

Research Paper

## **Complex fruit wine produced from dual culture fermentation of pineapple juice with *Torulaspora delbrueckii* and *Saccharomyces cerevisiae***

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This paper was originally presented at the International Conference on the Role of Universities in Hands-On Education, Chiang Mai, Thailand, August 2009.

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### **Abstract**

The use of *Torulaspora delbrueckii* in grape juice fermentation has been studied. However, the effect of this yeast on fruit wine made from pineapple is unknown. Characterization of the growth kinetics and the yeast's ability to complete fermentation of the resultant wines was undertaken for *T. delbrueckii*, *Saccharomyces cerevisiae* and dual cultures of *T. delbrueckii* and *S. cerevisiae*. Dual culture fermentation incorporating *T. delbrueckii* and *S. cerevisiae* permitted the modulation of yeast growth and ensured completion of the fermentation. The composition of pineapple wines made by monoculture and dual cultures were investigated. Pineapple wine produced by monocultures of *T. delbrueckii*, *S. cerevisiae* and dual cultures of *T. delbrueckii* and *S. cerevisiae* had distinct aroma characteristics. This work highlights the use of non-*Saccharomyces* yeasts and the importance of inoculation strategy for the production of a distinct flavour complexity in pineapple wine.

**Keywords:** Beverage, *Torulaspora delbrueckii*, winemaking, inoculation strategy, pineapple

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### **Introduction**

Wine is an alcoholic beverage made from grapes whose origins date back thousands of years. Wine is consumed in many countries around the world and is a profitable business. It was introduced to Thai society some decades ago, mostly as an imported

commodity and because of its high tax it was available mostly to consumers of the upper classes. Realizing an economic opportunity for the country, the government of Thailand in 2000 authorized some 2000 interested small enterprise producers to make Thai fruit wines. Only a relatively small amount of grape wine was commercially produced in comparison to imported wine, while wine made from various Thai fruit was produced in an overwhelmingly greater amount. Unfortunately, almost all of these small enterprises gave up winemaking a few years thereafter.

Today there are only a few producers left and the most plausible reason is that winemaking is an endeavour that relies heavily on scientific knowledge. The fermentation process of fruit juice into wine is a complex biochemical reaction involving microorganisms that requires study and practice. Lacking enough experience, a newcomer will have great difficulty consistently producing wine of good quality, and thus the consumers who desire complex and consistent taste in their fruit wines are rarely satisfied. Unfortunately this appears to be the reason that consumers labeled Thai fruit wine as low quality wines and ceased buying.

The research team of Rajamangala University of Technology Lanna (RMUTL) has been working on ways to produce good quality wines from various fruit available in Thailand. Preliminary results show the great potential for converting many Thai fruit into quality wine. However, each fruit has its own individual characteristics that require fruit-specific processing techniques. In order to achieve success for potential industries, further studies are needed to allow the gathering of knowledge and understanding of the fruit wine production process before an efficient product development cycle can be devised. Consistently good quality can then be guaranteed. The aim of this work was to evaluate the ability of a non-*Saccharomyces* yeast species, *Torulaspota delbrueckii*, to ferment pineapple juice. This paper reports on the impact that pure and dual cultures of *T. delbrueckii* and *S. cerevisiae* have on fermentation behaviour and analytical profile.

## **Methodology**

### ***Yeast strains and media***

*Saccharomyces cerevisiae* and *Torulaspota delbrueckii* came from the collection of the Section of Microbiology and Biochemistry, Geisenheim Research Center, Germany. Yeasts were grown at 25°C on YEPD medium; glucose, 20 g/l; yeast extract, 10 g/l; peptone, 20 g/l; and agar, 15 g/l.

### ***Yeast cells***

Total and viable cell numbers of yeasts were estimated microscopically by using a counting chamber slide. Cells (450 µl) were added to 50 µl of methylene blue solution (0.4% methylene blue, 10% ethanol and 0.4 M KH<sub>2</sub>PO<sub>4</sub>) and mixed. Blue cells were counted as dead cells, while cells without obvious colour were counted as live cells.

### ***Pineapple juice***

Pineapple juice was obtained from pineapple harvested in Lampang (2009). After peeling it was freshly pressed. Initial sugar was 14°Brix and acidity was 0.55% as estimated by refractometer and a titration method, respectively.

### **Fermentation conditions**

Triplicate experiments were carried out in 750 ml sterile bottles (650 ml pineapple juice per bottle) at 20°C, inoculated with 24 h pre-culture to obtain an initial level of  $1 \times 10^6$  cells/ml. Dual fermentation trials were simultaneously inoculated with  $1 \times 10^6$  *T. delbrueckii* cells/ml plus *S. cerevisiae*. The fermentation trial was terminated after 14 days. Fermentation kinetics were obtained by monitoring carbon dioxide production during yeast growth.

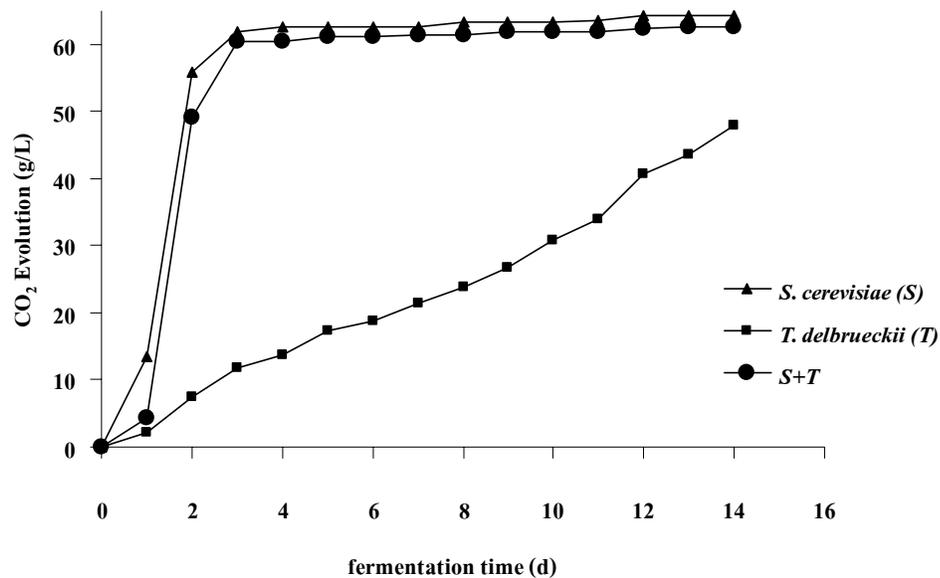
### **Pineapple juice and wine analysis**

