Research Article

Journal of Applied Science and Emerging Technology (JASET)
Published by Faculty of Applied Science, KMUTNB, Thailand
doi: 10.14416/JASET.KMUTNB.2025.01.004
Vol. 24, No. 1 [2025]: e255747

Application of Yeast Strain *Saccharomyces cerevisiae* to Produce Wine by Using Rice and Pineapple as Raw Material

Nutthawut Meesilp[1]*, and Yanapath Suksoem[1]

[1]Department of Applied Biology, Faculty of Science and Liberal Art, Rajamangala University of Technology Isan, Nakhon Ratchasima, 30000 Thailand

* Corresponding Author, E-mail: nutthawutm@hotmail.com DOI: 10.14416/JASET.KMUTNB.2025.01.004
Received 19 February 2024; Revised 13 January 2025; Accepted 21 January 2025

ABSTRACT

The purpose of this research was to produce fruit-flavored fermented glutinous rice wine by using yeast *S. cerevisiae* with rice and pineapple as raw materials for fermentation. Kiaw-ngu glutinous rice variety and pineapple were prepared as the mixture solutions with different ratios (rice : pineapple) as 100%–20% (v/v). Total 9 formulas of experiment were established for 7 days under anaerobic condition, and measured the characteristics of fermentative solutions in each day. The fermentation exhibited initial pH in acid ranges (4.50–4.70) and high total solid (15.0-21.9 °Brix). Alcohol value, clarity, and yeast population have gradually increased since 2 days of fermentation, whereas, pH and total solid had continuously decreased until 7 days. Especially, the mixture solution of ratio 80:20 exhibited the highest percentage of alcohol value as 9.77%, including, population *of S. cerevisiae* did not decline with average values as 7.51 log cfu/ml. On the other hand, pH, and total solid value have continuously decreased until 7 days of fermentation.

KEYWORDS: Yeast, *Saccharomyces cerevisiae*, Alcoholic beverages, Glutinous rice, Pineapple

Please cite this article as: N. Meesilp and Y. Suksoem, “Application of Yeast strain Saccharomyces cerevisiae to Produce Wine by Using Rice and Pineapple as Raw Material,” *Journal of Applied Science and Emerging Technology,* vol 24, no 1, pp. 1-9, ID. 255747, April 2025

1. INTRODUCTION

Wine is healthful alcoholic beverage with unique flavor and chemicals compositions containing water, ethanol, organic acids, phenolic compounds, proteins, polysaccharides, and numerous aroma compounds favored by consumers worldwide. Many popular fruits such as apple, pear, strawberry, cherries, plum, pineapple, oranges kiwi, banana, caja, mango, gabiroba, cocoa and cupuassu are widely utilized as majority raw materials to produce wine (Thungbeni et al., 2020). Colors, mouth feel, and aroma of wine have depended on composition of phenolics and volatile compounds (Zhai et al., 2023; Wang et al., 2022). Macromolecules of polysaccharides in wine compositions have the influence on wine colloidal exhibiting the interaction of phenolics and volatile compounds, including stability and organoleptic properties. Abundant polysaccharides found in several wine are arabinogalactan protein, type II rhamnogalacturonans, and mannoproteins (Zhai et al., 2023). The important role of polysaccharides is protective colloids increasing the clarity and stabilization by inhibiting or limiting aggregation, flocculation and precipitation of colloidal particles (Alcalde–Eon et al., 2014; Riou et al., 2002), including colloidal coloring matter and tannin precipitation at cold temperature or other unstable conditions (Zhai et al., 2023).

