Torulaspora delbrueckii for secondary fermentation in sparkling wine production

Manuscript Details

Manuscript number	FM_2017_948_R2
Title	Torulaspora delbrueckii for secondary fermentation in sparkling wine production
Article type	Research Paper

Abstract

In the search for the desired oenological features and flavour complexity of wines, there is growing interest in the potential use of non-Saccharomyces yeast that are naturally present in the winemaking environment. Torulaspora delbrueckii is one such yeast that has seen profitable use in mixed fermentations with Saccharomyces cerevisiae and with different grape varieties. T. delbrueckii can have positive and distinctive impacts on the overall aroma of wines, and has also been used at an industrial level. Here, T. delbrueckii was successfully used in pure and mixed secondary fermentations for sparkling wine. The two selected T. delbrueckii strains used completed the secondary fermentation ‘prise de mousse’ in these pure and mixed fermentations. The sparkling wines obtained with T. delbrueckii showed different aromatic compositions and sensory profiles to those of S. cerevisiae. T. delbrueckii strain DiSVA 130 showed high esters production and significantly high scores for some of the aromatic descriptors that positively influence the sensory profile of sparkling wine. Thus, the use of T. delbrueckii in pure and mixed fermentations is a suitable strategy to further develop the flavour complexity during secondary fermentation of sparkling wines.

Keywords	Sparkling wine; Torulaspora delbrueckii; aroma profile; bottle fermentation.
Corresponding Author	Maurizio Ciani
Corresponding Author's Institution	Polytechnic University of Marche
Order of Authors	Laura Canonico, Francesca Comitini, Maurizio Ciani

Submission Files Included in this PDF

File Name [File Type]

Cover let. Canonico et al..docx [Cover Letter]

Response to reviewers R2.docx [Response to Reviewers]

Highlights.docx [Highlights]

Canonico et al. Main R2.docx [Manuscript File]

Figure 1 R1.ppt [Figure]

fig. 2 (1).pptx [Figure]

Fig. 3 (1).pptx [Figure]

To view all the submission files, including those not included in the PDF, click on the manuscript title on your EVISE Homepage, then click 'Download zip file'.

Research Data Related to this Submission

There are no linked research data sets for this submission. The following reason is given:
Data will be made available on request

Dear Editor,Aline Lanvaud

thank you for the message. Sorry for the our misunderstanding, we didn't see the file of reviewer #3. We will correct the F1 manuscript and reply the comments of reviewer #3. The modification are highlighted in yellow. We would be glad if you would reconsider the revised manuscript, entitled, "***Torulaspora delbrueckii*** **for secondary fermentation in sparkling wine production** ". for inclusion in **FOOD MICROBIOLOGY**. .

Yours faithfully

Prof. Maurizio Ciani

Response to reviewers

Reviewer 1

The corrections were done.
I do not still understand why the maximum pressures varied from 5 to 6 bar (figure 1) while the residual sugars were similars.

The possible explanation to the variation the low sensitivity of the mechanic aphrometer that at high pressure (5-6 bar) are nots o precise.

Reviewer 3

The aim of the manuscript is study the use of two strains of Torulaspora, alone or mixed with a Saccharomyces strain as inoculum in the second fermentation of traditional method. Even the subject is interesting and there are some interesting data, methodology used and treatment of data have some lacks which need to be explained and improved to publish.

• Sensory analysis. The authors don't explain the attributes in the descriptive analysis and some of them represented in the figure 3 are not cited in the text. As example, there are an affective attribute as "Nicety". It would be interesting explain how the tasting panel was trained and which were the standard solutions for each attribute. It is quite surprising the attribute astringency, typical from red wines, in whites could more appropriate dryness?

Answer:
We commented the attributes that showed in general significant (statistical) differences among the sparkling wines. The trained testers using the descriptors in italian language so we translate the schedules. For Nicety we intend the" finesse of the perlage" in the mouth. Regarding the "astringency" attribute , in white wines is related to the sensation of unripe fruit and liked to sourness

• Overpressure measurement with aphrometer may be is not so accurate, in fact how the authors can explain the differences between treatments, it seems more than 1 bar.

Answer:
As answered to the reviewer 1 we agree that the aphrometer is not so accurate. Variations could be due to the reduced sensibility of the mechanic system in particular at high at high overpressure.

• In the PCA almost all the variability is explained by PC1 (96%), so Sc527 and Td130 quite similar. So, the improvement is not evident in this representation. On the contrary Sc527+Td313 is very different from the combination with the other Torulaspora. In my opinion, this representation could be improved may be displaying the OAV's and or including all the ethyl esters.

Answer:
We agree that Sc527 and Td130 are quite similar and the variability is explained only 2%. However the can be distinguished by isoamyl alcohol n-propanol and ethyl acetate production. We tried to elaborate the data of both analytical and sensorial analyses but the graphical was not improved .

• Conclusions are too speculative, 15ºC is not the usual temperature of fermentation in a sparkling wine, some producers do the fermentation even at 10ºC (A Review of Methode Champenoise Production, from Bruce W. Zoecklein, Virginia Cooperative Extension Service, 1989). So, the authors must include in the text "in our conditions" as they have not tested other temperatures, pH, sulfites,...)

Answer:
enclosed "in our conditions" in the conclusions

• The alcohol content (11,65%) is very high to produce sparkling wines, usually is 10 to 11%. Sulfites additions are not mentioned, it's well known the sensibility of Torulaspora to Sulphur dioxide.

Answer:
We added the SO2 concentration in the base wine production. The alcohol content could be quite high. However, in the central Italy but also in the north Italy the base wine for sparkling wine production generally have an initial alcohol content of about 11.5% vol. In our case we used Verdicchio variety base wine a typical variety of Marche region that exhibits in general higher alcohol content.

Specific comments:
Line 248: Explain units of acidity (as tartaric acid?).
Answer:
added in the text

Line 252: Who's the producer of tanniperle. I suppose is SOC, but it's not clear in the text.
Answer:
added in the text

Line 286: rewrite the sentence "After this period..." including more details of the process, as riddling and recorking (with crown?, cork?, muselette? ...)
Answer:
added details in the text

Line 661: In the sentence "In particular, Td130 was significantly high for some of the aromatic descriptors (white flowers, citrus, honey, odour intensity, softness), compared with the Sc527

[1] Olfactive Activity Values (the ratio of the concentration to the odour threshold)

pure fermentation. These data confirm..." most of the descriptors have not significance, as it is shown in the figure 3; and it's not clear if all the mixed and Torulaspora yeast have higher values with significance in th white flowers, the attribute with significance ($p<0.05$).

