Ann Microbiol (2015) 65:921–928
DOI 10.1007/s13213-014-0935-7

ORIGINAL ARTICLE

Cider fermentation with three *Williopsis saturnus* yeast strains and volatile changes

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Received: 7 April 2014 / Accepted: 16 June 2014 / Published online: 5 July 2014

Abstract *Williopsis saturnus* var. *subsufficiens* NCYC 2728, *W. saturnus* var. *saturnus* NCYC 22 and *W. saturnus* var. *mrakii* NCYC 500 were used to carry out cider fermentation to assess their impact on the volatile composition of cider. The changes of yeast cell population, °Brix and pH were similar among the three yeasts. Strain NCYC 500 grew best, with the highest cell population of 1.14×10^8 CFU ml^{-1}, followed by strains NCYC 2728 and NCYC 22 (8×10^7 CFU ml^{-1} and 3.19×10^7 CFU ml^{-1} respectively). Esters were the most abundant volatiles produced, followed by alcohols. Among the esters, ethyl acetate, 2-phenylethyl acetate, isoamyl acetate, *cis*-3-hexenyl acetate and hexyl acetate were the major volatiles. The major alcohols were ethanol, isoamyl alcohol, 2-phenylethyl alcohol and isobutyl alcohol. The three *Williopsis* yeasts transformed volatile compounds during cider fermentation with significant variations in terms of volatile production and degradation. This study implied that fermentation with *Williopsis* yeasts could result in cider with a more complex yet fruity aroma.

Keywords Cider · Fermentation · *Williopsis* · Volatiles · Flavor · Yeasts

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Introduction

Cider is a fermented alcoholic beverage made from fresh or concentrated apple juice through a process usually in the presence of added yeasts, in which the consumption of sugars by yeasts yields ethanol, carbon dioxide and flavor compounds. The fermentation can also be carried out spontaneously with indigenous yeast species that originate from the surface of the fruit and the surfaces of juice processing equipment. In spontaneous fermentation, the succession of yeast species occurs. Species of *Hanseniaspora*, *Kloeckera*, *Candida*, *Pichia*, and *Metschnikowia* dominate initially, but they gradually die as the ethanol concentration increases, and give way to more ethanol-tolerant *Saccharomyces cerevisiae* and *S. bayanus* to complete alcoholic fermentation (Fleet 2003; Suarez et al. 2007; Ciani and Comitini 2011).

Saccharomyces cerevisiae wine yeast is well known to enable the rapid, efficient and complete conversion of sugars to alcohols to realize complete fermentation (Kunkee and Bisson 1993; Heard 1999; Fleet 2003), whereas non-*Saccharomyces* yeasts bring about the benefits of the distinct aromatic properties in wine from controlled fermentation, and the flavor profiles of the wines may be altered favorably (Dequin et al. 2003). The yeast genus *Williopsis* was defined by Zender in 1925 and accommodates the saturn-shaped ascospore-forming species *Williopsis saturnus* (formerly *Hansenula saturnus*) (Yilmaztekin et al. 2008). Non-*Saccharomyces* species tend to be weakly fermentative, consuming less sugar and producing less ethanol under normal wine making conditions, and thus, are sometimes used as adjunct cultures with *S. cerevisiae* in simultaneous or sequential inoculations; the latter is normally required to accomplish complete ethanol fermentation (Jolly et al. 2003a, Jolly et al. 2003b).

Esters make the greatest contribution to the bouquet of alcoholic beverages (Rapp and Mandery 1986). A mixture

of ethyl caproate and ethyl caprylate (apple-like aroma) is a primary contributor to the characteristic fruity odors of fermentation bouquet, while isoamyl acetate imparts a banana-like aroma and 2-phenylethyl acetate contributes a fruity and flowery flavor (Rapp and Mandery 1986). Non-*Saccharomyces* wine yeasts are known as good producers of fruity acetate esters such as isoamyl acetate and 2-phenylethyl acetate (Rojas et al. 2001; Plata et al. 2003). Yeast species involved and a balance of ester formation by alcohol acetyltransferases and ester hydrolysis by esterases determine the ester concentration (Suomalainen 1971; Inoue et al. 1997).

Strains of *Williopsis saturnus* are known to produce high levels of acetate esters that impart fruity aroma, and have been well studied in various fruit-based alcoholic beverages including grape wine, mango wine, longan wine and papaya wine (Erten and Campbell 2001; Lee et al. 2010; Trinh et al. 2011; Li et al. 2012; Tanguler 2012, 2013). However, these yeast strains have not been investigated in cider fermentation. Therefore, the objective of this study was to assess the volatile changes during cider fermentation inoculated with three *Williopsis* yeasts strains (*W. saturnus* var. *subsufficiens* NCYC2728, *W. saturnus* var. *saturnus* NCYC 22 and *W. saturnus* var. *mrakii* NCYC 500). The information gained would be useful for selection of non-*Saccharomyces* yeasts for modulation of cider flavor.

Material and methods

Yeasts and microbiological growth media

Three *Williopsis* yeast strains, including *W. saturnus* var. *saturnus* NCYC 22, the *W. saturnus* var. *mrakii* NCYC 500 and *W. saturnus* var. *subsufficiens* NCYC 2728, were used in cider fermentation. All these strains were obtained from the National Collection of Yeast Cultures (Norwich, UK). The yeasts were inoculated into the sterile nutrient broth with the composition of 2 % (w/v) glucose, 0.25 % (w/v) yeast extract, 0.25 % (w/v) bacteriological peptone and 0.25 % (w/v) malt extract (pH 5.0). Pure active dried yeasts were incubated statically at 25 °C for up to 48 h and then stored in 1-ml sterile tubes at −80 °C as stock cultures before use.

