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Dynamic Changes of Physicochemical Properties of Pineapple Juice During Fermentation with Allochthonous and Autochthonous Yeasts under Different Conditions

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Abstract-The allochthonous yeasts are generally exploited as starter for fruit wines production. The unique abilities of these yeasts are useful to fermented beverages industry. The autochthonous yeasts, non-*Saccharomyces* yeasts have been reported to be wild yeasts that produce the unique flavors and exceptional quality when used for traditional wine fermentation. Pineapple juice is one of the juice contains sufficient nutrients for yeast to grow and ferment quality fruit wine. However, the juice contains proteolytic enzymes that may have an inhibitory effect on yeast during fermentation. Thus, species specific strains of yeast may be a key for pineapple juice fermentation. The objective of this research was to investigate the changes of physicochemical properties of fresh crushed Pattawia variety pineapple juice during fermentation with allochthonous and autochthonous yeasts under different conditions including; (i) shaking at 110 rpm in an orbital shaker, (ii) shaking at 110 rpm for 3 days then standing still, and (iii) without shaking. It was found that yeasts population of all conditions rapidly increased approximately 2 log cycles in day 1. After fermentation, allochthonous yeast could reduce total soluble solid of fresh crushed pineapple juice to 4.0–5.0 ^{o}brix through fermentation days while autochthonous yeast had relatively lower nutrients consumption rate as compared with the former. This study also demonstrated that different cultivation condition affected growth profiles and nutrients consumption behavior of yeasts resulting to changes of physicochemical properties of the juice. Based on fermentation profiles investigated, autochthonous pineapple yeast, *M. guilliermondii*, was potential for applications in making beverage derived from pineapple juice fermentation.

Keywords: Allochthonous yeast, autochthonous yeast, fermentation, fruit juice, pineapple

1. Introduction

The Pattawia variety pineapple (*Ananus comosus* (L.) Merr) is an economic tropical fruit of Thailand. Its flesh has pale yellow color, unique sweet and sour taste, and pleasant fruity flavors and aromas which were preferred by consumers. 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).

Typical wine fermentation, the non-*Saccharomyces* autochthonous yeasts dominate the ferment for the first few days before *Saccharomyces* takes over with rising ethanol concentrations (Bisson and Kunkee, 1993). These yeasts are reported to be wild yeasts that produce the unique flavors and exceptional quality when used for traditional wine fermentation. (Fleet *et al*., 2002). *Meyerozyma guilliermondii*, a telemorph of *Candida guilliermondii* or wine yeast, is a non-*Saccharomyces* autochthonous yeast that possesses highly adaptable specie and capable of producing ethanol from a variety of sugars (Maxwell *et al*., 2017). It obtained from various environmental samples, such as Ghanaian cocoa bean heap fermentations (Daniel *et al*., 2009), soil samples within the environments of a local distillery in Nigeria (Gidado *et al*., 2016) and spontaneous fermentation of Queen variety pineapple juice in Thailand (Chanprasartsuk *et al*., 2019).

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 (Chanprasartsuk and Prakitchaiwattana, 2016). In addition, their other abilities, flocculation, and tolerance to ethanol, low pH and high osmotic pressure, are useful to fermented beverages industry (Brice *et al*., 2018; Fleet *et al*., 2002).

The oxygen is necessary for increasing cell mass improving the overall uptake of nutrients. The presence of oxygen could convert yeast metabolism from fermentative to respiratory (Jouhten *et al*., 2008). The oxygen is exploited for their amino acids uptake, and sterol and unsaturated lipid biosynthesis (Ingledew *et al*., 1987; Rosenfeld *et al*., 2003). The yeasts growth rate under continuous aeration conditions were almost two times higher as compared with discontinuous aeration conditions (Cheong *et al*., 2007). Furthermore, the oxygen affects the formation of their generated products, such as esters, higher alcohols, medium-chain fatty acids, branched acids, aldehydes, and ketones which impact on complexity of aromas in final products (Varela *et al*., 2012). The different of cultivation methods could also had affected sensory qualities and characteristics of obtained final products. The objective of this research was to investigate the changes in kinetics of fresh crushed Pattawia pineapple juice inoculated with allochthonous and autochthonous yeasts under different cultivation methods.

2. Materials and Methods

2.1 Preparation of Yeast Culture

A commercial yeast strain, *S. cerevisiae* (Yeast culture collections, Department of food science, Burapha University, Thailand) as allochthonous yeast was used for pineapple inoculation. An indigenous yeast strain, *M. guilliermondii,* isolated from natural fermentation of Pattawia pineapple juice was used as autochthonous yeast. The strains identity was confirmed by morphological examination and molecular methods. The sequence analysis of the 26S rDNA D1/D2 of rDNA and primers were used as the diagnostic methods for examination according to (Kurtzman & Robnett, 1998). These yeasts were cultivated on Malt Extract Agar at 30^{o}C for 3-4 days for inoculum preparation.