Answer:
we explained in the text

Highlights

- *Torulaspora delbrueckii* in '*prise de mousse'* sparkling wine process was proposed

- Selected *T. delbrueckii* strains conducted and completed secondary fermentation of sparkling wine

- *T. delbrueckii* in'*prise de mousse'* is a suitable strategy to enhance sparkling wine aroma complexity

- *T. delbrueckii* strain DiSVA130 showed high esters production and best score for aromatic descriptors

Torulaspora delbrueckii for secondary fermentation in sparkling wine production

Laura Canonico, Francesca Comitini, Maurizio Ciani*

Dipartimento Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, Via Brecce Bianche, 60131 Ancona, Italy

***Corresponding author: Maurizio Ciani**

Dipartimento Scienze della Vita e dell'Ambiente

Università Politecnica delle Marche

Via Brecce Bianche

60131 Ancona, Italy

Tel: +39-071-2204987

E-mail: m.ciani@univpm.it

Other author emails:

Laura Canonico: l.canonico@univpm.it

Francesca Comitini:f.comitini@univpm.it

Abstract

In the search for the desired oenological features and flavour complexity of wines, there is growing interest in the potential use of non-*Saccharomyces* yeast that are naturally present in the winemaking environment. *Torulaspora delbrueckii* is one such yeast that has seen profitable use in mixed fermentations with *Saccharomyces cerevisiae* and with different grape varieties. *T. delbrueckii* can have positive and distinctive impacts on the overall aroma of wines, and has also been used at an industrial level. Here, *T. delbrueckii* was successfully used in pure and mixed secondary fermentations for sparkling wine. The two selected *T. delbrueckii* strains used completed the secondary fermentation '*prise de mousse*' in these pure and mixed fermentations. The sparkling wines obtained with *T. delbrueckii* showed different aromatic compositions and sensory profiles to those of *S. cerevisiae*. *T. delbrueckii* strain DiSVA 130 showed high esters production and significantly high scores for some of the aromatic descriptors that positively influence the sensory profile of sparkling wine. Thus, the use of *T. delbrueckii* in pure and mixed fermentations is a suitable strategy to further develop the flavour complexity during secondary fermentation of sparkling wines.

Keywords: Sparkling wine; non-*Saccharomyces*; *Torulaspora delbrueckii*; aroma profile; bottle fermentation

1. Introduction

In recent years, there has been increased demand for tailored wines with improved sensorial character and distinctive flavour complexity. In this regard, several studies have focussed on positive influences on wine complexity and quality of non-*Saccharomyces* yeast species in mixed fermentations with *Saccharomyces cerevisiae* starter strains (Ciani et al., 2010; Ciani and Comitini 2015; Jolly et al., 2014). Indeed, several non-*Sacccharomyces* species in simultaneous and sequential fermentations have been showed to increase some desired compounds, with the flavour complexity depending on the oenological features of the species/ strains introduced and the modalities of their use (Andorrà et al., 2012; Azolini et al., 2015; Comitini et al., 2011; García et al., 2016; Gobbi et al., 2013; Rojas et al., 2003; Saboudi et al., 2012; Viana et al., 2008).

Within these non-*Saccharomyces* wine yeasts, great attention has been focussed on *Torulaspora delbrueckii* because of its positive and distinctive impact on the overall aroma of a wine (Belda et al., 2017; Canonico et al., 2015; Loira et al., 2014; Renault et al., 2016). Indeed, *T. delbrueckii* is characterised by low production of undesirable compounds, such as acetic acid, while also increasing the metabolites that positively influence the analytical and sensorial profile of a wine (Belda et al., 2015, 2017; García et al., 2016; Renault et al., 2009; Taillandier et al., 2014). These data were also supported by evaluation of *T. delbrueckii* and *S. cerevisiae* sequential inoculation for different grape varieties, such as Soave, Sauvignon Blanc, Verdicchio, Palomino and Chardonnay, for wines at a pilot or an industrial scale (Azzolini et al., 2015; Canonico et al., 2015; Puertas et al., 2017).

Recently, the use of *T. delbrueckii* has been proposed in sequential fermentations with *S. cerevisiae* for the base wine for sparkling wine production. Gonzalez-Royo et al. (2015) demonstrated increased glycerol content, reduced volatile acidity, and positive impact on foam through sequential inoculation of *T. delbrueckii* with *S. cerevisiae*. Furthermore, Medina-Trujillo et al. (2017) investigated foam formation in sparkling wine, where the base wine fermentation process

was carried out by sequential inoculation of *T. delbrueckii* and *S. cerevisiae*. They showed that this *T. delbrueckii* sequential fermentation can have a positive impact on the final sparkling wine.

Sparkling wine is the result of secondary fermentation of the base wine by the Classic (traditional) method, or the Charmat (tank) method (Jackson, 2008; Stefenon et al., 2010; Torresi, et al., 2011; Pueyo and Martínez-Rodríguez, 2009). The production of high quality sparkling wines depends on the base wine composition (i.e., flavour, aroma, acidity) (Kemp et al., 2015), the aging stage (i.e., post-fermentation aroma), and the starter yeast used for the secondary fermentation process ('*prise de mousse*') (Vannier et al., 1999; Pozo-Bayón et al., 2003, 2009).

In the present study, we investigated the use of *T. delbrueckii* for secondary fermentation of the base wine, through evaluation of its impact on the analytical composition and aromatic profile of the sparkling wine produced.

2. Materials and methods

2.1. Yeast strains

S. cerevisiae strain DiSVA 527 (*Sc*527) and two *T. delbrueckii* strains DiSVA 130 (*Td*130) and DiSVA 313 (*Td*313) were used in the secondary fermentations for the sparkling wine production in this study, in pure and mixed cultures. These *T. delbrueckii* strains are maintained in the Yeast Collection of the Department of Life and Environmental Sciences (DiSVA) of the Polytechnic University of Marche (Italy). They were originally isolated from natural matrices from different environments, and they have been used as starter cultures in previous studies (Canonico et al., 2015; Canonico et al., 2016).

For short-term storage at 4 °C, the yeast strains were maintained on yeast extract (10 g L^{-1}), peptone (20 g L^{-1}), dextrose (20 g L^{-1}) (YPD) agar (15 g L^{-1}). For long-term storage at –80 °C, they were maintained in YPD broth (as for YPD agar, but without the agar) supplemented with 80% (w/v) glycerol.

2.2. Sparkling wine production

Production of the sparkling wine was carried out by the traditional method (i.e., bottle fermented) using a Verdicchio base wine (provided by Terre Cortesi Moncaro s.r.c.l, Montecarotto, Ancona, Italy). The base wine had the following main analytical composition: total acidity (as tartaric acid), 4.2 g L^{-1}; volatile acidity, 0.18 g L^{-1}; pH, 3.09; ethanol 11.65 % vol.; residual sugar, 0.29 g L^{-1}, total SO_2 15.0 mg L^{-1} . This was supplemented with 24 g L^{-1} sucrose (pressure expected c.a. 6 bar = 600kPa), 10 mg L^{-1} diammonium phosphate, and also with Tanniperle style untreated oak tannin (to provide a complete structure and to prevent reduction notes) (Enartis, Novara Italy), and with Actiperle cuve specified fermentation activant for '*prise de mousse*' (Station Oenotechnique De Champagne, France), both used following the manufacturer instructions. Verdicchio base wine was stabilised and sterile filtered using 0.45 µm membranes.

The starter strains were pre-cultured in the same Verdicchio base wine supplemented with 20 g L^{-1} sucrose for 10 days at 19 ±1 °C under static condition. The cells were then collected by centrifugation, washed three times with sterile distilled water, and inoculated into the bottles (750 mL) containing the Verdicchio base wine. The inoculations were designed for an initial yeast concentration of approximately 1 $\times 10^6$ cell mL^{-1}. The yeast cell numbers were estimated using a Thoma Zeiss cell chamber. These *Sc*527 and *Td*130/313 strains were used in both pure cultures and mixed cultures (inoculum ratio, 1:1).

