เรียนรู้เพื่อรับใช้สังคม

Autochthonous yeast as a potential starter culture for fresh crushed pineapple juice fermentation

On-ong Chanprasartsuk*, Pakarut Kansa, Wanchai Leethamwatkul
Department of Food Science, Faculty of Science, Burapha University, Muang, Chonburi, 20131 THAILAND
*Corresponding Author's Email: on_ong@buu.ac.th Telephone: +66 3810 3138

Rationale

Pineapple (*Ananas comosus* L. Merr.) is a fruit widely grown in tropical and sub-tropical countries. Its flesh has pale yellow color, unique sweet and sour taste, and pleasant fruity flavors and aromas which were preferred by consumers. Pineapple juice is considerably varied in sugar and acidity. Major carbohydrate constituents are sucrose, glucose and fructose while major acids are citric acid and malic acid (Sanewski et al., 2018). It is also a good sources of minerals, vitamin C, A and B (USDA), and bromelain enzyme that possesses proteolytic activity in the gastrointestinal tract and useful phytomedical application (Pavan et al., 2012).

In the beverage fermentative process, yeast plays an important role due to its high performance in the conversion of sugar into ethanol as well as aromatic esters and other metabolic products. The efficiency of the ethanol production is associated with the fermentative potential of the yeasts as well as the fermentation process optimization to improve the sensorial quality of the alcoholic beverages (Azhar et al., 2017). Typical fruit wine fermentation, the allochthonous yeast strains of *S. cerevisiae*, a commercial yeast strains, were often used as starter culture for fruit wines production since they could achieve complete fermentation of fruit juices, and the sugars are converted into alcohol, carbon dioxide, organic acids and secondary metabolites giving flavors and aromas in final fruit wines. To improve the fermentation, many researchers over the last few decades have tried to conduct a systematic investigation of yeasts associated with fruit juice fermentation. Tropical fruits are an important source of wild yeast strains, which these non-*Saccharomyces* yeasts are reported to produce the unique flavors and exceptional quality when used for traditional wine fermentation. (Fleet et al., 2002). Yeast ecology from the spontaneous fermentation of fruit juices has been studied in order to select and develop species-specific starter cultures as autochthonous yeast starters for high quality fruit wine production (Chanprasartsuk and Prakitchaiwattana, 2016). *Saccharomycodes ludwigii* is a non-*Saccharomyces* yeasts has been reported to isolated from sweet wines (Granchi et al, 2002; Loureiro and Malfeito-Ferreira, 2003) and wines at the end of the alcoholic fermentation or during storage (Romano et al., 1999). In addition, this non-*Saccharomyces* yeast has also been isolated from pineapple fruits (Chanprasartsuk et al., 2010). It can grow in the presence of high levels of ethanol and is adaptable to unfavorable conditions such as high concentrations of SO_2 (Vejarano, 2018). A selected *S'codes ludwigii* strain could produce high level of isobutyl alcohol, acetoin and ethyl acetate in Feijoa juice which characterized by a fresh odour with a fruity flavour, identified as flavour of apple and kiwi-fruit (Romano et al., 1999).

Research Objectives

The aim of this research was to investigate the changes in kinetics of fresh crushed Pattawia pineapple juice inoculated with allochthonous and autochthonous yeast.

Methodology

3.1 Preparation of pineapple juice

The Pattawia pineapple fruits (*Ananus comosus* (L.) Merr.) at harvesting stage were purchased from local market in Chonburi province (Eastern Thailand) used for all experiments. The pineapple fruits were washed and peeled. The pineapple flesh was crushed to obtain its juice. Crushed juices were filtered through a sterile cheesecloth. Then, the chemical characteristics, namely total soluble solid (Atago 2411-w06, Japan), total titratable acidity (as citric acid; AOAC, 2000), pH (Cyber Scan 1000 Euten, USA), nitrogen content (AOAC, 2000) and major amino acids (Bosch et al., 2006; van Wandelen and Cohen, 1997) of juice were analyzed. The crushed juices were decontaminated with the addition of potassium metabisulphite ($K_2S_2O_5$) to achieve a final concentration in the juice of 100 mg/L and collected in sterile Erlenmeyer flasks.

