Yeast ratio is a critical factor for sequential fermentation of papaya wine by Williopsis saturnus and Saccharomyces cerevisiae

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Summary

The growth kinetics and fermentation performance of Williopsis saturnus and Saccharomyces cerevisiae at ratios of 10:1, 1:1 and 1:10 (W:S) were studied in papaya juice with initial 7-day fermentation by W. saturnus, followed by S. cerevisiae. The growth kinetics of W. saturnus were similar at all ratios, but its maximum cell count decreased as the proportion of S. cerevisiae was increased. Conversely, there was an early death of S. cerevisiae at the ratio of 10:1. Williopsis saturnus was the dominant yeast at 10:1 ratio that produced papaya wine with elevated concentrations of acetate esters. On the other hand, 1:1 and 1:10 ratios allowed the coexistence of both yeasts which enabled the flavour-enhancing potential of W. saturnus as well as the ethyl ester and alcohol-producing abilities of S. cerevisiae. In particular, 1:1 and 1:10 ratios resulted in production of more ethyl esters, alcohols and 2-phenylethyl acetate. However, the persistence of both yeasts at 1:1 and 1:10 ratios led to formation of high levels of acetic acid. The findings suggest that yeast ratio is a critical factor for sequential fermentation of papaya wine by W. saturnus and S. cerevisiae as a strategy to modulate papaya wine flavour.

Introduction

In the recent years, increasing non-Saccharomyces yeasts have been recognized for their significant contributions and desirable effects on the sensory characteristics of the wine through the quantitative and qualitative diversity of the products and by-products of fermentation (Ciani and Maccarelli, 1998). However, these non-Saccharomyces yeasts are not vigorous or competitive fermenting microorganisms under oenological conditions; thus, they may be only employed as adjunct cultures in conjunction with strongly fermentative Saccharomyces cerevisiae strains for the completion of fermentation. Indeed, the use of mixed starters in winemaking enhanced the complexity of wine flavours and had advantages over the spontaneous and pure S. cerevisiae fermentations (Ciani et al., 2006; Rodríguez et al., 2010). Nevertheless, the impacts on wine aroma and quality by the multi-starter cultures are determined by the strains used and the inoculation strategy (Toro and Vazquez, 2002; Ciani et al., 2006).

Based on our knowledge, the majority of these studies was focused on the mixed-culture fermentations of selected non-Saccharomyces yeasts such as Candida, Torulaspora, Kloeckera and Hanseniaspora with S. cerevisiae (Ciani et al., 2006; 2010; Rodríguez et al., 2010). There are still numerous other non-Saccharomyces yeasts, which are potentially suited for making good quality wine, but remain less unknown because of a lack of research and development. The genus Williopsis (formerly Hansenula) used in this study was reported as being an important producer of esters, especially Williopsis saturnus strains that synthesized significant amounts of volatile branched-chain acetate esters (e.g. isoamyl acetate and isobutyl acetate) (Vandamme, 2003).

To date, only a few studies have evaluated the likelihood of W. saturnus in simultaneous fermentation with S. cerevisiae and indicated the improvement of aroma and characteristics of papaya and longan wine (Lee et al., 2010; Trinh et al., 2011). As for sequential fermentations, limited studies have been conducted. Interestingly, Clemente-Jimenez and colleagues (2005) and Rodríguez and colleagues (2010) emphasized that sequential fermentation is the most adequate strategy of strain combination, where the kinetic behaviour resembles a successful spontaneous fermentation and produces wine with differential aromatic quality, relative to simultaneous fermentation. Furthermore, several studies reported the limited contribution of non-Saccharomyces yeasts belonging to the genera Hanseniaspora, Kluyveromyces, Torulaspora and Williopsis in simultaneous mixed-culture fermentations due to their early growth arrest (Ciani et al., 2006; Moreira et al., 2008; Lee et al., 2010), whereas...
sequential fermentation allowed the persistence of non-
Saccharomyces yeasts with low fermentative power that
would extend or maximize their contact with the juice
matrix (Clemente-Jimenez et al., 2005; Ciani et al., 2006).
For these reasons, sequential fermentations of W. sat-
umus and S. cerevisiae were performed in our previous
study (Lee et al., 2012). However, the papaya wines pro-
duced did not acquire fermentation characteristics from
both yeasts due to the early growth arrest and low inocu-
lum level of S. cerevisiae (Lee et al., 2012). Hence, in the
present study, we studied sequential fermentation in
papaya wine by the utilization of different culture ratios of
W. saturnus and S. cerevisiae, especially ratios with
higher cell counts of S. cerevisiae than those used in the
previous study (Lee et al., 2012). We reported on the
fermentation behaviour and the metabolic interactions of
W. saturnus and S. cerevisiae in these sequential cultures
with respect to the production of ethanol and other volatile
compounds that would contribute to the organoleptic
characteristics of papaya wine.