2.2 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). 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 (TSS; ^{o}brix), total titratable acidity (%TTA as citric acid), pH nitrogen content (%w/v) and major amino acids (mg/100 mL) (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.

2.3 Inoculation of Yeast Culture to 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^{o}C) for 6 days with 3 different conditions including; (i) shaking at 110 rpm in an orbital shaker, (ii) shaking at 110 rpm for 3 days then standing still, and (iii) without shaking. These cultured juices were collected everyday for microbiological determination and physicochemical analysis. Yeasts population, pH, TSS, TTA and alcohol content (Vinometer, Alla^{â}) were investigated throughout the experiment. All experiments were conducted in triplicate.

3. Results and Discussion

The chemical characteristics of fresh crushed pineapple juice is shown in Table 1. The juice contained 12.50 %TSS, 0.42 %TTA as citric acid, pH 4.63 and 0.07 %w/v of nitrogen content. In previous reports, main sugar of pineapple juice was sucrose 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 TTA as citric acid and pH values of the juice were slightly different from other reports of researchers which were between 0.50-0.80% and 3.60-3.90, respectively. Citric and malic acids are the major organic acids of pineapple juice which found approximately 87 and 13% of total acid content, respectively (Chanprasartsuk *et al*., 2010; 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). For main amino acid contents, the high level of serine and proline had been found in pineapple juice. These results corresponding to a previous report of (Wen & Wrolstad, 2006) indicated that the amino acids identified in Pattawia pineapple juice were tryptophan, asparagine, proline, aspartic acid, serine, glutamic acid, a-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 & Daignan-Fornier, 2012). Thus, the amino profile as found in Pattawia pineapple juice as well as carbon source and also acidic condition demonstrated that pineapple juice itself could be suitable substrate for yeast to grow and convert to produce desirable secondary metabolites. These results indicated that Pattawia variety pineapple juice could be used as raw material for yeast cultivation without any nutrients adding. It contained many carbon sources, vitamins and minerals which the yeast could intake for their growth and reproduction.

The microbiological and chemical properties of pineapple juice inoculated with allochthonous and autochthonous yeasts under 3 different incubation methods monitored were shown in Figure 1. Based on the results of analysis, the yeast counts of inoculated juice under shaking at 110 rpm rapidly increased approximately 2 log cycles to 8.3-8.4 log cfu/mL in day 1 of cultivation. Then, the yeast count was dramatically tended to decline in day 3 to 4.0 log cfu/mL at the end of cultivation (Figure 1a). The similar yeast count results were also found in inoculated juice incubated with shaking at 110 rpm for 3 days then standing still. However, the decreasing rates of yeast population during the late cultivation were relatively slower than the former condition. The final yeast population of this cultivation method was 5.7±0.1 log cfu/mL (Figure 1b).

Table 1. Chemical characteristics of fresh crushed pineapple juice

Chemical characteristics	Values (± SD)
TSS (°brix)	12.50 (± 0.58)
TTA (% as citric acid)	0.42 (± 0.03)
pH	4.63 (± 0.03)
Nitrogen content (%w/v)	0.07 (± 0.00)
Main amino acids content (mg/100 mL)	
Serine	26.81
Proline	17.05
Aspatic acid	9.56
Glutamic acid	9.46
Alanine	6.92

These results corresponded to a significant reducing of TSS during their initial cultivation stages. The TSS of inoculated juices decreased to nearly 4 °brix in day 1, then remained through the cultivation.

For inoculated pineapple juice incubated without shaking (Figure 1c), the yeast population number had increased to 8.3 log cfu/mL in day 1 and still remained at 8.0 log cfu/mL until day 4, then gradually decreased throughout the cultivation. The final yeast reached a population level of 6.1±0.8 log cfu/mL which were relatively higher survival cells count than those of the inoculated juices incubated with shaking. The TSS of these juices were rapidly decreased to 5 °brix in day 1, then slightly decreased around 4 °brix at the end of cultivation. The TTA of all inoculated pineapple juice samples was relatively constant approximately 0.37-0.53 %w/v as citric acid in the pH range over the cultivation. The final alcohol contents of all experiments were between 4.0-5.0 %v/v.