3.2 Preparation of yeast culture

A commercial yeast strain, *S. cerevisiae* (Angle®, China) as allochthonous yeast was used for pineapple inoculation. An indigenous yeast strain, *S'codes ludwigii*, isolated from Pattawia pineapple fruits was used as autochthonous yeast. The yeast strain identity was confirmed by morphological examination and molecular methods. The sequence analysis of the 26S rDNA D1/D2 and ITS region of ribosomal DNA were used as the diagnostic methods for examination according to Kurtzman and Robnett (1998). These yeasts were cultivated on Malt Extract Agar at 30°C for 3 days for inoculum preparation.

3.3 Fermentation of fresh crushed pineapple juice

The inoculum yeast culture was prepared and inoculated to the prepared pineapple at initial population of 6 log cfu/mL (Chanprasartsuk et al., 2012). The inoculated juices were incubated at ambient temperature (30-32°C) for 6 days. These cultured juices were collected every day for microbiological determination and physicochemical analysis. Yeasts population, TSS, TTA, pH and alcohol content (Alla®, France) were investigated throughout the experiment. All experiments were conducted in triplicate.

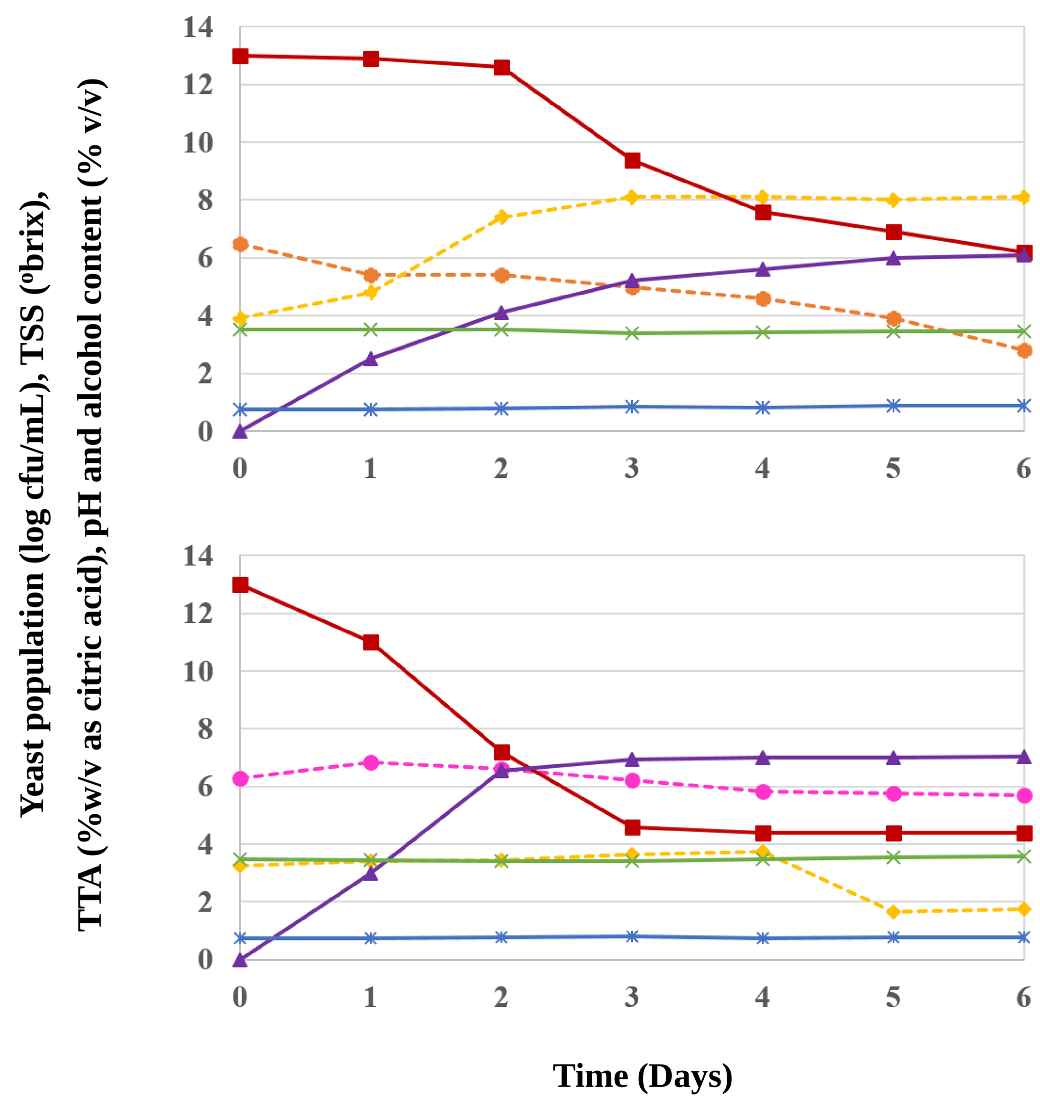
Results & Discussion

The chemical characteristics of fresh crushed pineapple juice is shown in Table 1. The juice contained 13.0 %TSS, 0.73 %TTA as citric acid, pH 3.29 and 0.07 %w/v of nitrogen content. In previous reports, sucrose was main sugar of pineapple juice which contained approximately 2/3 of total sugars and the rest sugars are glucose and fructose (Sairi et al. 2004). These assimilable sugars are sources of carbon and energy for yeast consumption (Fleet, 1998). The major organic acids of pineapple juice are citric and malic acids which found approximately 87 and 13% of total acid content, respectively (Sairi et al. 2004). Additionally, its nitrogen contents were adequate for yeast growth which should be more than 0.025 g/l (Ribéreau-Gayon et al., 2006). The high level of