

De-acidification of fresh whole pineapple juice wine by secondary malolactic fermentation with lactic acid bacteria

*Prakitchaiwattana, C., Boonin, K. and Kaewklin, P.

Department of Food Technology, Faculty of Science, Chulalongkorn University, Pathumwan, Bangkok 10330, Thailand

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Abstract

This study aimed to evaluate the de-acidification of fresh whole pineapple juice wine by secondary malolactic fermentation with lactic acid bacteria (LAB). Pineapple juice was primary fermented with a mixed yeast of *Saccharomyces ludwigii* S1 and *Hanseniaspora uvarum* TISTR5153 at 25–30°C for 7 d and then secondary LAB fermented with *Oenococcus oeni* LALVIN 31TM and/or *O. oeni* Enoferm® ALPHA at 25–30 oC for 4 weeks. Optimal secondary fermentation was found in the co-presence of both LAB, which decreased the malic acid content to 5.58 g/L forming lactic acid (4.39 g/L). The secondary ferment still contained 10% (v/v) alcohol but had a higher TTA (10.6 g/L) and pH (3.80). The sensory score of the wine after fermentation with both LAB isolates was increased and this was higher than when fermented with either LAB alone. Thus, secondary fermentation of pineapple wine using *O. oeni* could significantly improve the wine quality.

Keywords

De-acidification

Lactic acid bacteria

Pineapple wine

Starter culture

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Introduction

Fruit wines are produced from temperate and tropical fruits other than grapes (*Vitis vinifera*). Pineapple (*Ananas comosus* L. Merr.) is a significant economic agricultural plant that is widely grown in tropical areas, including within most regions of Thailand. This fruit can also be used to make wine since its juice is easily extracted (35–55% (v/v) juice yield), depending on the pineapple variety (Salvi and Rajput 1995), has a unique flavor, and a sufficient level of fermentable sugars, acids, nitrogen source, vitamins and minerals to support yeast growth and fermentation without the need to add exogenous yeast nutrients (Akamine 1976), similar to that for grape juice. Accordingly, pineapple juice has gained a high appeal in tropical wine making and has been used for the successful production of wines (Ayogu 1999; Chuaychusri *et al.* 2005). Unlike grapes, many tropical fruits, including pineapple, usually have a high acid content (Amerine and Ough 1980). Therefore, adding water to dilute the acidity, and then adjusting the sugar content and enhancing selected minerals to the diluted juice prior to fermentation has become the general practice (Akubor 1996). However, this leads to an inferior less fruity wine flavor. There have been some reports on the development of fermentation techniques with *S. cerevisiae* to produce better quality pineapple wines, and they have generally achieved an alcohol

range of 10.2–13.4% (v/v), but the pineapple wines with lower alcohol contents were found to be more acceptable in terms of their organoleptic properties (Ayogu 1999; Ruengrongpany 1996). Recently, the alternative ethanolic fermentation of pineapple wine from pasteurized 100% pineapple juice as the must with autochthonous *Saccharomyces (S'codes) ludwigii* and *Hanseniaspora uvarum* yeast isolates has been reported by Chanprasartsuk and research group (2010 a, b). In this fermentation of the undiluted (100% (v/v)) pineapple juice, *S'codes ludwigii* plays a major role in the ethanolic fermentation and helps to prolong the viability of *H. uvarum* during the initial fermentation stage, whilst *H. uvarum* enhances the complexity of the volatile compounds in the pineapple wine including through the generation of 2-phenylethyl acetate that provides a rose and flowery aroma. The mixed culture of *S'codes ludwigii* and *H. uvarum* increased the acceptability of the obtained pineapple wine, in terms of its aroma and taste, compared to the wine derived from a mixed culture of commercial *S. cerevisiae* and *H. uvarum*, but the overall liking score of the wine was still low due to its acidic taste (Chanprasartsuk *et al.* 2010a, b). To improve the acidic taste, the de-acidification of the ethanolic fermentation (wine) by subsequent secondary malolactic fermentation with lactic acid bacteria (LAB), which has generally been practiced in grape wine making, is a potential approach. This reaction is widely encouraged by

*Corresponding author.