Cider fermentation

Commercial apple juice (produced from a concentrate by the juice manufacturer) was purchased from a local supermarket in Singapore (Marigold® Apple 100 % juice, Malaysia Dairy Industries Pte Ltd.) with a composition of 12 g of total carbohydrate, 10.4 g of sugar, 0.8 g of dietary fiber and 1.2 mg of sodium per 100 ml, and there was no added sugar, preservative or flavoring according to the label.

Two-hundred and twenty ml of sterile apple juice were fermented in sterile 250-ml conical flasks after inoculation with 2 % (v/v) of 10^7–10^8 CFU ml^{-1} of respective yeast strain. Two independent fermentations for each strain were carried out for 14 days at 20 °C. During fermentation, samples were taken at intervals to measure total soluble solids (°Brix), cell count and pH by using a refractometer (ATAGO, Japan) and a pH meter (Metrolim, Switzerland). Yeast enumeration was conducted at the beginning and at the end of fermentation by spread plating method on potato dextrose agar (PDA).

Volatile analysis

Volatile compounds in apple juice and cider were analyzed by using headspace (HS) solid-phase microextraction (SPME) using a carboxen/polydimethylsiloxane fiber (85 μm) (Supelco, Inc., Bellefonte, PA) coupled with Agilent 7890A gas chromatography (GC)-5975 series mass spectrometry and flame ionization detector (FID) (Agilent Technology, USA). Five ml of each sample (adjusted to pH 2.5 using 1 M HCl) was tightly capped into a 20-ml glass vial and sealed with a Teflon/Silicone septum and was extracted by HS-SPME at 60 °C for 30 min with stirring at 250 rpm. After extraction, the fiber was inserted into the injection port of the GC set at 250 °C for 3 min to desorb the analytes from the fiber. A capillary column (DB-FFAP, Agilent Technology, USA) of 60 m × 0.25 mm I.D., coated with 0.25 μm film thickness of polyethylene glycol modified with nitroterephthalic acid, was used for separation and analysis. Oven temperature was initially programmed at 50 °C for 5 min, then was increased to 230 °C at a rate of 5 °C min^{-1} and held at this temperature for 20 min. Carrier gas was helium at 19.37 psi and a total flow rate of 42.7 ml min^{-1} was used. Mass spectra of unknown compounds were compared with those in the Wiley database (Agilent Technologies Inc.), and identification was checked and confirmed with the linear retention index values. GC-MSD ChemStation software G1701EA was used for data acquisition and analyses were carried out in duplicate. The results shown represent the mean values from two independent fermentations.

Statistical analysis

An analysis of variance (ANOVA) using Microsoft Office Excel (version 2003) was applied to day 14 experimental data (end of the fermentation period) to determine the significant differences in volatile compounds produced by three *Williopsis* yeasts. The results were significantly different if the associated *p* value was below 0.05 at the 95 % confidence level. The mean values and standard deviations were calculated from the data obtained from two independent fermentations. It must be pointed out that standard deviations in this

context referred to system errors (two replicate fermentations and duplicate analyses).

Result and discussion

Growth of yeasts, changes of total soluble solids, and pH during cider fermentation

The viable cell counts of all three yeast cultures reached the maximum cell populations at the end of the fermentation (day 14), where strain NCYC 500 showed the highest growth at 1.14×10^8 CFU ml^{-1}, followed by strain NCYC 2728 at 8×10^7 CFU ml^{-1} and by strain NCYC 22 at 3.19×10^7 CFU ml^{-1} (Table 1). The total soluble solids content decreased from about 12 °Brix to 7.14–8.9 °Brix depending on yeasts. Strain NCYC 500 with the highest cell population had a maximum residual soluble solid content of 8.9 °Brix, followed by strain NCYC 22 with a final °Brix of 7.6 and strain NCYC 2728 with a final °Brix of 7.15. These data indicate that there was no correlation between soluble solids reduction and cell count among different *Williopsis* yeasts, which could be attributed to strain variations in their ability to utilize sugars, as demonstrated elsewhere (Trinh et al. 2011). Overall, sugar consumption by three yeasts was weak, such that the ethanol content would be low (potential alcohol of approximately 1.8 to 3.0 % v/v). The pH increased from its initial value of 3.53 to a final value of 3.58–3.63.

Volatile compounds in apple juice

The volatile compounds in apple juice contained three acids, four alcohols, six aldehydes, nine esters and two ketones (Table 2). Among the volatiles, esters were the most abundant constituents in apple juice. C6 compounds (alcohol, aldehyde and corresponding acetate esters) are character-impact apple aroma compounds. However, not all volatiles detected were naturally present in apple juice. For example, furfural and 5-methyl-2-furfural are known thermal and/or light-induced compounds (Blanco-Gomis et al. 1991), whereas *cis*-3-hexenyl lactate and menthyl acetate have not been reported in apple juice and are considered to be added flavorings.

