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Torulaspora delbrueckii for secondary fermentation in sparkling wine production

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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.

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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, "***Torulaspota delbrueckii* for secondary fermentation in sparkling wine production**". for inclusion in **FOOD MICROBIOLOGY**. .

Yours faithfully

Prof. Maurizio Ciani

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5 Response to reviewers
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8

9 Reviewer 1
10

11 The corrections were done.

12 I do not still understand why the maximum pressures varied from 5 to 6 bar (figure 1) while the residual
13 sugars were similars.
14

15
16 The possible explanation to the variation the low sensitivity of the mechanic aphrometer that at high
17 pressure (5-6 bar) are not so precise.
18

19
20 Reviewer 3
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22
23 The aim of the manuscript is study the use of two strains of Torulaspora, alone or mixed with a
24 Saccharomyces strain as inoculum in the second fermentation of traditional method. Even the
25 subject is interesting and there are some interesting data, methodology used and treatment of
26 data have some lacks which need to be explained and improved to publish.
27

28
29 • Sensory analysis. The authors don't explain the attributes in the descriptive analysis
30 and some of them represented in the figure 3 are not cited in the text. As example,
31 there are an affective attribute as "Nicety". It would be interesting explain how the
32 tasting panel was trained and which were the standard solutions for each attribute. It
33 is quite surprising the attribute astringency, typical from red wines, in whites could
34 more appropriate dryness?
35

36 Answer:

37 We commented the attributes that showed in general significant (statistical) differences among the
38 sparkling wines. The trained testers using the descriptors in italian language so we translate the schedules.
39 For Nicety we intend the "finesse of the perlage" in the mouth. Regarding the "astringency" attribute, in
40 white wines is related to the sensation of unripe fruit and linked to sourness
41
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45 • Overpressure measurement with aphrometer may be is not so accurate, in fact how
46 the authors can explain the differences between treatments, it seems more than 1
47 bar.
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49 Answer:

50 As answered to the reviewer 1 we agree that the aphrometer is not so accurate. Variations could be due to
51 the reduced sensibility of the mechanic system in particular at high at high overpressure.
52

53 • In the PCA almost all the variability is explained by PC1 (96%), so Sc527 and Td130
54 quite similar. So, the improvement is not evident in this representation. On the
55 contrary Sc527+Td313 is very different from the combination with the other
56 Torulaspora. In my opinion, this representation could be improved may be displaying
57 the OAV's₁ and or including all the ethyl esters.
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62 Answer:

63 We agree that Sc527 and Td130 are quite similar and the variability is explained only 2%. However the can
64 be distinguished by isoamyl alcohol n-propanol and ethyl acetate production. We tried to elaborate the
65 data of both analytical and sensorial analyses but the graphical was not improved .
66

67 • Conclusions are too speculative, 15°C is not the usual temperature of fermentation in
68 a sparkling wine, some producers do the fermentation even at 10°C (A Review of
69 Methode Champenoise Production, from Bruce W. Zoecklein, Virginia Cooperative
70 Extension Service, 1989). So, the authors must include in the text “in our conditions”
71 as they have not tested other temperatures, pH, sulfites,...)
72

73 Answer:

74 enclosed “in our conditions” in the conclusions
75

76 • The alcohol content (11,65%) is very high to produce sparkling wines, usually is 10 to
77 11%. Sulfites additions are not mentioned, it's well known the sensibility of
78 Torulaspora to Sulphur dioxide.
79

80 Answer:

81 We added the SO₂ concentration in the base wine production. The alcohol content could be quite high.
82 However, in the central Italy but also in the north Italy the base wine for sparkling wine production
83 generally have an initial alcohol content of about 11.5% vol. In our case we used Verdicchio variety base
84 wine a typical variety of Marche region that exhibits in general higher alcohol content.
85

86 Specific comments:

87 Line 248: Explain units of acidity (as tartaric acid?).

88 Answer:

89 added in the text
90

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

92 Answer:

93 added in the text
94

95 Line 286: rewrite the sentence “After this period...” including more details of the process, as
96 riddling and recorking (with crown?, cork?, muselette? ...)