Rice (*Oryza sativa*) is vital cereal and important economic crop. This plant contains the major nutrient source of carbohydrate and other dietaries such as protein, lipid, vitamin, and fiber. In present, there are many risks involving in plantation and production of rice such as low price in markets, acid and saline soil, plant pathogen and pests, non–standard rice seed, non–disease resistant breed, drought, and water scarcity (Verma and Srivastav, 2020). Kiaw-ngu glutinous rice is a popular variety, which widely consumed in Thailand with important nutrients. Characteristics of rice grains is slender and long shape, including, they will be shinier and clearer after cooked than glutinous rice. Abundant vitamin E of rice playing as tocopherols (α-, γ-, and δ-tocopherols), and γ-oryzanol exhibits the antioxidant activity (Sudtasarn et al., 2019). Glutinous rice has explored the highest ethanol in conversion to acetic acid of vinegar fermentation with *S. cerevisiae* and *Acetobacter pasteurianus*, compared to Thai rice-polished, black fragrant rice, and black glutinous rice (Taweekasemsombut et al., 2021). Several rice products and their by–product from processing have been developed as human foods, and animal feeds such as vinegar, bakery, alcoholic beverage, rice bran oil, bran, and rice straw. Rice fermentation can be processed to several kind of foods and beverages such as traditional alcoholic drink (Sato) and fermented rice cake (Khao–Maak). Sato is an alcohol product manufactured from glutinous rice as raw material in fermentation process occurred by microbes in fungal group. These processes have occurred by two step as follow changing rice starch to sugar with molds, and then utilization of sugar for producing alcohol with yeast. Flavor and odor of Sato or Khao–Maak can be adapted by food additive, depended on varieties of rice and other mixtures such as banana, pea, and millet, including herbs as anti–pathogenic microbial agents such as pepper, garlic, and galangal (Tian et al., 2022). The semi–solid state fermentation of rice wine lacking amino acids and glucose due to complex structures would release the sub-units substances at late stage of fermentation slower than liquid–state fermentation of fruits juice (He et al., 2022). Thus, the amount of alcohol is the important indicators of quality of rice wine showing value lower. While, alcohol values of fruit wine are higher than only application of rice, which depended on types of juice as raw materials. They are used as carbon and nitrogen source in fermentative processes of microorganisms (Hirst and Richter, 2016; Wang et al., 2022, Wei et al., 2016).

Saccharomyces cerevisiae is the major yeast strain used in foods fermentation with or without alcohol products such as bakery, yeast extract, baker yeast, β–glucan, organic acids, alcoholic beverage, and kefir (Liszkowska and Berlowska, 2021). Moreover, other microorganisms that convert cereal starch into sugars, alcohol, and organic acids such as *Amylomyces* sp., *Penicillium* sp., *Aspergillus* sp., *Monascus* sp., *Actinomucor* sp., *Rhizopus oligosporus*, and *Mucor racemosus*, *S. fibuligera*, *Wickerhamomyces anomalus*, and *Pediococcus pentosaceus* (Hesseltine et al., 1988). Sugars found in fruit juice are used and continuously changed into alcohol including organic acid occurred by microorganisms that sugar can be reacted to form aldehydes, esters and other chemical components (Watanabe and Shimazu, 1980).

Pineapple (*Ananas comosus*) is monocotyledonous plant, which is consumed as fresh fruit or applied to manufacture various food products. This fruit has the special aroma and important nutrients. Chemical compositions of pineapple effecting on flavor quality of fruit due to rich in ester compounds consisting methyl–2–methylbutanoate, methyl hexanoate, methyl–3–(methylthiol)–propanoate, methyl octanoate, and 2–methoxy–4–vinyl phenol. Moreover, their phytochemicals and functional bioactive compounds such as antioxidants, organic acids, bromelain, phenolic compounds, and flavonoids have the benefit for health (Ali et al., 2020; Hossain and Rahman, 2011; Roda et al., 2017).

Rice and pineapple contained many beneficial compounds for health. Therefore, the aim of the research was to produce fruit-flavored fermented glutinous rice wine by using yeast *S. cerevisiae* with rice and pineapple as raw materials.

2. MATERIALS AND METHODS

2.1 Rice preparation

Kiaw-ngu glutinous rice variety, as raw material of wine production was prepared by washing with water, and then soaked in distilled water for 6 hours. Soaked rice was washed again for 2 times before steaming at 100 °C for 20–30 minutes, and then washed again following air dry for 10 minutes. Prepared rice 500 g. was blended, filled with sterilized distilled water 1,000.0 ml, and mixed with 0.2% (w/w) alpha-amylase (LD Carlson company) (EC 3.2.1.1) (National Center for Biotechnology Information, 2025) (1.18 g per 100.0 g of raw rice). Rice with enzyme was reacted by incubating at room temperature (25 °C) for 4 hours (Meng and Kim, 2020).

2.2 Yeast preparation

S. cerevisiae was prepared by culturing and purifying on Yeast Malt (YM) medium (yeast extract 3.0 g/l; malt extract 3.0 g/l; peptone 5.0 g/l; glucose 10.0 g/l; agar 15.0 g/l) with incubation at 30 °C for 24–48 hours. Starter of yeast inoculum was prepared by culturing with YM broth with incubation at 30 °C, 120 rpm for 24–48 hours. Yeast cell were harvested by using a centrifuge at 8,000 rpm for 10 minutes, and discarded a supernatant. Cell pellet was twice washed, and re–suspended with distilled water to solution. Turbidity of yeast cell suspension was adjusted with McFarland scale 0.5 (8 log cfu/ml).