Table 1 Cell counts of *Williopsis saturnus* before and after cider fermentation

Yeast strain	Cell count[a] (CFU ml^{-1})	Cell count[b] (CFU ml^{-1})	Cell count[c] (CFU ml^{-1})
NCYC 500	2.83×10^7	1.03×10^6	1.14×10^8
NCYC 2728	3.17×10^7	7.60×10^5	8.00×10^7
NCYC 22	3.65×10^7	7.24×10^5	3.19×10^7

NCYC 2728=*W. saturnus* var. *subsufficiens*; NCYC 22=*W. saturnus* var. *saturnus*; NCYC 500=*W. saturnus* var. *mrakii*

[a] Average initial cell count of the pre-culture

[b] Average cell count at the beginning of the fermentation

[c] Average cell count at the end of the fermentation

Volatile compound variations during cider fermentation and in final cider

During cider fermentation (day 0–14), several classes of volatile compounds, such as fatty acids, alcohols, aldehydes, esters and ketones, were transformed (Figs. 1–6) by the three strains of *W. saturnus*. Some volatile compounds in the apple juice including alcohols (hexanol, *cis*-3-hexanol, *trans*-2-hexenol), aldehydes (hexanal, 5-methyl-2-furfural), acetate esters (*cis*-3-hexenyl acetate, hexyl acetate, menthyl acetate), propanoate ester (*cis*-3-hexenyl lactate), ethyl esters (ethyl butanoate, ethyl 2-methylbutanoate) and ketones (beta-damascenone, furyl methyl ketone) were transformed (decreased or disappeared) during cider fermentation. Examples included disappearance of ethyl 2-methylbutanoate and *trans*-2-hexenol (Figs. 3 and 5), as well as depletion of hexanal and initial increases in hexanol and hexyl acetate (Table 2, Figs. 2 and 4). It is likely that esters initially present were hydrolyzed by yeast esterases and aldehydes were reduced to alcohols, followed by the conversion of alcohols to esters.

The changes of fatty acids among three *W. saturnus* strains showed similar trends, exemplified by a continuous increment of acetic and octanoic acids (Fig. 1). Strain NCYC 500 produced the least amount of fatty acids compared to strain NCYC 22 and strain NCYC 2728. There were statistical differences in the final concentrations of fatty acids at day 14 among or between the yeasts (Table 2).

Ethanol, isobutyl alcohol, isoamyl alcohol and 2-phenethyl alcohol were the major alcohols synthesized by the three yeasts, and these alcohols continuously increased during cider fermentation (Figs. 2 and 3). Ethanol is the most abundant product resulting from the fermentation of sugars by fermenting yeasts, but was found to be weakly produced by *Williopsis saturnus* yeasts (Erten and Campbell 2001). In this study, it was also observed that low levels of ethanol were produced, being in agreement with low sugar consumption. The lower production of ethanol would affect the overall smoothing effect on taste characteristics, losing the sensory characteristics of ethanol, i.e., body, sweetness, fullness and mouth-warming effect (Williams 1972).

Hexanol, *cis*-3-hexenol and *trans*-2-hexenol that were indigenous to the apple juice decreased or disappeared during cider fermentation. Interestingly, hexanol and *cis*-3-hexenol were initially produced by strain NCYC 500, possibly from the reduction of aldehydes and/or *trans-cis* isomerization (Figs. 2 and 3), resulting in their higher residual levels. Unlike the results reported by Viana et al. (2008), there were no

Table 2 Major volatile compounds (GC-FID peak area; Mean ×10^6±SD) found in apple juice and cider fermented with three *Williopsis saturnus* strains at day 14

