

Research Article

Fermentation of pineapple juice using wine yeasts: kinetics and characteristics

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Abstract

Fermentation behaviour of wine yeast in winemaking is the subject of considerable interest in relation to wine flavour complexity. The purpose of this study was to examine the fermentation characteristics of different yeast strains in pineapple winemaking. The sterilized pineapple juice was inoculated with yeasts to obtain an initial population of 1×10^6 cells/mL. The fermentation was carried out at 20°C and terminated at 14 days. Yeasts grew during 2 days of fermentation to give a maximum population of 10^7 cells/mL in both inoculated fermentation and natural fermentation. The fermentation rates of *Saccharomyces cerevisiae* and *Kluyveromyces thermotolerans* were rapid in comparison to *Torulasporadel brueckii*. Complete fermentation occurred only in the juice with inoculation of *S. cerevisiae* and *K. thermotolerans*. This research demonstrated how the different strains of yeast affect the kinetics and fermentation characteristics of pineapple wine.

Keywords: fruit wine, tropical, Thailand, *Saccharomyces cerevisiae*, *Kluyveromyces thermotolerans*, *Torulasporadel brueckii*, indigenous yeast

Introduction

Oenological technologies have been developed over thousands of years before anyone knew how fermentation worked. It is well known at present that yeast plays an important role in conversion of grape juice into wine. Today, most winemakers use commercially available strains of yeast for winemaking. These yeasts are usually sold in an active dry form which easily purchased in wine producing countries, e.g. France, Italy, Australia, Spain, Germany, United States, South Africa. The use of different wine yeast strains for grape winemaking has been widely studied and thus winemakers in these countries can readily identify appropriate wine yeasts for their wines. Several oenological technologies are applied to make fruit wines. However, there are many problems encountered by fruit winemakers that require solutions to improve fruit wine quality. Unfortunately, there is little research on fruit wines.

Thailand is the world's leading producer of pineapple [1]. Most of the pineapple produced annually is processed, canned, packaged and exported overseas. In 2000, Thailand had 17% of the world pineapple production market and over 40% of the exports of canned pineapple [2]. As it is normally available fresh, canned and juiced, it is interesting to look for an alternative pineapple product with greater value addition. Pineapple is a fruit possessing attractive flavour and refreshing sugar acid balance. Therefore, pineapple wine shows great potential for fruit white wine making [3-5]. Nonetheless, there are many aspects that have not yet been studied in order to meet consumer acceptability. The purpose of this research was to initiate studies to evaluate and better understand the behaviour of pineapple fermentation by wine yeasts in order to support pineapple winemaking with commercial potential.

Methods

Yeast strains and growth medium

Yeast strains were derived from the collection of the Section of Microbiology and Biochemistry, Hochschule Geisenheim University, 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.

Pineapple juice and fermentation

Pineapple juice was obtained from pineapple grown and harvested from Sadet District, Lampang, Thailand in 2011. After peeling, the fruit was cut and freshly pressed hydraulically (Sakaya Ltd, Thailand). The pineapple juice was sterilized at 121°C for 15 min. Triplicate experiments were carried out in 750-mL fermentation vessels containing 500 mL of the pineapple juice. The yeast inoculum was then aseptically transferred to the fermentation vessel to achieve the initial population of 1×10^6 cells/mL. The fermentation trial was terminated after 14 days. Fermentation kinetics were obtained by monitoring carbon dioxide production during yeast growth.

Determination of viable yeast cells

The viable yeast count was examined microscopically by a counting chamber slide following the protocol of the Department of Microbiology and Biochemistry, Hochschule Geisenheim University, Germany [6]. Cells (450 μ L) were added to 50 μ L of methylene blue solution (0.4% methylene blue, 10% ethanol and 0.4 M KH_2PO_4) and mixed. Blue cells were counted as dead cells, while cells without obvious colour were counted as live cells.

Determination of pH value

Acidity (pH) value of the juice and the fermented juice were determined by a pH meter (Model C831, Consort, Belgium) at room temperature.