2.3 Pineapple preparation

A fruit was washed with tap water, and peeled. Then, pineapple was sliced to small pieces and finely blended for 5 minutes. Pineapple juice was prepared by separating its residue with sterilized thin curtain linen fabric, and then pasteurized at

75 °C for 5 minutes followed by cooling immediately. Finally, pineapple juice with 100% concentration was prepared, and total solid has been estimated by using Hand refractometer.

2.4 Fermentation of wine

2.4.1 Formulas of production

The fermentation was established with 5 formulas with 3 replications based on various concentration of rice as follow 0%, 50%, 60%, 70%, and 80% (v/v), which combined with pineapple juice as 100%, 50%, 40%, 30%, and 20% (v/v), respectively. All formulas were fermented in sterilized glass bottles containing a final contents 1,000.0 ml and closed with airlock, and incubated at room temperature for 7 days. Fermentative suspensions were collected during 0-7 days after inoculation. The samples were measured their physical, chemical, and biological characteristics as follow the percentage of alcohol, total solid, pH value, clarity, yeast amount, color, and gas production.

2.4.2 Total solid measurement

Fermentative suspensions collected from each days during 0–7 days were measured the value of the total solid changing by using Hand refractometer, and expressed the value as Brix degree (°Brix).

2.4.3 Alcoholic estimation

Alcohol value of fermentative suspensions collected from each days during 0–7 days were measured by using Ebulliometer. The suspensions 50.0 ml were prepared in chamber of equipment, and heated until stable temperature. Likewise, distilled water was boiled until constant temperature. The suspensions have reached boiling point with steady temperature, read the values on a thermometer. Alcohol contents of samples were investigated by comparing the boiling point between water and wine products. On the disc, alcohol by volume on outer disc were observed, which corresponded to the boiling point on the inner disc and express as percentage of alcohol.

2.4.4 pH value

Samples of fermentation from 0–7 days were collected by using 15.0–20.0 ml of solution to measure the value of acid–base changing by using a digital pH meter.

2.4.5 Clarity measurement

Fermentative solutions by 10.0 ml were collected during 0–7 days. Fermentative suspensions were shaken homogeneous to mixture before collection of the samples. Then, the turbidity of suspensions were measured the value by using a spectrophotometer with an absorbance at OD 570 nm.

2.4.6 Yeast amount measurement

Fermentative samples during 0–7 days were shaken before collecting by 1.0 ml. Preparing diluents of fermentative solutions were done by using distilled water by 9.0 ml. Then, the optimal diluents of suspensions were taken to a Haemacytometer for counting amounts of yeast cells under light microscope at 40X lens. The result of yeast numbers from samples were presented in the unit of log cfu/ml.

3. RESULTS AND DISCUSSIONS

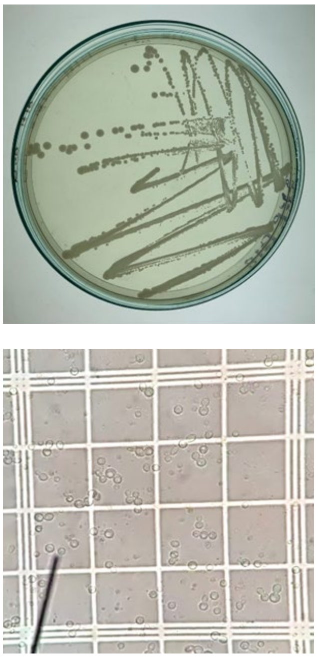
3.1 Yeast characteristic

The characteristics of yeast, *S. cerevisiae*, in the experiment have been proved the purity before application, which exhibited the colony morphology on YM medium after 24 hours as follow white, circular form, raised elevation, and entire margin. In addition, cell characteristics investigated under light microscope have exhibited the cylindrical, spherical, oval shape, and budding cell (Figure 1). Microorganisms could be apply to produce many kinds of foods, which enhanced nutritional properties of food products such as flavor, aroma and texture.

Products of fermented foods and beverage, including bioactive compounds such as alcohol, organic acid, peptides, enzyme, and anti-nutritive have been occurred by using the activities of microbial metabolisms (Wang et al., 2018, 2020). Final stage of fermentation was the important processes, which would release many bioactive and metabolite compounds such as gallic, protocatechuic, caffeic, ferulic, lactate, fumarate, succinate, malate, amino acids, peptides, fatty acids, and unsaturated fatty acids (Lee et al., 2016; Jiang et al., 2020). *S. cerevisiae* as important yeast has been used in food fermentation, especially alcoholic products. Its physiology endured to toxicity of ethanol making yeast continuously grew under the stress conditions (Parapouli et al., 2020). Likewise, *S. bayanus* and *S. ellipsoids* as other yeast strains contained the ability to produce ethanol in fermentation (Ekechukwu et al., 2020). In addition, yeast could be generally discovered in nature, especially fruits and vegetable, which allowed the spontaneous fermentation.

