Yeast Species and Bioactive-Compounds of Traditional Rice Wine Originated from Lombok Island, Indonesia

I Nyoman Sumerta*, Yeni Yuliani, Maya Komalasari, Ismu Purnaningsih, Atit Kanti

Research Center for Biology, Indonesian Institute of Sciences (LIPI)
Jl. Raya Jakarta Bogor Km. 46 Cibinong 16911, West Java, Indonesia
*Corresponding author: I Nyoman Sumerta, Email: i.nyoman.sumerta@lipi.go.id

Submitted: October 1, 2020; Revised: January 19, 2021; Accepted: February 4, 2021

ABSTRACT

In fermented beverages, yeasts have been exploited for many years and are well-known as alcohol producers. In Indonesian traditional beverages, however, information about microbiology and potential bioactive-compounds of rice wine produced by the local people, especially in Lombok, are limited. The present study described the compounds of traditional rice wine including yeast species and its produced compounds that have biological activity. The yeast in rice wine was isolated using three growth agar media by serial dilution, selected the yeast colonies for molecular identification, and performed gas chromatography tandem with mass spectrometry for profiling the chemical compounds of the rice wine. The result indicated that the rice wine sold without distillation still contained *Saccharomyces cerevisiae* as the main alcohol producer. Meanwhile, at least six bioactive-compounds such as l-(+)-Ascorbic acid 2,6-dihexadecanoate, performic acid, octadecanoic acid, sulfurous acid, tetratriacontane, and eicosane were detected and reportedly related to antimicrobial, antiviral, anticancer, and other pharmacological activities. These findings could be the first step of studies on exploring Indonesian's local rice wine as alcohol and bioactive-compound sources for health benefits.

Keywords: Alcoholic beverages; bioactive-compounds; Lombok; rice wine; yeast

INTRODUCTION

Rice wine is one of the most popular wines which are often found in eastern countries. It has various names such as *cheongju* and *makgeolli* in Korea, *tapuy* in Philippines, *sake* in Japan, *shaosingjiu* in China (Rhee *et al*., 2011), and *arak beras* or *brem* in Indonesia. These beverages are made of rice which is converted to sugars through a fermentation process by microbes, especially yeasts (Kwon *et al*., 2014). The presence of microbes in the fermentation process plays essential roles to determine the product quality, such as aroma and flavour (Kawarai *et al.,* 2007; Pires *et al*., 2014; De Vuyst *et al*., 2016) which construct the uniqueness of the fermented products. Furthermore, maintaining the composition of appropriate microbial species during the fermentation process could affect the rice wine quality (Hong *et al*., 2016).

Despite alcohol as the main product, rice wine also possesses many beneficial compounds for health. These compounds could provide nutritional information and health benefits as the advantages of the products from yeasts (De Vuyst *et al*., 2016) and serve for ethno-medicinal purposes (Ray *et al*., 2016). For example, wine in general is a source of phenolic and polyphenol compounds that could act as antioxidant (Snopek *et al.,* 2018; Rodríguez-Vaquero *et al*., 2020) and have antimicrobial activity to prevent many diseases (Daglia *et al.,* 2007; Jamir *et al*., 2019; Sánchez *et al*., 2019; Rodríguez-Vaquero *et al*., 2020). Likewise, rice wine has been reported to produce some bioactive compounds such as farnesol, squalene, and polyphenols showing antioxidant, anticancer, and antibacterial activities (Lee *et al.,* 2018; Cai *et al.,* 2019). By revealing its bioactive compounds, rice wine could be as a source of bioactive

DOI: http://doi.org/10.22146/agritech.60287
ISSN 0216-0455 (Print), ISSN 2527-3825 (Online)

compounds which enhance the quality of beverage products.

Indonesia has several traditional rice wines that have not been intensively explored yet. Various products such as *brem*, *tape ketan*, and *arak beras* are popular in this country. Those beverages are made traditionally and sold in the market without any distillation process or any sophisticated tools, resulting in the uniqueness of the organoleptic characters. A special character of the rice wine products is basically produced by different methods, raw materials, and microbial starters leading to various products or brands (Sujaya *et al.,* 2004; Hong *et al*., 2016; Mazzoli *et al*., 2017; Cai *et al*., 2019); these factors construct the distinctive compounds (Capece & Romano, 2019). Sujaya *et al*. (2004; 2011) revealed that *Saccharomyces cerevisiae* is predominantly found in traditional rice wine. However, there is no information about the bioactive compounds of traditional rice wine.

