Malaysian Journal of Chemical Engineering & Technology 6(2) (2023) 75–80

Malaysian Journal of Chemical Engineering & Technology

Journal Homepage: http://myjms.mohe.gov.my/index.php/mjcet

e-ISSN: 2682-8588

Fermentation of *Tacca leontopetaloides* starch with ragi tapai for bioethanol production

Suhaila Mohd Sauid *, Muhamad Afiq Ab Wahab, Mohamad Mohd Aliff Jamian, Ahmad Fahmi Mat Zain, Herlina Mustafa

School of Chemical Engineering, College of Engineering, Universiti Teknologi MARA, Selangor, Malaysia

*Corresponding email: suhaila.sauid@uitm.edu.my

Abstract

This study investigates the potential of *Tacca leontopetaloides* starch as an alternative raw material for bioethanol production. Ragi tapai was used as the saccharification and fermentation agent and different origin of ragi tapai was evaluated for the highest glucose production. Ragi tapai from Sabah (RTSab) produced significantly higher glucose as compared to other origins of ragi tapai and was then used in the rest of the study. Fermentation of ragi tapai with *T. leontopetaloides* starch was compared with the fermentation of ragi tapai - *Saccharomyces cerivisiae* co-culture. Glucose and ethanol concentrations were measured by spectrophotometric method. It was found that ragi tapai alone is more efficient in producing bioethanol as the concentration was the highest (17.4% v/v) with productivity reaching 4.5 g/l/h in comparison to only 3.7 g/l/h for co-culture fermentation. Glucose concentration also varied at higher level throughout the fermentation and peaked at 84.28 g/L. This study concludes that *T. leontopetaloides* starch is a potential raw material for bioethanol and use of ragi tapai is effective in producing bioethanol.

Article Info

https://doi.org/10.24191/mjcet.v6i2.21822

Article history:

Received date: 15 March 2023
Accepted date: 20 July 2023
Published date: 31 October 2023

Keywords:

Tacca leontopetaloids
Ragi tapai
Bioethanol
Starch

1.0 Introduction

The demand for alternative energy is constantly increasing due to environmental concerns and the depletion of fossil fuels. Bioethanol is considered as one of the most promising substitutes as it can be produced through fermentation and produces lower carbon dioxide emissions when combusted. From 2016 to 2022, the market for bioethanol fuel has grown dramatically, with a compound annual growth rate (CAGR) of 7.6% (Sharma et al., 2020). As of 2021, almost 27.3 million gallons of bioethanol are produced worldwide with the United States and Brazil being the main producers utilising corn and sugarcane, respectively as the raw materials (Renewable Fuel Association, 2022). However, the use of these stocks created many issues such as competition for land and water for food and agricultural complications such as drought (Aruwajoye et al., 2020; Ho et al., 2014). The use of agricultural biomass waste or other lignocellulosic materials as a substrate to produce bioethanol on the other hand has numerous challenges (Verma & Kumar, 2021). It includes the energy-intensive pretreatment steps to reduce the cellulose crystallinity and the removal of the lignin component before the sugar can be used in the fermentation (Madu et al., 2019). Therefore, the use of non-staple starchy materials as the raw materials is a more promising alternative.

Tacca leontopetaloides also known as Polynesian arrowroot is a wild plant known to have grown in the Pacific Island nations from species of flowering plants in the yam family, *Dioscoreaceae* (Ukpabi et al., 2009). The total carbohydrate content in *T. leontopetaloides* is 89.4% which is composed of 66.65% starch containing 22.7% amylose and 43.88% amylopectin (Utama et al., 2018). The characteristic of the *T. leontopetaloides* is that it looks like a potato and has hard tuber. In Malaysia, this plant is native and can be found in the East Coast of Peninsular. It is not being exploited nor harvested as food source by Malaysian locals, but it is consumed in Indonesia as substitute of

rice and the flour used in cake or pastry (Utama et al., 2018).