serine and proline had been found in pineapple juice. These results corresponding to a previous report of Wen and Wrolstad (2006) indicated that the amino acids identified in Pattawia pineapple juice were tryptophan, asparagine, proline, aspartic acid, serine, glutamic acid, α-alanine, aminobutyric acid, tyrosine, valine and isoleucine. These amino acids are not only the building blocks of proteins and peptides, but also play an important role in general metabolism of yeast cells (Ljungdahl and Daignan-Fornier, 2012).

Table 1 *Chemical characteristics of fresh crushed pineapple juice*

Chemical characteristics	Values (± SD)
TSS (°brix)	13.0 (± 0.0)
TTA (% as citric acid)	0.73 (± 0.02)
pH	3.29 (± 0.03)
Nitrogen content (%w/v)	0.07 (± 0.00)
Main amino acids content (mg/100 mL)	
Serine	26.81
Proline	17.05
Aspartic acid	9.56
Glutamic acid	9.46
Alanine	6.92

The microbiological and chemical properties of pineapple juice inoculated with allochthonous and autochthonous yeasts were shown in Figure 1. Based on the results of analysis, the yeast counts of inoculated juice with *S. cerevisiae* were dramatically tended to decline throughout fermentation (Figure 1a). Thus, the normal flora yeast could competitively grow and assimilate organic compounds of pineapple juice. Total soluble solid of pineapple juice started to decline in day 2 of fermentation. Generally, commercial yeast strain, *S. cerevisiae* is allochthonous yeast which can grow and achieve complete fermentation of fruit juices. The sugars in juice are converted into alcohol, carbon dioxide, organic acids and secondary metabolites giving flavors and aromas in final fruit wines (Chanprasartsuk and Prakitchaiwattana, 2016). The decreasing of allochthonous yeast population observed in this study could be due to the existence of bromelain enzyme of the juice, a specific type of protease, seemed to affect certain yeasts. The bromelain could theoretically inhibit fungal growth related to its proteolytic activity (López-García et al., 2012).



