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Research Article

Pineapple wine fermentation with yeasts isolated from fruit as single and mixed starter cultures

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Abstract

The main goal of this study was to investigate the fermentation profiles of pineapple wine from "Queen" pineapple juice with single and mixed starter cultures of yeasts isolated from pineapple fruit as starter cultures. The chemical properties of freshly crushed Queen pineapple juice were analyzed. pH, Total Soluble Solids (TSS), Total Titratable Acidity (TAA, as citric acid) and nitrogen content of fresh pineapple juice were 3.7±0.0, 18.0±0.1°Brix, 0.67±0.01 %w/v and 0.08±0.01%w/v, respectively. The pineapple juice was fermented with single and mixed starter cultures of *Saccharomyces cerevisiae*, *Saccharomycodes ludwigii* and *Hanseniaspora* isolate I, at 25°C for 10 days. Based on their fermentation characteristics, the mixed cultures of *S. cerevisiae* and *S'codes ludwigii*, *S. cerevisiae* and *Hanseniaspora* isolate I, and *S. cerevisiae*, *S'codes ludwigii* and *Hanseniaspora* isolate I could generate alcohol content in the final day of fermentation to 12.0, 12.0 and 13.0%(v/v), respectively. The mixed cultures of *S'codes ludwigii* and *Hanseniaspora* isolate I produced the highest alcohol content to 14.0 %(v/v) in the final day of fermentation and their fermentation profiles were similar to those of the batch of single *S. cerevisiae* and mixed culture of *S. cerevisiae* and *Hanseniaspora* isolate I.

Keywords: tropical fruit wine, autochthonous yeasts, Thailand, *Hanseniaspora*, *Saccharomycodes*.

Introduction

The pineapple variety "Queen" (*Ananas comosus* (L.) Merr. c.v. Phuket) is a popular fruit wildly consumed in Thailand. It is generally grown in the southern and some eastern parts of the country. Typically, Queen pineapple is favoured eaten fresh by consumers because of its unique characteristics. Its flesh is a deep yellow colour with good firmness and has a delicious taste characterized by sweetness and a little acidity. Additionally, it has very intense fruity

fragrances and aromatic flavours. Recently, interest in developing pineapple wine, one of the local pineapple products, has increased as a potential product for exportation. However, the production process specific to pineapple wine manufacture that is required to improve and standardize the quality, consistency and varieties of pineapple wine, is still very much at the initial stage of development. Autochthonous yeasts are reported to be wild yeasts that produce the unique flavours and exceptional quality when used for traditional wine fermentation [1].

The use of mixtures of different species and strains of yeasts as starter cultures (multi-starter cultures) to induce desirable fermentation of alcoholic beverages has been considered in previous research but application of this concept is still relatively new [2]. There are a few techniques in the application of multi-starter cultures for fermentation processes. Among these, simultaneous inoculation of the mixture of species is simply to control the fermentation and this has been reported in the research literature [3, 4, 5, 6, 7, 8]. Therefore, the main goal of this study was to investigate the fermentation properties of autochthonous yeasts isolated from pineapple fruit as single and mixed starter cultures in Queen pineapple juice fermentation.

Materials and Methods

Preparation of yeast cultures

Three yeast strains, commercial *S. cerevisiae* (Angle®, China), *S'codes ludwigii* isolated from pineapple fruit and *Hanseniaspora* isolate I isolated from spontaneous fermentation of pineapple juice [9] were used for pineapple wine production. The species of yeast isolates were identified by morphological examination, sequencing of the 26S rDNA D1/D2 and ITS region of ribosomal DNA [10] and RFLP of the ITS region [11]. These yeasts were cultured on Malt Extract Agar (Oxoid, England) at 25°C for 3-4 days for inoculum preparation.

Preparation of pineapple must

Pineapple samples of the "Queen" (*Ananas comosus* (L.) Merr. c.v. Phuket) at ripe stage were purchased from a local market in Chonburi Province (Eastern Thailand). The pineapple samples were cleaned with tap water, peeled and freshly crushed. The sugar concentration of crushed juice was increased to 22^{o}Brix by adding sucrose. It was then decontaminated with the addition of potassium metabisulphite ($K_2S_2O_5$) to achieve a final concentration in the juice of 100 mg l^{-1}.