In this research, the chemical compounds of rice wine which has pharmacological benefits and the yeast species in the final product of rice wine sold in the market were investigated. This research provided information about rice wine ingredients, including the yeast species and bioactive compounds which could be a genetic resource and a source of beneficial compounds for health in the future.

MATERIALS AND METHODS

Sampling Site and Sample Isolation

The rice wine sample was taken from a local vendor in Tragtag village, West Lombok Regency, West Nusa Tenggara Province, Indonesia. It was made of rice or sticky rice at a household scale, based on the existing local wisdom. The sample were taken from 40 mL of rice wine using a 50 mL vial tube then stored in a chiller before transported to the lab.

In the laboratory, the pH, sugar, and alcohol content (Refractometer) of the rice wine were tested. As for defining the yeast species, the wine sample was isolated by performing a serial dilution technique on three different media, namely YMA (10 g/L yeast extract - Bacto 21750, 5 g/L peptone - Oxoid LP0037, 3 g/L malt extract - Bacto 218630, 10 g/L glucose - Merck 108337, 20 g/L agar, 0.5 g chloramphenicol - Sigma C-0378), PDA (20 g/L potato dextrose agar - Himedia M096, 0.5 g chloramphenicol) and MEA (30 g/L malt extract - Bacto 218630, 20 g/L agar, 0.5 g chloramphenicol - Sigma C-0378) until reaching 10^5-10^7 dilution, then poured onto each agar. After incubation for 2-3 days, the single colony was picked up by sterilized toothpicks and subsequently purified several times. The single colony was then observed under a microscope (Olympus BX53). Finally, the pure yeast isolates were cryopreserved to 10% glycerol and 5% trehalose and deep-frozen to -80 °C.

Molecular Identification of Yeast Isolates

A single colony of yeast isolates was taken from deep-freeze preservation and was inoculated onto PDA. After 48 hours of incubation, approximately 1 µL of the cell colony was mixed into 50 µL of nuclease-free water and vortexed for several seconds. In order to gain the DNA template, the cell colony was then extracted by a boiling technique at 98 °C for 10 minutes (Packeiser *et al*., 2013). The PCR amplification of region D1/D2 of 26S ribosomal RNA gene referred to Sumerta and Kanti (2018) procedures using forward primer NL1 - CATATCAATAAGCGAAAAG and reverse primer NL4 - GGTCCGTGTTTCAAGACGG. After confirming the PCR products in 1% agarose gel, the amplicons were then sequenced to Macrogen, Republic of Korea All the obtained chromatogram trace files were converted into high-quality DNA sequences using SeqTrace (Stucky, 2012), while the homology analysis was aligned with type materials of 28S ribosomal RNA sequences from fungi type database and optimized by megablast algorithm in BLASTn. All the yeast strains isolated from the rice wine were deposited to Indonesian Culture Collection (InaCC), Research Center for Biology, Indonesian Institute of Sciences (LIPI) and their sequences were also submitted to GenBank for public references.

GC/MS Analysis

The preparation process adopted (Canelas *et al*., 2009) with some modifications. About 1 mL of the rice wine was filtered using the Millipore Millex Syringe filter with a pore size of 0.45 µm. The filtrate was then used for the analysis. The extraction process started by adding 2 mL of pre-cooled 50% (v/v) aqueous MeOH and 2 mL of pre-cooled $CHCl_3$. The mixture was vortexed for 10 minutes until two layers were well separated. The upper-phase and lower-phase were pooled into a new tube and dried using a vacuum concentrator (IKA-RV10). For the GC/MS analysis, the concentrated rice wine was re-suspended with 250 mL of 50% (v/v) aqueous MeOH and $CHCl_3$, then transferred to a silanized GC-vial for further analysis.