97 Answer:

98 added details in the text
99

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

102
103 ¹Olfactive Activity Values (the ratio of the concentration to the odour threshold)
104 pure fermentation. These data confirm...” most of the descriptors have not significance, as it is
105 shown in the figure 3; and it's not clear if all the mixed and Torulaspora yeast have higher
106 values with significance in th white flowers, the attribute with significance (p<0.05).
107

108 Answer:

109 we explained in the text
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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

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

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180 was carried out by sequential inoculation of *T. delbrueckii* and *S. cerevisiae*. They showed that this
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182 *T. delbrueckii* sequential fermentation can have a positive impact on the final sparkling wine.
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184 Sparkling wine is the result of secondary fermentation of the base wine by the Classic
185 (traditional) method, or the Charmat (tank) method (Jackson, 2008; Stefenon et al., 2010; Torresi, et
186 al., 2011; Pueyo and Martínez-Rodríguez, 2009). The production of high quality sparkling wines
187 depends on the base wine composition (i.e., flavour, aroma, acidity) (Kemp et al., 2015), the aging
188 stage (i.e., post-fermentation aroma), and the starter yeast used for the secondary fermentation
189 process (*prise de mousse*) (Vannier et al., 1999; Pozo-Bayón et al., 2003, 2009).
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197 In the present study, we investigated the use of *T. delbrueckii* for secondary fermentation of
198 the base wine, through evaluation of its impact on the analytical composition and aromatic profile
199 of the sparkling wine produced.
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203 204 205 **2. Materials and methods** 206

207 208 209 **2.1. Yeast strains** 210

211 *S. cerevisiae* strain DiSVA 527 (*Sc527*) and two *T. delbrueckii* strains DiSVA 130 (*Td130*) and
212 DiSVA 313 (*Td313*) were used in the secondary fermentations for the sparkling wine production in
213 this study, in pure and mixed cultures. These *T. delbrueckii* strains are maintained in the Yeast
214 Collection of the Department of Life and Environmental Sciences (DiSVA) of the Polytechnic
215 University of Marche (Italy). They were originally isolated from natural matrices from different
216 environments, and they have been used as starter cultures in previous studies (Canonico et al., 2015;
217 Canonico et al., 2016).
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227 For short-term storage at 4 °C, the yeast strains were maintained on yeast extract (10 g L⁻¹),
228 peptone (20 g L⁻¹), dextrose (20 g L⁻¹) (YPD) agar (15 g L⁻¹). For long-term storage at -80 °C, they
229 were maintained in YPD broth (as for YPD agar, but without the agar) supplemented with 80%
230 (w/v) glycerol.
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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 SO₂ 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 ×10⁶ cell mL⁻¹. The yeast cell numbers were estimated using a Thoma Zeiss cell chamber. These *Sc527* and *Td130/313* 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 \times 0.32 mm ID, \times 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 $^{\circ}\text{C}$, and the injection was in split mode (10:1; 60 s at 150 $^{\circ}\text{C}$). After 5 min at 40 $^{\circ}\text{C}$, the column temperature was raised by 5 $^{\circ}\text{C min}^{-1}$, up to 200 $^{\circ}\text{C}$. 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 $^{\circ}\text{C}$; detector temperature, 250 $^{\circ}\text{C}$; carrier gas, helium; flow rate, 2.5 mL min^{-1} . The temperature programme was: 50 $^{\circ}\text{C}$ for 5 min, then raised 3 $^{\circ}\text{C min}^{-1}$ to 220 $^{\circ}\text{C}$, and then 220 $^{\circ}\text{C}$ 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

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357 analysis using a scale from 1 to 10. This was carried out by a group of 10 trained tasters, on the
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359 basis of a list of descriptors related to both the aromatic notes (e.g., floral, fruity, toasty) and the
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361 main structural features (e.g., sweet, acidity, flavour, astringency, bitterness, olfactory persistence).
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363 Their data were combined, and the means were subjected to statistical analysis. The data processed
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365 in this way were used to provide information on both the contributions of each descriptor to the
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367 overall organoleptic quality of the sparkling wines, and the significant differences between the
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369 sparkling wines in relation to each descriptor.
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374 **2.5. Statistical analysis**