Ragi is a fermentation starter, normally in the form of dry circular shape biscuit and used in the making of tapai; which is defined as fermented glutinous rice or cassava. During fermentation, the cooked glutinous rice or cassava soften and is partially liquefied and has a sweet-sour taste. Tapai also have small amount of alcohol (Mohamed & Yusof, 2021). It is due to the existence of yeast species in ragi tapai (Law et al., 2011). Ragi tapai also have amylolytic bacteria and molds such as *Amylomyces rouxii, Endomycopsis, burtonii, Candida utilis, Candida pelliculosa, Rhizopus oryzae, Mucor sp*., *Lactobacillus plantarum* and *Bacillus licheniformis* (Gandjar, 2003; Law et al., 2011; Roslan et al., 2018).

The objective of this study is to investigate the capability of producing bioethanol from *T. leontopetaloides* using local ragi tapai. Four different sources of ragi tapai were evaluated for glucose production in this study. Ragi was used as saccharification agent to convert the *T. leontopetaloides* starch into glucose and eventually produced bioethanol. Then, the ragi tapai producing the highest glucose concentration was further tested for bioethanol production in the second part of this study. In this part, *Saccharomyces cerevisiae* was added as it is widely used in alcoholic beverage production (Parapouli et al., 2020).

2.0 Methodology

2.1 Materials

The *T. leotopelatoids* tubers were collected from Mersing, Johor. To extract the starch from the tubers, the tubers were peeled, and tuber fleshes were washed thoroughly to remove the layer of wax that found to be poisonous (Ubwa et al., 2011). The fleshes were grated, mixed with water and put in cloth to remove the solid fibres with the starch milk. The starch milk was let to precipitate for several hours. Then the clear upper layer water was removed leaving the sediment of starch. The wet starch then dried under the sun and grinded into powder. The ragi tapai starter was bought from different local markets in Johor, Kelantan, Perlis, and Sabah. Chemical used in this study were peptone (Merck), yeast extract (Merck), 3,5-Dinitrosalicylic acid solution and potassium dichromate (R&M).

2.2 Methods

2.2.1 Selection of Ragi Tapai

30 ml of 5% (w/v) *T. leontopetaloides* starch slurry was prepared in 100 ml shake flask. The temperature was raised to 70 °C for an hour. After obtaining the starch slurry with the appearance of translucent and gel-like consistency, the starch slurry was allowed to cool at room temperature until 37 °C to 40 °C. The ragi tapai culture suspension was prepared by dissolving 3 g of ragi tapai powder in 30 ml of 10% (w/v) liquid peptone and incubated in the incubator shaker (Infors Ecotron, Switzerland) for 30 mins at 37 °C and 200 rpm (Azmi et al., 2010). Then, 3 ml of ragi tapai culture suspension was pipetted into the starch slurry. All flasks were then kept in the incubator shaker at fixed condition of 30 °C and 50 rpm. Samples were taken to determine the glucose concentration from each flask after 20, 40, and 60 minutes of incubation. The highest glucose concentration obtained from all the four types of ragi tapai was selected for further experimental parameters. All experiments were done in triplicates.

2.2.2 Bioethanol fermentation

For the bioethanol fermentation, 3% (w/v) starch solution was prepared for 150 ml following the same method described in section 2.2.1. Then, the starch solution was inoculated with 15 ml ragi tapai culture suspension (preparation method described in 2.2.1) which equal to 10% of the working volume. The flask was then placed in the incubator shaker, shaken at 75 rpm and the temperature was maintained at 37 °C.

The same experiment was repeated with co-culturing the starch solution with ragi tapai and *S. cerevisiae* simultaneously. *S. cerevisiae* culture was prepared similarly to ragi tapai culture suspension. The inoculum for co-culture fermentation comprised of 7.5 ml ragi tapai suspension and *S. cerevisiae* culture respectively, making a total of 15 ml. The fermentation was done in triplicates. Samples were taken every two hours during the day only and once a day after 48 hours and up to 72 hours.