Email: cheunjit.P@chula.ac.th

Tel: +6622185515-6, Fax: +6622544314

the inoculation of the ethanolic ferment (wine) with commercial LAB strains of *Oenococcus oeni* (Dicks *et al.* 1995). However, there are very few reports about the application and efficiency of malolactic fermentation in fruit wines. In addition, as well as the high acidity of pineapple juice, that is derived mainly from citric and malic acids, it contains a relatively high and stable protease activity that may also have an inhibitory effect upon the yeast and/or LAB during their respective fermentations. Thus, this study aimed to evaluate the performance of two commercial malolactic fermenting LAB strains in the de-acidification of wine made from fresh whole pineapple juice to improve the pineapple wine quality.

Materials and Methods

Microbial strains

The yeasts strains used in this study for the primary (ethanolic) fermentation were *S'codes ludwigii* S1 isolates, isolated from naturally fermented pineapple juice (Chanprasartsuk *et al.* 2010a, b), obtained from the Department Food Technology Faculty of Science, Chulalongkorn University, Thailand. The *H. uvarum* TISTR 5153 strain was obtained from the Thailand Institute of Scientific and Technological Research. Yeasts were maintained on malt extract agar (MEA; Oxoid, England) at 4°C and subcultured until used. The two LAB isolates used for the secondary (malolactic) fermentation of the wine were *O. oeni* isolates LALVIN 31™ and Enoferm® ALPHA (Lallemand MBR®, Australia), and were maintained on de Man, Rogosa and Sharpe agar (MRS agar; Himedia) at 4°C and subcultured until use. All microbial cultures were cloned by restreaking on the agar media and a single colony was used to grow the starter inoculums culture.

Primary (ethanolic) fermentation

Whole pineapple fruits at the harvesting stage were freshly crushed, and the sugar concentration of the fresh juice sugar concentration was increased to 22°brix with sucrose. The juice was filtered and aliquoted at 3.2 L per 5-L sterile glass bottle with potassium metabisulphite (KMS) at a final concentration of 50 mg/L and then sealed with a bubbler airlock and left overnight for decontamination prior to use as the fermentation must. The yeast cultures (*S'codes ludwigii* and *H. uvarum*) were prepared by growth of a single colony in sterile 100% (v/v) pineapple juice (Tipco®, Thailand) in an orbital shaker (200 rpm) at 25–30°C for 20–24 h and then used to inoculate the prepared pineapple juice must at about 10⁶–10⁷ colony forming units (CFU)/

mL. The fermentation was conducted at 25–30°C for 1 week or until the alcohol level reached to 10% (Chanprasartsuk *et al.* 2010b).

Secondary (malolactic) LAB fermentation

The primary ethanolic ferment of pineapple juice was clarified by allowing it to sediment under refrigeration for 1–2 d before being racked at 2 L per 2.5-L sterile glass bottle, adding KMS to a final concentration of 25 mg/L, sealing with a bubbler airlock and leaving overnight for decontamination to yield the wine. For the de-acidification of this wine by secondary LAB fermentation, three different LAB ferments were evaluated; namely single isolates of *O. oeni* strains LALVIN 31™ (O1) or Enoferm® ALPHA (O2) or a mixed culture of equal levels of O1 and O2 (O1/O2). Each LAB starter culture was prepared in sterile 100% (v/v) pineapple juice under an anaerobic condition at 25–30°C for 3–5 d and then used to inoculate the pineapple wine (primary ethanolic ferment) at an initial level of about 10⁶ CFU/mL. Each LAB fermentation was performed in duplicate at 25–30°C for 1 month. The level of viable LAB cells, alcohol, TTA, reducing sugar and pH were investigated every week during the fermentation, whilst the level of organic acids was analyzed on the final day of fermentation.

Yeast and LAB cell densities

Estimation of the yeast and LAB cell density was enumerated as CFU/mL. For yeasts, each respective sample was serially diluted in 0.1% (w/v) peptone water and then for each dilution 0.1 mL was spread onto MEA plate in duplicate and incubated at 25–30°C for 2–4 d. Individual yeast colonies were counted on appropriate plates and the yeast population level evaluated as the CFU/mL. For LAB, the same procedure was followed only the diluted samples were spread onto MRS agar plates and incubated for 4–5 d.