Results and discussion

Evolution of biomass and enological properties

The evolution of W. saturnus and S. cerevisiae is shown
in Fig. 1. In all the yeast ratios, W. saturnus multiplied
incessantly, reaching the late log phase at day 7 and
remained stationary as fermentation progressed to com-
pletion until day 17 (Fig. 1). Although the growth kinetics
of W. saturnus was similar at different ratios, its maximum
cell count decreased slightly as the inoculated proportion
of S. cerevisiae was increased. On the other hand, S. cer-
evisiae decreased markedly upon inoculation at day 7 and
then remained relatively stable in the 10:1 ratio, while the
same yeast stayed almost constant throughout fermenta-
tion in the 1:1 and 1:10 ratios. As a consequence, high
viable cell densities of both yeasts coexisted and there
was no early death of W. saturnus.

These results differed from those of our previous study
(Lee et al., 2012) in which there was no succession of
yeasts in the sequential fermentation with the inoculation
of S. cerevisiae into the papaya juice partially fermented
by W. saturnus, and the fermentation was dominated by
W. saturnus. This was likely due to the higher ratio of
W. saturnus to S. cerevisiae (1000:1) used in the previous
study. Conversely, Toro and Vazquez (2002) revealed a
sharp decrease of Candida cantarellii upon the inocula-
tion of S. cerevisiae in sequential fermentation. The rapid
reduction of S. cerevisiae in the 10:1 ratio of W : S. could
be due to the killer-toxins (also known as mycocins) pro-
duced by W. saturnus, which are antagonistic against
Saccharomyces yeasts such as S. cerevisiae VL1 and
S. bayanus CVC-NF74 in yoghurt and cheese systems
(Liu and Tsao, 2009; 2010). Williopsis saturnus also
exhibits retardation and inhibition against other yeasts
such as Candida kefir and Kluyveromyces marxianus
(Liu and Tsao, 2009; 2010). Takasuka and colleagues
(1995) and Guyard and colleagues (2002) reported that
the Williopsis mycocins inhibit the growth of yeasts by
interfering with β-1,3 glucan synthesis, which disturbs the
synthesis of yeast cell walls and thus, resulting in cell lysis
and death. On the other hand, the persistence of both
yeasts in the 1:1 and 1:10 ratios could be due to the high
initial cell counts of S. cerevisiae that were able to over-
come the inhibitory effects caused by the mycocins of
W. saturnus. This hypothesis is supported by the findings

Fig. 1. Evolution of viable yeasts in papaya
wine sequential fermentation inoculated
with different ratios of W. saturnus var.
var. mrakii NCYC2251 and S. cerevisiae var.
bayanus R2. NCYC2251 (◊):R2 (♦) = 10:1;
NCYC2251 (△):R2 (▲) = 1:1; NCYC2251
(□):R2 (●) = 1:10. The data are presented
as the means ± standard deviation (n = 3).
Table 1. Physicochemical parameters of papaya wine (day 17) fermented with sequential cultures of W. saturnus and S. cerevisiae at different ratios (W. saturnus: S. cerevisiae).