Six bottles for each sparkling wine secondary fermentation trial were set up and crown corked of which two bottles per trial were plugged with an aphrometer (Oenoitalia group S.r.l.), to follow the evolution of overpressure during the bottle fermentation phase (i.e., the 'tirage'). For the secondary fermentation, the bottles were maintained at a temperature of 15 °C in a thermostat, as also during the storage period (12 months). After this period, the sparkling wines were disgorged and capped with cork and "muselette", for analysis and tasting.

2.3. Analytical determinations

The ethanol, pH and volatile acidity determinations were performed on the wines according to the Official European Union Methods (EC, 2000). The glucose and fructose (K-FRUGL) concentrations were determined using specific enzyme activity kits (Megazyme, Ireland), according to the manufacturer instructions. Acetaldehyde, ethyl acetate and the higher alcohols were quantified by direct injection into a gas–liquid chromatography system. After filtration through 0.2-μm membranes, 1 μL of each sample was spiked with 1-pentanol (as internal standard; 162 mg L^{-1}) and injected into a polyethylene glycol column (30 m × 0.32 mm ID, × 0.25 μm film thickness; Zebron ZB-WAX Plus; Phenomenex, Torrance, CA, USA). A gas chromatograph (GC-2014; Shimadzu, Kyoto, Japan) equipped with a flame ionisation detector was used, with nitrogen as the carrier gas (2.3 mL min^{-1}). The temperature of both the injector and detector was set to 220 °C, and the injection was in split mode (10:1; 60 s at 150 °C). After 5 min at 40 °C, the column temperature was raised by 5 °C min^{-1}, up to 200 °C. The volatile compounds were extracted using an ether: hexane (1:1, v/v) extraction technique, and evaluated by capillary gas–liquid chromatography. For quantification, the sparkling wines were spiked before their extraction with a known amount of 3-octanol as internal standard (1.6 mg L^{-1}). For the capillary gas–liquid chromatography, a glass 0.25-μm capillary column was used (length, 60 m; internal diameter, 0.32 mm; Supelcowax-10; Supelco, Bellefonte, PA USA). One microlitre was injected in split–splitless mode: 60 s splitless; injection temperature, 220 °C; detector temperature, 250 °C; carrier gas, helium; flow rate, 2.5 mL min^{-1}. The temperature programme was: 50 °C for 5 min, then raised 3 °C min^{-1} to 220 °C, and then 220 °C for 20 min. The compounds were identified and quantified by comparisons with external calibration curves for each compound.

2.4. Sensory analysis

At the end of the secondary fermentation of the base wine, the resulting sparkling wines were stored for 12 months. After this time, the products obtained by pure and mixed cultures underwent sensory

analysis using a scale from 1 to 10. This was carried out by a group of 10 trained tasters, on the basis of a list of descriptors related to both the aromatic notes (e.g., floral, fruity, toasty) and the main structural features (e.g., sweet, acidity, flavour, astringency, bitterness, olfactory persistence). Their data were combined, and the means were subjected to statistical analysis. The data processed in this way were used to provide information on both the contributions of each descriptor to the overall organoleptic quality of the sparkling wines, and the significant differences between the sparkling wines in relation to each descriptor.

2.5. Statistical analysis

Analysis of variance (ANOVA) was applied to the experimental data for the analytical characters of the sparkling wine. The means were analysed using the STATISTICA 7 software. The significant differences were determined using Duncan tests, and the data were considered significant if the associated P values were <0.05. The data from the sensory analysis were also subjected to Fisher ANOVA, to determine the significant differences ($p < 0.05$). Principal component analysis (PCA) was applied to discriminate among the means of the contents of the esters, higher alcohols and carbonyl compounds in the sparkling wine samples. The PCA was carried out using the Unscrambler 7.5 software (CAMO ASA, Oslo, Norway). The mean data were normalised, to neutralise any influence of hidden factors. The PCA provides a graphical representation of the overall differences in terms of the fermentation by-products of the final sparkling wines.

3. Results

3.1. Secondary fermentation kinetics

The secondary fermentation kinetics of the *Sc*527 and *Td*130/313 pure and mixed cultures are shown in Figure 1. The evolution and the final pressure values indicated that *Td*130/313 brought forward and completed the secondary fermentation. Indeed, all secondary fermentations (pure and

mixed) showed similar fermentation kinetics. However, the *Sc*527 pure culture showed the fastest fermentation kinetics until day 8 of the fermentations (3 bar), in comparison with the *Td*130/313 pure cultures and mixed cultures. After that, the *Sc*527 pure culture showed the slowest fermentation kinetics in comparison with the others. In contrast, the *Td*130/313 pure cultures showed the slowest fermentation kinetics until day 8, after which they showed faster fermentation kinetics than the other cultures. The *Sc*527+*Td*130/313 mixed fermentations showed intermediate evolution of pressure.

3.2. The main analytical characters of the sparkling wines

The data for the main analytical characters of these sparkling wines are reported in Table 1. All cultures in pure and mixed fermentations showed very similar ethanol (from 12.45 % vol. to 12.48 % vol.) and volatile acidity (from 0.23 g L^{-1} to 0.25 g L^{-1}). Also, the pHs were not significantly different among the sparkling wines produced. For the residual sugar, all of the sparkling wines showed total consumption of reducing sugars after bottle fermentation.

3.3. The main volatile compounds in the sparkling wines

To determine the influence of *Td*130/313 on the aromatic profiles of the sparkling wines, the main volatile compounds were assayed, and the results are summarised in Tables 2 and 3.

For the ester compounds (Table 2), the results showed a significant increase in ethyl butyrate (fruity, sweet) in the *Sc*527+*Td*313 mixed fermentation while the other sparkling wine trials have shown no significant differences among them. For ethyl acetate (pineapple, fruity, solvent), *Sc*527 alone showed significantly higher ethyl acetate production in comparison with the mixed fermentations. The *Td*130/313 pure fermentations showed comparable levels of ethyl acetate to that of *Sc*527. For phenyl ethyl acetate, these data did not show any significant differences. The *Sc*527+*Td*313 mixed cultures showed significantly higher production of ethyl hexanoate (fruity, green apple, brandy, wine-like), in comparison with the other sparkling wines produced. However,

the *Td*130/313 pure cultures and mixed cultures showed an increase in this fruit ester (ethyl hexanoate) when compared with the *Sc*527 pure cultures. A similar trend was seen for ethyl octanoate (fruity, strawberry, sweet). In both the *Td*130/313 pure fermentations and in the mixed fermentation of *Sc*527+*Td*313, there were significantly higher amounts of isoamyl acetate production (banana, fruity, sweet).

The data for the higher alcohol contents highlighted the different impacts of *Td*130/313 on the sparkling wine production in comparison with *Sc*527 (Table 2). Indeed, the sparkling wine inoculated with pure *Sc*527 showed the highest n-propanol, isobutanol and isoamylic alcohol (alcohol, ripe fruit), while the *Td*130/313 pure and mixed fermentations showed intermediate and lowest concentrations, respectively. The amylic alcohol and β-phenyl ethanol (rose, floral) did not show any significant differences among the sparkling wines, while the presence and fermentation activity of *Td*130/313 led to an increase in hexanol content. Furthermore, the *Td*130/313 fermentation activities in these pure and mixed fermentations led to significant reductions in acetaldehyde content, in comparison with the sparkling wines with pure *Sc*527 (Table 3).