Analysis of the pineapple juice and ferments

The reducing sugar content was determined by the Lane-Eynon method (AOAC, 1995), whilst the TTA was determined by titration with 0.1 N NaOH (AOAC, 1995) and the alcohol content was measured using a vinometer (Alla, France). For analysis of the organic acid levels, samples were first clarified by centrifugation and filtration through a 0.45 micron syringe filter. The filtrates were poured into a vial, capped and put in an autosampler tray for injection into the HPLC. The organic acid contents were then analyzed by HPLC (Waters™ 717 plus Autosampler with Waters™ 600 Controller, Waters Associates

Inc., USA) as reported (Davis *et al.* 1986; Bell *et al.* 1991) except with some modification. The analytical column (HPX-87H, 300 x 7.8 mm ion exclusion column, Bio-Rad, USA.) was run at 55 °C using 0.06% (v/v) orthophosphoric acid in water as the mobile phase at a flow rate of 0.5 mL/min. Organic acids were detected using a Waters™ 996 Photodiode Array Detector (Waters Associates Inc., USA.), and data were analyzed using the Millennium software program. The method was calibrated using a standard solution, comprised of a mixture of 5 g/L each of citric, tartaric, malic, succinic, lactic and formic acids, plus 0.1 g/L fumaric acid and 1% (v/v) acetic acid.

Sensory evaluation

The de-acidified wine, as in post-secondary LAB fermentation, was aseptically decanted (350 mL) into 400-mL amber glass bottles and KMS was added to a final concentration of 100 ppm before closing the bottle with easy-open cap. The bottled wine was stored at 4°C for 7 d before the acceptance test. The acceptability of the wines samples was evaluated using a nine-point hedonic scale for the “liking” of the overall wine, color, clarity, aroma and flavor, and a five-point Just About Right scale for the perceived sweetness, sourness, astringency, bitterness and degree of alcohol content. Both sensory testes were evaluated by 30 assessors.

Results and Discussion

Ethanol fermentation

A mixed culture of *S'codes ludwigii* and *H. uvarum* was used in the ethanolic fermentation of pineapple juice wine fermentation, as reported previously (Chanprasartsuk *et al.* 2010a, b). The pH did not significantly change during this fermentation. Alcohol was generated at an essentially linear rate during the 7-d fermentation period reaching 10% (v/v) at the final day of fermentation (Figure 1). The alcohol concentration in the final wine, at about 10% (v/v), was significantly lower than that previously found (12.9% (v/v)) in pineapple wine fermented from pasteurized 100% pineapple juices (Chanprasartsuk *et al.* 2010b). The *H. uvarum* strain used in this study was not an autochthonous strain, which might account for the lower total alcohol content due to its significantly lower ethanol fermentation ability than autochthonous strains. However, it was used in this co-culture to help increase the flavor complexity whereas *S'codes ludwigii* played the major role in alcohol production (Chanprasartsuk *et al.* 2010b). In addition, the residual SO₂ from the KMS-mediated

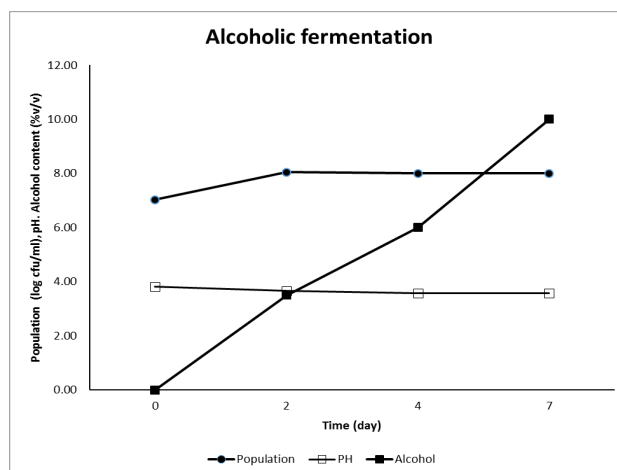


Figure 1. Changes in the (●) viable yeast population level, (■) ethanol content and (□) pH of the pineapple juice ferment during the ethanolic fermentation with a mixed culture of *S'codes ludwigii* and *H. uvarum* yeasts. Data are shown as the mean ± 1 SD, derived from 2 repeat

