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ORIGINAL PAPER

# Impact of sequential co-culture fermentations on flavour characters of Solaris wines

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**Abstract** Nowadays, the use of non-*Saccharomyces* yeasts in combination with *Saccharomyces cerevisiae* is being recognised to enhance the analytical composition of the wines. The aim of this work was to evaluate the influence of indigenous non-*Saccharomyces* yeasts on the flavour character of wines from the cool-climate grape cultivar Solaris in Denmark. The volatile and non-volatile compounds as well as the sensory properties of wines were evaluated. Solaris wines with *Hanseniaspora uvarum* sequentially inoculated with *S. cerevisiae* produced a larger amount of glycerol as well as heptyl acetate and 2-phenylethyl acetate. This co-culture fermentation also produced higher amounts of ethyl acetate and acetic acid, reducing the possibility of its use in winemaking. Three *Metschnikowia* strains, a *M. chrysoperlae* strain and two *M. fructicola* strains, gave a comparable production of volatile compounds. These wines were characterised by several *floral* and *fruity* attributes. The *Metschnikowia* strains turned out to be promising in winemaking from Solaris grapes.

**Keywords** Sequential fermentation · Non-*Saccharomyces* · Cool-climate grape cultivar · White wine · Volatile compounds · Sensory evaluation

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## Introduction

Wine fermentation is a complex process, where yeast strains play an essential role by converting sugars from the grapes into ethanol, carbon dioxide and by-products. Over the last decades, selected active yeast strains have commonly been used for wine fermentations. However, such approach limits the involvement of other species and yeast strains, thus reducing the complexity of the final product [1]. Recently, more attention has been given to take advantage of non-*Saccharomyces* yeasts in order to enhance the characteristics of a wine. Certain non-*Saccharomyces* species have potentials to introduce characteristics to the wine that may improve the aroma profile [2–4], enhance the glycerol content [5] and reduce the ethanol content [6].

Recently, some non-*Saccharomyces* yeast strains have been isolated and identified from local grapes in Denmark [7]. From them, *Metschnikowia fructicola* has been reported to be used against postharvest diseases of fruits [8–11]; *Metschnikowia chrysoperlae* has been reported to be isolated from the green lacewings [12]. None of these *Metschnikowia* species have been used for wine fermentation. *Hanseniaspora uvarum*, also known as *Kloeckera apiculata*, has been widely studied [2, 13–16]. However, its performance has not been evaluated for producing wines from cool-climate grapes.

Solaris is a white grape cultivar, which is dominantly planted in Denmark, England, and other regions in Northern Europe. The quality of Solaris grapes is appreciated in winemaking because of its stable yields and reliable berry ripening despite the cool climate. More importantly, Solaris grapes can produce balanced wines with fruity aroma profiles [17, 18]. In young white wine from Solaris grapes, the *floral* and *fruity* notes have been mainly attributed to

acetates and ethyl esters of short straight-chain fatty acids [17]. These compounds are strongly affected by the alcoholic fermentation.

The aim of this work was to evaluate the potential of using different indigenous non-*Saccharomyces,* inoculated with *S. cerevisiae* strains, to enhance the flavour characters of wines made from Solaris grapes.

## Materials and methods

### Yeast strains and inoculation cultures

Four strains of indigenous non-*Saccharomyces* species and a commercial *Saccharomyces cerevisiae* strain (Saint Georges S101, Bio Springer, France) were used. The *Metschnikowia chrysoperlae* (SF1-13) and *Metschnikowia fructicola A* (SF1-19) strains (not published), as well as the *Metschnikowia fructicola B* (RU9-4) and *Hanseniaspora uvarum* (RT9-7) strains [7], were previously isolated from Danish grapes and were available from the yeast culture collection at the Department of Food Science, University of Copenhagen, Denmark. Inoculation cultures were prepared by inoculating ($10^5$ cfu/mL) from an overnight culture and growing each yeast strain for 24 h in YGP medium (per litre: 5 g yeast extract, 10 g peptone, 10 g glucose, pH 5.6) at 25 °C with shaking (140 rpm).

### Fermentation trials

Alcoholic fermentations were performed in Solaris grape juice in 2-L blue-cap bottles fitted with a butyl stopper and a fermentation lock in tygon tubing containing 50 % (v/v) sterile glycerol. The grape juice (reducing sugar 18.0 % (w/v), total acid 9.6 g/L, pH 3.0) was obtained from the experimental vineyard at Pometet (Taastrup, University of Copenhagen) and was pasteurised and stored at 4 °C until use. For each sequential fermentation, a non-*Saccharomyces* yeast was inoculated ($10^6$ cfu/mL) followed by *S. cerevisiae* ($10^5$ cfu/ml) after 3 days. A pure fermentation was also conducted, inoculated with only *S. cerevisiae* ($10^5$ cfu/mL). All fermentations were carried out in duplicate. After around 20 days of fermentation, 75 mg/L sulphite was added to complete the fermentations. The finished young wine was used 2 months later for instrumental analysis and sensory evaluation.

### Oenochemical properties

Ethanol, pH, total acid, volatile acid, glycerol, sugars and organic acids were measured using a Fourier Transform Infrared Spectrophotometer (WineScan FT120, FOSS A/S, Hillerød, Denmark).