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376 Analysis of variance (ANOVA) was applied to the experimental data for the analytical characters of
377
378 the sparkling wine. The means were analysed using the STATISTICA 7 software. The significant
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380 differences were determined using Duncan tests, and the data were considered significant if the
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382 associated P values were <0.05 . The data from the sensory analysis were also subjected to Fisher
383
384 ANOVA, to determine the significant differences ($p <0.05$). Principal component analysis (PCA)
385
386 was applied to discriminate among the means of the contents of the esters, higher alcohols and
387
388 carbonyl compounds in the sparkling wine samples. The PCA was carried out using the
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390 Unscrambler 7.5 software (CAMO ASA, Oslo, Norway). The mean data were normalised, to
391
392 neutralise any influence of hidden factors. The PCA provides a graphical representation of the
393
394 overall differences in terms of the fermentation by-products of the final sparkling wines.
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400 **3. Results**

401 **3.1. Secondary fermentation kinetics**

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404 The secondary fermentation kinetics of the *Sc527* and *Td130/313* pure and mixed cultures are
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406 shown in Figure 1. The evolution and the final pressure values indicated that *Td130/313* brought
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408 forward and completed the secondary fermentation. Indeed, all secondary fermentations (pure and
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416 mixed) showed similar fermentation kinetics. However, the *Sc527* pure culture showed the fastest
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418 fermentation kinetics until day 8 of the fermentations (3 bar), in comparison with the *Td130/313*
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420 pure cultures and mixed cultures. After that, the *Sc527* pure culture showed the slowest
421
422 fermentation kinetics in comparison with the others. In contrast, the *Td130/313* pure cultures
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424 showed the slowest fermentation kinetics until day 8, after which they showed faster fermentation
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426 kinetics than the other cultures. The *Sc527+Td130/313* mixed fermentations showed intermediate
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428 evolution of pressure.
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432 433 **3.2. The main analytical characters of the sparkling wines** 434

435
436 The data for the main analytical characters of these sparkling wines are reported in Table 1. All
437
438 cultures in pure and mixed fermentations showed very similar ethanol (from 12.45 % vol. to 12.48
439
440 % vol.) and volatile acidity (from 0.23 g L⁻¹ to 0.25 g L⁻¹). Also, the pHs were not significantly
441
442 different among the sparkling wines produced. For the residual sugar, all of the sparkling wines
443
444 showed total consumption of reducing sugars after bottle fermentation.
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448 449 **3.3. The main volatile compounds in the sparkling wines**

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451 To determine the influence of *Td130/313* on the aromatic profiles of the sparkling wines, the main
452
453 volatile compounds were assayed, and the results are summarised in Tables 2 and 3.

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455 For the ester compounds (Table 2), the results showed a significant increase in ethyl butyrate
456
457 (fruity, sweet) in the *Sc527+Td313* mixed fermentation while the other sparkling wine trials have
458
459 shown no significant differences among them. For ethyl acetate (pineapple, fruity, solvent), *Sc527*
460
461 alone showed significantly higher ethyl acetate production in comparison with the mixed
462
463 fermentations. The *Td130/313* pure fermentations showed comparable levels of ethyl acetate to that
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465 of *Sc527*. For phenyl ethyl acetate, these data did not show any significant differences. The
466
467 *Sc527+Td313* mixed cultures showed significantly higher production of ethyl hexanoate (fruity,
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469 green apple, brandy, wine-like), in comparison with the other sparkling wines produced. However,
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475 the *Td130/313* pure cultures and mixed cultures showed an increase in this fruit ester (ethyl
476 hexanoate) when compared with the *Sc527* pure cultures. A similar trend was seen for ethyl
477 octanoate (fruity, strawberry, sweet). In both the *Td130/313* pure fermentations and in the mixed
478 fermentation of *Sc527+Td313*, there were significantly higher amounts of isoamyl acetate
479 production (banana, fruity, sweet).
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485
486 The data for the higher alcohol contents highlighted the different impacts of *Td130/313* on
487 the sparkling wine production in comparison with *Sc527* (Table 2). Indeed, the sparkling wine
488 inoculated with pure *Sc527* showed the highest n-propanol, isobutanol and isoamylic alcohol
489 (alcohol, ripe fruit), while the *Td130/313* pure and mixed fermentations showed intermediate and
490 lowest concentrations, respectively. The amylic alcohol and β -phenyl ethanol (rose, floral) did not
491 show any significant differences among the sparkling wines, while the presence and fermentation
492 activity of *Td130/313* led to an increase in hexanol content. Furthermore, the *Td130/313*
493 fermentation activities in these pure and mixed fermentations led to significant reductions in
494 acetaldehyde content, in comparison with the sparkling wines with pure *Sc527* (Table 3).
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505 For the carboxylic acids, the *Td130* pure cultures showed significantly higher production of
506 diethyl succinate in comparison with the other cultures. For the other carboxylic acids, the data did
507 not show any significant differences among the sparkling wines produced (Table 3).
508
509
510