sterilization would likely suppress fermentation, as seen in grape wine fermentation. Moreover, pineapple juice contains an abundant level of protease activity due to the presence of bromelain, a bioactive compound with significant biomedical properties (Bartholomew *et al.* 2003). This protease could be inhibitory to the yeasts, leading to a slower ethanolic fermentation rate relative to that for protease free juices including pasteurized pineapple juice. If so, then an alternative treatment of the juice will be required. The protease activity in the pineapple juice could be reduced by over 70% after thermal treatment while the activity level was not changed by KMS treatment (data not shown).

With respect to the four different primary ethanolic fermentation cultures (a single starter of commercial *S. cerevisiae* or *S'codes ludwigii*, and a mixed starter of either *S. cerevisiae* and *H. uvarum* or *S'codes ludwigii* and *H. uvarum*), they all yielded similar ferments (data not shown). However, when the final wine was subjected to sensory evaluation, the wine derived from the mixed culture of *S'codes ludwigii* and *H. uvarum* had a higher acceptance score relative to the other starter types (data not shown), and so was used in this study.

The organic acid contents in the resulting wine were not significantly changed from that in the initial pineapple juice except for the 3.0- and 1.13-fold increase in succinic acid (a minor component) and malic acid, respectively, and the 2.15-fold decreased citric acid level, the main organic acid in pineapple juice (Table 1). Citric acid has a marked influence on the pH and organoleptic quality of pineapple wine, and so this significant reduction (over 45%) in its level is interesting. Generally, in grape wine

Table 1. Organic acid levels in the pineapple juice, the primary ethanolic ferment (basic wine), and the secondary LAB malolactic ferments with three different LAB cultures (O1, O2 and O1/O2)

Organic acids	Acid content (g/L)				
	Pineapple juice	Basic wine	O1	O2	O1/O2
Citric	14.24 ± 0.20 ^a	6.60 ± 0.14 ^b	5.80 ± 0.01 ^c	5.60 ± 0.01 ^d	5.58 ± 0.02 ^d
Malic	9.41 ± 0.05 ^a	10.72 ± 0.01 ^b	8.11 ± 0.33 ^c	8.05 ± 0.16 ^c	6.94 ± 0.04 ^d
Lactic	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	4.42 ± 0.05 ^b	3.78 ± 0.00 ^c	4.39 ± 0.01 ^b
Tartaric	0.00 ± 0.00 ^{ns}	0.00 ± 0.00 ^{ns}	0.00 ± 0.00 ^{ns}	0.00 ± 0.00 ^{ns}	0.00 ± 0.00 ^{ns}
Succinic	0.57 ± 0.08 ^a	1.80 ± 0.05 ^b	1.97 ± 0.20 ^c	1.90 ± 0.05 ^c	1.98 ± 0.05 ^c
Fumaric	0.00 ± 0.00 ^{ns}	0.00 ± 0.00 ^{ns}	0.00 ± 0.00 ^{ns}	0.00 ± 0.00 ^{ns}	0.00 ± 0.00 ^{ns}
Formic	0.35 ± 0.24 ^a	0.30 ± 0.43 ^a	0.47 ± 0.10 ^{ab}	0.39 ± 0.05 ^a	0.48 ± 0.09 ^{ab}
Acetic	0.00 ± 0.00 ^{ns}	0.00 ± 0.00 ^{ns}	0.00 ± 0.00 ^{ns}	0.00 ± 0.00 ^{ns}	0.00 ± 0.00 ^{ns}

Data are shown as the mean ± 1 SD, derived from 2 repeats. Means within a row followed by a different lowercase superscript letter (a,b,c) are significantly different ($p \leq 0.05$). For O1, O2 and O1/O2 see materials and methods or Table 3.