For the carboxylic acids, the *Td*130 pure cultures showed significantly higher production of diethyl succinate in comparison with the other cultures. For the other carboxylic acids, the data did not show any significant differences among the sparkling wines produced (Table 3).

To determine the overall effects of *Td*130/313 in these pure and mixed secondary fermentations with the *S. cerevisiae* starter strain, the data on the volatile compounds were subjected to PCA analysis (Fig. 2). The sparkling wine distributions highlighted the effects of *Td*130/313, in both pure and mixed cultures. The *Td*130/313 pure cultures showed a different profile to those of the *Sc*527 pure cultures and mixed cultures (Fig. 2). Indeed, the processed data positioned the *Sc*527 pure culture in the bottom right of the biplot in Figure 2. This was characterised by n-propanol, ethyl acetate and isoamylic alcohol production. *Td*130 and *Sc*527+*Td*130 were positioned in the upper right of the biplot in Figure 2, while *Td*313 and *Sc*527+*Td*313 were positioned in the upper left. All of the *Td*130/313 sparkling wines (i.e., those

inoculated with both pure and mixed cultures) were characterised by hexanol, ethyl hexanoate, isoamyl acetate, ethyl octanoate and ethyl butyrate production. Moreover, mixed cultures showed greater differences of *Td*130/313 pure cultures in comparison with *Sc*527 cultures, which indicated interactions between these two species for the aromatic compounds they produce.

3.4. Sensorial analysis

The sparkling wines underwent sensory analysis and the data reported in Figure 3 showed significant differences for all of the sparkling wines analysed for some of the aromatic notes. In particular, for the main sensorial descriptors, the sparkling wines of all of the *Sc*527+*Td*130/313 mixed fermentations and the *Td*130/313 pure cultures were significantly different from those obtained with the *Sc*527 pure cultures. These data highlighted that *Td*130 was characterised for the sensorial attributes as white flowers, bread crust, sapidity and acidity showing significant differences from other sparkling wines with the exception of sapidity . In general, sparkling wines obtained by both the *Td*130/313 pure cultures and their mixed fermentations showed higher scores for the aromatic descriptors (white flowers, citrus, honey, odour intensity, softness) in comparison with the sparkling wines inoculated with *Sc*527. *Td*313 pure culture and *Sc*527+*Td*130/313 mixed fermentations showed significant intermediate scores for white flowers in comparison with *Sc*527 and *Td*130 pure cultures. Furthermore, the *Sc*527 sparkling wine was significantly different from the others, due to its high score for the descriptor for astringency.

4. Discussion

Already for some time, the use of *T. delbrueckii* was proposed as a wine starter to reduce the volatile acid in wines (Castelli, 1954). More recently, other positive oenological characters have been highlighted in terms of the production of compounds that positively influence the volatile compounds (e.g., esters, acetals, volatile thiols), or the body of the wine, such as glycerol,

polysaccharides or foam production in sparkling wines (Comitini et al., 2011; Belda et al., 2015; Gonzalez-Royo et al., 2014; Renault et al., 2015, 2016).

The use of *T. delbrueckii* in the production of sparkling wine was recently proposed in mixed cultures for the production of base wine (Gonzalez-Royo et al., 2014; Medina-Trujillo et al., 2017). To the best of our knowledge, there have been no previous studies into the use and the influences of non-*Saccharomyces* yeast during the secondary fermentation in sparkling wine production. Indeed, *S. cerevisiae* and/or *Saccharomyces bayanus* have been considered the best species to restart sluggish or stuck fermentations, or to carry out secondary fermentation in sparkling wine production (Perpetuini et al., 2016; Tofalo et al., 2016).

In this study, we assessed for the first time the contributions of *T. delbrueckii* to the secondary fermentation of a base wine for sparkling wine production. The selected *T. delbrueckii* strains used here (i.e., *Td*130/313) completed the secondary fermentation '*prise de mousse*' of the base wine, with 11.65% vol ethanol. In this context, some studies that have investigated oenological aptitudes have shown wide distributions of the fermentation power of *T. delbrueckii* that have ranged from 6% to 12% vol. ethanol (Comitini et al., 2011; Renault et al., 2009; van Breda et. al., 2013). The selection carried out on the strain populations, the adaptation phase for ethanol and sugar content in the pre-cultures, and the low temperature (15 °C) generally used for the '*prise de mousse*', might explain the fermentation performance during the secondary fermentation of the *Td*130/313 strains used in this study.

Indeed, the pressure evolution and the main oenological characters of the *Td*130/313 pure and mixed secondary fermentations showed complete consume of sugars and comparable kinetics (slightly slower in the first phase) to those of the *Sc*527 pure culture. The main differences with the use of *Td*130/313 here was for the volatile compounds and the sensorial profiles of the sparkling wines produced. In the secondary fermentation for sparkling wine production, *Td*130/313 confirmed previously reported behaviour in wines, in terms of the reduction in acetaldehyde and some higher alcohols, such as n-propanol and isoamylic alcohol (Bely et al., 2008; Canonico et al., 2015).

A positive impact of *Td*130/313 in both pure and mixed fermentations for sparkling wine was seen for ester production and the aroma profiles of the sparkling wines. Indeed, there were increases in ethyl butyrate, ethyl hexanoate, ethyl octanoate and isoamyl acetate. The impact of these aromatic compounds was confirmed by the sensorial analysis. Indeed, the sparkling wines produced by *Td*130/313 showed different sensorial profiles from the *S. cerevisiae* pure cultures.

All of the sparkling wines that were obtained by these pure and mixed fermentations were characterised by aromatic descriptors that positively influence the final product: the wine. In particular, *Td*130 was significantly high for some of the aromatic descriptors (white flowers, citrus, honey, odour intensity, softness), compared with the *Sc*527 pure fermentation. These data confirm that also during secondary fermentation, the tendency of *T. delbrueckii* is to increase esters formation in the wine (Renault et al., 2015; Chen and Liu, 2016).

There are few reports on the use in non-*Saccharomyces* yeasts in secondary fermentation for sparkling wine production. Only recently, two of non-*Saccharomyces* yeasts belonging to the species *Saccharomycodes ludwigii* and *Schizosaccharmomyces pombe* were assayed for secondary fermentation sparkling wine production using two different base (Ivit et al. 2017), These non-*Saccharomyces* led to sparkling wine with change in volatile acidity, volatile compounds and sensory profile in comparison with *S. cerevisiae*. The authors referred that the final sparkling wines did not show significant differences regarding to the overall quality among the strains tested indicating these non-*Saccharomyces* species can be furtherly studied to change specific characteristics of sparkling wines.