3.2 Alcohol measurement

Alcohol contents of fermentative production have been increased since 1 days until 7 days, which depended on various concentration of pineapple juice as shown in figure 1. All formulas of fermentation at 0 day lacked any alcohol values that they were used as the reference data with other days to compare the value changing. Then, alcohol contents have slowly increased after incubation for 1-2 day with equal value in all formulas. Each formula has been found the different values of alcohol after 3 days of incubation that the application of 100% pineapple juice has continuously increased alcohol showing the highest content, compared to others. All formulas have extremely contained alcohol contents since 3 days until 7 days, and the steady alcohol productions from yeast had been discovered at days 7. At the end of fermentation, high concentration of 80% and 70% rice (20% and 30% pineapple, respectively) exhibited the highest alcohol content, whereas, high concentration of 100% pineapple (0% rice) displayed the lowest content, compared to other formulas. Ethanol yield



A.

B.

Figure 1 Colony of *S. cerevisiae* on YM medium (A) and cell morphology under 40X of light microscope (B) at 24 hours after incubation.

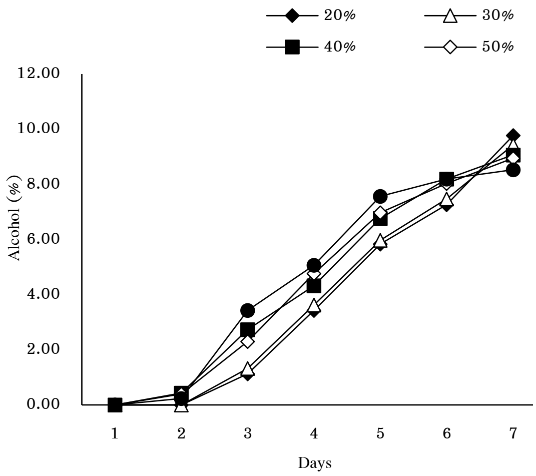


Figure 2 Alcohol contents from rice and pineapple fermentation with *S. cerevisiae* during 1-7 days (20%, 30%, 40%, 50%, and 100% of pineapple juice)

depended on the levels of sugar used as the carbon source for yeast growth. Macromolecules, starch of rice, as raw material of fermentation had to pre-treat with amylolytic enzymes to hydrolyze glycosidic linkages in starch molecule into sugar such as maltose and glucose (Rasiah and Rehm 2009; Magalhães and Souza, 2010). This reaction has occurred by the activities of α-amylase (α–1, 4–glucan–4–glucanohydrolase) and glucoamylase enzyme that they could be obtained from several mold strains such as *Rhizopus*, *Amylomyces* sp., and *Mucor* sp., *Aspergillus* sp. In addition, amylolytic enzymes as amylases could be applied in the processes of starch degradation and liquefaction to produce alcohol products of brewing and sugar industries (Sanghvi et al., 2011), including bread and baking, saccharification, textile desizing, paper, detergent (Metin et al., 2010). The recovery of ethanol from starch has been found as 56.9%, which depended on two molecules of ethanol per glucose repeating unit in starch (Sanchez et al., 1988). Amylose contents displayed as the important factor to recover ethanol. Waxy and low–amylose rice verities have been found the highest efficiency to recover ethanol. Waxy rice has been greatly developed as traditional rice wines and the Japanese mirin (Kaneko and Kumazawa, 2015), including, low–amylose rice has been used to produce Japanese rice wine sake and adjunct in beer brewing (Marconi et al., 2017). Gelatinization at low temperatures have contained low amylose content. Starch contents have depended on rice varies as follow waxy rice (1.4 - 1.9%), low–amylose rice (4.2 - 18.6%), and intermediate- and high-amylose rice (30.5 - 38.2%) (Sanchez et al., 1988). Modification of important factors as follow the ratio of rice and water, extending steaming time, strains of microbial starter, time of saccharification, and the moisture content of substrates during fermentation