Single and mixed cultures of pineapple juice fermentation

A commercial *S. cerevisiae*, the main yeast for general wine fermentation, was also used as the starter culture in this study. Inoculum cultures of three yeasts were prepared in sterile pineapple juice and used to inoculate pineapple juice fermentations. Each starter culture was inoculated to the prepared pineapple juice at initial population of 10^6 cell ml^{-1}. The pineapple juice fermentations were conducted at 25^{o}C for 10 days. These fermented pineapple juices were collected for microbiological determination, then kept at temperature -20°C awaiting chemical analysis. Yeasts population, pH (Cyber Scan 1000 Euten, USA), Total Soluble Solid (Atago 2411-w06, Japan), Total Titratable Acidity, alcohol content (Alla, France), concentrations of sugars and organic acids were investigated throughout the experiment.

Samples of the fermented pineapple juice were serially diluted in 0.1% peptone water. The yeasts in each dilution were enumerated and isolated by spread inoculation of 0.1 ml onto plates of MEA agar (Oxoid, England) and incubation at 25^{o}C for 2-4 days. This analysis was done in duplicate. Yeast colonies were counted to give populations as log cfu ml^{-1}.

Total titratable acidity of pineapple juice samples were titrated with 0.1 N NaOH using phenolphthalein as indicator, the results were expressed as percentage of citric acid. Sugars of the fermented pineapple juice were analyzed by HPLC (Waters 2690 Seperation Module, Waters Associates Inc., USA.), using the method of Raffo *et al* [12] and Gennaro *et al* [13], with some modification and detected by 410 Differential Refractive Index Detector (Waters Associates Inc., USA.). The data were analysed by a Millenium software program. Organic acids of the fermented pineapple juice were analyzed by HPLC (Waters 2695 Seperation Module, Waters Associates Inc., USA.), using the method of Lee [14] and Son *et al* [15], with some modification, and detected by a Waters 2487 Dual λ Absorbance Detector (Waters Associates Inc., USA.). The data were analyzed by an Empower software program.

Results and Discussion

Chemical properties of pineapple juice

The basic chemical characteristics of Queen pineapple juice are shown in Table 1. Based on the results of analysis, TSS of Queen pineapple juice was relatively high (18.1°Brix) compared with grape or other tropical fruit juices. The pH and TTA of pineapple juice were similar to those of grape juice, being 2.8-3.8 and 0.6-0.8 (%w/v), respectively [16]. Additionally, nitrogen content was adequate for yeast growth in the initial phase of fermentation, which should be more than 0.025 gL^{-1} [17].. This indicated that the juice could be used as raw material for wine fermentation since such condition of pineapple juice could allow yeast to grow and conduct fermentation. However, the main organic acid of pineapple juice is citric acid, which differs from grape juice. It is well known that tartaric acid is the main organic acid of grape juice. This difference could affect the fermentation profiles of yeast species. Therefore, the fermentation profile of the Queen pineapple juice was further evaluated.

Table 1. Basic chemical characteristics of freshly crushed pineapple juice.

Chemical characteristics	Value ± SD.
pH	3.7 ± 0.0
Total Soluble Solid (TSS, °Brix)	18.1± 0.1
Total Titratable acidity (TTA, as citric acid) (%w/v)	0.67 ± 0.01
Nitrogen content (%w/v)	0.08 ± 0.01

Fermentation profile of pineapple juice by single and mixed yeast starter cultures

The microbiological and chemical analyses were investigated throughout the fermentation. *S. cerevisiae*, the main yeast for general wine fermentation, was also used as the starter culture in this study. *S'codes ludwigii* and *Hanseniaspora* isolates I from pineapple fruit were selected to use as starter cultures in this study to investigate their fermentation potential. Table 2 shows the chemical analysis of final pineapple juice inoculated with single and mixed starter cultures. *S. cerevisiae* as a control starter could produce an alcohol content up to 12.3%(v/v), *S'codes ludwigii* could produce the final alcohol content up to 12.3%(v/v), which is similar to *S. cerevisiae*. In addition, it showed other fermentation characteristics, namely sugar utilization and organic acid production, which were similar to *S. cerevisiae*. On the other hand, low alcohol production was observed from the batch of *Hanseniaspora* isolate I. It could produce the final alcohol content in pineapple juice at just 6.0%(v/v). This result agreed with other research, which reported that *H. uvarum* could generate low ethanol content during the micro-fermentation of other fruit juice [18].