511 To determine the overall effects of *Td130/313* in these pure and mixed secondary
512 fermentations with the *S. cerevisiae* starter strain, the data on the volatile compounds were
513 subjected to PCA analysis (Fig. 2). The sparkling wine distributions highlighted the effects of
514 *Td130/313*, in both pure and mixed cultures. The *Td130/313* pure cultures showed a different
515 profile to those of the *Sc527* pure cultures and mixed cultures (Fig. 2). Indeed, the processed data
516 positioned the *Sc527* pure culture in the bottom right of the biplot in Figure 2. This was
517 characterised by n-propanol, ethyl acetate and isoamylic alcohol production. *Td130* and
518 *Sc527+Td130* were positioned in the upper right of the biplot in Figure 2, while *Td313* and
519 *Sc527+Td313* were positioned in the upper left. All of the *Td130/313* sparkling wines (i.e., those
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534 inoculated with both pure and mixed cultures) were characterised by hexanol, ethyl hexanoate,
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536 isoamyl acetate, ethyl octanoate and ethyl butyrate production. Moreover, mixed cultures showed
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538 greater differences of *Td130/313* pure cultures in comparison with *Sc527* cultures, which indicated
539
540 interactions between these two species for the aromatic compounds they produce.
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543 544 545 **3.4. Sensorial analysis**

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547 The sparkling wines underwent sensory analysis and the data reported in Figure 3 showed
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549 significant differences for all of the sparkling wines analysed for some of the aromatic notes. In
550
551 particular, for the main sensorial descriptors, the sparkling wines of all of the *Sc527+Td130/313*
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553 mixed fermentations and the *Td130/313* pure cultures were significantly different from those
554
555 obtained with the *Sc527* pure cultures. These data highlighted that *Td130* was characterised for the
556
557 sensorial attributes as white flowers, bread crust, sapidity and acidity **showing significant**
558
559 **differences from other sparkling wines with the exception of sapidity**. In general, sparkling wines
560
561 obtained by both the *Td130/313* pure cultures and their mixed fermentations showed higher scores
562
563 for the aromatic descriptors (white flowers, citrus, honey, odour intensity, softness) in comparison
564
565 with the sparkling wines inoculated with *Sc527*. ***Td313* pure culture and *Sc527+Td130/313* mixed**
566
567 **fermentations showed significant intermediate scores for white flowers in comparison with *Sc527***
568
569 **and *Td130* pure cultures.** Furthermore, the *Sc527* sparkling wine was significantly different from
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571 the others, due to its high score for the descriptor for astringency.
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577 **4. Discussion**

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581 Already for some time, the use of *T. delbrueckii* was proposed as a wine starter to reduce the
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583 volatile acid in wines (Castelli, 1954). More recently, other positive oenological characters have
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585 been highlighted in terms of the production of compounds that positively influence the volatile
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587 compounds (e.g., esters, acetals, volatile thiols), or the body of the wine, such as glycerol,
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593 polysaccharides or foam production in sparkling wines (Comitini et al., 2011; Belda et al., 2015;
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595 Gonzalez-Royo et al., 2014; Renault et al., 2015, 2016).

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598 The use of *T. delbrueckii* in the production of sparkling wine was recently proposed in
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600 mixed cultures for the production of base wine (Gonzalez-Royo et al., 2014; Medina-Trujillo et al.,
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602 2017). To the best of our knowledge, there have been no previous studies into the use and the
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604 influences of non-*Saccharomyces* yeast during the secondary fermentation in sparkling wine
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606 production. Indeed, *S. cerevisiae* and/or *Saccharomyces bayanus* have been considered the best
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608 species to restart sluggish or stuck fermentations, or to carry out secondary fermentation in
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610 sparkling wine production (Perpetuini et al., 2016; Tofalo et al., 2016).