| Day 0 | Day 10:1 | Day 1:1 | Day 1:10 |
|-------|----------|---------|----------|
| pH    | 3.53 ± 0.03<sup>a</sup> | 3.54 ± 0.01<sup>a</sup> | 3.53 ± 0.03<sup>a</sup> | 3.56 ± 0.01<sup>a</sup> |
| °Brix | 11.00 ± 0.07<sup>b</sup> | 6.60 ± 1.00<sup>b</sup> | 3.71 ± 0.10<sup>c</sup> | 3.65 ± 0.17<sup>c</sup> |
| Ethanol (ml l<sup>−1</sup>) | 0.06 ± 0.00<sup>d</sup> | 13.84 ± 0.84<sup>d</sup> | 38.31 ± 2.02<sup>d</sup> | 39.71 ± 1.97<sup>d</sup> |
| Sugars (g l<sup>−1</sup>) | | | | |
| Fructose | 41.62 ± 1.98<sup>e</sup> | 22.63 ± 5.97<sup>e</sup> | ND | ND |
| Glucose | 46.07 ± 2.14<sup>e</sup> | 11.91 ± 7.26<sup>e</sup> | ND | ND |
| Organic acids (g l<sup>−1</sup>) | | | | |
| Acetic acid | ND | 0.45 ± 0.05<sup>e</sup> | 0.67 ± 0.02<sup>e</sup> | 0.83 ± 0.04<sup>e</sup> |
| Citric acid | 4.51 ± 0.20<sup>e</sup> | 2.90 ± 0.13<sup>e</sup> | 3.42 ± 0.22<sup>e</sup> | 3.39 ± 0.15<sup>e</sup> |
| Malic acid | 5.50 ± 0.34<sup>e</sup> | 4.11 ± 0.18<sup>e</sup> | 4.30 ± 0.11<sup>e</sup> | 3.71 ± 0.25<sup>e</sup> |
| Oxalic acid | 0.04 ± 0.00<sup>e</sup> | 0.07 ± 0.01<sup>e</sup> | 0.05 ± 0.00<sup>e</sup> | 0.07 ± 0.01<sup>e</sup> |
| Pyruvic acid | 0.86 ± 0.10<sup>e</sup> | 0.88 ± 0.01<sup>e</sup> | 0.97 ± 0.01<sup>e</sup> | 0.89 ± 0.07<sup>e</sup> |
| Succinic acid | 3.17 ± 0.19<sup>e</sup> | 4.09 ± 0.05<sup>e</sup> | 2.78 ± 0.08<sup>e</sup> | 3.68 ± 0.22<sup>e</sup> |
| Tartaric acid | 0.90 ± 0.05<sup>e</sup> | 0.77 ± 0.01<sup>e</sup> | 0.34 ± 0.01<sup>e</sup> | 0.39 ± 0.04<sup>e</sup> |

<sup>a,b,c,d</sup> Statistical analysis at 95% confidence level with same letters in the same row indicating no significant difference.

ND, not detected.

in Liu and Tsao (2010), which showed that the inhibitory effect of W. saturnus is regulated by the initial cell count of the target yeast and is effective especially at lower levels of the target yeast.

Total soluble solids (°Brix), sugar consumption, organic acids, ethanol and pH changes are presented in Table 1. Generally, the papaya wine produced by the sequential fermentation of 1:1 ratio had most of the physicochemical properties similar to that produced by the 1:10 ratio, except for acetic, malic, oxalic and succinic acids (Table 1). Among the fermentations, the 1:10 ratio produced papaya wine with the highest ethanol content of 39.71 ml l<sup>−1</sup> (Table 1). This was in agreement with the kinetic trends observed in Lee and colleagues (2010). Volatiles that were initially present, especially fatty acids, sulfur compound and esters (e.g. butyric acid, benzyl isothiocyanate and methyl butyrate) responsible for the typical papaya flavour (Pino et al., 2003), were metabolized to trace levels (Table 2).