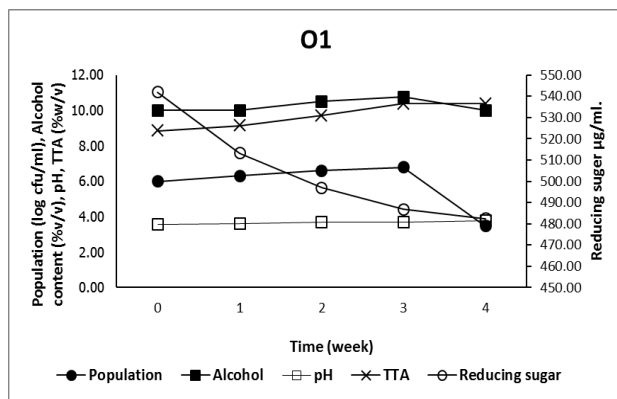
fermentation, citric acid is produced by yeasts during the ethanolic fermentation at around 0.1–0.4 g/L and then further broken down by LAB during the malolactic fermentation (Ribereau-Gayon *et al.* 2006a). In contrast, in pineapple juice, the level of this acid was reduced when fermented with the selected yeasts used in this study. It seems that these yeasts can potentially initiate the de-acidification process along with ethanolic fermentation of this high citric acid containing fruit juice, and so reduce the sharp sour taste in the pineapple wine. Many yeasts species, including Baker's yeasts, can utilize citrate for their growth and respiration on intermediates of the tricarboxylic acid cycle as their sole carbon source, and that organic acids can simply diffuse (in the uncharged form) across the cell wall of yeasts. However, the difference between citrate utilizing and non-utilizing yeasts is typically not due to changes in major metabolic pathways, but differences in the citrate permeability of the intact cells (Barnett and Kornberg 1960; Cole and Keenan 1986; Mira *et al.* 2010; Piper *et al.* 2001). Note, however, that the metabolic pathway of citrate metabolism in yeasts is not well characterized relative to that in bacteria, particularly the LAB. In addition, apart from the pathway as just those described, the reduction of acid could be due to the adsorption of the acid molecules by yeast cell walls during fermentation.

Regardless, *S'codes ludwigii* is native to pineapple fruits and so could be well adapted to the stress from high acidity and to uptake citrate (at this high concentration) as a carbon source. If correct, this notion is relatively novel and requires further systematic investigation for substantiation and characterization.

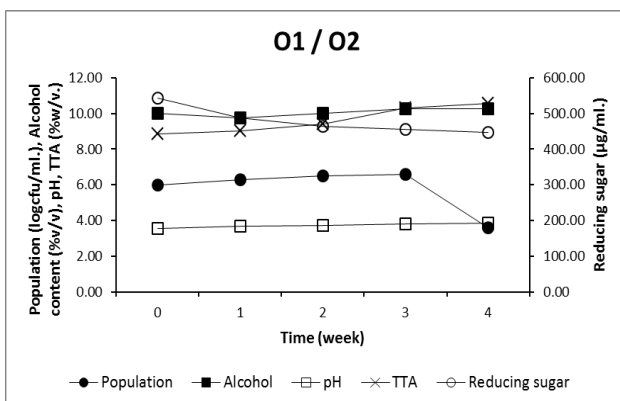
Secondary LAB (malolactic) fermentation with single and double (mixed) LAB strains

As previously mentioned, the pineapple wines with lower alcohol contents were found to be more acceptable in terms of their organoleptic properties in the sensory tests. Thus, this study selected the pineapple wine containing 10% (v/v) alcohol for the secondary LAB (malolactic) fermentation. The initial properties of the wine were 10.0% (v/v) alcohol, 0.54 g/L of reducing sugars and 8.84 g/L of TTA with a pH of 3.57 (Table 1). In the secondary LAB fermentation, the profile of the wine after fermentation with O1, O2 and the mixed O1/O2 LAB cultures were relatively similar with no significant change in the alcohol, reducing sugar and TTA levels, but a slight increase in the pH and changes in some of the organic acid levels (Table 1). With respect to the organic acid levels, a slight reduction in the citric and malic acid levels and increase in lactic and succinic acid levels was noted (Table 1). Over the 4-week fermentation period, the density of viable LAB cells increased over the first 2 (O2) or 3 (O1 and O1/O2) weeks followed by rapidly falling, whilst the TTA and pH increased steadily and the reducing sugars decreased over the 4-week fermentation period (Figure 2). There was no marked difference in these changes between the ferments from the three different LAB cultures, except for a more dramatic decrease in the final reducing sugar level in the mixed O1/O2 LAB ferment compared to those fermented with the O1 or O2 cultures alone.