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613 In this study, we assessed for the first time the contributions of *T. delbrueckii* to the
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615 secondary fermentation of a base wine for sparkling wine production. The selected *T. delbrueckii*
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617 strains used here (i.e., *Td130/313*) completed the secondary fermentation ‘*prise de mousse*’ of the
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619 base wine, with 11.65% vol ethanol. In this context, some studies that have investigated oenological
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621 aptitudes have shown wide distributions of the fermentation power of *T. delbrueckii* that have
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623 ranged from 6% to 12% vol. ethanol (Comitini et al., 2011; Renault et al., 2009; van Breda et al.,
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625 2013). The selection carried out on the strain populations, the adaptation phase for ethanol and
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627 sugar content in the pre-cultures, and the low temperature (15 °C) generally used for the ‘*prise de*
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629 *mousse*’, might explain the fermentation performance during the secondary fermentation of the
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631 *Td130/313* strains used in this study.

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634 Indeed, the pressure evolution and the main oenological characters of the *Td130/313* pure
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636 and mixed secondary fermentations showed complete consume of sugars and comparable kinetics
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638 (slightly slower in the first phase) to those of the *Sc527* pure culture. The main differences with the
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640 use of *Td130/313* here was for the volatile compounds and the sensorial profiles of the sparkling
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642 wines produced. In the secondary fermentation for sparkling wine production, *Td130/313* confirmed
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644 previously reported behaviour in wines, in terms of the reduction in acetaldehyde and some higher
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646 alcohols, such as n-propanol and isoamyl alcohol (Bely et al., 2008; Canonico et al., 2015).
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652 A positive impact of *Td130/313* in both pure and mixed fermentations for sparkling wine
653 was seen for ester production and the aroma profiles of the sparkling wines. Indeed, there were
654 increases in ethyl butyrate, ethyl hexanoate, ethyl octanoate and isoamyl acetate. The impact of
655 these aromatic compounds was confirmed by the sensorial analysis. Indeed, the sparkling wines
656 produced by *Td130/313* showed different sensorial profiles from the *S. cerevisiae* pure cultures.
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662 All of the sparkling wines that were obtained by these pure and mixed fermentations were
663 characterised by aromatic descriptors that positively influence the final product: the wine. In
664 particular, *Td130* was significantly high for some of the aromatic descriptors (white flowers, citrus,
665 honey, odour intensity, softness), compared with the *Sc527* pure fermentation. These data confirm
666 that also during secondary fermentation, the tendency of *T. delbrueckii* is to increase esters
667 formation in the wine (Renault et al., 2015; Chen and Liu, 2016).
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676 There are few reports on the use in non-*Saccharomyces* yeasts in secondary fermentation for
677 sparkling wine production. Only recently, two of non-*Saccharomyces* yeasts belonging to the
678 species *Saccharomyces ludwigii* and *Schizosaccharomyces pombe* were assayed for secondary
679 fermentation sparkling wine production using two different base (Ivit et al. 2017), These non-
680 *Saccharomyces* led to sparkling wine with change in volatile acidity, volatile compounds and
681 sensory profile in comparison with *S. cerevisiae*. The authors referred that the final sparkling wines
682 did not show significant differences regarding to the overall quality among the strains tested
683 indicating these non-*Saccharomyces* species can be furtherly studied to change specific
684 characteristics of sparkling wines.
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695 In conclusion, **in our conditions** these data highlight that the use of *T. delbrueckii* in pure or
696 mixed fermentations is a suitable strategy to improve flavour production during secondary
697 fermentation, and thus to obtain sparkling wines with a composition of aroma compounds and a
698 sensory profile different from those of the *S. cerevisiae* starter strains. This *Td130* strain (i.e., *T.*
699 *delbrueckii* DiSVA 130) provided significant high scores for some of the aromatic descriptors
700 (which were supported by high esters production) that positively influenced the sparkling wine
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711 sensory profiles. The use of these selected *T. delbrueckii* (Td130/313) strains in secondary
712 fermentation for sparkling wine production represents a new approach to obtain an innovative
713 product. Further investigations are needed to identify the best applications in terms of the traditional
714 method used here and the Charmat method, to enhance the oenological characters of the *T.*
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720 *delbrueckii* yeast species in the secondary fermentation of sparkling wines.
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723 **Acknowledgments**