Among the volatiles, ethanol and higher alcohols were the major compounds produced (Tables 1 and 2). The kinetic changes of these alcohols were similar in all the fermentations, where the alcohols increased gradually during the early stage of fermentation by W. saturnus and increased rapidly upon the inoculation of S. cerevisiae, then either remained stable or declined slightly (Fig. 2). 2-Ethylhexanol indigenous to the juice was utilized by the yeasts (data not shown). The sequential fermentation of 10:1 ratio consistently produced the lowest amounts of alcohols, whereas the 1:1 and 1:10 ratios produced comparable amounts of ethanol and higher alcohols except for isobutyl and 2-phenylethyl alcohols (Tables 1 and 2). The 1:10 ratio produced significantly higher concentrations of these alcohols than the 1:1 ratio (Table 2). This could be ascribed to the greater inoculum size and viable yeast count of S. cerevisiae (Fig. 1), and its higher metabolic ability to produce higher alcohols (Lee et al., 2010). Among the higher alcohols, 2-phenylethyl alcohol exceeded its corresponding odour threshold value of 10 mg l<sup>−1</sup> (Table 2), especially for the 1:10 ratio with 64.47 mg l<sup>−1</sup> 2-phenylethyl alcohol, which is expected to impart more floral and rose-like notes.

Higher alcohols are important precursors for the formation of fruity esters. The ratio of the contents of higher alcohols to esters is known to influence the sensory properties of fermented beverages. Particularly, wines with

Evolution of volatiles and aroma qualities of papaya wines

Numerous volatiles (e.g. alcohols, aldehydes, esters, fatty acids, monoterpenes, ketones and volatile phenols) contributing to the sensory properties of papaya wine were produced and further transformed by the different ratios of W. saturnus and S. cerevisiae. Selected volatiles in the final papaya wines were analysed (Table 2). Some of these volatiles increased continuously, while others increased initially and then remained unchanged or declined gradually, being similar to the kinetic trends observed in Lee and colleagues (2010). Volatiles that were initially present, especially fatty acids, sulfur compound and esters (e.g. butyric acid, benzyl isothiocyanate and methyl butyrate) responsible for the typical papaya flavour (Pino et al., 2003), were metabolized to trace levels (Table 2).
increased contents of esters possess an enhanced fruity flavour that could be improved if the higher alcohol contents were to decrease (Moyano et al., 1994). A new sulfur-containing alcohol, 2-(methylthio)ethanol, was produced in all fermentations especially at 1:1 and 1:10 ratios (Fig. 2), which is reported for the first time in papaya wine and could be derived from L-methionine catabolism by the yeasts. This volatile sulfur compound has been commonly detected in other wines such as white wines, Tinta Negra Mole red wine and Italian sparkling wines (Perestrelo et al., 2006; Fedrizzi et al., 2010). The heavy sulfur compound cannot be eliminated and may impart French bean and cauliflower-like aroma to wine near its flavour threshold of 250 μg l⁻¹ (Darriet et al., 1999). However, Perestrelo and colleagues (2006) reported that most of the sulfur compounds identified in wines are usually found at levels below their threshold values. It is not known whether all the yeast ratios used in this study would result in any flavour impact due to 2-(methylthio)ethanol in the papaya wine.

Volatile fatty acids are another important group of volatiles produced by the yeasts (Table 2). The kinetic changes of the volatile fatty acids were similar in all the fermentations with trends comparable to the alcohols in Fig. 2, except for butyric acid that was metabolized (Table 2). The sequential fermentation of 1:1 ratio produced the highest amount of most fatty acids, except for acetic, isobutyric, hexanoic and benzoic acids (Table 2). The 1:10 ratio would have been expected to produce the most C8, C10 and C12 fatty acids, given that S. cerevisiae is known to be the main producer of these acids. These results indicate some kind of interaction between W. saturnus and S. cerevisiae at 1:1 ratio that favoured production of these fatty acids and this interaction merits further research. The sequential fermentation of 1:10 ratio produced the highest amount of acetic acid (991.64 mg l⁻¹), followed by the 1:1 and 1:10 ratios with 872.17 mg l⁻¹ and 494.15 mg l⁻¹ acetic acid, respectively (Table 2), which were in line with the acetic acid results obtained by HPLC (Table 1). This could be in part due to the hydrolysis by S. cerevisiae of some acetate esters such as ethyl acetate produced by W. saturnus. The high level of acetic acid produced in all fermentations (Table 2), especially those in the 1:1 and 1:10 ratios
may be expected to exert some adverse effects (e.g. acidic, vinegar and pungent flavours) on the aromatic quality of the papaya wine, but this was not confirmed in sensory evaluation presented below. The results of our study differed from those of Kapsopoulou and colleagues (2007), who highlighted that sequential fermentation reduced the acetic acid content of wine. This discrepancy could be attributed to the domination of *S. cerevisiae* in their sequential fermentation and different non-*Saccharomyces* yeast (*Kluyveromyces thermotolerans*) used in the latter study.