Table 2. Chemical analysis of final pineapple juice fermented by single and mixed starter cultures.

Yeast species	pH	TSS (^{0}Brix)	TTA (as citric acid, %w/v)	%Alcohol (v/v)	Sugars (g 100ml^{-1})		
					Glucose	Fructose	Sucrose
S. cerevisiae (commercial strain; Sc)	3.9	8.4	0.67	12.3	0.00	0.81	0.41
S'codes ludwigii (Sl)	3.9	9.0	0.67	12.3	0.00	0.41	0.31
Hanseniaspora isolate I (H-I)	3.6	11.6	0.75	6.0	0.92	5.67	0.13
Sc + Sl	3.7	6.6	0.71	12.0	0.00	0.43	0.15
Sc + H-I	3.9	7.0	0.72	12.0	0.00	0.52	0.26
Sl + H-I	3.9	7.0	0.72	14.0	0.41	1.31	0.00
Sc + Sl + H-I	3.9	6.6	0.74	13.0	0.64	0.45	0.00

Figure 1 shows the patterns of fermentation with mixed starter cultures. The fermentation pattern with mixed cultures of *S. cerevisiae* and *Hanseniaspora* isolate I (Figure 1A), during day 1 and day 2, the populations of both yeasts increased approximately 2 log cycles. After day 2, the *S. cerevisiae* and *Hanseniaspora* isolate I population were slightly decreased, approximately 2 log cycles through to the last day. The alcohol content was observed at the early stage of fermentation and rapidly increased to an amount of 12% (v/v) on the last day of fermentation. *S. cerevisiae* produced invertase enzymes which rapidly degraded sucrose. Consequently, the rapid decrease of TSS in substrate was observed, and then they were utilized for alcohol production. For the batch with mixed cultures of *S'codes ludwigii* and *Hanseniaspora* isolate I (Figure 1B), the populations of both yeasts increased approximately 2 log cycles at the initial stage of fermentation and were stable throughout the fermentation. During day 1 and day 2, the alcohol production pattern was similar to the batch with mixed cultures of *S. cerevisiae* and *Hanseniaspora* isolate I. After day 2, the alcohol production shifted to generally increase throughout the fermentation, allowing the final alcohol increase to a maximum amount of 14% (v/v) on the last day of fermentation. From the previous research, Chanprasartsuk *et al* [19] investigated the fermentation profile of Smooth Cayenne pineapple juice with the same yeast cultures used in this study. The fermentation profile of Queen pineapple juice in this study was relatively similar to the Smooth Cayenne pineapple juice reported. Noticeably, in this study, the population of *Hanseniaspora* isolate I of mixed starter cultures was relatively higher than commercial *S. cerevisiae* and *S'codes ludwigii* survived throughout the pineapple juice fermentation. Numerous research on grape juice fermentation have reported that non-*Saccharomyces* yeast, especially *Kloeckera apiculata* (*Hanseniaspora*) and *Candida stellata*, survive during fermentation at a significant level [20, 21]. Additionally, these yeasts are capable of anaerobic and aerobic growth and may persist during the fermentation, competing with *Saccharomyces* for nutrients [22].