### Volatile composition analysis

Volatile aroma compounds were collected in a dynamic headspace sampling (DHS) system using Tenax-TA traps. The collected volatiles were thermally desorbed and analysed by gas chromatography–mass spectrometry (GC–MS) as reported by Liu et al. [17]. Separation of volatiles was carried out on a DB-Wax column (30 m × 0.25 mm × 0.25 μm). The GC–MS data processing was carried out using the software MSD Chemstation G1701EA (Version E.01.00.237, Agilent Technologies Inc., Palo Alto, CA, USA). Identification of volatiles was made by the probability based on matching of mass spectra with those available of a commercial database (Wiley275.L, G1035A, Agilent Technologies, Inc.). To support the identification, linear retention indices (LRI) were calculated and compared to retention indices of authentic standards or reported LRI values in the literature. The results from volatile analysis were presented as peak areas of the compounds identified.

### Sensory analysis

The sensory evaluation was performed in the sensory laboratory at the University of Copenhagen. A trained panel consisting of ten assessors (two males and eight females; mean age = 36 years) was recruited. All assessors had been generally trained in sensory evaluation of different food matrices, including wine. They were paid for their participation.

A modified Flash Profile method [19] was used to assess the samples in four sessions. A Napping followed by an attribute generation step was integrated as a way to help assessors get familiar with the product space, as well as focus on the attributes that discriminate the samples. The final attribute list was built after a repeated Napping task. The number of sensory attributes was restricted to 15. Assessors were then asked to rank the flavour intensities of the samples according to each attribute of their own list. A repeated ranking session with the same final list was conducted. A blind repeated sample (wine fermented with single *S. cerevisiae*) was used to evaluate the reliability of the panel. Thus, six wines in total were presented simultaneously to the assessors at room temperature. An overview of the sensory procedure is shown in Fig. 1. Assessors could smell or taste the samples as many times as they wanted. Sensory assessments took place in sensory booths designed according to ISO/ASTM guidelines.

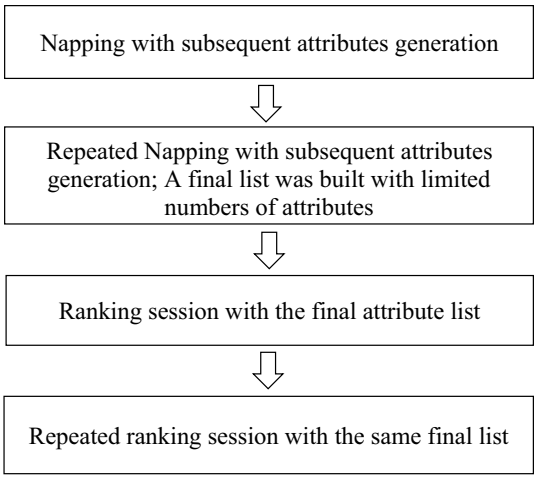


**Fig. 1** Procedure of the modified Flash Profile

### Data analysis

The variation in volatiles and non-volatiles measured in the wines was assessed by one-way analysis of variance (ANOVA) using SAS JMP (version 7.0, SAS Institute Inc., Cary, USA) with Tukey HSD means comparison test post hoc comparisons (5 % level).

For the sensory data, the panel's performance was tested by Friedman test. Attributes that were found not to discriminate the products significantly were excluded for multivariate analysis. Generalized Procrustes Analysis (GPA) [20] was applied for the consensus configuration between the sensory maps of the assessors. The software XLSTAT (Addinsoft, New York, NY) was used.

## Results and discussion

### Oenochemical analysis

Table 1 shows the results from oenochemical analysis. As can be seen, samples showed significant differences for all parameters. The ethanol content of the wines ranged from 10.7 to 11.8 % (v/v). The content of volatile acid was between 0.2 and 0.3 g/L with the exception of *H. uvarum/S. cerevisiae* wine producing 0.6 g/L of volatile acid. Although this range of volatile acid content is normal for white wine [21], there is a higher risk for *H. uvarum/S. cerevisiae* to produce excessive volatile acid, which could impart a vinegar-like character to wines. All non-*Saccharomyces* yeasts produced significantly higher content of glycerol than single *S. cerevisiae* fermentation. For the wine acidity, non-*Saccharomyces* yeasts lowered the content of total acid and correspondingly increased the pH. The glucose in all wines was consumed fully (data not shown in Table 1), while the fructose especially in those with non-*Saccharomyces* was not fully fermented. For instance, the wines with the *Metschnikowia* strains had 10–15 g/L of residual fructose. This could be due to production of antimicrobial compounds and/or exhaust of one or more nutrients during fermentation. It should also be noted that, rather surprisingly, the single *S. cerevisiae* culture consumed the most amount of sugars but produced the lowest amount of ethanol, without increasing the amounts of, for example, glycerol and acetic acid (i.e. volatile acidity). Further experiments are required to elucidate these issues.

### Volatile analysis

A total of 82 volatile compounds were identified in the Solaris wines. Values and ANOVA results are presented in Table 2.