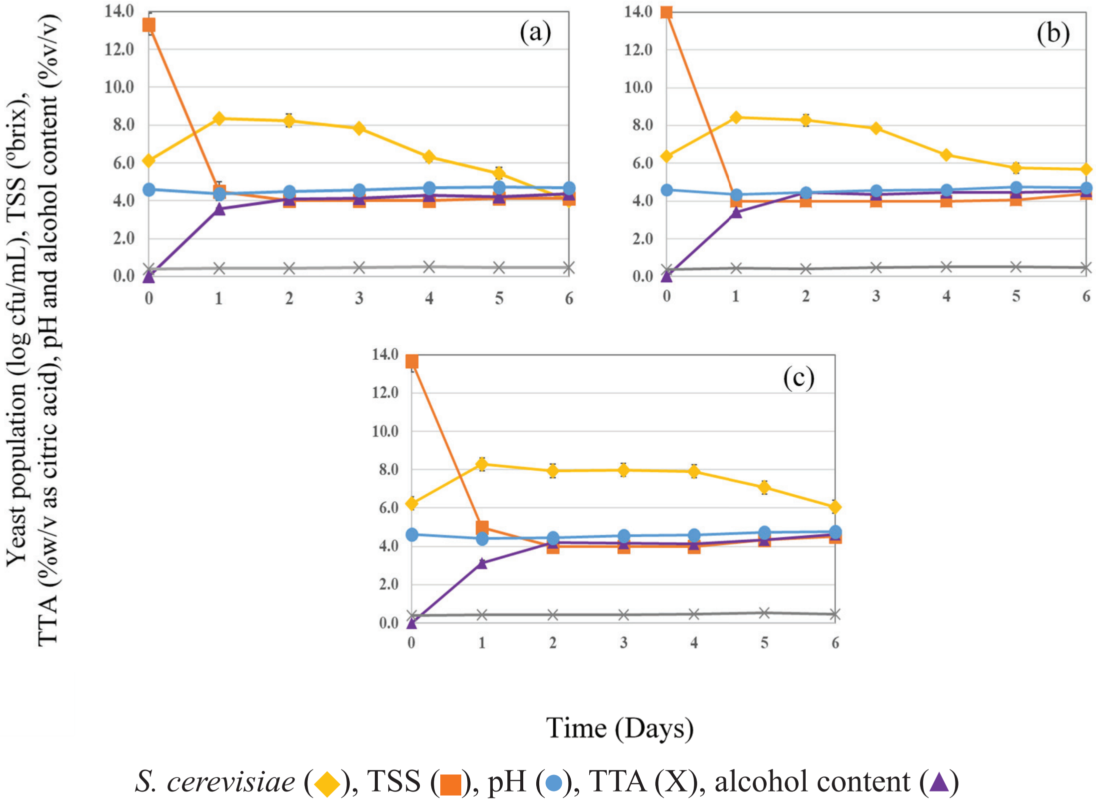
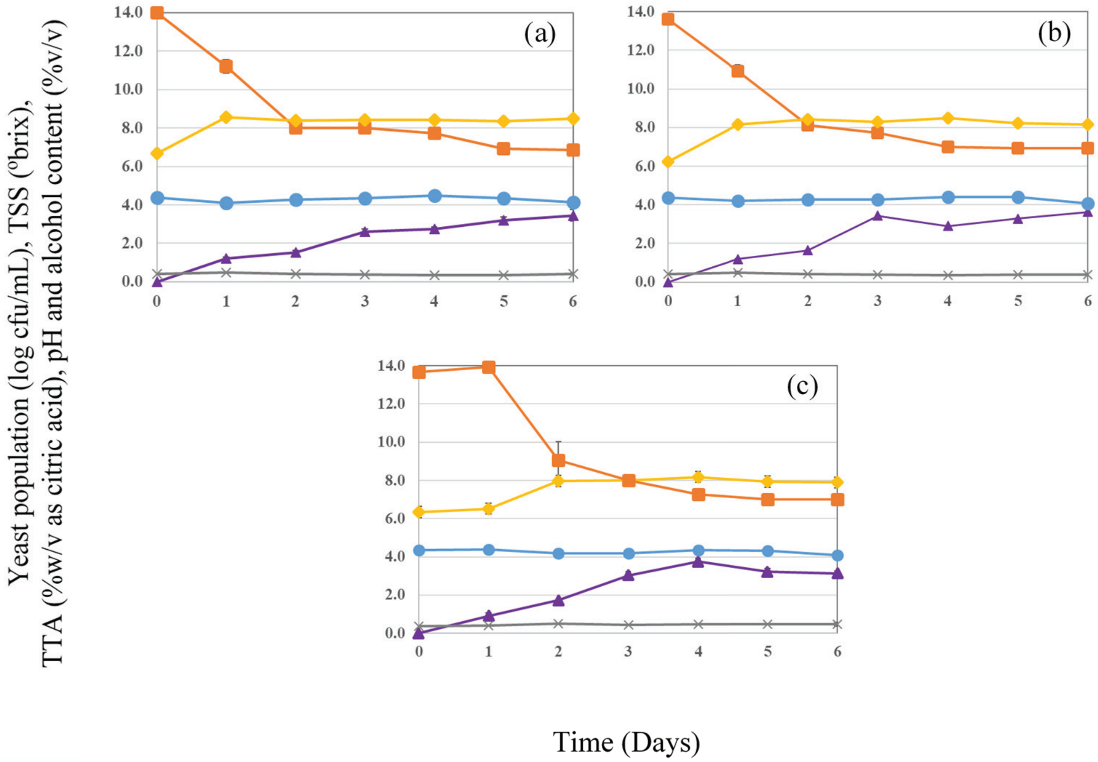