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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)
<i>S. cerevisiae</i>	527	12.45±0.00 ^a	0.24 ±0.02 ^a	3.21 ±0.00 ^a	0.23 ±0.04 ^{bc}
<i>T. delbrueckii</i>	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
<i>S. cerevisiae</i> + <i>T. delbrueckii</i>	527 + 130	12.46±0.03 ^a	0.23 ±0.014 ^a	3.20 ±0.00 ^a	0.20 ±0.04 ^c
<i>S. cerevisiae</i> + <i>T. delbrueckii</i>	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 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	Ethyl	Phenyl	Ethyl	Ethyl	Isoamyl	n-Propanol	Isobutanol	Amyl	Isoamylic	β-Phenyl	Hexanol
	butyrate	acetate	ethyl acetate	hexanoate	octanoate	acetate			alcohol	alcohol	ethanol	
Base wine	0.200	32 ±2	0.007	0.025	0.077	0.206	56 ±1	14±1	14 ±0	114±2	6 ±1	0.052
	±0.113		±0.006	±0.009	±0.010	±0.016						±0.005
<i>S. cerevisiae</i> 527	0.348	91 ±8 ^a	0.011	0.035	0.012	0.333	91±1 ^a	21±1 ^a	13 ±2 ^a	152 ±4 ^a	9 ±2 ^{ab}	0.082
	±0.103 ^b		±0.013 ^a	±0.004 ^d	±0.004 ^c	±0.029 ^c						±0.004 ^d
<i>T. delbrueckii</i> 130	0.427	84 ±1 ^{ab}	0.008	0.048	0.041	0.399	84 ±2 ^b	17 ±1 ^b	11±1 ^a	142±3 ^b	13±1 ^a	0.163
	±0.049 ^{ab}		±0.001 ^a	±0.001 ^c	±0.002 ^a	±0.011 ^{ab}						±0.008 ^a
<i>T. delbrueckii</i> 313	0.237	80 ±4 ^{abc}	0.004	0.066	0.039	0.428	84±0 ^b	18 ±0 ^b	11. ±2 ^a	144 ±2 ^b	10±1 ^{ab}	0.119
	±0.057 ^c		±0.002 ^a	±0.001 ^b	±0.001 ^a	±0.006 ^a						±0.007 ^{bc}
<i>S. cerevisiae</i> 527 + <i>T. delbrueckii</i> 130	0.400	76 ±2 ^{bc}	0.002	0.097	0.038	0.342	79 ±2 ^c	15 ±0 ^c	9 ±1 ^a	128±2 ^c	11 ±1 ^{ab}	0.098
	±0.025 ^b		±0.000 ^a	±0.004 ^a	±0.001 ^a	±0.006 ^{bc}						±0.027 ^{cd}
<i>S. cerevisiae</i> 527 + <i>T. delbrueckii</i> 313	0.675	72 ±1 ^c	0.002	0.069	0.032	0.452	72 ±1 ^d	16 ±0 ^c	10±1 ^a	119±1 ^d	9 ±1 ^b	0.145
	±0.243 ^a		±0.000 ^a	±0.002 ^b	±0.001 ^b	±0.061 ^a						±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
<i>S. cerevisiae</i> 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
<i>T. delbrueckii</i> 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
<i>T. delbrueckii</i> 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
<i>S. cerevisiae</i> 527 + <i>T. delbrueckii</i> 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
<i>S. cerevisiae</i> 527 + <i>T. delbrueckii</i> 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).