Determination of titratable acidity

Distilled water (25 mL) and phenolphthalein indicator (2-3 drops) were added to the juice or fermented juice (5 mL). Phenolphthalein solution (2-3 drops) was used as indicator. Then the juice or the fermented juice was slowly titrated with the 0.1 N NaOH. The point of neutrality was reached when the indicator changed from colourless to pink. Total acidity (%) was expressed as citric acid based on 1 mL of 0.1M NaOH was equivalent to 0.0070g citric acid [7].

Determination of total soluble solids

The total soluble solids (TSS) was estimated by a hand refractometer (N-1 α , Atago, Tokyo, Japan). The results were expressed as degree Brix ($^{\circ}$ Brix).

Determination of alcohol content

The alcohol content in the sample was measured by ebulliometry [8]. The ebulliometer (Dujardin – Salleron, Noizay, France) was used to determine the boiling temperatures of distilled water and the sample. The alcohol content was indicated by using the temperatures to read directly from the calculation disk of the ebulliometer.

Determination of free alpha amino nitrogen (FAN)

Ninhydrin colorimetric method was used to measure free alpha amino nitrogen in the juice and the fermented juice. The modified method of Chomsri [6] was applied for analysis.

Optical microscopy

Enumeration of yeast during pineapple juice fermentation was examined using a microscope. Fermented pineapple juice during fermentation was inspected using an optical microscope (1000x) to observe the yeast growth.

Determination of fermentation kinetics

During pineapple juice fermentation, the weight of each container was measured to monitor carbon dioxide evolution, a measure of the fermentation progress [6]. Estimation of fermentation kinetic parameters are described as follows.

R_x	Rate of cell formation (cell/mL/d)
R_{EtOH}	Rate of alcohol formation (g/L/d)
R_{CO_2}	Rate of carbon dioxide formation (g/L/d)
R_s	Rate of sugar consumption (g/L/d)
R_{FAN}	Rate of FAN consumption (mg/L/d)
$Y_{p/s}$	yield coefficient of ethanol on sugar (g ethanol/ g sugar)
$Y_{FAN/s}$	yield coefficient of FAN on sugar (mg N/g sugar)

Results and Discussion

Figure 1 shows pure and indigenous yeast growths during fermentation of pineapple juice. Fermentations were carried out using pineapple juice containing 145 g/L of sugar and 0.46% of acidity as citric acid. Maximum propagation rates of *S. cerevisiae*, *T. delbrueckii* and *K. thermotolerans* were obtained at the 2nd day of fermentation while the indigenous yeast concentrations were slowly increased until the 6th day of fermentation. The pure yeast maintained cell concentrations of about 10^7 cell/mL for 2-4 days and declined 0.6-1.2 logarithmic units lower than the maximum cell concentrations at the end of fermentation. In comparison to the natural yeast, yeast cell concentration reduced sharply after 6 days of fermentation and there was a significant increase of cell concentration on the 10th day. This could reflect the interaction of microflora in pineapple juice influencing the behaviour of yeast growth in the spontaneous fermentation.