Class	Compound	Apple juice	NCYC 22	NCYC 500	NCYC 2728	Organoleptics
Acid	Acetic acid	0.65±0.02	3.83±0.92^{a}	1.92±0.12^{b}	1.54±0.24^{c}	Acidic, vinegar
	Hexanoic acid	–	0.11±0.04^{ab}	0.07±0.01^{b}	0.10±0.01^{a}	Fatty, cheesy, sour
	Octanoic acid	0.11±0.03	1.62±0.19^{a}	0.49±0.07^{b}	1.77±0.44^{a}	Fatty, cheesy
	Decanoic acid	–	0.71±0.12^{a}	0.17±0.03^{b}	0.55±0.16^{a}	Sour, fatty
	2-Ethylhexanoic acid	0.32±0.08	–	–	–	–
Alcohol	Ethanol	1.36±0.36	971±331^{a}	535±53^{b}	1013±220^{a}	Strong alcoholic
	Propanol	–	2.56±0.57^{a}	1.01±0.03^{b}	0.88±0.1^{c}	Alcoholic, musty
	Isobutyl alcohol	–	1.40±0.37^{a}	2.43±0.10^{b}	4.22±0.64^{c}	Ether, winey
	Isoamyl alcohol	–	10.40±0.47^{a}	10.67±0.56^{a}	10.42±0.82^{a}	Alcoholic, fruity
	Hexanol	1.46±0.04	0.52±0.02^{a}	4.61±0.11^{b}	2.47±0.15^{c}	Green, grassy
	cis-3-Hexenol	4.66±0.13	0.69±0.03^{a}	3.22±0.14^{b}	1.66±0.06^{c}	Green, grassy
	trans-2-Hexenol	4.19±0.08	–	–	–	Green, grassy
	2-Phenylethanol	–	2.48±0.13^{a}	4.37±1.43^{b}	4.27±0.21^{b}	Floral, rose
	Eugenol	–	0.84±0.04^{a}	0.73±0.18^{a}	1.21±0.12^{b}	Sweet, clove, woody
Aldehyde	Benzaldehyde	–	0.39±0.12^{a}	0.36±0.08^{a}	0.30±0.07^{a}	Almond-like odor
	Hexanal	5.00±0.06	–	–	–	Green, beany
	trans-2-Hexenal	92.13±0.78	–	–	–	Green, leafy
	Furfural	29.35±0.80	–	–	–	Bitter almond, sweet
	5-Methyl-2-Furfural	0.16±0.02	0.05±0.01^{a}	0.06±0.002^{b}	0.057±0.018^{ab}	Spice, caramel, maple
	4-Methylbenzaldehyde	0.49±0.02	–	–	–	–
	3,4-Dimethylbenzaldehyde	0.59±0.06	3.73±0.66^{a}	1.00±0.18^{b}	3.10±0.36^{a}	–
Ester	Ethyl acetate	7.47±0.46	119.73±8.19^{a}	53.30±2.21^{b}	70.51±1.51^{c}	Sweet, fruity
	Ethyl butanoate	10.04±0.12	–	–	–	Pineapple, sweet, fruity
	Ethyl 2-methylbutanoate	7.24±0.11	–	–	–	Berry, fruity
	Butyl acetate	–	1.40±0.087^{a}	0.18±0.024^{b}	0.55±0.057^{c}	Fruity, banana
	Isoamyl acetate	–	26.22±1.15^{a}	40.22±3.03^{b}	45.16±2.13^{c}	Sweet, fruity, banana
	Hexyl acetate	65.95±0.31	11.19±1.52^{a}	9.20±1.01^{a}	10.00±0.18^{a}	Fruity, floral
	cis-3-Hexenyl acetate	113.44±0.11	15.63±0.48^{a}	6.47±0.68^{b}	9.93±0.45^{c}	Fruity, berry
	trans-2-Hexenyl acetate	1.43±0.02	–	–	–	Fruity, green
	cis-3-Hexenyl lactate	3.86±0.11	–	–	–	Green, leafy, melon
	Ethyl octanoate	–	1.34±0.27^{a}	0.55±0.018^{b}	3.22±0.63^{c}	Fruity, winey
	Furfuryl acetate	–	2.51±0.16^{a}	0.60±0.24^{b}	1.61±0.059^{c}	Sweet, fruity
	Menthyl acetate	15.12±0.28	2.74±0.39^{a}	3.78±0.52^{b}	3.03±0.56^{ab}	Fruity, minty
	2-Phenylethyl acetate	0.51±0.08	56.03±3.59^{a}	28.31±1.58^{b}	38.86±1.24^{c}	Rose-like, sweet, honey
	Ethyl dodecanoate	–	0.26±0.037^{a}	0.23±0.01^{b}	0.46±0.063^{c}	–
	2-Phenylethyl propanoate	–	0.15±0.009^{a}	0.22±0.009^{b}	0.319±0.035^{c}	Rose-like, fruity, honey
Ketone	2-Furylmethyl ketone	0.28±0.01	0.129±0.032^{a}	0.19±0.011^{b}	0.18±0.017^{b}	–
	beta-Damascenone	0.71±0.04	0.33±0.031^{a}	0.25±0.002^{b}	0.24±0.03^{b}	Rose-like, cooked apple

$^{a, b, c}$ Statistical analysis at 95 % confidence level with same letters indicating no significant difference

significant differences for isoamyl alcohol between the three strains, whereas the amount of 2-phenylethyl alcohol produced by strains NCYC 500 and NCYC 2728 were approximately twofold higher than that of strain NCYC 22 (Table 2). Aromatic eugenol has been found in cider before (Xu et al. 2007; Fan et al. 2011), but its origin in cider is still not clear. Figure 3 shows that eugenol increased consistently during cider fermentation, indicating its biological origin. Eugenol was probably glycosidically bound and was released by glycosidases from the yeasts. *Williopsis* and other non-*Saccharomyces* cider yeasts are reported to contain glycosidase activities (Li et al. 2012; Pando et al. 2012).

The most abundant volatile compounds in cider were esters that could be categorized into acetate esters, ethyl esters and

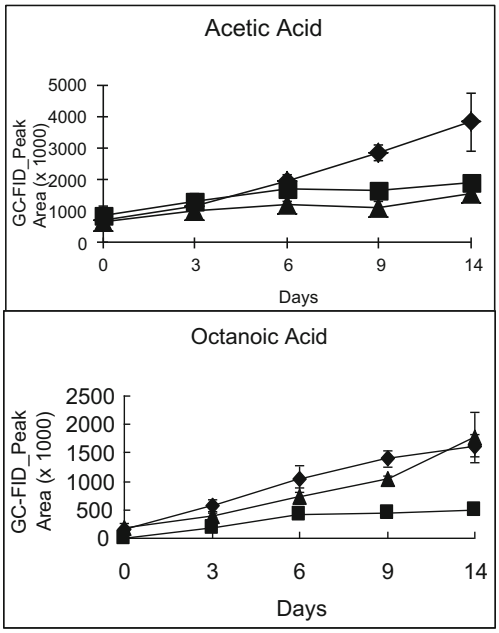


Fig. 1 Changes of acids during cider fermentation by three *Williopsis* yeast strains: *W. saturnus* var. *saturnus* NCY C22 (◆), *W. saturnus* var. *mrakii* NCYC 500 (■), and *W. saturnus* var. *subsufficiens* NCYC 2728 (▲)

propanoate esters (Table 2, Figs. 4–5). All these esters had similar dynamic patterns of formation and degradation during cider fermentation amongst the three strains of *W. saturnus* yeasts. *cis*-3-hexenyl acetate, hexyl acetate, ethyl 2-methylbutanoate, menthyl acetate and *trans*-2-hexenyl acetate initially present in the apple juice were degraded, whereas butyl acetate, 2-phenylethyl acetate and isoamyl acetate were formed. Strains NCYC 22 and 2728 initially produced *cis*-3-hexenyl acetate and hexyl acetate, followed by almost