### Esters

Esters were the largest group in terms of the number of volatiles in the Solaris wines. Significant differences between samples were observed for most of the esters. Various esters were produced by yeasts during fermentation with ethyl esters and acetates being the major esters. The highest levels of ethyl hexanoate (*fruity, apply peel*), ethyl (*Z*)-3-hexenoate and ethyl heptanoate were found in wines sequentially

**Table 1** Oenochemical parameters of Solaris wines with different inoculations: sequential fermentations *M. chrysoperlae/S. cerevisiae* (Mc-Sc), *M. fructicola A/S. cerevisiae* (Mf.A-Sc), *M. fructicola B/S. cerevisiae* (Mf.B-Sc), *H. uvarum/S. cerevisiae* (Hu-Sc), and single fermentation inoculated with *S. cerevisiae* (Sc)

| | Mc-Sc[a] | Mf.A-Sc | Mf.B-Sc | Hu-Sc | Sc | Significance[b] |
|---|---|---|---|---|---|---|
| Ethanol (% v/v) | 11.4$^{b}$ | 11.8$^{a}$ | 11.3$^{b}$ | 11.1$^{c}$ | 10.7$^{d}$ | *** |
| pH | 3.1$^{b}$ | 3.2$^{a}$ | 3.1$^{b}$ | 3.0$^{c}$ | 2.8$^{d}$ | *** |
| Total acid (g/L) | 9.1$^{c}$ | 8.9$^{d}$ | 9.1$^{c}$ | 9.4$^{b}$ | 10.2$^{a}$ | *** |
| Volatile acid (g/L) | 0.3$^{b}$ | 0.3$^{b}$ | 0.3$^{b}$ | 0.6$^{a}$ | 0.2$^{c}$ | *** |
| Glycerol (g/L) | 6.4$^{b}$ | 6.8$^{a}$ | 6.2$^{bc}$ | 6.1$^{c}$ | 4.2$^{d}$ | *** |
| Malic acid (g/L) | 4.0$^{b}$ | 3.9$^{bc}$ | 3.9$^{bc}$ | 3.8$^{c}$ | 4.7$^{a}$ | *** |
| Tartaric acid (g/L) | 3.3$^{b}$ | 3.2$^{b}$ | 3.3$^{b}$ | 3.4$^{b}$ | 3.8$^{a}$ | *** |
| Fructose (g/L) | 14.6$^{a}$ | 14.1$^{b}$ | 10.0$^{c}$ | 5.2$^{d}$ | 3.8$^{e}$ | *** |

[a] Different letters in the same row represent significant differences at $p < 0.05$ level

[b] The significance levels between samples. *** $p < 0.001$

**Table 2** Volatile compounds (mean peak areas/$10^5$) identified in the Solaris wines with different inoculations: sequential fermentations *M. chrysoperlae/S. cerevisiae* (Mc-Sc), *M. fructicola A/S. cerevisiae* (Mf.A-Sc), *M. fructicola B/S. cerevisiae* (Mf.B-Sc), *H. uvarum/S. cerevisiae* (Hu-Sc), and single fermentation inoculated with *S. cerevisiae* (Sc)