In conclusion, in our conditions these data highlight that the use of *T. delbrueckii* in pure or mixed fermentations is a suitable strategy to improve flavour production during secondary fermentation, and thus to obtain sparkling wines with a composition of aroma compounds and a sensory profile different from those of the *S. cerevisiae* starter strains. This *Td*130 strain (i.e., *T. delbrueckii* DiSVA 130) provided significant high scores for some of the aromatic descriptors (which were supported by high esters production) that positively influenced the sparkling wine

sensory profiles. The use of these selected *T. delbrueckii* (*Td*130/313) strains in secondary fermentation for sparkling wine production represents a new approach to obtain an innovative product. Further investigations are needed to identify the best applications in terms of the traditional method used here and the Charmat method, to enhance the oenological characters of the *T. delbrueckii* yeast species in the secondary fermentation of sparkling wines.

Acknowledgments

L. Canonico was supported by Polytechnic University of Marche Postdoctoral contract (D.R. n^ 1057 del 20.10.2016).

References

Andorrà, I., Berradre, M., Mas, A., Esteve-Zarzoso, B., Guillamón, J.M., 2012. Effect of mixed culture fermentations on yeast populations and aroma profile. Food Sci. Technol. 49, 8–13.

Azzolini, M., Tosi, E., Lorenzini, M., Finato, F., Zapparoli, G., 2015. Contribution to the aroma of white wines by controlled *Torulaspora delbrueckii* cultures in association with *Saccharomyces cereviasiae*. World J. Microbiol. Biotechnol. 31, 277–293.

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.

Belda, I., Ruiz, J., Beisert, B., Navascués, E., Marquina, D., Calderón, F., Rauhut, D., Benito, S., Santos, A., 2017. Influence of *Torulaspora delbrueckii* in varietal thiol (3-SH and 4-MSP) release in wine sequential fermentations. Int. J. Food Microbiol. 257, 183–191.

Bely, M., Stoeckle, P., Masneuf-Pomarède, I., Dubourdieu, D., 2008. Impact of mixed *Torulaspora delbrueckii–Saccharomyces cerevisiae* culture on high-sugar fermentation. Int. J. Food Microbiol. 122, 312–320.

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.

Canonico, L., Agarbati, A., Comitini, F., Ciani, M., 2016. *Torulaspora delbrueckii* in the brewing process: A new approach to enhance bioflavour and to reduce ethanol content. Food Microbiol. 56, 45–51.

Castelli, T., 1954. Les agents de la fermentation vinaire. Arch. Microbiol. 23, 323-342.

Chen, D., Liu, S.Q., 2016. Impact of simultaneous and sequential fermentation with *Torulaspora delbrueckii* and *Saccharomyces cerevisiae* on non-volatiles and volatiles of lychee wines. LWT - Food Sci. Technol. Int. 65, 53–61.

Ciani, M., Comitini, F., 2015. Yeast interactions in multi-starter wine fermentation. Curr. Opin. Food Sci. 1, 1–6.

Ciani, M., Comitini, F., Mannazzu, I., Domizio, P., 2010. Controlled mixed culture fermentation: a new perspective on the use of non-*Saccharomyces* yeasts in winemaking. FEMS Yeast Res. 10, 123–133.

Comitini, F., Gobbi, M., Domizio, P., Romani, C., Lencioni, L., Mannazzu, I., Ciani, M., 2011. Selected non-*Saccharomyces* wine yeasts in controlled multistarter fermentations with *Saccharomyces cerevisiae*. Food Microbiol. 28, 873–888.

EC, 2000. Community reference methods for the analysis of spirits drinks. Off. J. Eur. Commun. Reg. EC No2870/00. Brussels.

García, M., Esteve-Zarzoso, B., Arroyo, T., 2016. Non-*Saccharomyces* yeasts: biotechnological role for wine production, in: Grape and Wine Biotechnology. InTech. DOI: 10.5772/64957.

Gobbi, M., Comitini, F., Domizio, P., Romani, C., Lencioni, L., Mannazzu, I., Ciani, M., 2013. *Lachancea thermotolerans* and *Saccharomyces cerevisiae* in simultaneous and sequential co-fermentation: a strategy to enhance acidity and improve the overall quality of wine. Food Microbiol. 33, 271–281.

González-Royo, E., Pascual, O., Kontoudakis, N., Esteruelas, M., Esteve-Zarzoso, B., Mas, A., Canals, J.M., Zamora, F., 2015. Oenological consequences of sequential inoculation with non-*Saccharomyces* yeasts (*Torulaspora delbrueckii* or *Metschnikowia pulcherrima*) and *Saccharomyces cerevisiae* in base wine for sparkling wine production. Eur. Food Res. Technol. 240, 999–1012.

Jackson, R.S., 2008. Wine science principles and applications (789 pp.) (3rd ed.). London, UK: Academic Press.

Jolly, N.P., Varela, C., Pretorius, I.S., 2014. Not your ordinary yeast: non-*Saccharomyces* yeast in wine production uncovered. FEMS Yeast Res. 14, 215–237.

Kemp, B., Alexandre, H., Robillard, B., Marchal, R., 2015. Effect of production phase on bottle-fermented sparkling wine quality. J. Agric. Food Chem. 63, 19–38.

Ivit, N. N., Loira, I., Morata, A., Benito, S., Palomero, F., Suárez-Lepe, J. A., 2017. Making natural sparkling wines with non-*Saccharomyces* yeasts. Eur. Food Res. Technol. doi.org/10.1007/s00217-017-3015-y.

Loira, I., Vejarano, R., Bañuelos, M.A., Morata, A., Tesfaye, W., Uthurry, C., Villa, A., Cintora, I., Suárez-Lepe, J.A., 2014. Influence of sequential fermentation with *Torulaspora delbrueckii* and *Saccharomyces cerevisiae* on wine quality. LWT - Food Sci. Technol. 59, 915–922.

Medina-Trujillo, L., González-Royo, E., Sieczkowski, N., Heras, J., Canals, J.M., Zamora, F., 2017. Effect of sequential inoculation (*Torulaspora delbrueckii*/ *Saccharomyces cerevisiae*) in the first fermentation on the foaming properties of sparkling wine. Eur. Food Res. Technol. 243, 681–688.

Perpetuini, G., Di Gianvito, P., Arfelli, G., Schirone, M., Corsetti, A., Tofalo, R., Suzzi, G., 2016. Biodiversity of autolytic ability in flocculent *Saccharomyces cerevisiae* strains suitable for traditional sparkling wine fermentation. Yeast, 33, 303–312.

Puertas, B., Jiménez, M.J., Cantos-Villar, E., Cantoral, J.M., Rodríguez, M.E., 2017. Use of *Torulaspora delbrueckii* and *Saccharomyces cerevisiae* in semi-industrial sequential inoculation to improve quality of Palomino and Chardonnay wines in warm climates. J. Appl. Microbiol. 3, 733–746.

Pozo-Bayón, M.A., Hernández, M.T., Martín Álvares, P.J., Polo, M.C., 2003. Study of low molecular weight phenolic compounds during the aging of sparkling wines manufactured with red and white grape varieties. J. Agric. Food Chem. 51, 2089–2095.

Pozo-Bayón, M.A., Martín-Álvarez, P.J., Moreno-Arribas, M.V., Andujar-Ortiz, I., Pueyo, E., 2010. Impact of using Trepat and Monastrell red grape varieties on the volatile and nitrogen composition during the manufacture of rosé Cava sparkling wines. Food Sci. Technol. 43, 1526–1532.