Esters constitute the other major fermentation-derived volatiles that include acetate esters, ethyl esters and other medium to long-chain esters (Table 2). The kinetic changes of esters varied with the ester type. Most of the ethyl esters increased slowly at the initial stage of fermentation by *W. saturnus*, followed by substantial increases upon the inoculation of *S. cerevisiae* and then either remained stable or experienced a steady or sharp decline (Fig. 3). Acetate esters, on the other hand, increased substantially during the initial stage of fermentation and decreased sharply upon the inoculation of *S. cerevisiae*, except for ethyl acetate and 2-phenylethyl acetate in the 10:1 and 1:1 ratios (Fig. 3). The evolution or net accumulation of esters in wine is the result of the balance between yeast ester-synthesizing enzymes and esterase enzymes promoting their hydrolysis in the respective yeasts (Lilly et al., 2006). The results of the present study differed from the findings in our previous study (Lee et al., 2012); in the latter study, there was no significant modification of esters with the inoculation of *S. cerevisiae* into the papaya wine partially fermented by *W. saturnus*. It is reported that the volatiles produced by one of the yeasts can be metabolized by the other (Ciani et al., 2010) and redox interactions existed between yeasts (Cheraiti et al., 2005).

The sequential fermentation of 1:1 ratio produced the highest amount of ethyl esters and other miscellaneous esters, except for ethyl hexanoate, ethyl octanoate and acetate esters (Table 2). This correlated with the higher volatile fatty acid production in the 1:1 ratio (Table 2), which are essential precursors for ethyl ester formation (Saerens et al., 2008). The sequential fermentation of 10:1 ratio, on the other hand, produced the highest concentrations of most acetate esters, whereas the 1:10 ratio had the highest amount of 2-phenylethyl acetate, ethyl hexanoate and ethyl octanoate (Table 2). The high viable yeast population of *W. saturnus* against *S. cerevisiae* in the 10:1 ratio accounted for the higher acetate ester production, as *W. saturnus* is a good producer of acetate esters (Park et al., 2009; Trinh et al., 2011). This is in agreement with the lower levels of higher alcohols in the 10:1 ratio (Table 2), which served as precursors, together with acetyl-CoA, for acetate esters (e.g. isoamyl acetate) synthesis by the action of alcohol acetyltransferase (Park et al., 2009). *Saccharomyces cerevisiae*, the principal wine yeast, is a known potent producer of ethyl esters that contribute pleasant fruity and floral odours to wine aroma. Surprisingly, the 1:10 ratio with the highest *S. cerevisiae* did not produce the uppermost amount of most ethyl esters (Table 2). This could be due to the coexistence of both yeasts in the 1:10 ratio (Fig. 1), which may modulate the ester formation capability of *S. cerevisiae*.

![Fig. 2. Changes of higher alcohols and 2-(methylthio)ethanol during papaya wine sequential fermentation inoculated with different ratios of *W. saturnus* and *S. cerevisiae*. 10:1 ratio (♦); 1:1 ratio (▲); 1:10 ratio (■).](image-url)
gestion is supported by the findings in Cheraiti and colleagues (2005) in that one species or strain in mixed-culture fermentation may impact on the metabolic behaviour of another strain.