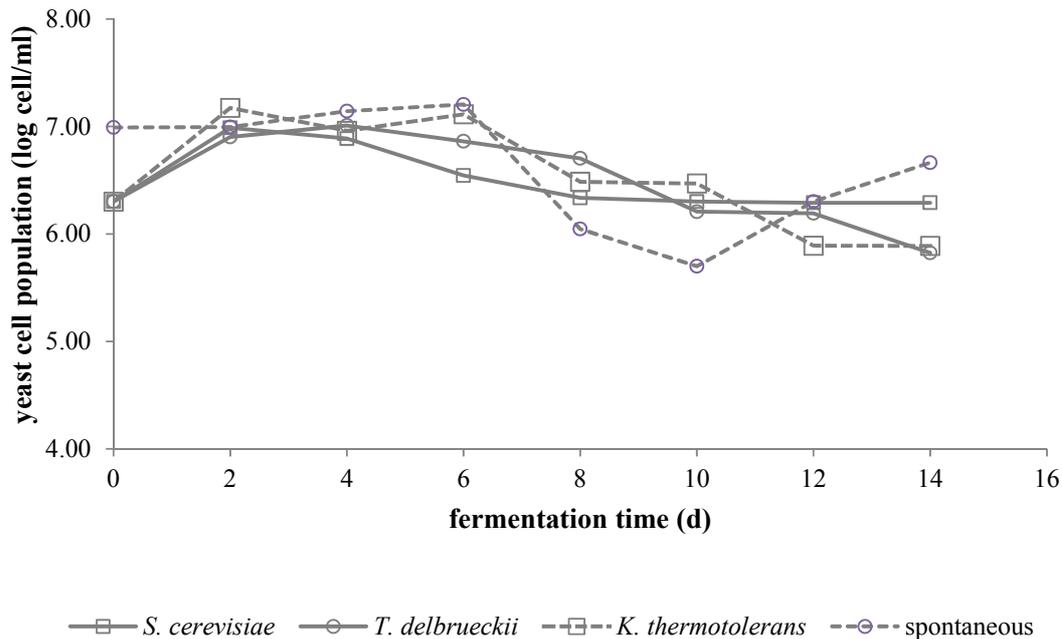


Figure 1. Yeast growth during fermentation of pineapple juice with wine yeasts.

From an inspection of micrographs shown in Figures 2 and 3, it can be seen that similar yeast cell sizes and shapes were observed in the micrographs obtained from pineapple juice fermented with pure yeast. The cell yeast density appearing on the micrographs was related to yeast cell concentration during fermentation as reported previously. Different yeast cell sizes and shapes and other microorganisms appeared in the spontaneous fermentation. Furthermore, distinct shapes like apiculate yeast, e.g. *Hansenispora*, were found in the images of the spontaneous fermentation. This suggested that apiculate yeast might be associated with the spontaneous fermentation in this study. A recent study by Chanprasartsuk *et al.* [4] discovered *Hansenia sporauvarum* and *Pichia guilliermondii* were the main yeast species observed on the fruit skins and *P. guilliermondii* was likely to initiate the fermentation, with *H. uvarum* becoming more prevalent and persistent in playing a role in the fermentation system. The study also indicated that the other wine yeasts, in particular *Saccharomyces* yeasts, were not found in any of the natural fermentation systems of pineapple juice.

During the fermentation, the yeasts involved in the pure and spontaneous fermentation consumed sugar and released primary products, i.e. ethanol and carbon dioxide. The profiles of sugar consumption and carbon dioxide liberation of *S. cerevisiae* and *K. thermotolerans* were similar in the pineapple juice fermentation but were different to *K. thermotolerans* in the spontaneous fermentation (Figure 4). Indigenous yeasts utilized sugar and produced carbon dioxide in slightly higher amounts than *T. delbrueckii*. This result would be expected from the yeasts possessing high fermentability present in the microflora of pineapple juice.

