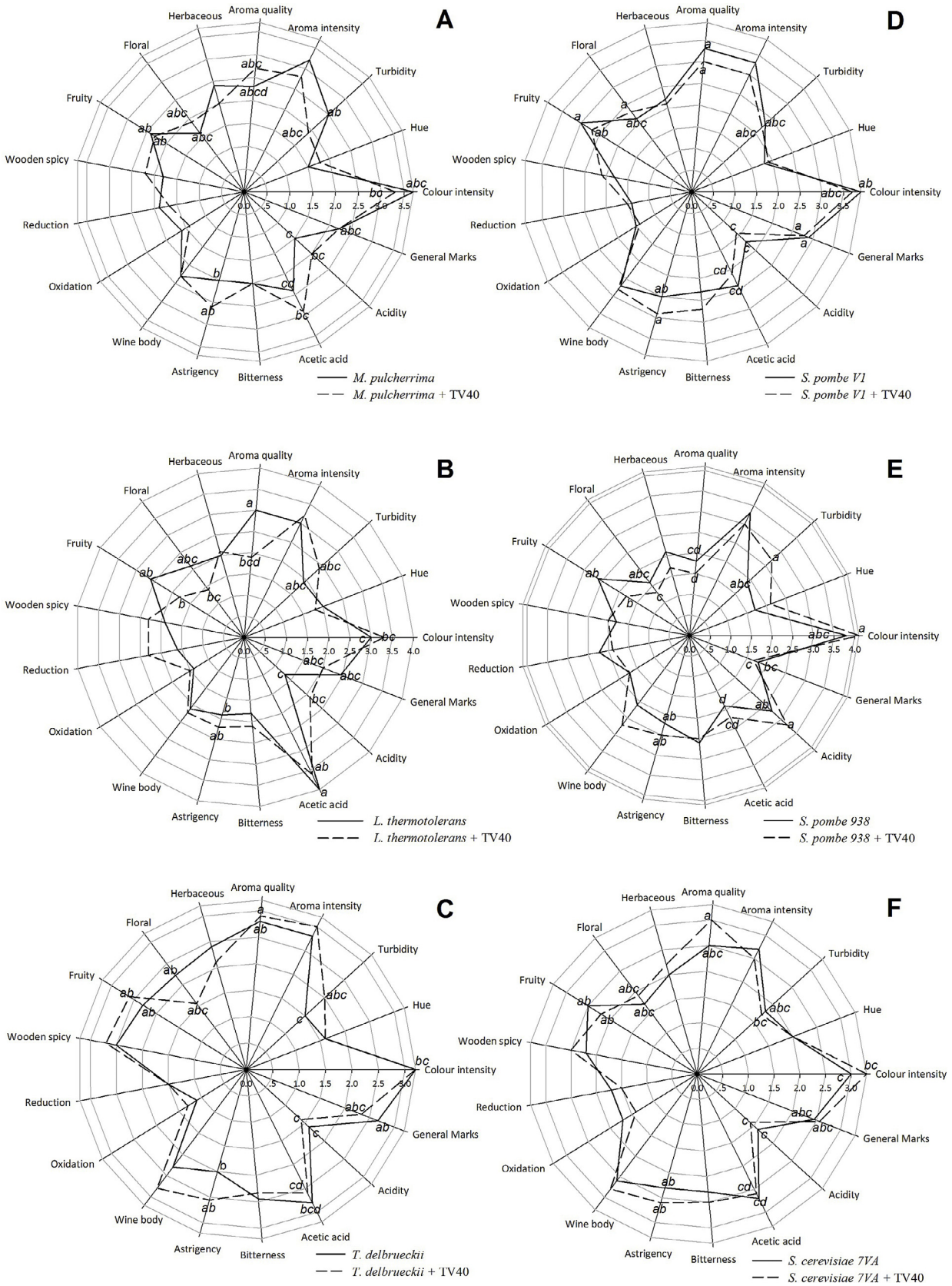

Fig. 4. Radar plots of sensory analysis for different fermentation scenarios, the influence of oenological tannin was also investigated. (A) Sensory properties of the sequential fermentations by *M. pulcherrima*. (B) Sensory properties of the sequential fermentations by *L. thermotolerans*. (C) Sensory properties of the sequential fermentations by *T. delbrueckii*. (D) Sensory properties of single strain fermentation by *S. pombe* V1. (E) Sensory properties of the single strain fermentations of *S. pombe* 938. (F) Sensory properties of the single strain fermentations by *S. cerevisiae* 7VA. Grape seed tannin, Tanicol Vintage 40 g/hL (TV40) was applied at the beginning of fermentation for each yeast strain. Different letters on each index showed significant differences ($p < 0.05$) for twelve treatments.

ethyl lactate is related to lactic acid metabolism. Based on the analysis of Table 1, *L. thermotolerans* produced high level of lactic acid and result in excess of ethyl lactate (14 mg/L as its intolerant level), which could affect the sensory properties. *S. pombe* possesses a special ability to produce more 2, 3-butanediol, which contributes the fruity aroma described as banana to wines. Nevertheless, *S. cerevisiae* 7VA is an advantaged yeast strain to release more 2-phenylethanol, which is characterized as floral aroma.