2.2.3 Glucose quantification

For the glucose quantification method, 2 ml of samples was taken from the flask and centrifuged (Eppendorf 5415 R, Germany) at 10,000 rpm for 5 mins. The supernatant was then diluted with distilled

water at ratio 1:2. Next, 3,5-Dinitrosalicylic acid (DNSA) reagent was added into the mixture at 1:1 ratio and vortexed thoroughly. The mixture was boiled for 5 mins and let to cool before diluted with distilled water and measured using spectrophotometer (HACH DR2700, Germany) at wavelength 540 nm**.** The optical density (OD) values were compared with the glucose standard curve for glucose concentration produced.

2.2.4 Biomass concentration

2 ml of the sample was taken from the shaken flasks by using a pipette and put in centrifuge tube. Supernatant and cell were separated by centrifugation at 10,000 rpm for 15 mins. After supernatant removal, cell was dried in the oven with the temperature of 60 °C until constant weight was achieved 24 hours. The cell dry weight or biomass concentration was calculated.

2.2.5 Bioethanol quantification

For the ethanol quantification method, 2 ml of the fermentation broth was taken from the flask and centrifuged at 10,000 rpm for 15 minutes. 0.2 ml of from the supernatant was taken and mixed with 5 ml of potassium dichromate solution. The mixture was heated up to 60 °C for 20 mins and let to cool before diluted with distilled water to final volume of 10 ml. The mixture then was measured using spectrophotometer (HACH DR2700, Germany) at wavelength 600 nm**.** The OD values obtained were compared with the ethanol standard curve for ethanol concentration produced.

3.0 Results and discussion

3.1 Microscopic Observation of Ragi Tapai

The ragi tapai culture suspensions for different origins were observed under microscope and shown in Fig. 1. The shape of the cells for all tapai from Perlis, Johor, and Kelantan appeared as sphere shape similar to shape observed in ragi and tuak by Kofli and Dayaon (2010). Meanwhile, ragi tapai from Sabah shown in Fig. 1 (d) had a rod shape. Based on the oval shape of Fig. 1 (a-c), these bacteria could be *Enterococcus faecium* or *Saccharomyces cerevisiae* which usually found in ragi tapai, while the rod shape could be *Lactobacillus plantarum* or *Bacillus licheniformis* that have been found in several types of starter culture and fermented foods (Roslan et al., 2018; Sujaya et al., 2010; Thanh et al., 2008).

3.2 Selection of Ragi Tapai

In the next part of the study, ragi tapai culture suspension was inoculated in the starch solution. Samples were taken after 20, 40, and 60 mins. It was expected that glucose would be produced, as ragi tapai contains a lot of amylolytic bacteria and moulds. Fig. 2 compares the concentration of glucose produced at time 20, 40, and 60 mins of fermentation for all origins of ragi tapai. From this figure, it shows that all types of ragi tapai produced glucose indicating that the *T. leontopetaloides* starch was digestible by the ragi tapai microorganisms. Ragi tapai from Sabah produced the highest glucose concentration at 31.51 g/l after 60 mins while ragi tapai from Johor produced the lowest amount of glucose at only 4.14 g/l. Both ragi tapai from Kelantan and Perlis produced slightly higher glucose than that ragi tapai from Johor. From these preliminary results, ragi tapai from Sabah was selected to be used for the next part of this study.

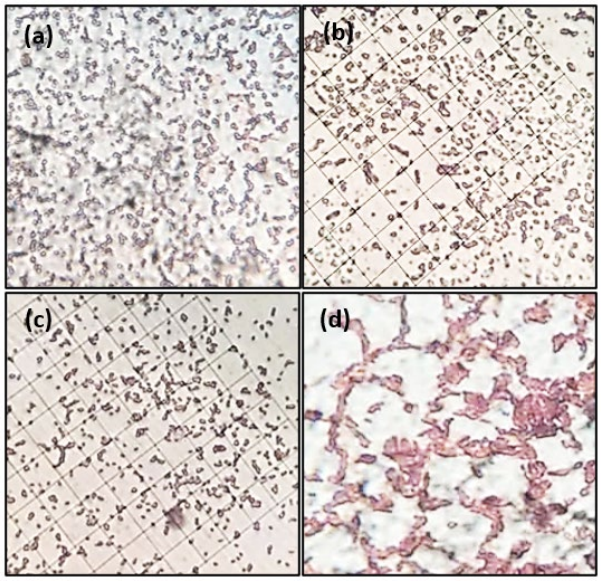

Fig. 1: Ragi tapai microorganisms observed under microscope (100 x magnifications) for ragi tapai from (a) Perlis (b) Johor (c) Kelantan (d) Sabah