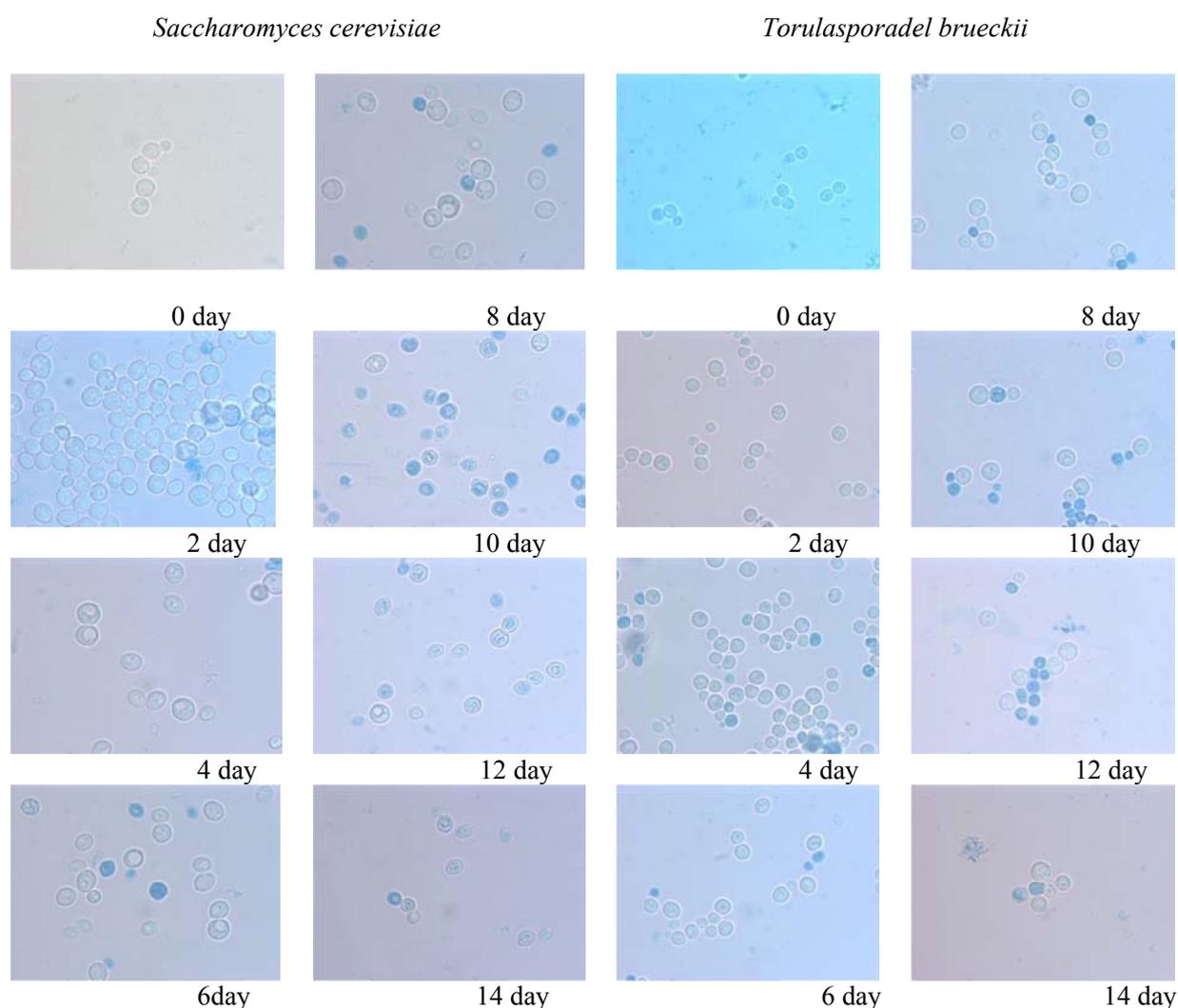


Figure 2. Micrographs of *Saccharomyces cerevisiae* and *Torulaspordel brueckii* evolution in pineapple juice fermentation, —bar = 10 μ m

Yeast assimilable nitrogen (YAN) is essential to the growth and metabolism of yeast during fermentation. In grape juice amino acids are a major source of YAN for yeast. For quantification of YAN in this study, free alpha amino nitrogen (FAN) was measured. The uptake of YAN by *S. cerevisiae* and *K. thermotolerans* was greater than *T. delbrueckii* and the indigenous yeast in pineapple juice fermentation. The result indicated that the nitrogen requirement distinguished the strains of *K. thermotolerans* and *T. delbrueckii*. Nitrogen requirements of yeast, particularly *S. cerevisiae* have been extensively reviewed [9-15]. Concentration of total amino acids in grape juice varied greatly between 65-2,721 mg/L [16-17], with the YAN content approximately 50-350 mg/L. Arginine and proline are the primary and secondary amino acids in grape juice, respectively. In contrast, asparagines, a preferentially utilized amino acid, is the most abundant in pineapple juice and available as YAN with the concentration of 63 times greater than in grape juice [18]. Proline classified as a yeast-non-assimilable nitrogen (YNAN) is present as a small amount in pineapple