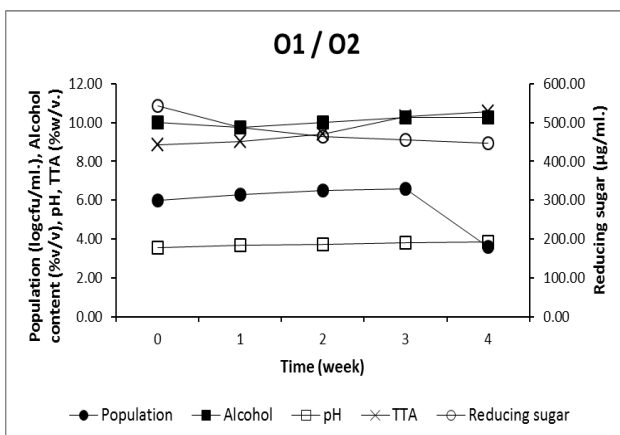
The reduction in the malic acid levels in all secondary LAB ferments were significantly greater than that for citric acid, with the highest reduction of malic acid and reducing sugar levels being found in the mixed O1/O2 LAB culture ferment. This reflects the positive interaction between the O1 and O2 LAB



(A)



(B)



(C)

Figure 2. The (●) number of viable LAB, (■) ethanol concentration, (□) pH, (x) TTA and (○) reducing sugar level in the pineapple wine during the secondary malolactic fermentation with the (A, B) single LAB cultures of (A) *Oenococcus oeni* LALVIN 31™ (O1) and (B) *Oenococcus oeni* Enoferm® ALPHA (O2), or (C) the mixed O1/O2 culture. Data are shown as the mean \pm 1 SD, derived from 2 repeats

strains in the pineapple wine secondary malolactic fermentation, where they both support each other in sugar and malic acid utilization. These commercial LAB strains could seemingly utilize malic acid as their main carbon source in pineapple wine, as in grape wine, and so the partial de-acidification (increased pH and TTA) of pineapple wine by

malolactic fermentation was achieved using these two LAB strains alone or together to a final pH of 3.71–3.85 and TTA of 10.4–10.6 g/L. The pH and TTA of wine are important since they both influence the astringency and sour taste whilst a balanced pH and acidity also enhance the fruit character of a wine. In grape wines, the pH level for table wine ranges between 3.1–3.4 and 3.3–3.6 for white and red wines, respectively, with a preferred TTA level of 7–9 and 6–8 g/L for white and red wines, respectively (Romano *et al.* 2003). The major acid in grape and pineapple wines is different. In grape wine, tartaric acid is the main one (2 to > 6 g/L), and is not affected by ethanolic or malolactic fermentation, followed by malic acid (1–6.5 g/L) that can be 100% converted to lactic acid by secondary malolactic fermentation by LAB. With respect to grape wine, citric acid, a minor acid in grapes, is produced by yeasts during ethanolic fermentation and degraded by the LAB in the secondary malolactic fermentation, but still typically constitutes up to 10% of the total acid content (0.1–0.7 g/L) in the final grape wine. Lactic acid is mainly produced in the malolactic fermentation and can reach levels up to 3 g/L, whereas succinic acid is mainly produced during the ethanolic fermentation and is present in the final wine at about 1 g/L (Ribereau-Gayon *et al.* 2006a, b).