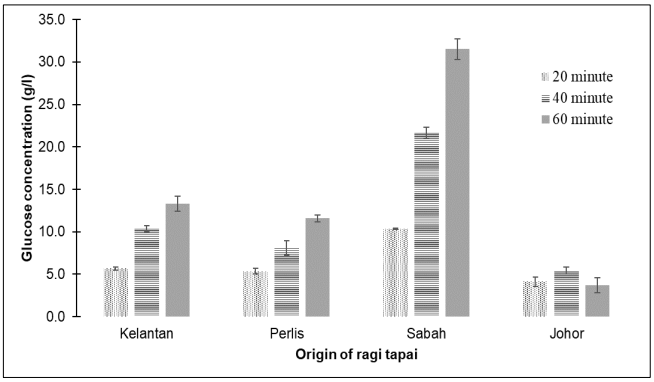

Fig. 2: Glucose concentration produced by different origin of ragi tapai

3.3 Bioethanol fermentation

In the second part of this study, *T. leontopetaloides* starch underwent simultaneous saccharification and fermentation (SSF) with ragi tapai or co-cultured the ragi tapai with *S. cerevisiae* in the production of bioethanol. As ragi tapai contains mixed culture of amylolytic microorganisms and yeast (Gandjar, 2003; Law et al., 2011; Roslan et al., 2018), ragi tapai was able to saccharify *T. leontopetaloids* starch into glucose and then simultaneously fermenting it into bioethanol. As shown in Fig. 3, at the early stage of fermentation, the biomass concentrations were higher for both fermentations and dropped gradually to a varying value of below 10 g/L. The high biomass concentration at the beginning could be contributed by the powder contained in the ragi tapai suspension. Comparing both fermentations, fermentation of only ragi tapai has higher biomass concentration than co-culture fermentation.

The fermentation of ragi tapai also yielded higher glucose concentrations, with the highest concentration reaching 84.28 g/L. After 12 hours, the glucose concentrations fluctuated between 62.51 g/L and 79.24 g/L. The glucose concentrations obtained in this study was close to the concentrations obtained by Azmi et al. (2010) for their fermentation of ragi tapai with cassava starch. Furthermore, significant amounts of glucose remained in the broth, suggesting that the fermentation rate was low.

The same trend was also observed for the *ragi tapai* and *S. cerevisiae* co-culture fermentation (Fig. 4). However, the glucose concentrations obtained were significantly lower. The low glucose concentration might be due to the co-culture itself. For co-culture fermentation, the volume of inoculum added in to start the fermentation was 7.5 ml for ragi tapai and *S. cerevisiae,* respectively to make the total volume of inoculum equal to 10% of the working volume. This differed from the fermentation using only ragi tapai, as the entire 10% inoculum volume (15 ml) consisted entirely of ragi tapai culture. As *S. cerevisiae* is non-amylolytic yeast (Jamai et al., 2007); glucose production was solely dependent on the ragi tapai culture. Since the volume of ragi tapai was reduced by half in co-culture fermentation, the production of amylase by the amylolytic microorganisms might be lower, thus reducing glucose production. Furthermore, as glucose is required for cell growth, maintenance and/or ethanol formation the glucose produced was rapidly consumed by both ragi tapai microorganisms and the added *S. cerevisiae* (Diong et al., 2016; Pervez et al., 2014). This might explain the lower glucose concentration in the broth. However, from 30 hours onwards, the glucose concentrations showed an increasing trend reaching almost 60 g/L by the end of fermentation. This suggested that glucose was not being consumed and continued to be produced. The increasing trend of glucose concentration in the last 24 hour for both fermentations might yield from the enzymatic activity of amylase secreted from the amylolytic molds such as *Aspergillus and Rhizopus* groups in the ragi tapai (Diong et al., 2016; Wu et al., 2015).