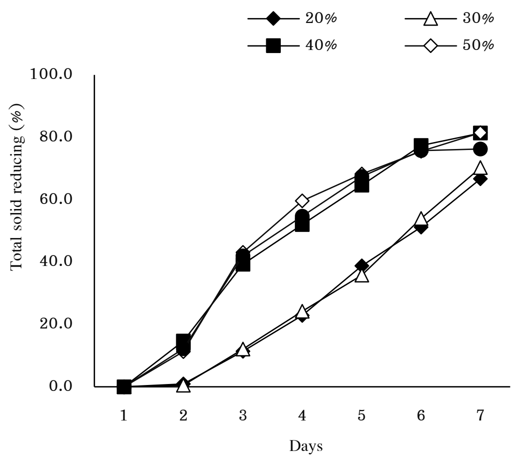


Figure 3 Total solid reducing of rice and pineapple fermentation with *S. cerevisiae* during 1-7 days (20%, 30%, 40%, 50%, and 100% of pineapple juice).

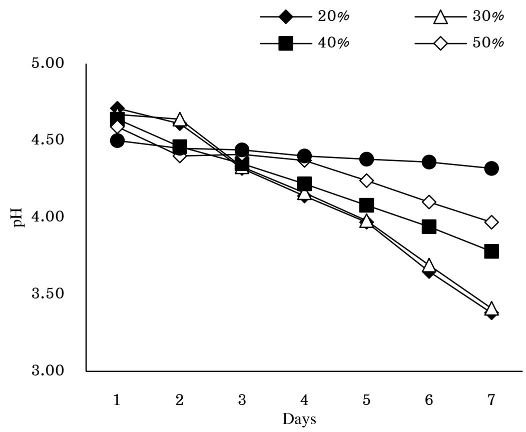


Figure 4 pH changing of rice and pineapple fermentation with *S. cerevisiae* during 1-7 days (20%, 30%, 40%, 50%, and 100% of pineapple juice).

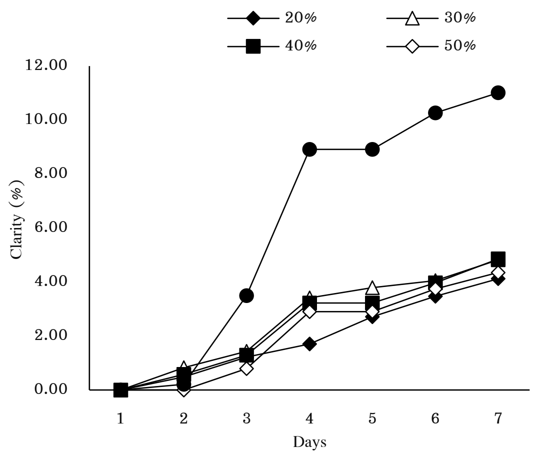


Figure 5 Clarity changing of rice and pineapple fermentation with *S. cerevisiae* during 1-7 days (20%, 30%, 40%, 50%, and 100% of pineapple juice).

have affected to increase yield of alcohol products (16-18%) (Chay et al 1986).

3.3 Total solid

The initial values as 15.0-21.9 °Brix has been found at 0 day. High concentration as 80% and 70% rice mixed 20% and 30% pineapple, respectively, exhibited the highest total solid value as shown in figure 2. Total solid values of fermentation have gradually decreased since 1–7 day after inoculation, especially, rapidly reduced after 1 day (100%, 50%, and 40% pineapple) and 2 days (20%, and 30% pineapple) until 7 days. The formulas of 40% and 50% pineapple exhibited the highest reduction of total solid (85.1%) in fermentative solution at a final day. Soluble sugar contents in liquid–fermentations have affected on high initial total solid of wine fermentation, and reduced by microbial activities in metabolisms for their growth, including conversion of sugar to alcohol products (He et al., 2022; Thungbeni et al., 2020). Total solid contents of liquid-fermentation decreasing have indicated that sugar from pineapple and rice have been used by yeast.

3.4 pH changing

The initial pH as 4.40-4.80 have been found in each formula of rice and pineapple fermentations as shown in figure 4. The highest concentration of 100% pineapple exhibited little reduction of pH from 4.50 (1 days) to 4.32 (7 days), compared to other formulas. Whereas, the concentration of 20%, 30%, and 50% pineapple (80%, 70%, and 50% rice, respectively) demonstrated extreme pH decreasing into lower value than 100% fruit application, especially 20 and 30% pineapple expressed the lowest pH as 3.38 and 3.41, respectively. Low pH (3.8–4.0) occurring in fermentation processes could rapidly allow alcoholic product presence, which inhibited undesirable microorganisms. This condition was not only control many spoilage organisms, but also increase the optimum environments for yeast growth. The combination of pineapple juice and rice as mixture of wine have operated pH into the appropriate value to perform a successful fermentation by yeast during 0–7 days. Metabolic activities of sugar by yeast have produced carbon–dioxide to form carbonic acids resulting the reduction of pH value into acid range (Roda et al., 2017).