In the pineapple wine, the pH and TTA in the pineapple wine obtained in this study after secondary LAB (malolactic) fermentation were relatively high compared to that in grape wines, which might be due to the different major acids present in these two musts. Malic acid was found at the highest concentration in the post-secondary LAB fermentation (6.9–8.1 g/L), and was the second most common organic acid in fresh pineapple juice after citric acid but was increased in concentration in the primary ethanolic ferment (unlike citric acid that was decreased) and then decreased in the secondary LAB fermentation. In contrast, citric acid, the major acid in pineapple juice (up to 14 g/L), was significantly decreased in the primary ethanolic fermentation (2.16-fold to 6.6 g/L) but was not reduced very much more in the secondary LAB fermentation, being at 5.6–5.8 g/L in the final wine. Tartaric acid, which is the main acid that gives the sour taste in grape wine, was not detected in any pineapple juice or subsequent ferment in this study. Apart from naturally existing compounds in pineapple juice, such as amino acids, that are significantly different from that in grape juice, the different acid profiles of pineapple wine might be a key factor in the different sour taste character and/or acid balance taste. This aspect requires further characterization.

Table 2. Characters of the pineapple wine obtained from the mixed *S'codes ludwigii* and *H. uvarum* primary ethanolic ferment (basic wine) and after the secondary LAB malolactic fermentation by the O1, O2 and O1/O2 LAB cultures

Character	Basic wine	O1	O2	O1/O2
pH	3.57 ± 0.01 ^a	3.78 ± 0.02 ^b	3.71 ± 0.01 ^b	3.85 ± 0.02 ^c
Alcohol (% (v/v))	10.0 ± 0.04 ^a	10.00 ± 0.00 ^a	10.25 ± 0.35 ^a	10.25 ± 0.35 ^a
Reducing sugar (mg/L)	542.0 ± 1.8 ^c	482.3 ± 0.0 ^b	500.9 ± 0.0 ^b	447.3 ± 0.0 ^b
TTA (g/L)	8.84 ± 0.54 ^a	10.37 ± 1.00 ^b	10.57 ± 1.00 ^b	10.57 ± 1.00 ^b

Data are shown as the mean ± 1 SD, derived from 2 repeats. Means within a row followed by a different lowercase superscript letter (^{a,b,c}) are significantly different ($p \leq 0.05$). For O1, O2 and O1/O2 see materials and methods or Table 3.

Table 3. The mean liking scores of the final pineapple wines (post -secondary malolactic fermentation), as evaluated by the nine-point Hedonic Scale

Malolactic LAB	Color	Clarity	Aroma	Overall
<i>O. oeni</i> LALVIN 31™ (O1)	6.10 ± 1.40 ^a	5.93 ± 1.44 ^a	6.13 ± 1.35 ^{ab}	5.73 ± 0.91 ^a
<i>O. oeni</i> Enoferm® ALPHA (O2)	5.30 ± 1.73 ^b	5.77 ± 1.17 ^a	5.73 ± 0.87 ^a	5.50 ± 1.11 ^a
O1/O2	6.43 ± 1.38 ^a	6.27 ± 1.20 ^a	6.57 ± 0.90 ^b	6.80 ± 0.92 ^b

Data are shown as the mean ± 1 SD, derived from 30 people (sensory taste panel). Means within a column followed by a different lowercase superscript letter (a,b,c) are significantly different ($p \leq 0.05$).

Apart from malic acid, the levels of other organic acids, such as citric and tartaric acids, have been reported to be reduced by fermentation these commercial LAB strains (Liu 2009). However, in this study the malic acid levels were reduced by only around 20% of the initial level, which indicates a poor performance of these commercial LAB isolates in reducing the malic acid level under these conditions and so a poor de-acidification of the pineapple wine. It is likely that these LAB strains were not well adapted to grow and perform malolactic fermentation, which might be due to the high level of citric acid, protease activity and a lack of specific nutrients. Although the citric acid level was reduced during the ethanolic fermentation, the residual acid level was not efficiently reduced further by the secondary LAB fermentation (de-acidification). Accordingly, new LAB strains that are better adapted to pineapple juice, such as naturally isolated LAB isolates from pineapples, should be further evaluated so as to develop a more optimal culture and/or co-culture for the efficient de-acidification of pineapple wine. Appropriate LAB strains should be able survive