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1206 **Figure captions**
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1210 **Figure 1.** Fermentation kinetics of the sparkling wines produced in the pure and mixed secondary
1211 fermentations. Pure fermentations of *S. cerevisiae* DiSVA 527 (—■—), and *T. delbrueckii* DiSVA
1212 130 (—▲—) and DiSVA 313 (···●···). Mixed fermentations of *S. cerevisiae* DiSVA 527 plus *T.*
1213 *delbrueckii* DiSVA 130 (—▲—), and plus *T. delbrueckii* DiSVA 313 (···●···).
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1221 **Figure 2.** Principal component analysis based on the data for the volatile compounds in the
1222 sparkling wines produced by the *S. cerevisiae* DiSVA 527 (*Sc527*) and *T. delbrueckii* DiSVA 130
1223 (*Td130*) and DiSVA 313 (*Td313*) pure fermentations and mixed fermentations.
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1230 **Figure 3.** Sensory analysis of the sparkling wines produced in the pure and mixed secondary
1231 fermentations. Pure fermentations of *S. cerevisiae* DiSVA 527 (—■—), and *T. delbrueckii* DiSVA
1232 130 (—▲—) and DiSVA 313 (···●···). Mixed fermentations of *S. cerevisiae* DiSVA 527 plus *T.*
1233 *delbrueckii* DiSVA 130 (—▲—) and plus *T. delbrueckii* DiSVA 313 (···●···). *, Significantly
1234 different (Fisher ANOVA; p-value 0.05).
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Figure 1