| Code | Compounds | Calculated LRI[a] | Reported LRI[b] | Mc-Sc[c] | Mf.A-Sc | Mf.B-Sc | Hu-Sc | Sc | Significance[d] |
|---|---|---|---|---|---|---|---|---|---|
| Esters | Ethyl esters | | | | | | | | |
| 1 | Ethyl acetate | 875 | 907 | $10000^{ab}$ | $10000^{ab}$ | $9900^{bc}$ | $12000^{a}$ | $7600^{c}$ | *** |
| 2 | Ethyl propanoate | 942 | 951 | $1000^{b}$ | $1000^{b}$ | $960^{b}$ | $3500^{a}$ | $950^{b}$ | *** |
| 3 | Ethyl 2-methylpropanoate | 951 | 955 | $97^{b}$ | $97^{b}$ | $100^{b}$ | $360^{a}$ | $400^{a}$ | *** |
| 4 | Ethyl butanoate | 1034 | 1028 | 7000 | 7300 | 6900 | 6600 | 6400 | ns |
| 5 | Ethyl 3-methylbutanoate | 1073 | 1060 | $21^{b}$ | $29^{b}$ | $28^{b}$ | $18^{b}$ | $110^{a}$ | *** |
| 6 | Ethyl pentanoate | 1143 | 1148 | $24^{a}$ | $25^{a}$ | $23^{ab}$ | $14^{bc}$ | $12^{c}$ | ** |
| 7 | Ethyl (*E*)-crotonate | 1168 | 1152 | 140 | 130 | 130 | 190 | 150 | ns |
| 8 | Ethyl hexanoate | 1252 | 1220 | $7900^{a}$ | $7700^{a}$ | $8100^{a}$ | $5400^{b}$ | $5500^{b}$ | *** |
| 9 | Ethyl (*E*)-4-hexenoate | 1307 | – | $2^{b}$ | $2^{b}$ | $2^{b}$ | $20^{a}$ | $20^{a}$ | *** |
| 10 | Ethyl (*Z*)-3-hexenoate | 1316 | 1296 | $7^{a}$ | $7^{a}$ | $8^{a}$ | $2^{b}$ | $2^{b}$ | *** |
| 11 | Ethyl (*E*)-3-hexenoate | 1320 | 1301 | $18^{b}$ | $21^{b}$ | $28^{a}$ | $2^{c}$ | $1^{c}$ | *** |
| 12 | Ethyl heptanoate | 1346 | 1351 | $83^{a}$ | $64^{a}$ | $65^{a}$ | $26^{b}$ | $19^{b}$ | *** |
| 13 | Ethyl 2-hydroxypropanoate | 1357 | 1358 | 600 | 560 | 500 | 540 | 570 | ns |
| 14 | Ethyl octanoate | 1448 | 1436 | 8500 | 8500 | 8800 | 7100 | 8500 | ns |
| 15 | Ethyl nonanoate | 1545 | 1541 | 21 | 18 | 21 | 15 | 21 | ns |
| 16 | Ethyl decanoate | 1652 | 1636 | 6000 | 5900 | 6300 | 5200 | 6600 | ns |
| 17 | Ethyl benzoate | 1684 | 1690 | $17^{b}$ | $83^{a}$ | $21^{b}$ | $15^{b}$ | $18^{b}$ | *** |
| 18 | Diethyl succinate | 1689 | 1689 | $26^{ab}$ | $22^{b}$ | $22^{b}$ | $17^{b}$ | $37^{a}$ | ** |
| 19 | Ethyl 9-decenoate | 1703 | 1694 | $2600^{a}$ | $2400^{a}$ | $2400^{a}$ | $2600^{a}$ | $1700^{b}$ | ** |
| 20 | Ethyl dodecanoate | 1797 | 1842 | $1800^{ab}$ | $1500^{ab}$ | $2100^{a}$ | $1400^{ab}$ | $1100^{b}$ | * |
| | Acetates | | | | | | | | |
| 1 | Methyl acetate | 810 | | 64 | 54 | 57 | 61 | 77 | ns |
| 2 | Propyl acetate | 960 | 969 | $2000^{a}$ | $2000^{a}$ | $1700^{a}$ | $2100^{a}$ | $580^{b}$ | *** |
| 3 | 2-Methylpropyl acetate | 1008 | 1015 | $5600^{a}$ | $5300^{a}$ | $5500^{a}$ | $3000^{b}$ | $1900^{c}$ | *** |
| 4 | Butyl acetate | 1077 | 1075 | $390^{ab}$ | $500^{a}$ | $300^{b}$ | $290^{b}$ | $110^{c}$ | *** |
| 5 | 3-Methylbutyl acetate | 1136 | 1117 | $17000^{ab}$ | $19000^{a}$ | $19000^{a}$ | $17000^{ab}$ | $14000^{b}$ | ** |
| 6 | Pentyl acetate | 1180 | 1180 | $110^{a}$ | $120^{a}$ | $100^{a}$ | $110^{a}$ | $45^{b}$ | *** |
| 7 | Hexyl acetate | 1291 | 1270 | $4700^{ab}$ | $5000^{ab}$ | $4500^{ab}$ | $5700^{a}$ | $4200^{b}$ | * |
| 8 | (*Z*)-3-Hexenyl acetate | 1323 | 1327 | $270^{a}$ | $250^{a}$ | $220^{a}$ | $130^{b}$ | $72^{b}$ | *** |
| 9 | Heptyl acetate | 1383 | 1366 | $39^{b}$ | $39^{b}$ | $28^{b}$ | $98^{a}$ | $36^{b}$ | *** |
| 10 | 2-Ethylhexyl acetate | 1391 | – | $40^{a}$ | $38^{a}$ | $17^{b}$ | $13^{bc}$ | $7^{c}$ | *** |
| 11 | 2-Phenylethyl acetate | 1798 | 1829 | $3100^{a}$ | $2800^{b}$ | $2700^{b}$ | $3200^{a}$ | $2700^{b}$ | *** |
| | Other esters | | | | | | | | |
| 1 | Methyl hexanoate | 1191 | 1188 | $170^{ab}$ | $180^{ab}$ | $200^{a}$ | $110^{b}$ | $130^{ab}$ | * |
| 2 | 3-Methylbutyl butanoate | 1279 | 1267 | $18^{ab}$ | $21^{ab}$ | $23^{a}$ | $12^{b}$ | $25^{a}$ | * |
| 3 | 2-Methylpropyl hexanoate | 1364 | – | $43^{a}$ | $39^{a}$ | $53^{a}$ | $13^{b}$ | $14^{b}$ | *** |
| 4 | Methyl octanoate | 1396 | 1389 | 180 | 190 | 210 | 150 | 220 | ns |
| 5 | 3-Methylbutyl hexanoate | 1468 | 1475 | $230^{ab}$ | $210^{ab}$ | $250^{a}$ | $130^{b}$ | $160^{ab}$ | * |
| 6 | Propyl octanoate | 1522 | – | 33 | 29 | 33 | 24 | 25 | ns |
| 7 | 2-Methylpropyl octanoate | 1560 | – | $24^{a}$ | $20^{ab}$ | $28^{a}$ | $9^{c}$ | $15^{bc}$ | *** |
| 8 | Methyl decanoate | 1604 | 1608 | $66^{ab}$ | $66^{ab}$ | $79^{ab}$ | $59^{b}$ | $110^{a}$ | * |
| 9 | 3-Methylbutyl octanoate | 1733 | 1658 | $210^{ab}$ | $210^{ab}$ | $260^{a}$ | $100^{b}$ | $270^{a}$ | ** |
| 10 | Propyl decanoate | 1668 | – | 12 | 12 | 14 | 17 | 16 | ns |
| 11 | 3-Methylbutyl decanoate | 1796 | 1868 | 67 | 61 | 92 | 28 | 94 | ns |
| Higher alcohols | | | | | | | | | |
| 1 | 1-Propanol | 1046 | 1037 | $670^{a}$ | $510^{ab}$ | $540^{a}$ | $100^{c}$ | $350^{b}$ | *** |