As for the bioethanol production, ragi tapai fermentation produced higher bioethanol, peaking at 17.4% (v/v) concentration at 26 hours. After that, bioethanol concentration dropped to 5.97% (v/v) and the concentration fluctuated between 4.96% and 6.76% (v/v) until the end of the fermentation. These values are comparable with ethanol concentration obtained by J.R Hernandez et al. (2011) for their fermentation with corn starch. However, the bioethanol concentration was slightly lower for the co-culture fermentation as

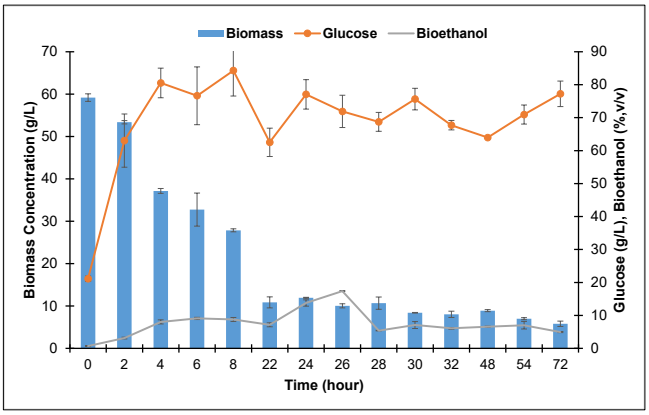

Fig. 3: Biomass, glucose and bioethanol profile for *T. leontopetaloides* starch fermentation with ragi tapai

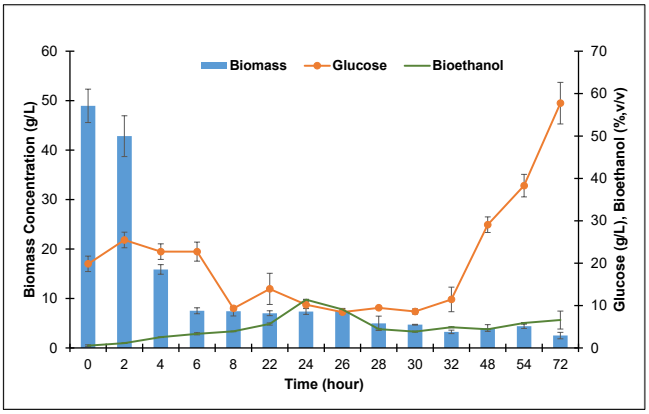

Fig. 4: Biomass, glucose and bioethanol profile for fermentation of *T. leontopetaloides* starch with ragi tapai and *S. cerevisiae* co-culture

compared to the fermentation with only ragi tapai. The highest bioethanol production was 11.4% (v/v) with yield equivalent of $Y_{P/S}$ = 1.12% of ethanol produced/gram of glucose consumed and then it dropped and varied between 4.05% and 6.6% (v/v) until the end of the fermentation. The lower bioethanol production could be resulted from the lower biomass and glucose produced. This trend was also observed by Ediviani et al. (2014) that found the bioethanol produced was higher for fermentation with ragi tapai only. Furthermore, the reduction of ethanol concentration after 26 hours might be due to conversion of ethanol to other by-products as suggested by Azmi et al. (2010). In addition, the ethanol loss also could be due to evaporation as the fermentation was conducted at 37 °C, which is significant for ethanol concentration higher than 6% (Abdel-Banat et al. 2010).

4.0 Conclusions

In this study, *T. leontopetaloides* starch was fermented with different origin of ragi tapai. Ragi tapai originated from Sabah showed the highest glucose and bioethanol concentration was 84.28 g/L and 17.4% (v/v), respectively. However, co-culturing the starch with ragi tapai and *S. cerevisiae* demonstrated a reduction in both glucose and bioethanol production. Based on the findings in this study, it shows that local non-food source, *T. leontopetaloides* starch, can be used as an alternative starch in the production of bioethanol. As bioethanol is currently produced using corn, *T. leontopetaloides* can be grown and used in bioethanol production, thus eliminating the food competition issue with corn.