CA and PCA (Fig. 3) were jointly carried out to evaluate volatile compounds. Two groups were classified by CA at scale 10 (Fig. 3A). The volatile compounds of. *S. pombe* trials are considered to be different from others. Meanwhile, *S. cerevisiae* 7VA were so dominated to the sequential inoculation that non-*Saccharomyces* fermentations (*M. pulcherrima*, *L. thermotolerans*, *T. delbrueckii*) showed close connections with 7VA. PCA results (Fig. 3B) indicates the development of volatile compounds related with tannin treatment and yeast strain. Apparently, most of the alcohols, excluded methanol and 2-phenylethanol, are mapped close to PC 1 (48.6%), which indicates an integrated factor of grape seed tannin and yeast strain (*S. cerevisiae 7VA* is an important variance since it took part in all sequential fermentations). These two factors effectively contributed to alcohols development. Furthermore, three important esters, isoamyl acetate, ethyl acetate and ethyl lactate are also positively related to tannin treatment and *S. cerevisiae 7VA*. Nevertheless, the use of oenological tannin was unacted on the development of acetaldehyde, acetoin, 2, 3-butanediol and 2-phenylethyl acetate, which are close to PC 2 (16.8%). In conclusion, *S. cerevisiae* 7VA was a dominated factor in sequential fermentations and could significantly influence the development of volatile compounds during AF. Meanwhile, the pre-fermentative use of oenological tannin can be effective to improve the aroma complexity of sequential fermentations.

3.6. Sensory analysis

Fig. 4 illustrated effects of oenological tannin on sensory properties of different fermentations. Wine samples fermented by *L. thermotolerans* showed a remarkable impression of acetic acid (Fig. 4B). It is due to *L. thermotolerans* possess a strong ability to produce high amount of L-lactic acid during AF (Kapsopoulou et al., 2007) and develop the highest level of total acidity in all fermentations (refer to Table 1). The wines of *S. pombe* got an excellent mark on colour intensity (Fig. 4D, E), which also verified the relevant results in Table 2 to some extent. In Fig. 4 C and E, oenological tannin increased wine turbidity of *T. delbrueckii* and *S. pombe 938*. *T. delbrueckii, S. pombe V1* and *S. cerevisiae 7VA* showed good performance on aroma quality (Fig. 4C, D, F) where the 938 was given the lowest mark (Fig. 4E). These strains can be considered as top three yeasts which effectively contributed to aroma quality, floral and fruity. The result can be correlated with the modification of aromatic compounds. For instance, higher alcohols, acetate esters, and ethyl esters were promoted by the three strains and positively influenced the aroma properties (Lu et al., 2016). Moreover, oenological tannin effectively increased the aroma quality of *S. cerevisiae 7VA*. However, this effect was not significant to non-*Saccharomyces* strains and even was negative to *L. thernotolerans* (Fig. 4B). Interestingly, the fermentations with *T. delbrueckii*, *S. pombe V1* and *S. cerevisiae 7VA* showed high general mark and the property of low acidity. It is not significant in other wine samples. On the other hand, the application of oenological tannin could be negative on floral and fruity impression of *L. thermotolerans* as well as *S. pombe* 938 trials (Fig. 4B, E). Meanwhile, pre-fermentative use of oenological tannin significantly increased wine astringency, which could be related to the increase of total polyphenols (Table 2) in each fermentation.

4. Conclusion

In terms of yeasts fermentative kinetic, the influence of low amount of oenological tannin (40 g/hL) on non-*Saccharomyces* yeasts growth kinetics is not significant. Wild *S. pombe* strains, 938 and V1 showed similar fermentation ability to *S. cerevisiae*, both of them can independently conduct the whole alcoholic fermentation. However, *M. pulcherrima, L. thermotolerans* and *T. delbrueckii* have to be carried out sequential inoculation with *S. cerevisiae* 7VA, which is the dominant factor to influence wine components and sensory properties. The anthocyanin composition and volatile compounds of *S. pombe* fermentation are different from those in sequential fermentations. Regarding the sensory analysis, *T. delbrueckii, S. pombe V1* and *S. cerevisiae 7VA* were considered as top three yeast strains which positively contributed to wine aroma quality. However, the application of oenological tannin could be negative on floral and fruity impression of *L. thermotolerans* as well as *S. pombe 938*. On the other hand, oenological tannin was verified can be positive for the development of vinylphenolic pyranoanthocyanins, which is effective to improve colour stability and protect the wines from being oxidized. Pre-fermentative use of oenological tannin was also particularly benefit to the development of higher alcohols in the fermentations involving *S. ceresivisiae*. Nevertheless, sensory analysis showed that the low amount of oenological tannin could be less effective to modify the aroma impression of non-*Saccharomyces* wines.

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