Pueyo, E., Martínez-Rodríguez, A.J., 2009. Sparkling wines and yeast autolysis, in: Moreno-Arribas, M.V., Polo, M.C. (Eds.), Chapter 3A. Wine Chemistry and Biochemistry. 1 edition. Springer, 735.

Renault, P., Miot-Sertier, C., Marullo, P., Hernández-Orte, P., Lagarrigue, L., Lonvaud-Funel, A., Bely, M., 2009. Genetic characterization and phenotypic variability in *Torulaspora delbrueckii* species: potential applications in the wine industry. Int. J. Food Microbiol. 134, 201–210.

Renault, P., Coulon, J., de Revel, G., Barbe, J.C., Bely, M., 2015. Increase of fruity aroma during mixed *T. delbrueckii*/ *S. cerevisiae* wine fermentation is linked to specific esters enhancement. Int. J. Food Microbiol. 207, 40–48.

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. Frontiers Microbiol. 7.

Rojas, V., Gil, J.V., Piñaga, F., Manzanares, P., 2003. Acetate ester formation in wine by mixed cultures in laboratory fermentations. Int. J. Food Microbiol. 86, 181–188.

Sadoudi, M., Tourdot-Marechal, R., Rousseaux, S., Steyer, D., Gallardo-Chacon, J.J., Ballester, J., Vichi, S., Guerin-Schneider, R., Caixach J., Alexandre, H., 2012. Yeast-yeast interactions revealed by aromatic profile analysis of *Sauvignon Blanc* wine fermented by single or co-culture of non-*Saccharomyces* and *Saccharomyces* yeasts. Food Microbiol. 32, 243–253.

Stefenon, C.A., Colombo, M., Bonesi, C.D.M., Marzarotto, V., Vanderlinde, R., Salvador, M., Henriques, J.A.P., 2010. Antioxidant activity of sparkling wines produced by Champenoise and Charmat methods. Food Chem. 119, 12–18.

Taillander, P., Lai, Q.P., Julien-Ortiz, A., Brandam, C., 2014. Interaction between *Torulaspora delbrueckii* and *Saccharomyces cerevisiae* in wine fermentation: influence of inoculation and nitrogen content. World J. Microbiol. Biotechnol. 30, 1959–1967.

Tofalo, R., Perpetuini, G., Di Gianvito, P., Arfelli, G., Schirone, M., Corsetti, A., Suzzi, G., 2016. Characterization of specialized flocculent yeasts to improve sparkling wine fermentation. J. Appl. Microbiol. 120, 1574–1584.

Torresi, S., Frangipane, M.T., Anelli, G., 2011. Biotechnologies in sparkling wine production. Interesting approaches for quality improvement: a review. Food Chem. 129, 1232–1241.

van Breda, V., Jolly, N., van Wyk, J., 2013. Characterisation of commercial and natural *Torulaspora delbrueckii* wine yeast strains. Int. J. Food Microbiol. 163, 80–88.

Vannier, A., Brun, O.X., Feinberg, M.H., 1999. Application of sensory analysis to champagne wine characterization and discrimination. Food Qual. Prefer. 10, 101–107.

Viana, F., Gil, J.V., Genoves, S., Valles, S., Manzanares, P., 2008. Rational selection of non-*Saccharomyces* wine yeasts for mixed starters based on ester formation and enological traits. Food Microbiol. 25, 778–785.

Table 1. Main analytical characters of the sparkling wines produced by the pure and mixed fermentations.

Yeast species	DiSVA strain	Ethanol % vol	Volatile acidity (g acetic acid L^{-1})	pH	Residual sugar (g L^{-1})
S. cerevisiae	527	12.45±0.00[a]	0.24 ±0.02[a]	3.21 ±0.00[a]	0.23 ±0.04[bc]
T. delbrueckii	130	12.47±0.01[a]	0.23 ±0.01[a]	3.21 ±0.00[a]	0.46 ±0.04[c]
	313	12.48±0.02[a]	0.25 ±0.00[a]	3.21 ±0.00[a]	0.36 ±0.03[a]
S. cerevisiae + *T. delbrueckii*	527 + 130	12.46±0.03[a]	0.23 ±0.014[a]	3.20 ±0.00[a]	0.20 ±0.04[c]
S. cerevisiae + *T. delbrueckii*	527 + 313	12.46±0.01[a]	0.25 ±0.02[a]	3.21 ±0.00[a]	0.29 ±0.03[ab]

Data are means ±standard deviations.

Data with different superscript letters ([a], [b], [c]) within each column are significantly different (Duncan tests; $P < 0.05$). Analytical characters of the base wine: ethanol, 11.65% vol; volatile acidity, 0.18 as g acetic acid L^{-1}; pH, 3.09; residual sugar, 0.29 g L^{-1}.

Table 2. Main volatile esters and alcohols in the sparkling wines produced by the pure and mixed fermentations after 12 months aging.

Fermentation trial	Esters (mg L^{-1})						Alcohols (mg L^{-1})					
	Ethyl butyrate	**Ethyl acetate**	**Phenyl ethyl acetate**	**Ethyl hexanoate**	**Ethyl octanoate**	**Isoamyl acetate**	**n-Propanol**	**Isobutanol**	**Amyl alcohol**	**Isoamylic alcohol**	**β-Phenyl ethanol**	**Hexanol**
Base wine	0.200 ±0.113	32 ±2	0.007 ±0.006	0.025 ±0.009	0.077 ±0.010	0.206 ±0.016	56 ±1	14±1	14 ±0	114±2	6 ±1	0.052 ±0.005
S. cerevisiae 527	0.348 ±0.103^{b}	91 ±8^{a}	0.011 ±0.013^{a}	0.035 ±0.004^{d}	0.012 ±0.004^{c}	0.333 ±0.029^{c}	91±1^{a}	21±1^{a}	13 ±2^{a}	152 ±4^{a}	9 ±2^{ab}	0.082 ±0.004^{d}
T. delbrueckii 130	0.427 ±0.049^{ab}	84 ±1^{ab}	0.008 ±0.001^{a}	0.048 ±0.001^{c}	0.041 ±0.002^{a}	0.399 ±0.011^{ab}	84 ±2^{b}	17 ±1^{b}	11±1^{a}	142±3^{b}	13±1^{a}	0.163 ±0.008^{a}
T. delbrueckii 313	0.237 ±0.057^{c}	80 ±4^{abc}	0.004 ±0.002^{a}	0.066 ±0.001^{b}	0.039 ±0.001^{a}	0.428 ±0.006^{a}	84±0^{b}	18 ±0^{b}	11. ±2^{a}	144 ±2^{b}	10±1^{ab}	0.119 ±0.007^{bc}
S. cerevisiae 527 + *T. delbrueckii 130*	0.400 ±0.025^{b}	76 ±2^{bc}	0.002 ±0.000^{a}	0.097 ±0.004^{a}	0.038 ±0.001^{a}	0.342 ±0.006^{bc}	79 ±2^{c}	15 ±0^{c}	9 ±1^{a}	128±2^{c}	11 ±1^{ab}	0.098 ±0.027^{cd}
S. cerevisiae 527 + *T. delbrueckii 313*	0.675 ±0.243^{a}	72 ±1^{c}	0.002 ±0.000^{a}	0.069 ±0.002^{b}	0.032 ±0.001^{b}	0.452 ±0.061^{a}	72 ±1^{d}	16 ±0^{c}	10±1^{a}	119±1^{d}	9 ±1^{b}	0.145 ±0.007^{ab}

Data are data means ±standard deviations. Statistical analysis did not include the base wine.