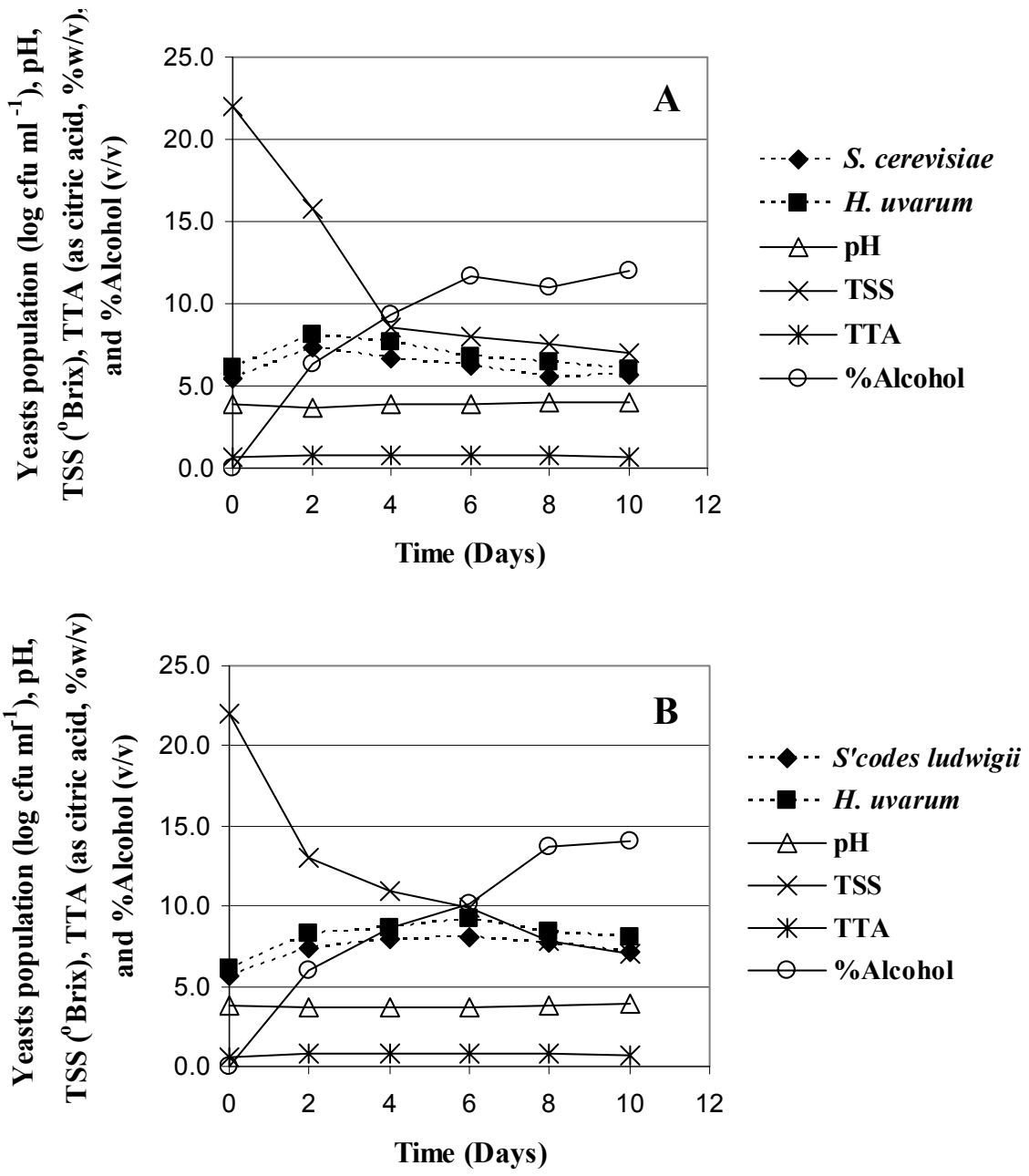

Figure 1. Changes of yeast population, pH, TSS, TTA and %alcohol of pineapple juice during fermentation with mixed starter cultures.
A: *S. cerevisiae* and *Hanseniaspora* isolate I; B: *S'codes ludwigii* and *Hanseniaspora* isolate I

For sugar determination (Table 2), sucrose, glucose and fructose were utilized throughout the fermentation in all batches of single and mixed cultures. Nevertheless, glucose remained relatively higher than other batches throughout the fermentation of single *Hanseniaspora* isolate I, which could be due to its slow rate of fermentation. The previous reports have stated that *Hanseniaspora* yeasts could not use sucrose as a carbon source for their growth and fermentation [23, 24], which clearly differed from the sugar assimilation pattern of *S.*

cerevisiae and *S'codes ludwigii*. However, the decreasing of sucrose content occurred from the spontaneous inversion of sucrose to be glucose and fructose in low pH conditions [25].

Conclusions

The results obtained from this study demonstrated that Queen pineapple juice could be a good substrate for yeast fermentation. The yeast isolates *S'codes ludwigii* and *Hanseniaspora* used as mixed starter cultures could perform appropriate alcoholic fermentation for Queen pineapple wine production. However, organoleptic properties, including volatile compounds composition of the resulting wine, should be further evaluated.

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