**Table 2** continued

| Code | Compounds | Calculated LRI[a] | Reported LRI[b] | Mc-Sc[c] | Mf.A-Sc | Mf.B-Sc | Hu-Sc | Sc | Significance[d] |
|---|---|---|---|---|---|---|---|---|---|
| 2 | 2-Methyl-1-propanol | 1100 | 1099 | 5100$^{a}$ | 4200$^{ab}$ | 4900$^{ab}$ | 2400$^{c}$ | 3600$^{bc}$ | *** |
| 3 | 1-Butanol | 1158 | 1145 | 1500$^{a}$ | 1400$^{a}$ | 1200$^{a}$ | 480$^{b}$ | 560$^{b}$ | *** |
| 4 | 3-Methyl-1-butanol | 1225 | 1205 | 17000$^{b}$ | 17000$^{b}$ | 17000$^{b}$ | 15000$^{c}$ | 20000$^{a}$ | *** |
| 5 | 3-Methyl-3-butanol | 1264 | 1263 | 18 | 16 | 15 | 17 | 17 | ns |
| 6 | 1-Pentanol | 1268 | 1255 | 95$^{a}$ | 98$^{a}$ | 77$^{ab}$ | 67$^{b}$ | 61$^{b}$ | ** |
| 7 | 2-Heptanol | 1336 | 1273 | 22 | 17 | 21 | 21 | 17 | ns |
| 8 | 3-Methyl-1-pentanol | 1342 | 1325 | 140$^{b}$ | 150$^{b}$ | 130$^{b}$ | 77$^{c}$ | 290$^{a}$ | *** |
| 9 | 1-Hexanol | 1369 | 1360 | 2600$^{b}$ | 2800$^{b}$ | 2400$^{b}$ | 3500$^{a}$ | 3400$^{a}$ | *** |
| 10 | (*E*)-3-Hexen-1-ol | 1377 | 1386 | 110$^{a}$ | 99$^{ab}$ | 88$^{ab}$ | 84$^{ab}$ | 69$^{b}$ | * |
| 11 | 3-Ethoxy-1-propanol | 1388 | 1409 | 12$^{a}$ | 12$^{a}$ | 10$^{a}$ | 14$^{a}$ | 3$^{b}$ | ** |
| 12 | (*Z*)-3-Hexen-1-ol | 1394 | 1391 | 59$^{a}$ | 58$^{a}$ | 49$^{ab}$ | 40$^{b}$ | 36$^{b}$ | ** |
| 13 | (*Z*)-2-Hexen-1-ol | 1425 | 1400 | 35$^{a}$ | 30$^{a}$ | 27$^{a}$ | 16$^{b}$ | 15$^{b}$ | *** |
| 14 | 2-Ethylhexanol | 1498 | 1487 | 20 | 17 | 15 | 19 | 18 | ns |
| 15 | 2,3-Butanediol | 1551 | 1523 | 90 | 130 | 88 | 47 | 35 | ns |
| 16 | 1-Octanol | 1567 | 1553 | 21$^{b}$ | 18$^{b}$ | 20$^{b}$ | 21$^{b}$ | 56$^{a}$ | *** |
| 17 | 1-Decanol | 1772 | 1765 | 11$^{c}$ | 8$^{c}$ | 11$^{bc}$ | 31$^{b}$ | 89$^{a}$ | *** |
| 18 | Benzylalcohol | 1896 | 1865 | 4$^{b}$ | 3$^{b}$ | 5$^{b}$ | 22$^{a}$ | 4$^{b}$ | *** |
| 19 | 2-Phenylethanol | 1936 | 1925 | 2000$^{ab}$ | 1800$^{b}$ | 1800$^{b}$ | 1500$^{b}$ | 2900$^{a}$ | ** |
| Aldehydes | | | | | | | | | |
| 1 | 3-Methylbutanal | 904 | 910 | 48 | 41 | 49 | 34 | 63 | ns |
| 2 | Hexanal | 1081 | 1084 | 13 | 13 | 9 | 11 | 10 | ns |
| 3 | Octanal | 1302 | 1280 | 12$^{a}$ | 5$^{ab}$ | 5$^{ab}$ | 3$^{b}$ | 3$^{b}$ | * |
| 4 | Nonanal | 1400 | 1385 | 39$^{a}$ | 23$^{ab}$ | 17$^{ab}$ | 14$^{b}$ | 14$^{b}$ | * |
| 5 | Decanal | 1508 | 1484 | 43$^{a}$ | 33$^{ab}$ | 27$^{ab}$ | 18$^{b}$ | 15$^{b}$ | ** |
| 6 | Benzaldehyde | 1537 | 1495 | 30 | 20 | 19 | 12 | 13 | ns |
| Ketones | | | | | | | | | |
| 1 | 2-Heptanone | 1185 | 1170 | 37 | 28 | 34 | 25 | 33 | ns |
| 2 | 3-Hydroxy-2-butanone | 1297 | 1287 | 440$^{a}$ | 450$^{a}$ | 330$^{a}$ | 67$^{b}$ | 28$^{b}$ | *** |
| 3 | 6-Methyl-5-hepten-2-one | 1350 | 1340 | 13$^{ab}$ | 8$^{b}$ | 13$^{ab}$ | 19$^{ab}$ | 23$^{a}$ | * |
| 4 | 2-Undecanone | 1609 | 1543 | 32$^{a}$ | 26$^{a}$ | 24$^{a}$ | 10$^{b}$ | 10$^{b}$ | *** |
| Fatty acids | | | | | | | | | |
| 1 | Acetic acid | 1458 | 1450 | 2$^{b}$ | 20$^{b}$ | 520$^{b}$ | 1800$^{a}$ | 910$^{b}$ | *** |
| 2 | 2-Methylpropanoic acid | 1576 | – | 0$^{b}$ | 3$^{b}$ | 19$^{b}$ | 90$^{a}$ | 99$^{a}$ | *** |
| 3 | Butanoic acid | 1637 | 1619 | 0 | 1 | 1 | 8 | 16 | ns |
| 4 | 3-Methylbutanoic acid | 1679 | – | 0$^{b}$ | 0$^{b}$ | 26$^{b}$ | 41$^{ab}$ | 93$^{a}$ | ** |
| 5 | Hexanoic acid | 1797 | 1829 | 0$^{c}$ | 0$^{c}$ | 0$^{c}$ | 150$^{b}$ | 340$^{a}$ | ** |
| 6 | Octanoic acid | 1786 | – | 1$^{b}$ | 1$^{b}$ | 4$^{b}$ | 64$^{ab}$ | 430$^{a}$ | * |
| Terpenes | | | | | | | | | |
| 1 | *(Z)*-Rose oxide | 1364 | 1337 | 22 | 24 | 25 | 30 | 34 | ns |
| 2 | Neroloxide | 1484 | 1479 | 80$^{b}$ | 92$^{b}$ | 94$^{b}$ | 140$^{a}$ | 150$^{a}$ | ** |
| 3 | Hotrienol | 1618 | 1623 | 120 | 120 | 97 | 99 | 110 | ns |
| Acetal | | | | | | | | | |
| | 2,4,5-Trimethyl-1,3-dioxolane | 927 | 956 | 1400$^{a}$ | 1300$^{a}$ | 1000$^{a}$ | 230$^{b}$ | 160$^{b}$ | *** |
| C13-norisoprenoid | | | | | | | | | |
| | *β*-Damascenone | 1798 | 1813 | 28 | 31 | 28 | 29 | 27 | ns |