The organic-compounds were analyzed using Shimadzu GCMS-QP 2010 Ultra and Restek Rtx-5MS capillary columns as the static phase. Injection volume was about 5 µL at an injector temperature of 250 °C, ion source temperature of 200 °C, and interface temperature of 270 °C. The column temperature was programmed from 50 °C which was raised to 100 °C (7°C/min) for 3 minutes and then increased to 200 °C (10 °C/min) for 2 minutes. Finally, the column temperature reached 270 °C

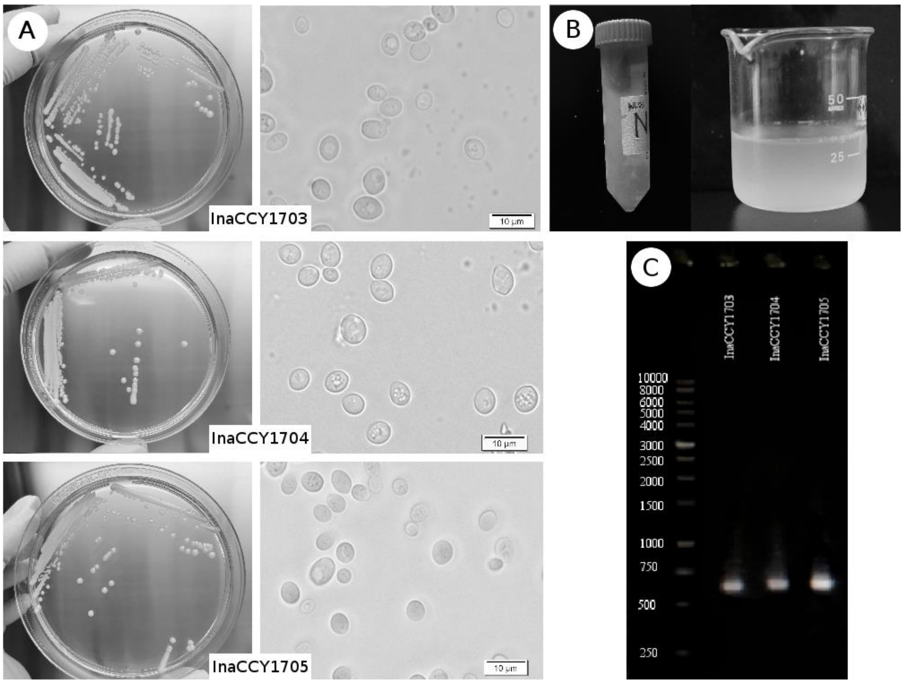

Figure 1. (A) The colony morphology of the obtained isolates on agar plates and cell morphology under the microscope observation; (B) The fresh samples of rice wine; (C) Electrophoresis visualization of D1/D2 region of 26S RNA gene

(5 °C/min). The interpretation of the detected compounds was attached to the machine database referring to the National Institute Standard and Technology (NIST) - https://chemdata.nist.gov/.

RESULT AND DISCUSSION

Yeast species in rice wine

Rice wine is one of ubiquitous alcoholic beverages in Lombok Island. This beverage is commonly sold by the local people in household shops or traditional markets. Lombok's rice wine is traditionally made of rice or sticky rice and fermented using the local knowledge without any sophisticated appliances or any distillation process. The sample of rice wine contained 4.5% alcohol and 7 % sugar withpH of 3 and remained with a milky in colour (Figure 1B). These properties suggested that the product was only produced by filtration at the end of the fermentation and still contained insoluble solids suspension. A filtration process only removes solid materials but does not stop the fermentation process, thus affecting the quality especially flavour and shortening the shelf life of a beverage as the metabolism of the yeasts is still ongoing (de la Fuente-Blanco *et al.*, 2016). This condition could also promote the growth of spoilage microorganisms such as acetic acid bacteria and lactic acid bacteria which decrease the pH or flavour of rice wine (Bartowsky & Henschke, 2008; Rhee *et al.*, 2011).

The yeast species that was still found in the final product of the rice wine sold in the market was isolated by using a serial dilution technique on three isolation media. The morphology of the yeast colonies on agar plates were similar to that of under a microscope observation (Figure 1A). The molecular identification was then performed to ensure the taxonomic position of the three obtained isolates. Based on the molecular homology on D1/D2 area of 26S ribosomal RNA gene, all of the obtained isolates were closely related to *Saccharomyces cerevisiae* (Table 1). This sequence area was about 600 bp visualized on the electrophoresis result as shown in Figure 1C.