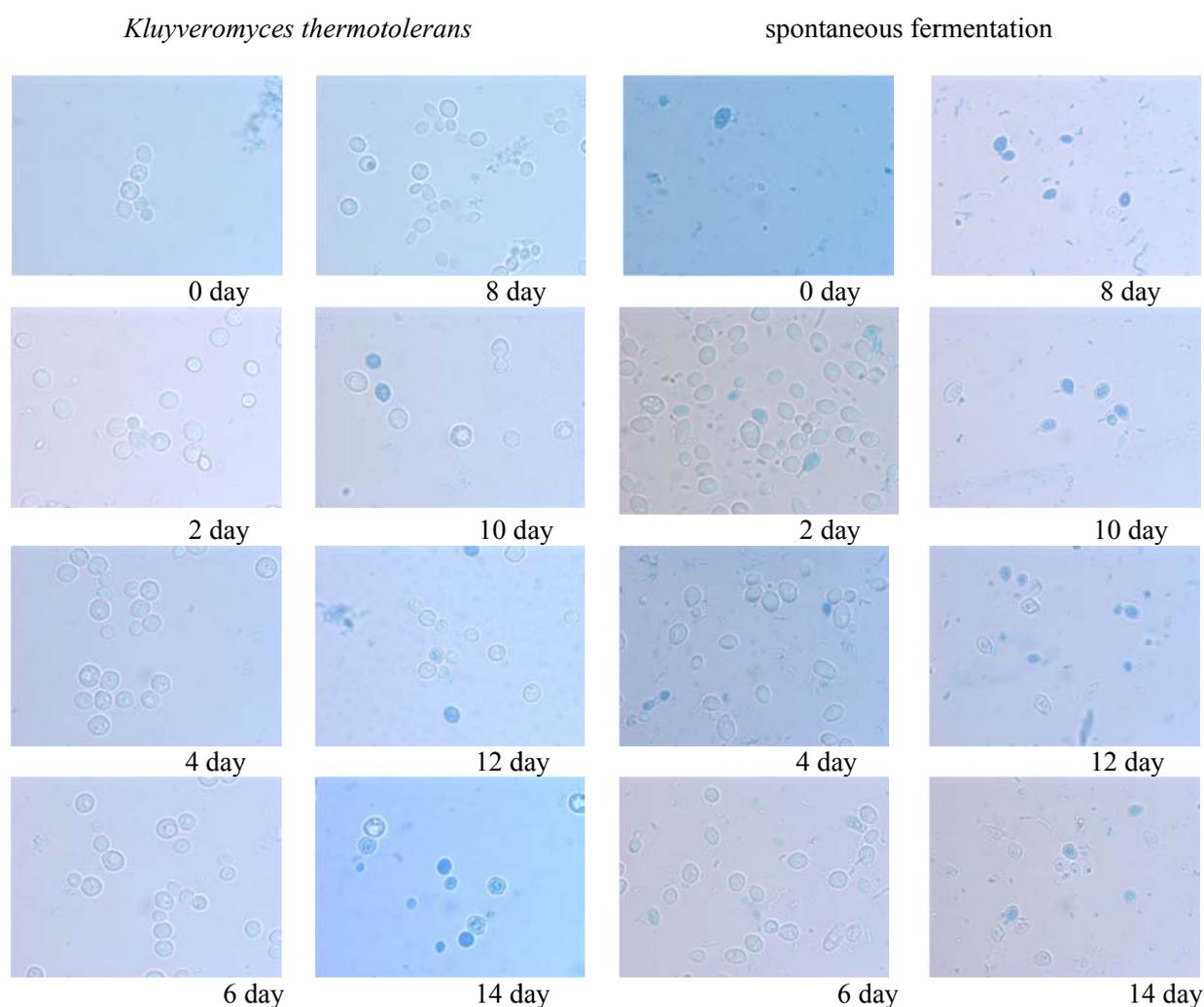


Figure 3. Micrographs of *Kluyveromyces thermotolerans* and natural yeast evolution in pineapple juice fermentation, — bar = 10 μ m

Considering FAN, measurements in this study revealed approximately 1700 mg/L (290 mg FAN/L) of amino acids in pineapple juice, compared with the average of amino acid content in grape juice of 1500 mg/L (260 mg FAN/L) [16]. It is generally agreed that a concentration exceeding 140 mg N/L is adequate for a low risk of sluggish or stuck fermentation [19, 20]. This means that pineapple juice used in this trial was an adequate and efficient source of YAN to support yeast growth during its fermentation.