Figure 1. Changes of allochthonous yeast populations, TSS, TTA, pH and alcohol content of inoculated pineapple juice during incubation at ambient temperature for 6 days with 3 different conditions; (a) shaking at 110 rpm, (b) shaking at 110 rpm for 3 days then standing still, and (c) without shaking

The pineapple juice inoculated with autochthonous yeast results were showed in (Figure 2). Under shaking at 110 rpm, the rapid growth of yeast approximately 2 log cycles in the initial cultivation stage of inoculated juice cultivated was observed (Figure 2a and 2b). The yeast count of fermented juice under shaking at 110 rpm for 6 days was rather steady around 8.4 cfu/mL until the end of cultivation. The similar yeast count results were also found in inoculated juice incubated with shaking at 110 rpm for 3 days then standing still. Whereas, the slightly decreasing of yeast count was observed in the final cultivation stage of the fermented juice under shaking at 110 rpm for 3 days then standing still. These results were different from those of fermentation with allochthonous *S. cerevisiae* which clearly decline in the final stage of cultivations (Figure 1). The autochthonous yeast count results were corresponded with the reduction of TSS of pineapple juice. The TSS of inoculated pineapple juices gradually decreased to 8 °brix in day 2, then slightly decreased through the end of cultivation. For inoculated pineapple juice incubated without shaking (Figure 2c), the yeast population number had reached maximum at 8.0 log cfu/mL in day 2 and remained over the cultivation. The slightly increasing of TSS was observed in day 1, then dramatically decreases to 9 °brix in day 2, then gently decreased through the cultivation. The final TSS was 7 °brix at the end of cultivation. The TTA and pH of all inoculated pineapple juice samples were between 0.36-0.49 %w/v as citric and 4.2-4.4, respectively. The final alcohol contents of all experiments were between 3.1-3.6 %v/v.



M. guilliermondii (◆), TSS (■), pH (●), TTA (X), alcohol content (▲)

Figure 2. Changes of autochthonous yeast populations, TSS, TTA, pH and alcohol content of i noculated pineapple juice during incubation at ambient temperature for 6 days with 3 different conditions; (a) shaking at 110 rpm, (b) shaking at 110 rpm for 3 days then standing still, and (c) without shaking

With respect to the results of analysis, Both of allochthonous and autochthonous yeasts could uptake organic substances in fresh crushed pineapple juice as their energy sources for their growth and reproduction. Thus, their TSS had significantly declined in the initial state of cultivation. As stated above, sucrose is a main sugar of pineapple juice which containing approximately 2/3 of total sugars and the rest sugars are glucose and fructose. A commercial *S. cerevisiae* could synthesize and release invertase enzyme that catalyzes the hydrolysis of sucrose into a mixture of monosaccharides, glucose and fructose. From a previous report of (Plascencia-Espinosaa *et al*., 2014), *M. guilliermondii* could generate invertase enzyme possessing a potential for numerous industrial applications since the enzyme was active at high sucrose concentration with thermostability. In addition, not only natural carbon sources contained in pineapple juice, but also have many amino acids as nitrogen sources that could stimulate the yeasts growth during cultivation. Alanine, arginine, asparagine, aspartate, glutamate, glutamine, and serine were reported as preferred nitrogen sources of *S. cerevisiae* yeast (Ljungdahl & Daignan-Fornier, 2012). L-serine is an amino acid playing role in protein synthesis one such metabolite involved in multiple pathways. In addition, it also directed to the synthesis of amino acids such as L-cysteine and glycine (Kanou *et al*., 2020).