The harvested stage of the fermentation process contributed to the view of the isolated microbial community (Kim *et al.*, 2010) since each microbe species had different ranges of tolerance on various ecological factors such as pH. In this study, the final product of the rice wine sold in the market only contained one yeast species which might have the highest tolerance characteristics. Basically, yeasts have been more effective as alcohol producer than other microbial groups (Walker & Walker, 2018; Vamvakas & Kapolos, 2020) while *S. cerevisiae* is the yeast species that is commonly found in many alcoholic beverages (Walker & Walker, 2018) and reportedly could tolerate a high alcohol concentration

(Vamvakas & Kapolos, 2020). This species could lengthen the fermentation process to obtain a higher alcohol level and it is usually found at the end of a fermentation process (Sujaya *et al.*, 2004). However, a longer fermentation process could disrupt the organoleptic properties such as flavour, pH, and aroma (de la Fuente-Blanco *et al.*, 2016).

Profile of Chemical Compounds of Rice Wine Retrieved from GC/MS analysis

In addition to alcohol as the main product, Lombok's rice wine also produced various chemical compounds based on gas chromatography tandem with mass spectrometry (GC/MS) results. By using a combination of methanol and chloroform, this method could expand the detected compound coverage in the mass spectrometry as a detector such as amino acids, polar organic compounds, and non-polar fatty acids (Khoomrung *et al.,* 2015). This combination reportedly allows polar and non-polar compounds and is common for exploring general compounds (Canelas *et al.*, 2009; Khoomrung *et al.,* 2015; Haleyur *et al.*, 2016). Furthermore, this method was also supported by using Restek Rtx-5MS GC-MS Columns known as a general column for many purposes to detect wider coverage of the compounds in the rice wine.

Some compounds are an important part of foods and beverages to enhance the value for consumption or industry such as aroma and flavor. In this study, a medium spectrum peak over 30 minutes retention time was obtained (Figure 2). Those spectrums were affiliated to 10 compounds in fatty acids and hydrocarbons while the hydrocarbon, ethane as the highest % area following fatty acids, Tetratriacontane and l-(+)-Ascorbic acid 2,6-dihexadecanoate (Table 2). Some of those organic compounds were volatile (VOCs) such as ethane, benzene, anhydro 5-mercapto-3-methyl-1,2,3,4-oxatriazolium hydroxide, performic acid as they had shorter retention times, meaning low boiling points. These VOCs could become aromatic hydrocarbons constructing a specific odor of the beverages (Alvim *et al.*, 2017).

Predicted Bioactive Compounds of Rice Wine

After referring to the National Institute Standard and Technology (NIST) database and some references journals, six of ten compounds were known to exhibit biological activity as well as pharmacological characteristics (Figure 3). The most prevailing compounds were l-(+)-Ascorbic acid 2,6-dihexadecanoate and Octadecanoic acid or stearic acid. Both of those compounds have reportedly

Table 1. Yeasts species isolated from rice wine

No	Isolate codes	InaCC codes	GenBank accession	Name of species	Similarity index (%)
1	Y19LM010	InaCCY1703	MT810009	*Saccharomyces cerevisiae*	99.65
2	Y19LM011	InaCCY1704	MT810010	*Saccharomyces cerevisiae*	100
3	Y19LM019	InaCCY1705	MT810011	*Saccharomyces cerevisiae*	100

Table 2. A list of the chemical compounds of rice wine referring to GC/MS database (NIST)

No.	Ret. Time	% Area	Name
1.	3.698	42.63	Ethane, 1,1'-oxybis[2,2-dimethoxy-
2.	3.928	7.15	Benzene, 1,1'-(1,5-hexadiene-1,6-diyl)bis-
3.	4.065	1.59	Anhydro 5-mercapto-3-methyl-1,2,3,4-oxatriazolium hydroxide
4.	7.406	2.21	Performic acid, trimethylsilyl derivative
5.	24.388	17.55	l-(+)-Ascorbic acid 2,6-dihexadecanoate
6.	27.809	2.85	Octadecanoic acid
7.	29.800	20.27	Tetratriacontane
8.	29.967	2.27	Sulfurous acid, octadecyl 2-propyl ester
9.	30.067	1.86	Eicosane, 2,6,10,14,18-pentamethyl-
10.	30.879	1.62	1-Cyclohexyldimethylsilyloxy-3,5-dimethylbenzene