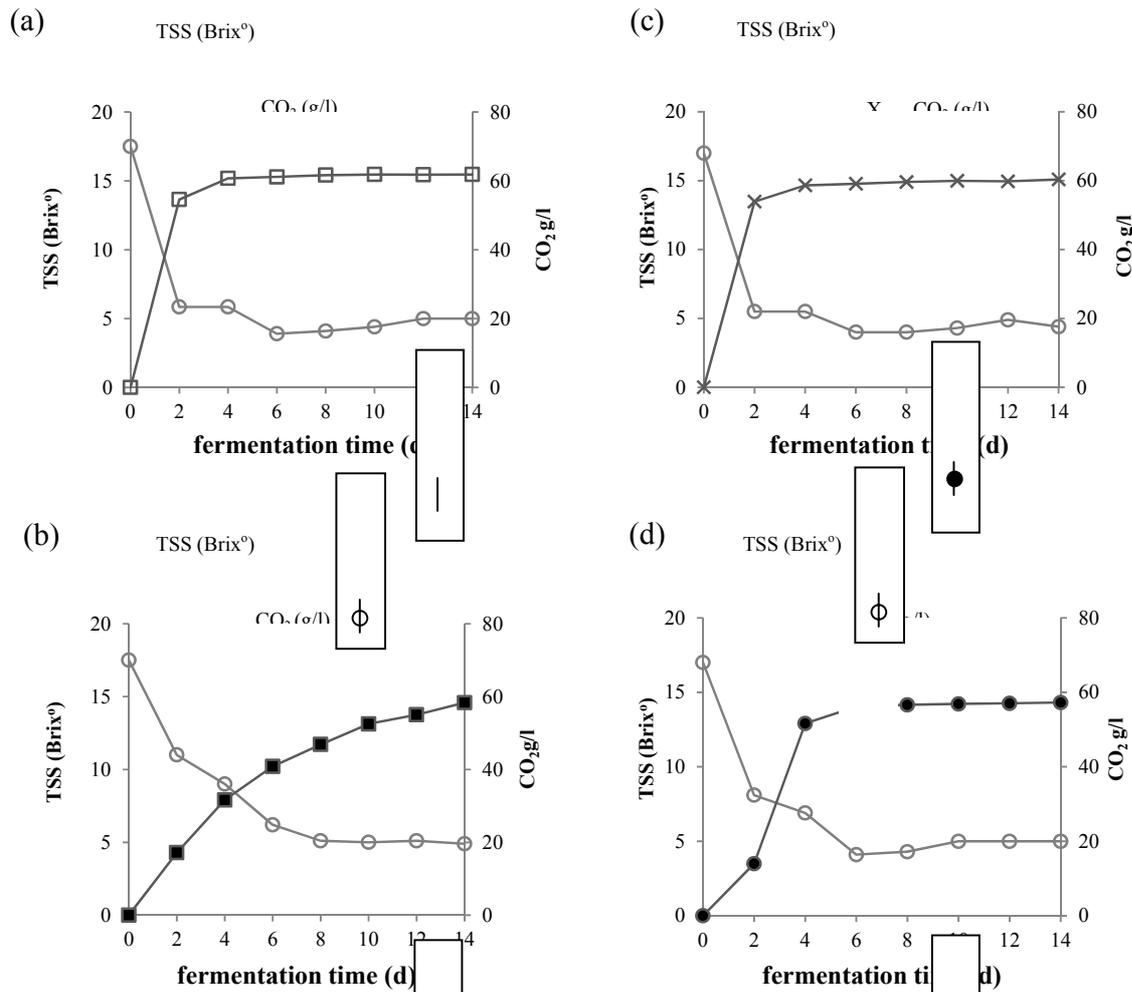


Figure 4. Fermentation profile of pineapple juice fermented with wine yeasts for 14 days, *S. cerevisiae* (a), *T. delbrueckii* (b), *K. thermotolerans* (c) and spontaneous fermentation (d).