Ethyl hexanoate and ethyl octanoate were reported as the odour-active compounds in papaya wine (Pino and Queris, 2011). The concentrations of these ethyl esters in the 1:1 and 1:10 ratios were higher than their threshold values, suggesting that they can contribute pleasant fruity, floral and honey-like flavours to the final wine bouquet (Luebke, 1980). Other ethyl esters (ethyl decanoate and ethyl dodecanoate) produced by both the 1:1 and the 1:10 ratios were also higher than the threshold values. Similarly, these ethyl esters can add pleasant and fruity notes to the papaya wine, but may impart rancid and soapy flavours to the wine bouquet when their concentration was too high (Li et al., 2012). On the other hand, the concentrations of acetate esters in all the fermentations could contribute to the floral (rose) and fruity (banana) notes (Luebke, 1980), especially for the 10:1 and 1:10 ratios with the highest amount of isoamyl acetate and 2-phenylethyl acetate respectively (Table 2). However, the high concentration of ethyl acetate produced by all the ratios was considered detrimental to the wine quality, as ethyl acetate at high levels (200 mg l\(^{-1}\)) exerts a solvent-like aroma (Etievant, 1991).

Principal component analysis (PCA) was applied to the ethanol (Table 1) and volatile compounds (Table 2) to discriminate the common characteristics as well as to reveal the diversity in the volatile composition among the papaya wines. The PCA result indicates distinctive volatile compositions and clear separation among the papaya wines (Fig. 4). The papaya wine produced by the sequential fermentation at 10:1 ratio was mainly characterized by ethyl acetate and those volatiles associated with papaya juice (e.g. butyric acid and benzaldehyde). Conversely, the sequential fermentation at 1:1 ratio was associated with more medium-chain fatty acids and ethyl esters such as ethyl decanoate, ethyl dodecanoate and ethyl tetradecanoate. The papaya wine produced by sequential fermentation at 1:10 ratio was distinguished with a high percentage of acetic acid, ethyl hexanoate, ethyl octanoate, isobutyl octanoate, ethanol and higher alcohols.

**Sensory analysis**

The papaya wine produced by the 10:1 ratio had most of the sensory attributes similar to the other ratios, but there are substantial differences among the ratios that resulted in the differentiation of aroma profiles (Fig. 5). Wine fermented by the 1:10 ratio had more noticeable yeasty, sweet and fusel notes than the 10:1 ratio (Fig. 5), which was probably due to the high levels of 2-phenylethyl acetate, ethyl esters and higher alcohols (Table 2). On the other hand, the wine produced by the 1:1 ratio possessed less buttery and cocoa notes regardless of the significant amount of 3-hydroxy-2-butanone detected (data not shown). There were no significant differences in the aroma profiles in all the papaya wines regardless of the different ratios, which differed from those found for the volatile compounds determined by GC-MS/FID (Tables 1 and 2) and PCA result (Fig. 4). This might be attributed to the complex nature of the papaya wine matrix where the non-volatile compounds such as phenolic compounds,
organic acids and carbohydrates, or other volatile compounds that significantly impact on aroma volatility and perception (Guth and Fritzler, 2004).

In conclusion, the ratio of \textit{W. saturnus} NCYC2251 to \textit{S. cerevisiae} R2 was crucial for the survival of yeasts which had significant impacts on the production of volatile compounds such as alcohols, fatty acids and esters. Among the yeast ratios, the 1:1 and 1:10 ratios (\textit{W.}: \textit{S.}) enabled the coexistence of both yeasts and enhanced the production of desirable volatile compounds through synergistic effects. The use of sequential fermentation with \textit{W. saturnus} and \textit{S. cerevisiae} at a sufficiently higher ratio of the latter provides a feasible strategy to alter the papaya wine volatile profile.