[a] Linear retention indices (LRI) calculated according to the retention time

[b] LRI reported in Flavournet and Pherobase for DB-Wax capillary GC column

[c] Different letters in the same row represent significant differences at $p < 0.05$ level. No letters were added if there was no significant difference between samples

[d] The significance levels between samples. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; *ns* not significant

fermented with three *Metschnikowia* strains and *S. cerevisiae*, indicating a genera tendency. Specifically, *M. fructicola A* was a stronger producer of ethyl benzoate with approximately four times higher levels than the rest wines. This compound has a pleasant odour described as *sweet, wintergreen, fruity, medicinal, cherry and grape*. *M. fructicola B* produced a larger amount of ethyl (*E*)-3-hexenoate. The wine fermented with *H. uvarum/S. cerevisiae* showed a significantly higher level of ethyl acetate than that in single *S. cerevisiae* wine. This result is in agreement with previous studies using *H. uvarum* in winemaking from other grape cultivars [2, 14, 22]. *H. uvarum/S. cerevisiae* also produced the highest values of ethyl propanoate (*fruity and yeast*). A substantially larger value of ethyl 3-methylbutanoate was observed in wine with single *S. cerevisiae* fermentation. Furthermore, the most ethyl 2-methylpropanoate was found in the single *S. cerevisiae* wine as well as the *H. uvarum/S. cerevisiae wine.* It has been stated that ethyl esters of branched short-chain fatty acids are less correlated with pleasant flavour for young white Solaris wine [17, 23].

It is generally admitted that acetates exhibit *floral* and *fruity* odours and thus are essential for young wine. Apart from methyl acetate, all acetates revealed significant differences between samples. Furthermore, all acetates, except methyl acetate and heptyl acetate, showed higher levels in wines with sequential fermentations compared to those obtained by single *S. cerevisiae*. The three *Metschnikowia* strains showed considerably increased production of 2-methylpropyl acetate and (*Z*)-3-hexenyl acetate. Furthermore, *M. chrysoperlae* and *M. fructicola A* produced more 2-ethylhexyl acetate. A substantially higher level of heptyl acetate was found in *H. uvarum/S. cerevisiae* wine in contrast to other wines. The *H. uvarum* and *M. chrysoperlae* strains also had a larger capability of producing 2-phenylethyl acetate. This compound contributes a desirable aspect to the bouquet of wine [24, 25].