3.5 Clarity measurement

Turbidity values of rice and pineapple mixture solution have depended on the concentration of pineapple juice as shown in figure 5. Formula of high concentration, 100% pineapple juice, without rice supplement exhibited the highest turbidity percentage of suspension at the final days as 11.1%, compared to other formulas. Whereas, application of 20% pineapple (80% rice) in mixture solution demonstrated the lowest turbidity value at the final days as 4.12%. The clarity values in solution of fermentation have regularly increased since 1 days until 7 days. Clarity as one of wine physical characteristic must be modified before consuming. Biological process, bromelain as a cysteine protease has been found in many fruits and their stem tissue, especially pineapple (Mohan et al., 2016; Sunday, 2018; Zhai et al., 2023). Bromelain has performed the enzymatic activity to inhibit browning reaction that it contained a combination with different kinds of proteases with the role similar to other enzymes such as phosphatases, glucosidases, peroxidases, cellulases. In addition, bromelain could be applied to other foods processing such as meat tenderization consisting the hydrolysis of muscle and tissue, clarification of beer, anti–browning agent in fruit, and development of good qualitative dough production in the bakery industry (Arshad et al., 2014). Moreover, the reactions such as oxidation, condensation, and polymerization have also

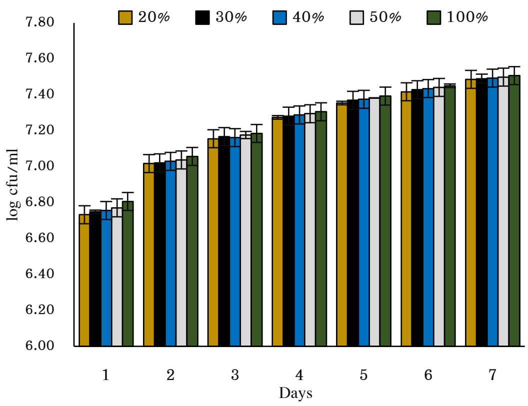


Figure 6 Amount of *S. cerevisiae* in rice and pineapple fermentation during 1-7 days (20%, 30%, 40%, 50%, and 100% of pineapple juice).

caused to make color change, decrease in lightness of products during storage for long times.

3.6 Yeast estimation

Amount of *S. cerevisiae* growing in the mixture of rice and pineapple fermentations have continuously increased during 1–7 days as shown in figure 6. Yeast exhibited the exponential growth phase rapidly within short time after incubation for 1 days at room temperature, which did not decline in cell values until 7 days. At the final day of fermentation exhibited the average value as 7.51 log cfu/ml more than 1 day (average 6.71 log cfu/ml). Especially, the application of 100% pineapple showed higher growth of yeast cell than other formulas. Yeast growths in the mixtures of rice and pineapple juice during fermentation have directly affected on a variation of alcohol contents.

5. CONCLUSION

Alcohol contents of the fermentation have occurred at 2 days after inoculation. The application of 80% rice with 20% pineapple juice exhibited the highest value as 9.77%, while, 100% pineapple displayed the lowest value as 8.53% at the final days. Application of pineapple juice depended on the level of concentrations has assisted the clarity of fermentative solution of rice wine.

ACKNOWLEDGMENT

This research was supported by plant genetic conservation project under the Royal Initiative of Her Royal Highness Princess Maha Chakri Sirindhorn and faculty of science and liberal art, Rajamangala University of technology Isan.

REFERENCES

Alcalde-Eon, C., Garcia-Estevez, I., Puente, V., & et al. (2014). Color stabilization of red wines. A chemical and colloidal approach. *Journal of Agricultural and Food Chemistry, 62*(29), 6984–6994. https:// doi.org/10.1021/jf4055825.

Ali, M. M., Hashim, N., Abd Aziz, S., & Lasekan, O. (2020). Pineapple (*Ananas comosus*): A comprehensive review of nutritional values, volatile compounds, health benefits, and potential food products. *Food Research International, 137*, 1–13. https://doi.org/10.1016/j.foodres.2020.109675.

Arshad, Z. I. M., Amid, A., Yusof, F., & et al. (2014). Bromelain: an overview of industrial application and purification strategies. *Applied Microbiology and Biotechnology, 98*, 7283–7297. https://doi.org/10.1007/s00253-014-5889-y

Chay, P. B., Atutubo, E. O., & Sanchez, P. C. (1986). Optimization of solid substrate fermentation in rice wine production. *Philippine Agriculture. 69*,159–172.