Data with different superscript letters ($^{a, b, c, d}$) within each column are significantly different (Duncan test; $P < 0.05$).

Table 3. Main volatile carbonyl compounds and carboxylic acids in the sparkling wines produced by the pure and mixed fermentations after 12 months aging.

Fermentation trial	Carbonyl compounds (mg L^{-1})		Carboxylic acids (mg L^{-1})			
	Acetaldehyde	Acetoin	Diethyl succinate	Butyric acid	Octanoic acid	Decanoic acid
Base wine	73.33 ±1.43	ND	0.030 ±0.002	0.032 ±0.012	0.409 ±0.025	0.043 ±0.000
S. cerevisiae 527	26.58 ±0.86^{a}	ND	0.008 ±0.006^{c}	0.032 ±0.024^{ab}	0.007 ±0.010^{ab}	0.021 ±0.003^{a}
T. delbrueckii 130	22.43 ±1.10^{b}	ND	0.123 ±0.038^{a}	0.046 ±0.009^{a}	0.031 ±0.004^{a}	0.026 ±0.027^{a}
T. delbrueckii 313	23.20 ±0.54^{b}	ND	0.005 ±0.004^{c}	0.005 ±0.005^{b}	0.020 ±0.019^{ab}	0.070 ±0.036^{a}
S. cerevisiae 527 + T. delbrueckii 130	19.45 ±0.95^{c}	ND	0.022 ±0.020^{c}	0.014 ±0.016^{ab}	0.012 ±0.004^{ab}	0.071 ±0.017^{a}
S. cerevisiae 527 + T. delbrueckii 313	22.47 ±1.12^{b}	ND	0.072 ±0.028^{b}	0.044 ±0.004^{a}	0.002 ±0.001^{b}	0.018 ±0.001^{a}

ND, not detected

Data are data means ±standard deviations. Statistical analysis did not include the base wine.

Data with different superscript letters ($^{a, b, c}$) within each column are significantly different (Duncan test; $P < 0.05$).

Figure captions

Figure 1. Fermentation kinetics of the sparkling wines produced in the pure and mixed secondary fermentations. Pure fermentations of *S. cerevisiae* DiSVA 527 ( ), and *T. delbrueckii* DiSVA 130 ( ) and DiSVA 313 ( ). Mixed fermentations of *S. cerevisiae* DiSVA 527 plus *T. delbrueckii* DiSVA 130 ( ), and plus *T. delbrueckii* DiSVA 313 ( ).

Figure 2. Principal component analysis based on the data for the volatile compounds in the sparkling wines produced by the *S. cerevisiae* DiSVA 527 (*Sc*527) and *T. delbrueckii* DiSVA 130 (*Td*130) and DiSVA 313 (*Td*313) pure fermentations and mixed fermentations.

Figure 3. Sensory analysis of the sparkling wines produced in the pure and mixed secondary fermentations. Pure fermentations of *S. cerevisiae* DiSVA 527 ( ), and *T. delbrueckii* DiSVA 130 ( ) and DiSVA 313 ( ). Mixed fermentations of *S. cerevisiae* DiSVA 527 plus *T. delbrueckii* DiSVA 130 ( ) and plus *T. delbrueckii* DiSVA 313 ( ). *, Significantly different (Fisher ANOVA; p-value 0.05).

Figure 1

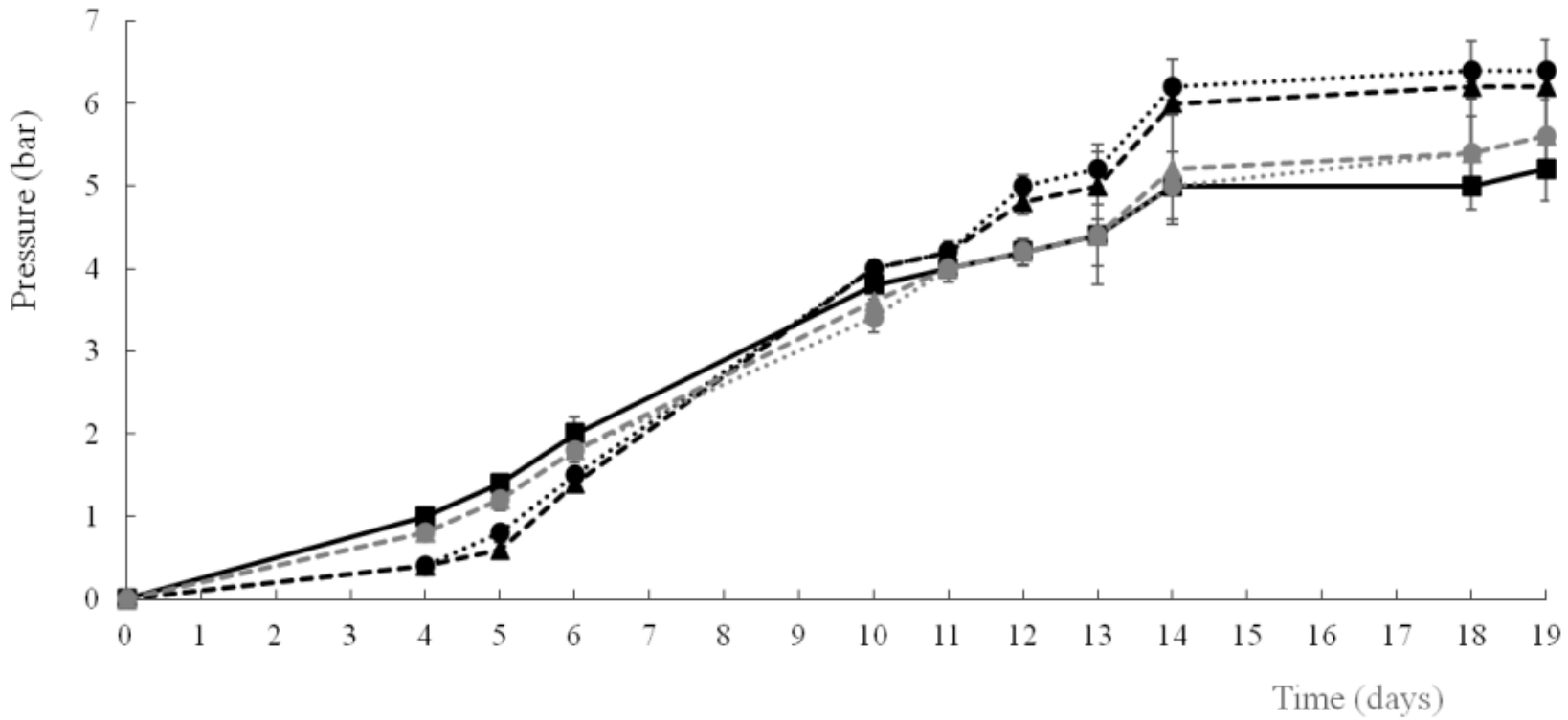

Pressure (bar)
0
1
2
3
4
5
6
7
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Time (days)