## Higher alcohols

Higher alcohols were another important group of volatile compounds in the wines. All alcohols, except 3-methyl-3-butanol, 2-heptanol, 2-ethylhexanol and 2,3-butanediol, showed significant differences between samples. There was higher production of 3-methyl-1-butanol and 2-phenylethanol in the wine with single *S. cerevisiae* in contrast to those fermented by sequential cultures. 3-Methyl-1-butanol has an unpleasant odour with descriptor *nail polish*, while 2-phenylethanol has been described by *honey, rose and spicy* attributes [26]. Significant increases of 3-methyl-1-pentanol, 1-octanol and 1-decanol were also observed in the single *S. cerevisiae* wine. Balanced contents of aliphatic higher alcohols contribute to aromatic complexity, whereas excessive concentration can result in wines with a strong, pungent smell and taste [27, 28]. Single *S. cerevisiae* wine as well as *H. uvarum/S. cerevisiae* wine also gave rise to higher levels of 1-hexanol, which usually contributes to *grass* and *green* flavours [29]. On the contrary, the *Metschnikowia* strains, in their sequential cultures with *S. cerevisiae,* were higher producers of 1-butanol and (*Z*)-2-hexen-1-ol.

## Aldehydes and ketones

There were 6 aldehydes and 4 ketones identified in the Solaris wines. The *M. chrysoperlae* wine produced higher levels of octanal, nonanal and decanal compared to the *S. cerevisiae* wine. The three *Metschnikowia* strains produced significantly higher values of 3-hydroxy-2-butanone and 2-undecanone. 3-Hydroxy-2-butanone is usually considered to have a *buttery* note, while 2-undecanone has *fruity* and *floral* notes [30, 31].

## Fatty acids

In terms of fatty acids detected in this study, single *S. cerevisiae* produced the highest levels of 3-methylbutanoic acid, hexanoic acid and octanoic acid in contrast to the rest wines. Furthermore, single *S. cerevisiae* wine together with *H. uvarum/S. cerevisiae* wine also produced more 2-methylpropanoic acid. *H. uvarum* was a strong producer of acetic acid with at least two times higher values than the other wines. It has been widely reported that *H. uvarum* produced high levels of acetic acid [13, 27]. The concentration of ethyl acetate is strongly influenced by acetic acid content [32]. It is thus reasonable that *H. uvarum* produced the significantly highest values of both compounds. However, there was not found any general connection between the levels of acetates and the levels of acetic acid (Table 2). Nor was it so that the wines with the highest levels of ethyl esters linked to the ethanol level of the wines (neither positive nor negative correlation) (Tables 1 and 2). Apparently, the levels of aroma compounds formed were only to a limited degree determined by precursor levels. Thus, the species differences in enzymatic regulation appeared to be more important.

## Terpenes and other volatiles

Terpenes are originally derived from the grape berries. Three terpenes were identified in the Solaris wines. As expected, there were no significant differences between samples for *(Z)*-rose oxide and hotrienol. However, neroloxide exhibited the highest values in *H. uvarum/S. cerevisiae* wine and single *S. cerevisiae* wine. Neroloxide was described with *oil* and *flower* notes.

2,4,5-Trimethyl-1,3-dioxolane and *β*-damascenone were also detected in the wines. The *Metschnikowia* strains

produced higher levels of 2,4,5-trimethyl-1,3-dioxolane than the other two wines. This compound has been often considered as an indicator of oxidation. There was no significant difference between samples for $\beta$-damascenone, which is derived from the grape berries.

## Sensory analysis

The chemical compositions in wines influenced the sensory properties. The GPA plot of significant attributes is shown in Fig. 2. The first two dimensions accounted