Total acidity was determined by a titration method [1]. Ethanol concentrations (% vol.) were measured by an ebulliometer. Fructose, glucose, sucrose concentrations were analyzed by HPLC. The sensory properties of aroma were also evaluated in fresh pineapple wine.

## **Results and Discussion**

### **Fermentation behaviour**

Fermentation was performed in pineapple juice containing 140 g/l sugar. The influence of different yeasts on the fermentation kinetics and on the two main oenological characteristics is shown in Fig. 1 and Table 1. The results of the fermentation kinetics confirmed that *S. cerevisiae* was the yeast capable of completely fermenting after 14 days, while *T. delbrueckii* only fermented to 70% of *S. cerevisiae* level. The alcoholic fermentation of *T. delbrueckii* continued slowly over 30 days after inoculation (not shown). *T. delbrueckii* had a slower fermentation rate in comparison to *S. cerevisiae*. Similar results were also reported in grape juice [2, 3].



**Figure 1. Fermentation profile of pineapple juice at 20°C.**

In pineapple juice *S. cerevisiae* produced ethanol in the final product at the same concentration as the dual culture inoculation of *S. cerevisiae* and *T. delbrueckii*. The higher ethanol concentration in the *S. cerevisiae* (8.4% vol.) was not the only difference. In comparison with the *S. cerevisiae* and dual fermentation of *S. cerevisiae* and *T. delbrueckii*, the monoculture fermentation of *T. delbrueckii* had a lower ethanol/sugar yield.

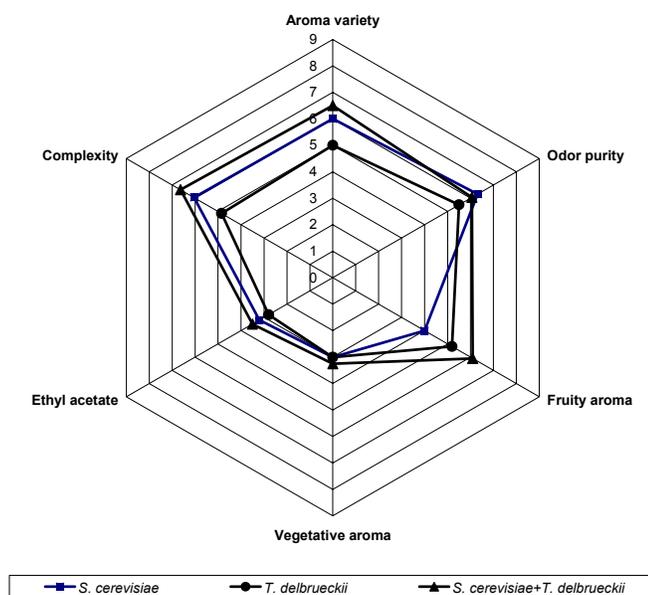
**Table 1. Oenological characters of pure and dual fermentation at 20°C.**

	<i>S. cerevisiae</i>	<i>T. delbrueckii</i>	Dual fermentation
Residual sugar (g/l)	< 1	18.50	< 1
Ethanol (% vol.)	8.4	5.7	8.4

As was expected, inoculating pineapple juice with *T. delbrueckii* yeast resulted in a sluggish fermentation. Although this yeast species produced lower ethanol yields, it gave a desirable secondary metabolite profile. Bely *et al* [4] found that low acetic acid production is a positive feature for grape juice fermentation, and therefore the combination of *T. delbrueckii* species and *S. cerevisiae* could improve the quality of wine made from pineapple, while ensuring that the fermentation would be complete. However, further investigation into the multi-starter fermentation is required to better determine what is the metabolite profile of these yeast strains in pineapple juice fermentation.

#### ***Sensory properties of pineapple wine aroma***

The sensory properties of pineapple wine aroma are shown in Fig 2. Inoculated mixed yeasts in pineapple juice produced wines with different sensory aroma properties when compared to wine made from pure fermentations.



**Figure 2. Aroma sensory properties with different inoculation treatments.**

Sensory descriptive analysis was used to describe and quantify the differences in aroma of pineapple wine as a result of different inoculation treatments. Using mixed cultures of *T. delbrueckii* and *S. cerevisiae* resulted in complexity in the pineapple wine. Similarly, *T. delbrueckii* was used to increase the sensory variety of wine made from grapes as reported by Grossmann *et al.* [5] and Sommer *et al.* [6].

### Conclusion

This work describes the first trial of a research project aimed at studying the multi-starter inoculation in making fruit wines. The results enhance our understanding of the behaviour of non-*Saccharomyces* and foreshadow the potential application to fruit juice winemaking. The use of multi-starter inoculation should be an alternative to control the extent of the fermentation with the goal of enhancing the flavour complexity of the wine. Experimental work for this research project was conducted in the laboratory and the next step is development to the industrial scale.

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