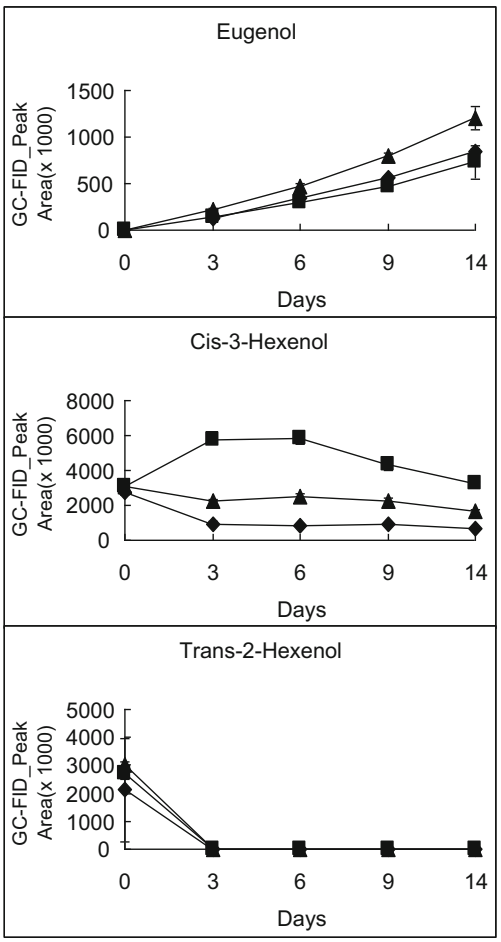
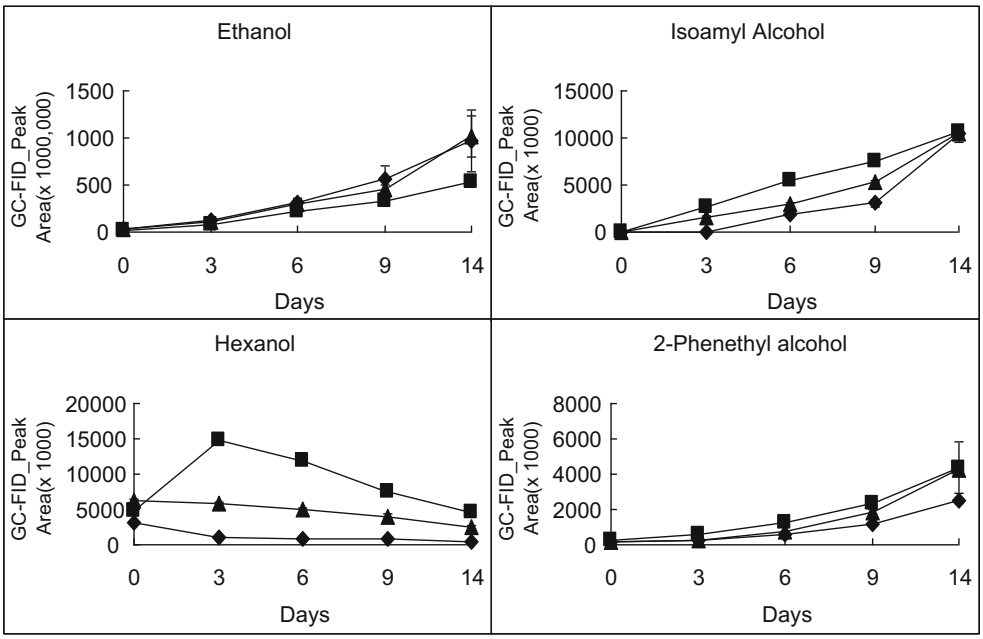


Fig. 3 Changes of other alcohols during cider fermentation by three *Williopsis* yeast strains: *W. saturnus* var. *saturnus* NCYC 22 (◆), *W. saturnus* var. *mrakii* NCYC 500 (■), and *W. saturnus* var. *subsufficiens* NCYC 2728 (▲)

Fig. 2 Changes of alcohols during cider fermentation by three *Williopsis* yeast strains: *W. saturnus* var. *saturnus* NCYC 22 (◆), *W. saturnus* var. *mrakii* NCYC 500 (■), and *W. saturnus* var. *subsufficiens* NCYC 2728 (▲)