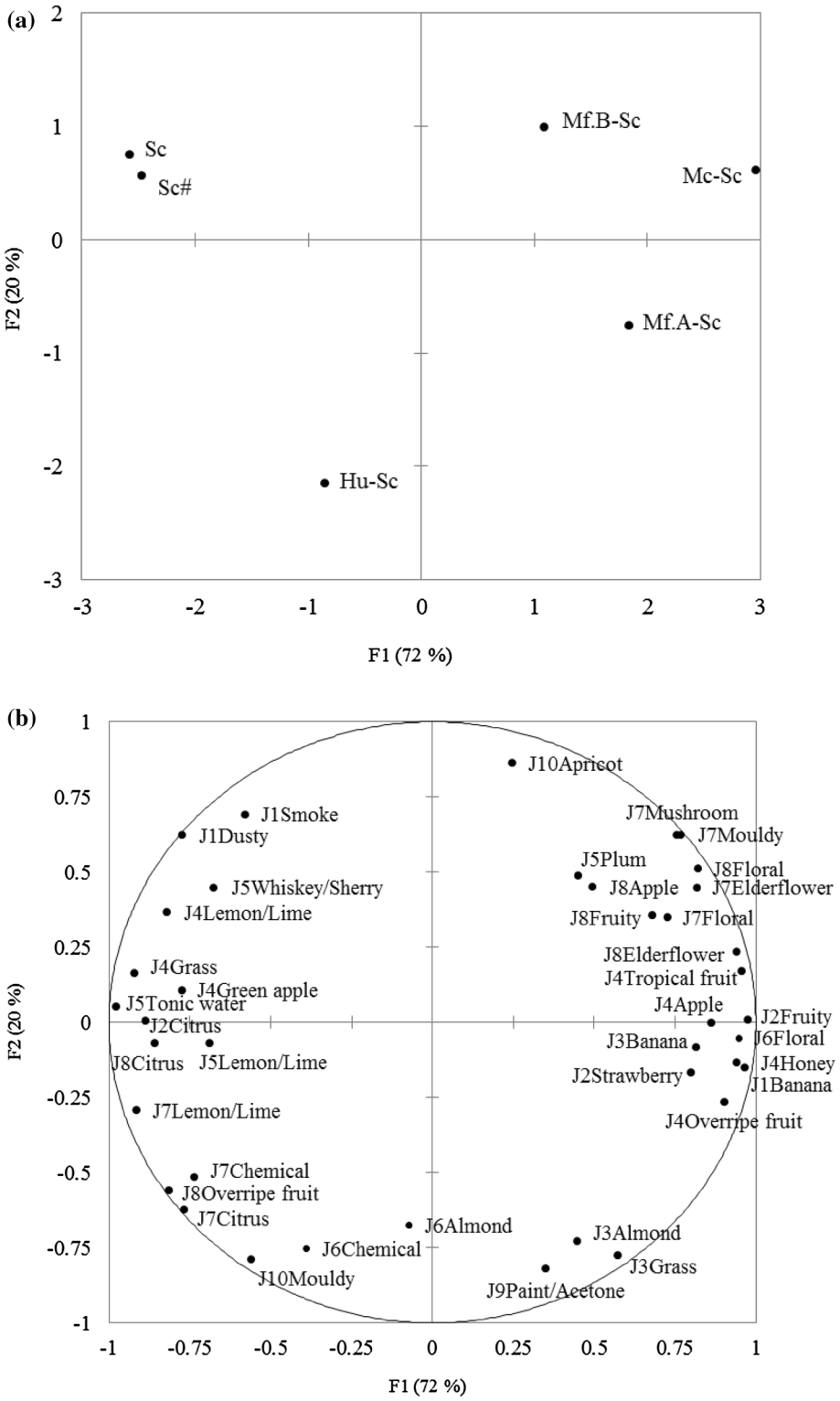


**Fig. 2** GPA plots obtained from the modified Flash Profile. **a** Consensus configuration plot of wine samples with different inoculations: sequential fermentations *M. chrysoperlae/S. cerevisiae* (Mc-Sc), *M. fructicola A/S. cerevisiae* (Mf.A-Sc), *M. fructicola B/S. cerevisiae* (Mf.B-Sc), *H. uvarum/S. cerevisiae* (Hu-Sc), single fermentation inoculated with *S. cerevisiae* (Sc) and its blind replicate (Sc#); **b** Variable plot of significant attributes used by each assessor. Codes J1–J10 referred to Judge 1–Judge 10

for 92 % of the total explained variance (72 % and 20 %, respectively). As can be seen in the configuration plot, the wines were positioned in three groups: the wines fermented with *Metschnikowia* strains were positioned in the positive side of dimension 1 and the wines with single *S. cerevisiae* fermentation were in the negative side of dimension 1, whereas the *H. uvarum/S. cerevisiae* wine was located in the negative side of dimension 2. The *Metschnikowia* wines and the single *S. cerevisiae* wines contributed to differences in a higher level in dimension 1, while *H. uvarum* contributed more in dimension 2. It is worth noting that the two blind repeated samples (*S. cerevisiae* wine) were close to each other, representing a good level of accuracy by the sensory panel. The single *S. cerevisiae* wines were mainly described with *lemon/lime*, *grass*, *green apple* and *whisky/sherry* attributes. In contrary, the wines fermented with the *Metschnikowia* strains were characterised by some *fruity* and *floral* flavour notes, such as *apple*, *tropical fruit*, *elderflower* and *banana*. This could be a result of increased content of acetate esters and ethyl esters of short-chain fatty acids in these wines. Besides, *H. uvarum* wine was described with *almond*, *chemical*, *mouldy* and *acetone* attributes. The *acetone* note appeared to be correlated with an excessive level of ethyl acetate.

## Conclusions

This study evaluated sequential yeast inoculation in fermentations of Solaris white wines. The non-*Saccharomyces*, *H. uvarum* produced a larger amount of glycerol, heptyl acetate and 2-phenylethyl acetate when sequentially inoculated with *S. cerevisiae* yeast, while also producing higher levels of acetic acid and ethyl acetate. This wine was described with *chemical*, *mouldy* and *acetone* flavour attributes. The three *Metschnikowia* strains, a *M. chrysoperlae* and two *M. fructicola*, had rather similar production of volatile compounds, such as higher levels of 2-methylpropyl acetate and (*Z*)-3-hexenyl acetate compared to the wine with single inoculation of *S. cerevisiae*. These three wines were characterised by *floral* and *fruity* attributes, especially the wine with *M. chrysoperlae* was closely associated with *fruity, tropical fruit* and *elderflower* attributes. The *Metschnikowia* strains turned out to be possible candidates for producing Solaris wines with some more pleasant flavour notes. However, it is worth mentioning that further studies are needed to optimise the use of *Metschnikowia* species for a larger-scale wine production.

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**Conflict of interest** The authors declare no conflict of interest.

**Compliance with ethics requirements** This article does not contain any studies with human or animal subjects.

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