Ekechukwu, C. C., Ikele, C. C, Okafor, C. A., & et al. (2021). Comparative assessment of three yeast samples for wine production from pineapple. *Multidisciplinary Science Journal, 3*(1), 1–8.

He, Y., Yuan, G., Wang, C., & et al. (2022). Correlation analysis between starch and glucose content in rice wine brewing. *Food and Fermentation Industries, 48*, 28–36. https://doi.org/10. 13995/j.cnki.11-1802/ts.028723

Hesseltine, C. W., Rogers, R. & Winarno, F. G. (1988). Microbiological studies on amylolytic oriental fermentation starters. *Mycopathologia, 101,* 141–155.https://doi.org/10.1007 /BF00437031.

Hirst, M. B., & Richter, C. L. (2016). Review of aroma formation through metabolic pathways of *Saccharomyces cerevisiae* in beverage fermentations. *American Journal of Enology and Viticulture*, *67*, 361–370. https://doi.org/10.5344/ajev.2016.15098.

Hossain, M. A., & Rahman, S. M. M. (2011). Total phenolics, flavonoids and antioxidant activity of tropical fruit pineapple. *Food Research International, 44*(3), 672–676. https://doi.org/ 10.1016/j.foodres.2010.11.036.

Jiang, L., Su, W., & Mu, Y. (2020). Major metabolites and microbial community of fermented black glutinous rice wine with different starters. *Frontiers in Microbiology, 11,* 1–13. https:// doi.org/10.3389/fmicb.2020.00593.

Kaneko, S., & Kumazawa, K. (2015). Aroma compounds in Japanese sweet rice wine (Mirin) screened by aroma extract dilution analysis (AEDA). *Bioscience, Biotechnology, and Biochemistry, 79*(3), 484–487. http://dx.doi.org/10.1080/09168451.2014.980218.

Lee, D. E., Lee, S., Jang, E. S., Shin, H. W., Moon, B. S., & Lee, C. H. (2016). Metabolomic profiles of Aspergillus oryzae and Bacillus amyloliquefaciens during rice koji fermentation. *Molecules, 21*(6), 773. https://doi.org/10.3390/molecules21060773.

Liszkowska, W., & Berlowska, J. (2021). Yeast fermentation at low temperatures: Adaptation to changing environmental conditions and formation of volatile compounds. *Molecules, 26*, 1–21. https://doi.org/10.3390/molecules26041035.

Marconi, O., Sileoni, V., Ceccaroni, D., & Perretti, G. (2017). The use of rice in brewing. Chapter 4. *Advances in International Rice Research*, 49–66. http://dx.doi.org/10.5772/66450.

Meng, L. W., & Kim, S. M. (2020). Effects of different carbohydrates on the physicochemical properties of rice flour, and the quality characteristics of fermented rice cake. *Food Science and Biotechnology, 29*(4), 503–512. https://doi.org/10.1007/s10068-019-00693-7.

Metin, K., Koc, O., Ateşlier, B. B., & Biyik, H. H. (2010). Purification and characterization of α-amylase produced by Penicillium citrinum HBF62. *Afr. J. Biotechnol*, *9*(45), 7692-7701.

Mohan, R., Sivakumar, V., Rangasamy, T., & Muralidharan, C. (2016). Optimisation of bromelain enzyme extraction from pineapple (Ananas comosus) and application in process industry. *American Journal of Biochemistry and Biotechnology*, *12*(3), 188-195. https://doi.org/10.3844/ajbbsp.2016.188.195.

National Center for Biotechnology Information. PubChem Enzyme Summary for Enzyme 3.2.1.1, *Alpha–amylase (EC 3.2.1.1)*. Retrieved Jan, 7, 2025, https://pubchem.ncbi.nlm.nih.gov/protein/EC:3.2.1.1.

Parapouli, M., Vasileiadis, A., Afendra, A. S., & Hatziloukas, E. (2020). Saccharomyces cerevisiae and its industrial applications. *AIMS Microbiology, 6*(1), 1–31. http://doi.org.10.3934/microbiol.2020001

Rasiah, I. A., & Rehm, B. H. (2009). One-step production of immobilized α-amylase in recombinant Escherichia coli. *Applied and environmental microbiology*, *75*(7), 2012-2016. https://doi.org/10.1128/AEM.02782-08.

Riou, V., Vernhet, A., Doco, T., & et al. (2002). Aggregation of grape seed tannins in model wine–effect of wine polysaccharides. *Food Hydrocolloids, 16*(1), 17–23. https://doi.org/10.1016/S0268-005X(01)00034-0.