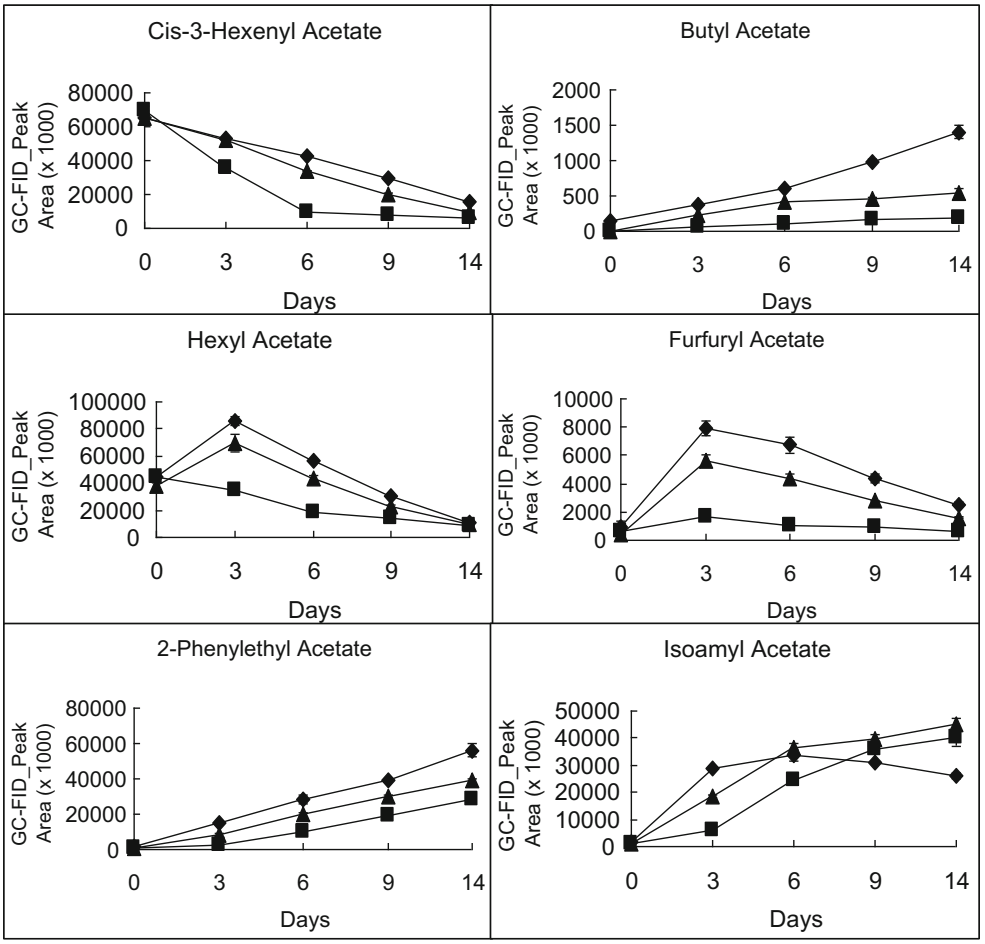


Fig. 4 Changes of acetate esters during cider fermentation by three *Williopsis* yeast strains: *W. saturnus* var. *saturnus* NCYC 22 (◆), *W. saturnus* var. *mrakii* NCYC 500 (■), and *W. saturnus* var. *subsufficiens* NCYC 2728 (▲)

complete degradation. The indigenous volatile compounds that impart the distinct characteristic apple aroma were mostly degraded by *W. saturnus* yeasts and were retained to some content, such as hexyl acetate and *cis*-3-hexenyl acetate (a major ester compound in the final cider).

In line with reports by Rojas et al. (2001, 2003), our findings also showed that acetate esters such as isoamyl acetate and 2-phenylethyl acetate were formed in relatively high concentrations by non-*Saccharomyces* yeasts. Moreover, non-*Saccharomyces* yeasts proved to be good producers of isoamyl acetate and 2-phenylethyl acetate and the genera *Hanseniaspora* and *Pichia* were better producers of acetate esters (Viana et al. 2008). Similar results were obtained in our study (Table 2), where the three strains of *W. saturnus* produced most of the acetate esters such as isoamyl acetate, 2-phenylethyl acetate, *cis*-3-hexenyl acetate, hexyl acetate, furfuryl acetate and butyl acetate. It was possible that furfural (a thermal product in the juice) was reduced to furfuryl alcohol by the three yeast strains, and furfuryl acetate was derived from the alcoholysis reaction between acetyl Co-A and furfuryl alcohol catalyzed by the alcohol acetyl Co-A transferase from the yeasts.

Among the ethyl esters, ethyl butanoate and ethyl 2-methylbutanoate that were naturally present in the apple juice declined continuously during cider fermentation, whereas ethyl acetate and ethyl octanoate were generated by the three strains of *W. saturnus*, but the ethyl esters differed statistically in their final levels between or among the yeasts (Fig. 5). Ethyl acetate was the main ester, produced in the greatest amount (39.9–292.8 mg L^{-1}) in wine that was synthesized by selected non-*Saccharomyces* yeast strains (Viana et al. 2008). Ethyl and acetate esters are the key contributors of fruity aroma to wine (Rapp and Mandery 1986). Non-*Saccharomyces* yeasts are generally low producers of ethyl esters relative to *Saccharomyces cerevisiae* yeasts.