S. cerevisiae (●); *S'codes ludwigii* (●); Normal flora yeast (◆); TSS (■); TTA (✱); pH (×); alcohol content (▲)

Figure 1 Fermentation profiles during alcoholic fermentation with *S. cerevisiae* (a) and *S'codes ludwigii* (b)

The clearly different yeast count results were found in inoculated juice with *S'codes ludwigii*. The autochthonous yeast population increased for 1 log cfu/ml in the initial stage of fermentation, and it was a main yeast throughout fermentation. It could utilize organic compounds and reduced TSS of pineapple juice since day 1 from 13.0 to 4.4 °brix in day 4 of fermentation. The final population of *S'codes ludwigii* was 5.7±0.1 log cfu/mL (Figure 1b). The survival of *S'codes ludwigii* in fresh crushed pineapple juice during fermentation could be caused it is an indigenous yeast strain which associated with pineapple fruits. This autochthonous yeast strain could relatively better adapt to the environment of proteolytic juice compared to allochthonous yeast (Capece et al., 2019). Chemical characteristics of final fermented juices inoculated allochthonous and autochthonous yeasts compared to fresh crushed pineapple juice were indicated in Table 2. The TTA of fermented pineapple juice samples was relatively constant between 0.77-0.87 %w/v as citric acid in the pH range over the fermentation. The final alcohol content of fermented juice fermented with autochthonous yeast approximately was 7.0 %v/v.

Table 2 *Chemical characteristics of fresh crushed pineapple juice and its ferments*

Chemical characteristics	Values (± SD)		
	Pineapple juice	*S. cerevisiae*	*S'codes ludwigii*
TSS (°brix)	13.0 (± 0.0)	6.2 (± 0.0)	4.4 (± 0.0)
TTA (% as citric acid)	0.73 (± 0.02)	0.87 (± 0.03)	0.77 (± 0.02)
pH	3.29 (± 0.03)	3.46 (± 0.02)	3.57 (± 0.01)
Alcohol content (%v/v)	0.0 (± 0.0)	6.1 (± 0.2)	7.0 (± 0.1)

Conclusion

Autochthonous pineapple yeast, *S'codes ludwigii*, could grow and utilize organic substances and reduced TSS of fresh crushed pineapple juice, as well as achieve complete the fermentation. Thus, the further analysis of its nutrients and VOCs generated during the fermentation and its final products are needed. This study presented the potential of autochthonous pineapple yeast, *S'codes ludwigii*, for applications of alcoholic beverages fermentation derived from fresh crushed pineapple juice.