**Experimental procedures**

**Preparation of yeast cultures and papaya juice**

\textit{Williopsis saturnus} var. \textit{markii} NCYC2251 and \textit{S. cerevisiae} var. \textit{bayanus} Lavin R2 were obtained from National Collection of Yeast Cultures (Norwich, UK) and Lallemand (Brooklyn Park, Australia) respectively. \textit{Williopsis saturnus} was propagated and maintained according to the procedure...
described in Lee and colleagues (2010), while \textit{S. cerevisiae} (freeze-dried form) was stored at \(-80^\circ\text{C}\) before use. Papayas of the Sekaki cultivar were washed, juiced and centrifuged at \(32 \times 140\) g for 15 min at \(4^\circ\text{C}\). The initial sugar concentration of papaya juice was \(11.11^\circ\text{Brix}\) (containing 41.62 g of fructose and 46.07 g of glucose per litre of juice) and the pH was 4.98. The juice was brought to pH 3.5 by the addition of 1 M DL-malic acid and sanitized by adding 100 mg l\(^{-1}\) potassium metabisulphite (K\(_2\)S\(_2\)O\(_5\)); moreover, sterility check was performed by plate counting.

**Fermentation conditions**

Triplicate sequential fermentations were carried out with aliquots of 280 ml\(^{-1}\) sanitized papaya juices at \(20^\circ\text{C}\) by inoculation with \(-10^5\) cfu ml\(^{-1}\) \textit{W. saturnus} (pre-culture grown in the same medium at \(25^\circ\text{C}\) for 96 h) for 7 days. After 7 days (late log phase of \textit{W. saturnus} with a viable cell count of \(-10^7\) cfu ml\(^{-1}\)), fermentations were inoculated with \(-10^6\) cfu ml\(^{-1}\), \(-10^7\) cfu ml\(^{-1}\) and \(-10^8\) cfu ml\(^{-1}\) of \textit{S. cerevisiae} to obtain ratios of 10:1, 1:1 and 1:10 (\textit{W. saturnus}\textit{S. cerevisiae}) respectively. Before inoculation, \textit{S. cerevisiae} (freeze-dried form) was reconstituted in sterile nutrient broth (Lee et al., 2012) and concentrated by centrifugation to obtain an initial density of \(1.17 \times 10^{10}\) cfu ml\(^{-1}\). All fermentations were maintained up to 17 days under static conditions.

**Yeast enumeration and analytical determinations**

Enumeration of wine yeasts was performed by plating on potato dextrose agar (PDA) (39 g l\(^{-1}\), Oxoid, Basingstoke, Hampshire, England) that allowed \textit{W. saturnus} to be morphologically distinguished from \textit{S. cerevisiae} colonies. Plates were incubated at \(25^\circ\text{C}\) for 2 days before counting. The total soluble solids (\(^\circ\text{Brix}\) and pH values were measured using a refractometer (ATAGO, Japan) and pH meter (Metrohm, Switzerland) respectively. Sugars determinations were carried out on a Zorbax carbohydrate column (Agilent, Santa Clara, CA, USA) using a mixture of acetic and water (80:20 v/v) as the mobile phase at a flow rate of 1.4 ml min\(^{-1}\), and connected to a low temperature evaporative light scattering detector (ELSD-LT). Organic acids were analysed by a Supelcogel C-610 H column (300 \(	imes\) 7.8 mm, Supelco, Bellefonte, PA, USA) using 1 ml l\(^{-1}\) sulfuric acid as the mobile phase at a flow rate of 1.4 ml min\(^{-1}\) and \(-10^3\) cfu ml\(^{-1}\). All fermentations were maintained up to 17 days under static conditions.

**Sensory evaluation**

The sensorial evaluation of papaya wines was done by a panel of eight well-trained flavourists (three females and five males) from Firmenich Asia (Singapore) who are experienced in wine tasting and in aroma evaluation. There were eight sensory descriptors for the papaya wine aroma: acidic, alcoholic, buttery, cocoa, fruity, fusel, sweet and yeasty. The wine samples were coded and presented randomly to the panel, and the aroma intensity on each sensory descriptor was rated on a hedonic scale from 0 (uncharacteristic) to 5 (very strong).

**Statistical analysis**

Analysis of variance (ANOVA) using SPSS 17.0 software for Windows (SPSS, Chicago, IL) was applied to the experimental data to determine significant differences between the samples. The statistical level of significance was set at \(P < 0.05\). PCA was performed using the software Matlab R2008a (Mathworks, Natick, MA, USA).

**Conflict of interest**

None declared.

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