The aldehyde compounds such as hexanal, furfural and 5-methyl-2-furfural in the apple juice (the latter two were likely the products of juice heat treatment) decreased significantly or were completely catabolized throughout the fermentation process, while benzaldehyde increased (Table 2, Fig. 6). In addition, ketones like beta-damascenone and furyl methyl ketone were degraded continuously during cider fermentation

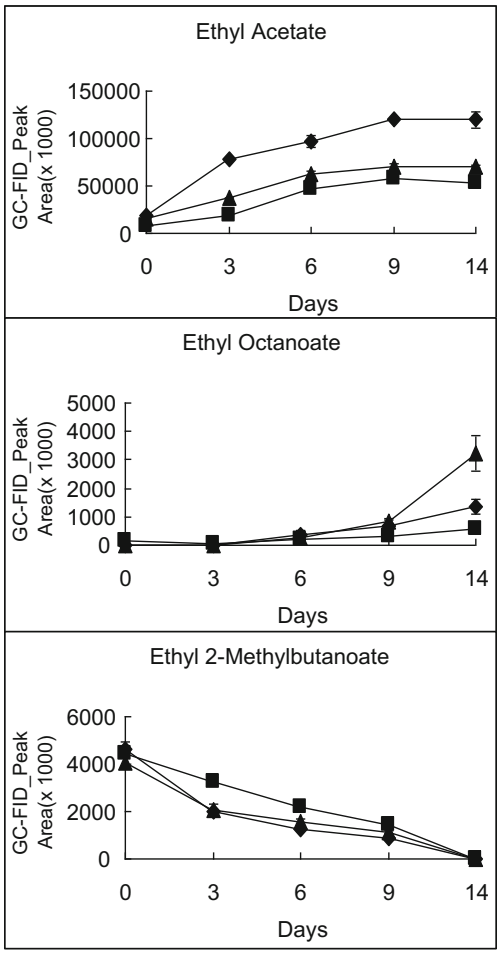


Fig. 5 Changes of ethyl esters during cider fermentation by three *Williopsis* yeast strains: *W. saturnus* var. *saturnus* NCYC 22 (◆), *W. saturnus* var. *mrakii* NCYC 500 (■), and *W. saturnus* var. *subsufficiens* NCYC 2728 (▲)

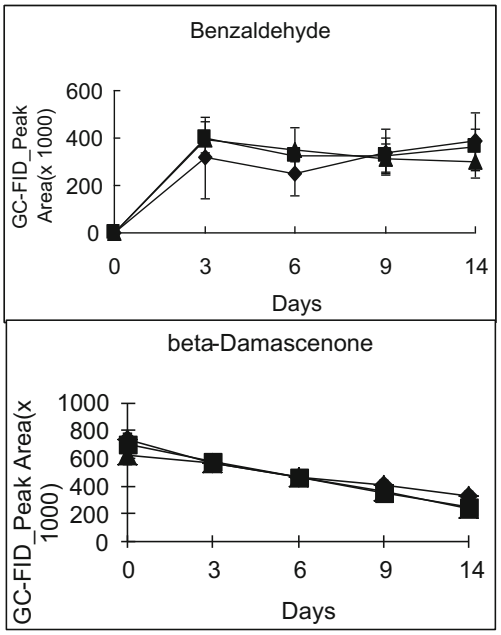


Fig. 6 Changes of carbonyls during cider fermentation by three *Williopsis* yeast strains: *W. saturnus* var. *saturnus* NCYC 22 (◆), *W. saturnus* var. *mrakii* NCYC 500 (■), and *W. saturnus* var. *subsufficiens* NCYC 2728 (▲)

(Fig. 6). Furfural, 5-methyl-2-furfural and furyl methyl ketone reportedly pose potential health risks (Stich et al. 1981) and their decrease after fermentation would be beneficial for consumer health. It is likely that aldehydes and ketones acted as electron acceptors and were reduced to their corresponding alcohols that can be converted into esters such as furfuryl acetate and hexyl acetate (Fig. 4).

The three strains of *W. saturnus* played an essential role in transforming the vast array of volatile compounds in apple juice and cider. The kinetic changes of formation and utilization of the volatile compounds had similar characteristics, yet with some variations, with statistical differences at day 14 between or among the yeasts. Non-*Saccharomyces* wine yeasts are good producers of acetate esters (Rojas et al. 2001; Plata et al. 2003). We also showed that the volatile compounds like ethyl acetate, butyl acetate, hexyl acetate, *cis*-3-hexenyl acetate, furfuryl acetate and 2-phenylethyl acetate, which contributed fruity and sweet-aroma to cider, were formed in the greatest amounts by strain NCYC 22, followed by strains NCYC 2728 and NCYC 500. However, isoamyl acetate, which gave the fruity, sweet and banana-like aroma to cider, was produced in the highest concentrations by strain NCYC 2728 , while strain NCYC 22 produced the least amount of it.

Williopsis saturnus strains are metabolically active and their metabolites are expected to improve the wine quality and flavor via ester-synthesizing activities, although they are weak ethanol producers. Based on their sugar consumption, *W. saturnus* strains can form lower levels of ethanol and higher alcohols, such as propanol, isobutyl alcohol, isoamyl alcohol and active amyl alcohols, than *Saccharomyces cerevisiae* (Romano et al. 1992; Zironi et al. 1993). Non-*Saccharomyces* yeasts are capable of producing wine with a more complex aroma, which may improve wine quality and have a significant and favorable effect on the sensory characteristics (Ciani and Maccarelli 1998; Egli et al. 1998; Jolly et al. 2003a; Romano et al. 1992), but are not able to complete fermentation due to their low ethanol tolerance. Therefore, further research has to be carried out using non-*Saccharomyces* wine yeasts as part of mixed starters together with *Saccharomyces cerevisiae* to complete alcoholic fermentation (Jolly et al. 2003b; Rojas et al. 2003; Romano et al. 1992).