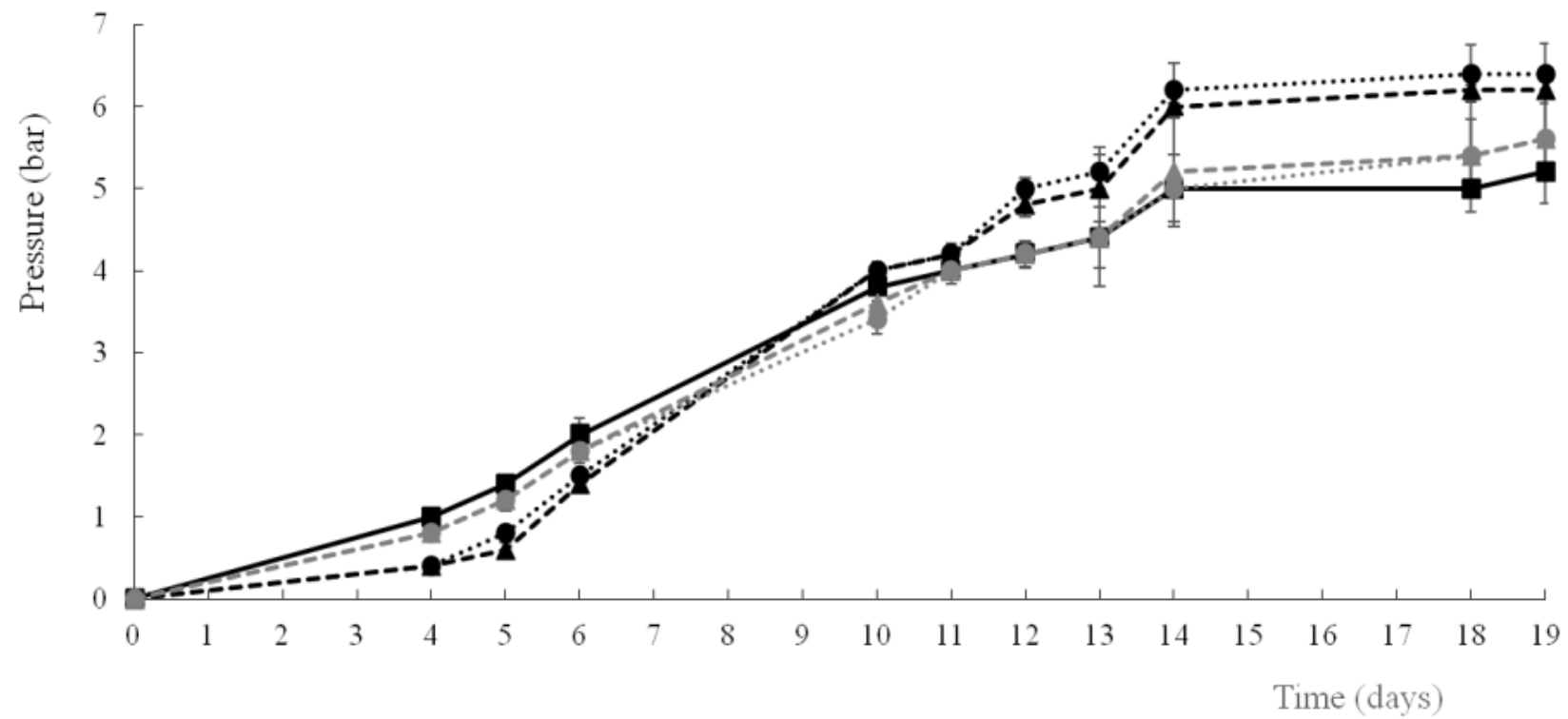


Figure 2

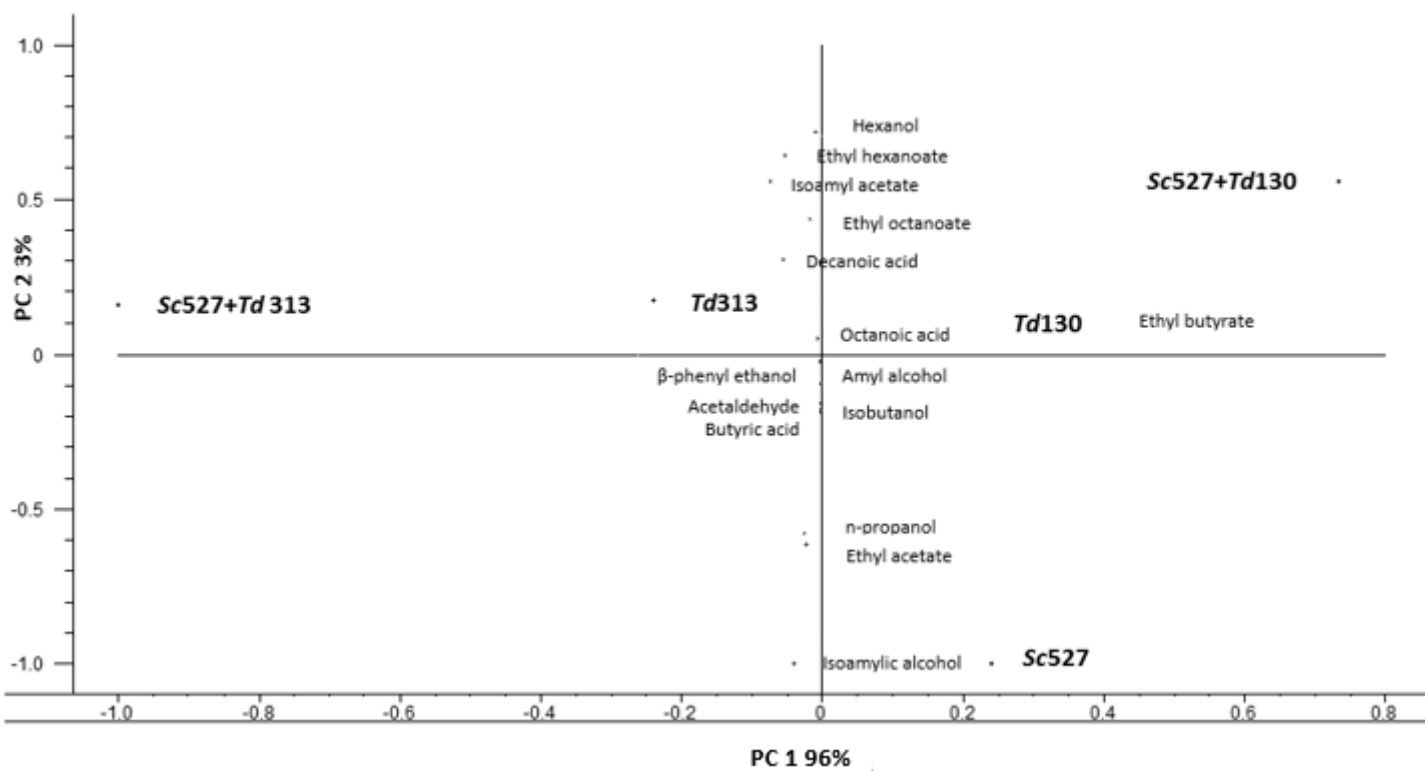


Figure 3