References

AOAC. (2000). In: *Official methods of analysis of AOAC international*, 17th. Maryland, USA: AOAC International.
Azhar, S.H.M., Abdulla, R., Jambo, S.A., Marbawi, H., Gansau, J.A., Faik, A.A.M. & Rodrigues, K.F. (2017). Yeasts in sustainable bioethanol production: A review. *Biochemistry and Biophysics Reports*, 10, 52-61.
Bosch, L., Alegría, A. & Farré, R. (2006). Application of the 6-aminoquinolyl-N-hydroxysccinimidyl carbamate (AQC) reagent to the RP-HPLC determination of amino acids in infant foods. *Journal of Chromatography B*, 831(1-2), 176-183.
Capece, A., Pietrafesa, R., Siesto, G., Romaniello, R., Condelli, N. & Romano, P. (2019). Selected indigenous *Saccharomyces cerevisiae* strains as profitable strategy to preserve typical traits of primitivo wine. *Fermentation*, 5, 87.
Chanprasartsuk, O., Pheanudomkitlert, K. & Toonwai, D. (2012). Pineapple wine fermentation with yeasts isolated from fruit as single and mixed starter cultures. *Asian Journal of Food and Agro-Industry*, 5(02), 104-111.
Chanprasartsuk, O. & Prakitchaiwattana, C. (2016). Impact of allochthonous and autochthonous yeast starters: Case studies in fruit wine fermentations. In: *Food microbiology: Fundamentals, challenges and health implications*. (edited by E. Perkins). Pp.117-160. New York, USA: Nova Science Publisher, Inc.
Chanprasartsuk, O., Prakitchaiwattana, C., Sanguandeekul, R. & Fleet, H.G. (2010). Autochthonous yeasts associated with mature pineapple fruits, freshly crushed juice and their ferments; and the chemical changes during natural fermentation. *Bioresource technology*, 101(19), 7500-7509.
Fleet, G.H., Prakitchaiwattana, C., Beh, A. & Heard, G.M. (2002). In: Ciani, M. (Ed.), The yeast ecology of wine grapes. *Biodiversity and biotechnology of wine yeasts.* Pp.1-18. Kerala, India: Research Signpost.
Granchi, L., Ganucci, D., Messini, A. & Vincenzini, M. (2002). Oenological properties of *Hanseniaspora osmophila* and *Kloeckera cortices* from wines produced by spontaneous fermentations of normal and dried grapes. *FEMS Yeast Research*, 2, 403-407.
Kurtzman, C.P. & Robnett, C.J. (1998). Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. *Antonie Van Leeuwenhoek*, 73, 331-371.
Ljungdahl, P.O., & Daignan-Fornier, B. (2012). Regulation of Amino Acid, Nucleotide, and Phosphate Metabolism in *Saccharomyces cerevisiae*. *Genetics*, 190(3), 885-929.
López-García, B., Hernández, M. & Segundoe, B.S. (2012). Bromelain, a cysteine protease from pineapple (*Ananas comosus*) stem, is an inhibitor of fungal plant pathogens. *Letters in Applied Microbiology*, 55(1), 62-67.
Loureiro, V. & Malfeito-Ferreira, M. (2003). Spoilage yeasts in the wine industry, *International journal of food microbiology*, 86, 23-50.
Pavan, R., Jain, S., Shraddha & Kumar, A. (2012). Properties and Therapeutic Application of Bromelain: A Review. *Biotechnology Research International*, 2012, 976203.
Ribéreau-Gayon, P., Dubourdieu, D., Donéche, B. & Lonvaud, A. (2006). *Handbook of enology: the microbiology of wine and vinifications*, vol. 1, 2nd. Pp.53-77. Chichester, England: John Wiley and Sons, Ltd.
Romano, P., Marchese, R., Laurita, C., Saleano, G. & Turbanti, L. (1999). Biotechnological suitability of *Saccharomycodes ludwigii* for fermented beverages. *World journal of microbiology and biotechnology*, 15, 451-454.
Sairi, M., Yih, L.J. & Sarmidi, M.R. (2004). Chemical composition and sensory analysis of fresh pineapple juice and deacidified pineapple juice using electrodialysis. Corpus ID, 39239943.
Sanewski, G.M., Bartholomew, D.P. & Paull, R.E. (2018). *The pineapple: botany, production and uses*, 2nd. Wallingford, UK: CABI Publishing.
USDA. Pineapple, all varieties, raw nutrition facts and analysis per serving. [online]. Available: https://www.nutritionvalue.org/Pineapple%2C_all_varieties%2C_raw_nutritional_value.html (15 April 2021).
van Wandelen, C. & Cohen, S.A. (1997). Using quaternary high-performance liquid chromatography eluent systems for separating 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate-derivatized amino acid mixtures. *Journal of Chromatography A*, 763(1-2), 11-22.
Vejarano, R. (2018). *Saccharomycodes ludwigii*, control and potential uses in winemaking processes. *Fermentation*, 4(3), 71; https://doi.org/10.3390/ fermentation4030071.
Wen, L. & Wrolstad, R.E. (2002). Phenolic composition of authentic pineapple juice. *Journal of Food Science*, 67(1), 155-161.