References

Blanco-Gomis D, Gutiérrez-Alvarez MD, Sopena-Naedo L, Mangas-Alonso JJ (1991) High performance liquid chromatographic determination of furfural and hydroxymethylfurfural in apple juices and concentrates. Chromatographia 32:45–48

Ciani M, Comitini F (2011) Non-*Saccharomyces* wine yeasts have a promising role in biotechnological approaches to winemaking. Ann Microbiol 61:25–32

Ciani M, Maccarelli F (1998) Oenological properties of non-*Saccharomyces* wine yeasts associated with wine-making. World J Microbiol Biotechnol 14:199–203

Dequin S, Salmon JM, Nguyen HV, Blondin B (2003) Wine yeasts. In: Boekhout T, Robert V (eds) Yeasts in food: beneficial and detrimental aspects. Berhr's Verlag, Hamburg, pp 389–412

Egli CM, Edinger WD, Mitrakul CM, Henick-Kling T (1998) Dynamics of indigenous and inoculated yeast populations and their effect on the sensory character of Riesling and Chardonnay wines. J Appl Microbiol 85:779–789

Erten H, Campbell I (2001) The production of low-alcohol wines by aerobic yeasts. J Inst Brew 107:207–215

Fan W, Xu Y, Han Y (2011) Quantification of volatile compounds in Chinese cider by stir bar sorptive extraction (SBSE) and gas chromatography–mass spectrometry (GC-MS). J Inst Brew 117:61–66

Fleet GH (2003) Yeasts in fruit and fruit products. In: Boekhout T, Robert V (eds) Yeasts in food. Woodhead Publishing Ltd., Cambridge, UK, pp 267–287

Heard GM (1999) Novel yeasts in winemaking—looking to the future. Food Aust 51:347–352

Inoue Y, Trevanichi S, Fukuda K, Izawa S, Wakai Y, Kimura A (1997) Roles of esterase and alcohol acetyltransferase on production of isoamyl acetate in *Hansenula mrakii*. J Agri Food Chem 45:644–649

Jolly NP, Augustyn OPH, Protorius IS (2003a) The use of *Candida pulcherrima* in combination with *Saccharomyces cerevisiae* for the production of Chenin Blanc wine. S Afr J Enol Viticul 24:63–69

Jolly NP, Augustyn PPH, Protorius IS (2003b) The effect of non-*Saccharomyces* yeasts on fermentation and wine quality. S Afr J Enol Viticul 24:55–62

Kunkee RE, Bisson LF (1993) Wine-making yeasts. In: Rose AH, Harrison JS (eds) The yeasts. Academic, London, pp 69–128

Lee P-R, Ong Y-L, Yu B, Curran P, Liu S-Q (2010) Evolution of volatile compounds in papaya wine fermented with three *Williopsis saturnus* yeasts. Int J Food Sci Technol 45:2032–2041

Li X, Yu B, Curran P, Liu S-Q (2012) Impact of two *Williopsis* yeast strains on the volatile composition of mango wine. Int J Food Sci Technol 47:808–815

Pando R, Lastra A, Suarez B (2012) Screening of enzymatic activities in non-Saccharomyces cider yeasts. J Food Biochem 36:683–689

Plata C, Millan C, Mauricio JC, Ortega JM (2003) Formation of ethyl acetate and isoamyl acetate by various species of wine yeasts. Food Microbiol 20:217–224

Rapp A, Mandery H (1986) Wine aroma. Experientia 42:873–884

Rojas V, Gil JV, Pinaga F, Manzanares P (2001) Studies on acetate ester production by non-*Saccharomyces* wine yeasts. Int J Food Microbiol 70:283–289

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

Romano P, Suzzi G, Comi G, Zironi R (1992) Higher alcohol and acetic acid production by apiculate wine yeasts. J Appl Microbiol 73:126–130

Stich HF, Rosin MP, Wu CH, Powrie WD (1981) Clastogenicity of furans found in food. Cancer Lett 13:89–95

Suarez B, Pando R, Fernandez N, Querol A, Madrera R (2007) Yeast species associated with the spontaneous fermentation of cider. Food Microbiol 24:25–31

Suomalainen H (1971) Yeast and its effect on the flavor of alcoholic beverages. J Inst Brew 77:164–177

Tanguler H (2012) Evaluation of *Williopsis saturnus* inoculum level on fermentation and flavor compounds of white wines made from Emir (*Vitis vinifera* L.) grown in Anatolia. Food Biotechnol 26:351–368

Tanguler H (2013) Influence of temperatures and fermentation behavior of mixed cultures of *Williopsis saturnus* var. *saturnus* and *Saccharomyces cerevisiae* associated with wine making. Food Sci Technol Res 19:781–193

Trinh T-T-T, Yu B, Curran P, Liu S-Q (2011) Dynamics of volatile compounds during longan juice fermentation by three yeasts from the genus *Williopsis*. Acta Alimentaria 40:385–395

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

Williams AA (1972) Flavor effects of ethanol in alcoholic beverages. Flav Ind 3:604–607

Xu Y, Fan W, Qian M (2007) Characterization of aroma compounds in apple cider using solvent-assisted flavor evaporation and headspace solid-phase microextraction. J Agric Food Chem 55:3051–3057

Yilmaztekin M, Erten H, Cabaroglu T (2008) Production of isoamyl acetate from sugar beet molasses by *Williopsis saturnus* var. *saturnus*. J Inst Brew 114:34–38

Zironi R, Romano P, Suzzi G, Battistutta F, Comi G (1993) Volatile metabolites produced in wine by mixed and sequential cultures of *Hansenizspora guilliermondii* or *Kloeckera apiculata* and *Saccharomyces cerevisiae*. Biotechnol Lett 15:235–238