Furthermore, this study also showed that the simultaneous saccharification and fermentation of *T. leontopetaloides* starch with ragi tapai could reduce the use of enzymes thus reducing the cost of bioethanol fermentation. For future work, investigation on the effect of starch concentration, temperature and inoculum size can be conducted with industrial grade yeast or other ethanol-producing microorganisms to increase the yield of bioethanol production.

Contribution statement

Suhaila Mohd Sauid: Conceptualisation, supervision, methodology, formal analysis, writing-review and editing and validation; **Muhamad Afiq Ab Wahab:** Methodology, formal analysis, investigation; **Mohamad Mohd Aliff Jamian:** Methodology, formal analysis, investigation; **Ahmad Fahmi Mat Zain:** Methodology, formal analysis, investigation, writing-original draft; **Herlina Mustafa:** Methodology, formal analysis, investigation.

Conflict of interest

The authors declare no competing financial interests.

Acknowledgement

The present study was made possible through funding from Ministry of Sciences, Technology and Innovation, UiTM Internal grant 600-IRMI/MyRA 5/3/LESTARI (084/2017). Continuous support from Universiti Teknologi MARA is gratefully acknowledged.

References

Abdel-Banat, B. M. A., Hoshida, H., Ano, A., Nonklang, S., & Akada, R. (2010). High-temperature fermentation: How can processes for ethanol production at high temperatures become superior to the traditional process using mesophilic yeast? *Applied Microbiology and Biotechnology*, *85*(4), 861–867. https://doi.org/10.1007/s00253-009-2248-5

Aruwajoye, G. S., Kassim, A., Saha, A. K., & Gueguim Kana, E. B. (2020). Prospects for the improvement of bioethanol and biohydrogen production from mixed starch-based agricultural wastes. *Energies*, *13*(24). https://doi.org/10.3390/en13246609

Azmi, A. S., Ngoh, G. C., Mel, M., & Hasan, M. (2010). Ragi tapai and Saccharomyces cerevisiae as potential coculture in viscous fermentation medium for ethanol production. *African Journal of Biotechnology*, *9*(42), 7122–7127.

Diong, K. C., Ngoh, G. C., & Chua, A. S. M. (2016). Transformation of Starchy Lignocellulosic Biomass to Ethanol using Ragi Tapai Synergized with Microwave Irradiation Pretreatment. *BioResources*, *11*(1), 1991–2006. https://doi.org/10.15376/BIORES.11.1.1991-2006

Ediviani, W., Octavia, S., & Yasman, Y. (2014). Effectiveness of Powder and Solid Ragi (Starter) from Dago (Bandung, West Java, Indonesia) Traditional Market on Production of Bioethanol from Rice Straw Hydrolyzate. *Makara Journal of Science*, *18*(1), 18–25. https://doi.org/10.7454/mss.v18i1.3045

Gandjar, I. (2003). TAPAI from cassava and cereals. *Proceedings of the 1st International Symposium and Workshop on Insight into World of Indigenous*

Fermented Foods for Technology Development and Food Safety, 1–10. http://www.agriqua.doae.go.th/worldfermentedfood/I_10_Gandjar.pdf

Ho, D. P., Ngo, H. H., & Guo, W. (2014). A mini review on renewable sources for biofuel. *Bioresource Technology*, *169*, 742–749. https://doi.org/10.1016/J.BIORTECH.2014.07.022

Hernandez, J. R., Capareda, S. C., Portilllo, O., Hays, D. B., & Rooney, W. L. (2011). Simultaneous Saccharification and Fermentation (SSF) of High Digestible Grain Sorghum for Ethanol Production. *Biological Engineering Transactions*, *4*(1), 3–15. https://doi.org/10.13031/2013.37173

Jamai, L., Ettayabi, K., El Yamani, J., & Ettayabi, M. (2007). Production of ethanol from starch by free and immobilized Candida tropicalis in the presence of α-amylase. *Bioresource Technology*, *98*(14), 2765–2770. https://doi.org/10.1016/j.biortech.2006.09.057

Kofli, N. T., & Dayaon, S. H. M. (2010). Identification of microorganism from ragi for bioethanol production by API kit. *Journal of Applied Sciences*, *10*(21), 2751–2753. https://doi.org/10.3923/jas.2010.2751.2753

Law, S. V., Abu Bakar, F., Mat Hashim, D., & Abdul Hamid, A. (2011). Popular fermented foods and beverages in Southeast Asia. *International Food Research Journal*, *18*(2), 475–484.