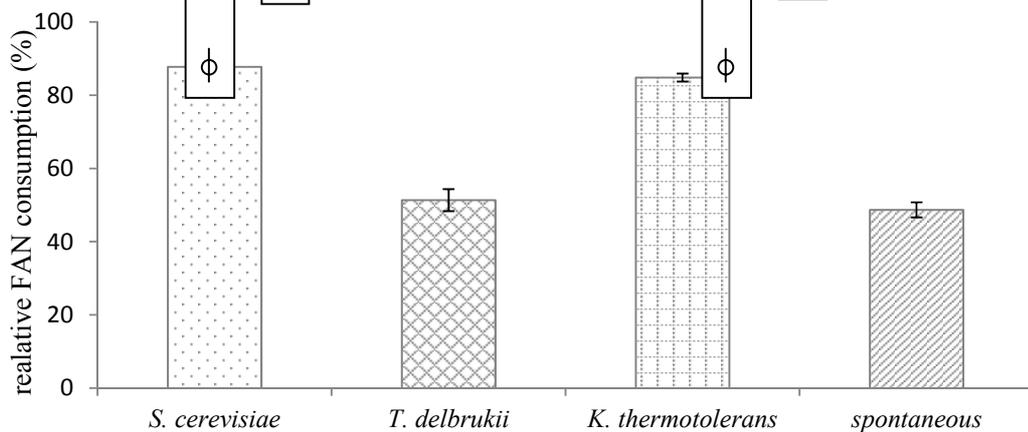


Figure 5. Relative FAN consumption of wine yeasts in pineapple juice fermentation.

Fermentation kinetics of wine yeasts and natural yeasts in the pineapple juice varied significantly among yeast species (Table 1). The maximum cell formation rate of 6.45×10^6 cell/mL/d was attained in pineapple juice fermented with *K. thermotolerans*. Rates of sugar consumption corresponded to rates of alcohol formation and carbon dioxide formation. The yeast species with the fastest rate of sugar consumption in pineapple juice fermentation was *S. cerevisiae*, which completed fermentation within five days. *T. delbrueckii* had the slowest fermentation and *S. cerevisiae* and *K. thermotolerans* fermented faster than *T. delbrueckii* in pineapple juice. This fermentative behaviour was also observed in grape juice fermentation of other research [6].

Table 1. Comparative fermentation kinetic parameters of pineapple juice fermentation using wine yeasts.

Treatment	R_x (cell/mL/d)	R_{EtOH} (g/L/d)	R_{CO_2} (g/L/d)	R_s (g/l/d)	R_{FAN} (mg/L/d)	$Y_{FAN/s}$ (mg N/g sugar)	$Y_{p/s}$ (g ethanol/g sugar)
<i>S. cerevisiae</i>	3.86×10^6	15.54	14.86	30.40	18.35	2.11	0.51
<i>T. delbrueckii</i>	2.05×10^6	4.41	4.21	8.62	10.73	1.25	0.51
<i>K. thermotolerans</i>	6.45×10^6	10.28	9.83	20.10	17.75	2.06	0.51
spontaneous	1.05×10^6	7.50	7.18	14.68	10.18	1.21	0.51

Conclusion

The present study shows different kinetics of wine yeast in pineapple juice fermentation. Most interesting is the finding that pineapple juice had an adequate and efficient yeast assimilable nitrogen source for fermentation. Yeast assimilable nitrogen affects the production of the major metabolites arising from sugar fermentation which influences aroma and flavour of the wine. Although more studies are need, the preliminary data of this study confirmed the potential of pineapple juice to make wine. Scale-up and optimization of the fermentation process and the associated kinetics, characteristics and organoleptic evaluations are also necessary.

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