Roda, A., Lucini, L., Torchio, F., & Moutounet, M. (2017). Metabolite profiling and volatiles of pineapple wine and vinegar obtained from pineapple waste. *Food Chemistry, 229*, 734–742. https://doi.org/10.1016/j.foodchem.2017.02.111.

Sanchez, P. C., Juliano, B. O., Laude, V. T., & et al. (1988). Non waxy rice for tapuy (rice wine) production. *Cereal Chemistry, 65*(3), 240–243.

Sanghvi, G. V., Koyani, R. D., & Rajput, K. S. (2011). Isolation, optimization, and partial purification of amylase from Chrysosporium asperatum by submerged fermentation. *Journal of Microbiology and Biotechnology, 21*(5), 470–476. https://doi.org/10.4014/jmb.0910.10014.

Souza, P. M., & Magalhães, P. O. (2010). Application of microbial α-amylase in industry–A review. *Brazilian Journal of Microbiology, 41*(4), 850–861. https://doi.org/10.1590/S1517-83822010000400004.

Sudtasarn, G, Homsombat, W, Chotechuen, S, & Chamarerk ,V. (2019). Quantification of Tocopherols, Tocotrienols and γ-Oryzanol Contents of Local Rice Varieties in Northeastern Thailand. *Journal of Nutritional Science and Vitaminology (Tokyo)*, *65*(Supplement), S125-S128. https://doi.org/10.3177/jnsv.65.S125.

Sunday, A. (2018). Effect of Clarifying Agents (Gelatin and Kaolin) on Fruit Wine Production. *International Journal of Agriculture Innovations and Research, 6*(4), 130–132.

Taweekasemsombut, S., Tinoi, J., Mungkornasawakul, P., & Chandet, N. (2021). Thai Rice Vinegars: Production and Biological Properties. *Applied Sciences*, *11*(13), 5929. https://doi.org/10.3390/app11135929

Thungbeni, T, Samir, T. E., & Samir, W. (2020). Effect of different levels of wine yeast and sugar in wine production from pineapple (*Ananas comosus*). *International Journal of Current Microbiology and Applied Sciences, 9(12)*, 681–693. https://doi.org/10.20546/ijcmas.2020.912.082.

Tian, S., Zeng, W., Fang, F., Zhou, J., & Du, G. (2022). The microbiome of Chinese rice wine (Huangjiu). *Current Research in Food Science*, *5*, 325-335. https://doi.org/10.1016/j.crfs.2022.01.007.

Verma, D. K, & Srivastav, P. P. (2020). Bioactive compounds of rice (*Oryza sativa* L.): review on paradigm and its potential benefit in human health. *Trends in Food Science and Technology, 97*, 355–65. http://doi.org.10.1016/j.tifs.2020.01.007.

Wang, B., Wu, Q., Xu, Y., & et al. (2020). Synergistic effect of multiple saccharifying enzymes on alcoholic fermentation for Chinese Baijiu production. *Applied Environmental Microbiology, 86(8)*, 1–12. https://doi.org/10.1128/AEM.00013-20.

Wang, B., Wu, Q., Xu, Y., & Sun, B. (2018). Specific volumetric weight-driven shift in microbiota compositions with saccharifying activity change in starter for Chinese Baijiu fermentation. *Frontiers in Microbiology*, *9*, 2349. https://doi.org/10.3389/fmicb.2018.02349.

Wang, C., Yuan, G., He, Y., Tang, J., Zhou, H., & Qiu, S. (2022). The formation of higher alcohols in rice wine fermentation using different rice cultivars. *Frontiers in Microbiology, 13*, 1–19. https://doi.org/10.3389/fmicb.2022.978323.

Watanabe, M., & Shimazu, Y. (1980). Effect of yeasts on botrytised wine making. *Journal of fermentation technology, 58*(3), 227–235.

Wei, X. L., Liu, S. P., Yu, J. S., & et al. (2016). Innovation Chinese rice wine brewing technology by biacidification to exclude rice soaking process. *Journal of Bioscience and Bioengineering, 123*(4), 460–465. https://doi.org/10.1016/j.jbiosc.2016.11.014.

Zhai, H. Y., Li, S. Y., Zhao, X., Lan, Y. B., Zhang, X. K., Shi, Y., & Duan, C. Q. (2023). The compositional characteristics, influencing factors, effects on wine quality and relevant analytical methods of wine polysaccharides: A review. *Food Chemistry, 403,* 1–12. https://doi.org/10.1016/j.foodchem.2022.