Madu, J. O., Fabunmi, T. E., & Agboola, B. O. (2019). Effects of chemical treatments on the bioethanol yield and composition of Isoberlinia doka waste. *SN Applied Sciences*, *1*(3), 1–8. https://doi.org/10.1007/s42452-019-0223-z

Mohamed, H. N., & Yusof, N. A. I. (2021). Identification of volatile flavor compounds in fermented glutinous rice (Tapai) using gas chromatography. *AIP Conference Proceedings*, *2370*(1), 060014. https://doi.org/10.1063/5.0062351

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

Pervez, S., Aman, A., Iqbal, S., Siddiqui, N. N., & Ul Qader, S. A. (2014). Saccharification and liquefaction of cassava starch: an alternative source for the production of bioethanol using amylolytic enzymes by double fermentation process. *BMC Biotechnology*, *14*(1), 49. https://doi.org/10.1186/1472-6750-14-49

Renewable Fuel Association. (2022). *Global Ethanol Production by Country or Region* (Issue December, p. 2020). https://afdc.energy.gov/data/10331

Roslan, R., Rehan, M. M., Kamarudin, K. R., Noor, H. M., Huda-Faujan, N. U. R., & Radzi, S. M. (2018). Isolation and identification of amylolytic bacteria from ragi. *Malaysian Applied Biology*, *47*(2), 83–88.

Sharma, B., Larroche, C., & Dussap, C. G. (2020). Comprehensive assessment of 2G bioethanol production. *Bioresource Technology*, *313*, 123630. https://doi.org/10.1016/J.BIORTECH.2020.123630

Sujaya, I. N., Nocianitri, K. A., & Asano, K. (2010). Diversity of bacterial flora of Indonesian ragi tape and their dynamics during the tape fermentation as determined by PCR-DGGE. *International Food Research Journal*, *17*(2), 239–245.

Thanh, V. N., Mai, L. T., & Tuan, D. A. (2008). Microbial diversity of traditional Vietnamese alcohol fermentation starters (banh men) as determined by PCR-mediated DGGE. *International Journal of Food Microbiology*, *128*(2), 268–273. https://doi.org/10.1016/j.ijfoodmicro.2008.08.020

Ubwa, S. T., Anhwange, B. A., & Chia, J. T. (2011). Chemical Analysis of Tacca leontopetaloides Peels. *American Journal of Food Technology*, *6*(10), 932–938. https://doi.org/10.3923/ajft.2011.932.938

Ukpabi, U. ., Ukenye, E., & Olojede, A. . (2009). Raw-Material Potentials of Nigerian Wild Polynesian Arrowroot (Tacca leontopetaloides) Tubers and Starch. In *Journal of Food Technology 7* (Vol. 4, pp. 135–138).

Utama, G. L., Sugandi, W. K., & Lembong, E. (2018). Dilution Ratio of Tacca leontopetaloides in the Production of Bioethanol with the Inoculation of Indigenous Yeasts Consortium. *Journal of the Japan Institute of Energy*, *97*(9), 270–273. https://doi.org/10.3775/jie.97.270

Verma, N., & Kumar, V. (2021). Microbial conversion of waste biomass into bioethanol: current challenges and future prospects. *Biomass Conversion and Biorefinery*. https://doi.org/10.1007/s13399-021-01824-z

Wu, Q., Chen, B., & Xu, Y. (2015). Regulating yeast flavor metabolism by controlling saccharification reaction rate in simultaneous saccharification and fermentation of chinese maotai-flavor liquor. *International Journal of Food Microbiology*, *200*, 39–46. https://doi.org/10.1016/j.ijfoodmicro.2015.01.012