Figure 2

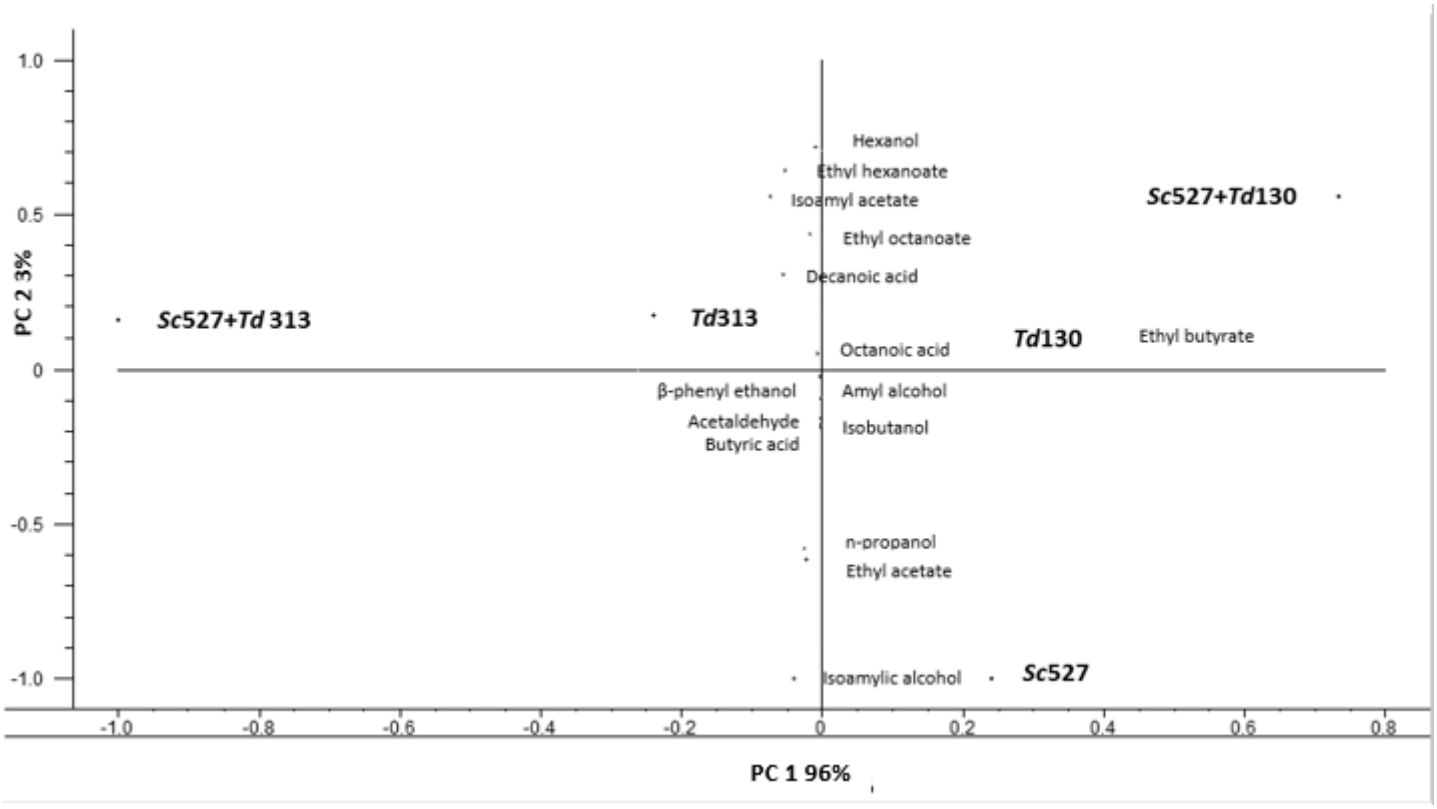
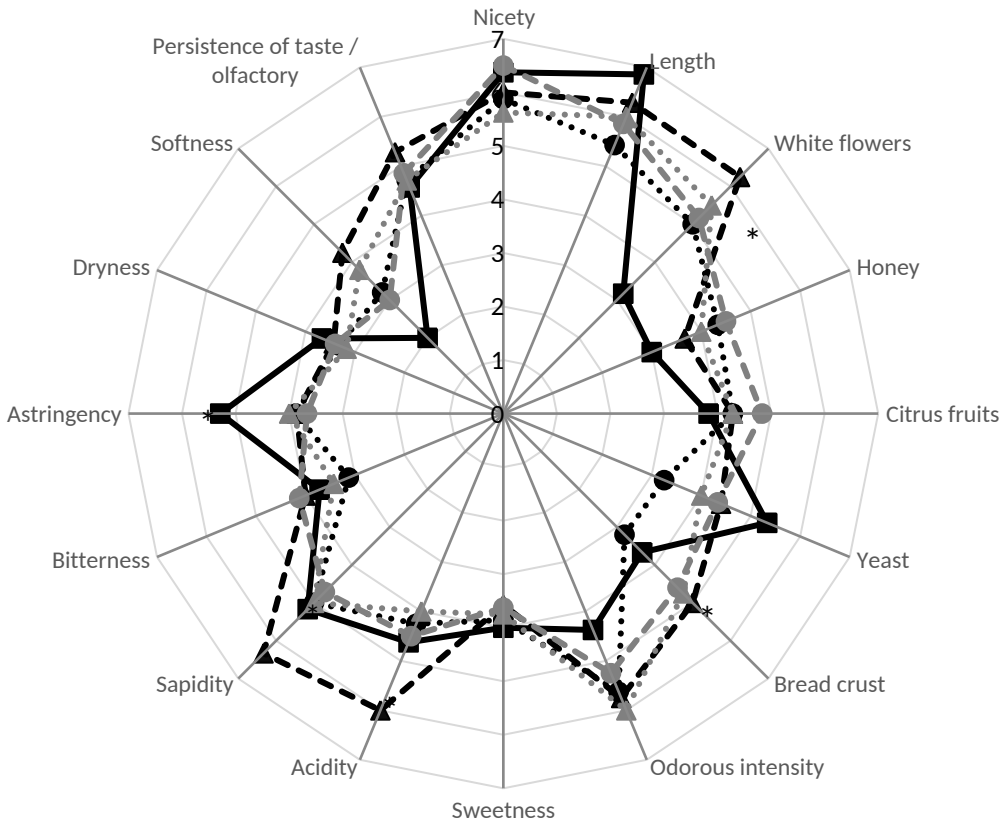

1.0
0.5
0
-0.5
-1.0
PC 2 3%
-1.0
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
PC 1 96%
Hexanol
Ethyl hexanoate
Isoamyl acetate
Ethyl octanoate
Decanoic acid
Octanoic acid
β-phenyl ethanol
Amyl alcohol
Acetaldehyde
Isobutanol
Butyric acid
n-propanol
Ethyl acetate
Isoamylic alcohol
Sc527+Td130
Sc527+Td 313
Td313
Td130
Ethyl butyrate
Sc527

Figure 3


Nicety
Length
White flowers
Honey
Citrus fruits
Yeast
Bread crust
Odorous intensity
Sweetness
Acidity
Sapidity
Bitterness
Astringency
Dryness
Softness
Persistence of taste / olfactory
7
5
4
3
2
1
0
*
*
*