the high alcohol concentration, low pH, extreme fermentation temperature and the presence of SO₂ (Davis *et al.* 1988; Drici-Cachon *et al.* 1996). Some *Lactobacilli* species have been reported as alternative LAB for malolactic fermentation (G-Alegria *et al.* 2004; Lerm *et al.* 2011; Pozo-Bayon *et al.* 2005), including *L. plantarum* (du Toit *et al.* 2010) and the commercial *L. plantarum* V22R isolate (Fumi *et al.* 2010). This species shows a more diverse enzyme activity profile than *O. oeni* (Matthews *et al.* 2004; Mtshali *et al.* 2010; Spano *et al.* 2005), which could play an important role in modification of the wine aroma profile (Guerzoni *et al.* 1995; Matthews *et al.* 2004; Swiegers *et al.* 2005). In addition, *L. plantarum* produces citrate lyase, an enzyme responsible for catabolism of citrate to aromatic volatile metabolites (Lerm *et al.* 2011). There have been reports on the application of different LAB strains for malolactic fermentation in fruit wines. For example, *L. plantarum* could complete malolactic fermentation of loquat wine at a SO₂ and ethanol concentration of less than 70 mg/L and 12.7% (v/v), respectively, but was incomplete when the pH value was < 3 (Zhigang

et al. 2011). However, the changes in the organic acid profile during the secondary malolactic fermentation of loquat wine were not reported in their study. Interestingly, *L. plantarum* has been reported to be a normal flora of pineapple juice that can be used for the reduction of residual sugar and citric acid levels in pineapple juice ferments (Di Cagno et al. 2010; Hansawat and Prakitchaiwattana 2013). This indicates the possibility of the further development of specific (autochthonous) LAB cultures for a better secondary malolactic (de-acidification) fermentation stage of pineapple wine.

Sensory analysis

After the secondary (malolactic) LAB fermentation of the pineapple wine the pH and TTA values were significantly increased whilst the reducing sugar level was slightly reduced. Among the three wines derived from the secondary fermentation with the different LAB cultures, that from the mixed O1/O2 LAB culture had a significantly higher pH and lower reducing sugar level than those from the single O1 or O2 LAB ferments. The taste balance in wine reflects the interaction and harmony between the alcohol/sugar and acidity content, where a wine with low acidity has a distinctly flat taste, and that with a high acidity has a sour taste. The pH, however, can be very different at similar TTA levels depending on the amount and proportion of the acid types and salts (Munyon and Nagel 1977). Therefore, to find an appropriate balance the pineapple wines were subjected to sensory analysis. The wine from the mixed O1/O2 LAB fermentation was more acceptable than those from either of the single LAB ferments in all categories assayed on the nine-point Hedonic scale; namely the color, clarity, flavor and overall taste (Table 3). For the perception of sourness, bitterness and degree of alcohol, assessed using the five-point Just About Right scale, the wine fermented by the O2 LAB culture having lowest liking score reflected too high degree of bitterness and sourness (data not shown). Thus, the level of acidity would appear to have a greater influence on the overall acceptability of the pineapple wine. The organoleptic feel of the wine from the mixed O1/O2 LAB fermentation, with the highest overall liking score, reflected the more balanced composition of this wine. Comparing the "overall liking" of the pineapple wines before and after the secondary LAB fermentation, it was found that the overall liking score was significantly increased from 5.8 to 6.8 after the secondary LAB fermentation (data not shown). This indicates that the de-acidification of the wine by malolactic fermentation with the two commercial LAB isolates

(O1 and O2) could significantly improve the pineapple wine quality. The protease activity level in the pineapple wine remained at about 70% and 60% of that in the fresh juice after the primary ethanolic and secondary LAB fermentations, respectively (data not shown). This could help the wine to have a unique character, whilst it could be known as an enzymatic wine having medicinal properties, which is not found in most other fruit wines. Moreover, the protease may perhaps enhance the wine clarification and inhibit some spoilage microorganisms.

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