Although these yeasts could utilize nutrients in pineapple juice as its energy source, their consumption rates were different. Noticeably, the relatively higher consumption rates at the initial cultivation stage of *S. cerevisiae* than those of *M. guilliermondii* in pineapple juice in all incubation methods were observed. The TSS of pineapple juices inoculated with *S. cerevisiae* rapidly decreased to 4^{o}brix within 1 day of cultivation. The relatively high nutrient consumption rates of *S. cerevisiae* could contribute a lack of nutrients condition in pineapple juice and ethanol toxicity of the high ethanol production leading to its population declining in day 3-4 of cultivation. Whereas, the population numbers of *M. guilliermondii* were constant approximately 8.0 log cfu/mL over the end of fermentation. The TSS of the juices inoculated with *M. guilliermondii* were gradually decreased and seemly constant nearly 7 ^{o}brix at the end of cultivation which relatively higher than those of *S. cerevisiae*. These results could be due to its low fructose consumption rate ability (Khattab & Kodaki, 2016). These yeasts intake nutrients not only for their growth and cell manipulation, but also for the production of alcohol content and other volatile organic compounds in the juices. Corresponding to their final TSS, *S. cereviaise* could utilized more nutrients in pineapple juice to generate relatively higher alcohol content than those of *M. guilliermondii*. As stated in previous report, *M. guilliermondii* performed a higher conversion of glucose to ethanol than fructose preceded by the highest conversion of sucrose during fermentation of sucrose, glucose, and fructose mixtures (Khattab & Kodaki, 2016). The slow nutrient consumption rate of *M. guilliermondii* could be useful to numerous secondary metabolites production which these compounds *act* afterward as *flavor* factors in the final *juices*.

The shaking is an aeration process that oxygen was added into the pineapple juice during propagation which have effected on the growth of yeast. These yeasts are the facultative anaerobe that can grow equally well aerobically and anaerobically in the presence of nutrients. (Cheong *et al*., 2007) reported that yeasts grown under continuous aeration conditions were almost two times higher as compared with discontinuous aeration conditions. The oxygen is necessary for increasing cell mass improving the overall uptake of nutrients. Yeast cells have used oxygen to synthesize unsaturated fatty acids and sterols which form the cell membrane during propagation, however it could lead oxidative stress to yeast cell. Nevertheless, during aerobic metabolism, reactive oxygen species (ROS) including hydroxyl radical (OH^-), hydrogen peroxide and (H_2O_2) and superoxide anion (O_2^-) which are highly damaging have been generated. These reactive molecules could have destroyed protein, DNA and lipids of yeast cell leading to a decline in the number of viable cells and enter the death phase (Brown *et al*., 2018). The initial growth rate of *M. guilliermondii* significantly was affected by the condition of cultivation. The slight increasing of yeast count of inoculated pineapple juices incubated without shaking in day 1 were observed. Thus, their TSS were started to decline in day 2 of incubation. These results were relative different from those of *S. cerevisiae*. No significant effect of shaking on their initial growth rate were observed, however their nutrient consumption rate of the juices incubated without shaking was slightly slower than those of the juices incubated with shaking. Notably, their growth curve of inoculated pineapple juices incubated with shaking entered the death phase in day 3. The behavior of yeast cells could lead to the different characteristics of final pineapple juice, especially their flavor and volatile organic compounds.

Besides of yeast growth, oxygen affects the formation of volatile organic compounds, such as esters, higher alcohols, medium-chain fatty acids, branched acids, aldehydes, and ketones (Varela *et al*., 2012). Thus, the different of cultivation methods could also had affected the complexity of aromas in final pineapple juice products. The alcohol content production in all inoculated pineapple juice incubated with and without shaking was observed. The ability of alcohol production in the juices of *S. cerevisiae* was relatively slightly higher than those of *M. guilliermondii*. These results were also obtained in the production of high fructose glucose syrup with *M. guilliermondii*. Its ability of ethanol production was relatively low during sugar beet molasses fermentation (Khattab & Kodaki, 2016). These unique characteristics of autochthonous yeast, *M. guilliermondii* can be useful to an application of the production of novel beverages from pineapple juices.

4. Conclusion

Allochthonous and autochthonous yeasts could utilize organic substances and reduced TSS of fresh crushed pineapple juice during cultivation with and without shaking condition. Although the autochthonous pineapple yeast, *M. guilliermondii*, had relatively lower consumption rate but it could survival through the cultivation. Its gentle consumption rate in pineapple juice allowing the yeast cell to generate secondary metabolites particularly, VOC contributing to the complexity of VOCs in their final products. Thus, the further analysis of their nutrients and VOCs generated during the cultivation and their final products are needed. This research presented the potential of autochthonous pineapple yeast, *M. guilliermondii*, for applications of industrial beverage production,

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