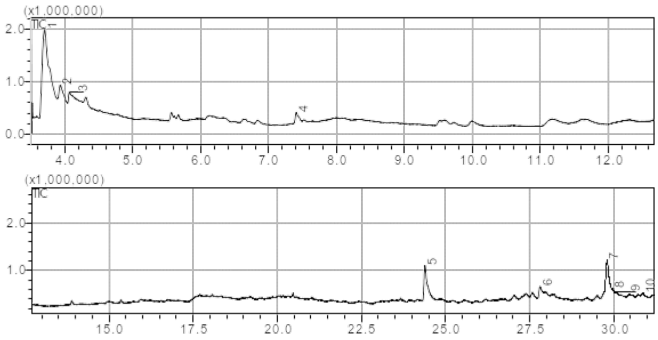

Figure 2. Profiles of GC/MS chromatograms of the rice wine

had multi-functions for pharmacology and are commonly found in plants (Ramya *et al.*, 2015). A vitamin C derivative, l-(+)-Ascorbic acid 2,6-dihexadecanoate, could act as an anticoagulant, antioxidant, anti-inflammatory, antitumor, anticonvulsant, antidiabetic, antidiarrheic, antiglaucoma, antifertility, antigastric, antimalaria, antiprotozoal, antiatheroscelerotic, anti-fatigue, antihepatic, antihypertensive, antiplague, antiproliferative, antiseptic, anti-stroke, antituberculous, hypolipidemic, neuroprotective, neurotransmitter, termiticide, and antiviral agent (Ramya *et al.*, 2015). Meanwhile, a saturated long-chain fatty acid, Octadecanoic acid, could play a role as an anticancer and anti-inflammatory (Manivannan *et al.*, 2017), anesthetic, anthelmintic, anticoagulant, anti-fatigue, antiaging, antiamoebic, anti-anaemic, antianxiety, antiasthmatic, antibacterial, antibiotic, antioxidant, antidiabetic, anti-bronchitis, anticalculi, anticarcinogenic, anticataract, anticonvulsant, antianalgesic, anti-diarrheic, antifertility, antigastric, antihemorrhagic, antimalaria, antiobesity, antistress, and antiviral agent (Ramya *et al.,* 2015). These compounds have quite robust functions for pharmacology and would be potential for further uses.

The remaining bioactive-compounds possess antimicrobial and anticancer activities. Tetratriacontane is a volatile compound that has antimicrobial activity (Karabay-Yavasoglu *et al.*, 2007; Swamy *et al.*, 2017) similar to sulfurous acid which could inhibit the growth of certain bacteria (Caballero *et al.,* 2003). Antimicrobial activity is also shown in performic acid which is known as disinfectant in the medical and food industry (Gehr *et al.*, 2009). This compound could eliminate bacteria and viruses such as Salmonella spp., spores and cysts (Karpova *et al.*, 2013) and is quite popular as a natural disinfectant (Gehr *et al.,* 2009). Lastly, eicosane is a fatty acid that is responsible as an antibacterial (Kumar, 2011) and antifungal agent (Ahsan *et al.*, 2017).

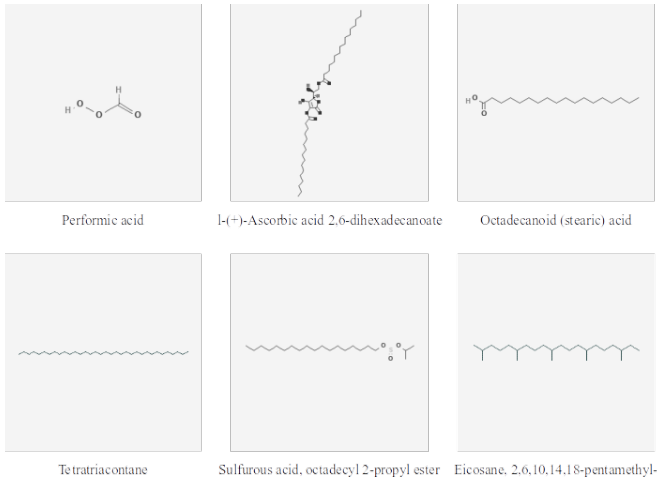

Figure 3. Molecular structure of the bioactive-compounds of rice wine retrieved from PubChem Database (NCBI, 2020)

All of those bioactive compounds are really beneficial for pharmaceutical purposes, so making rice wine as an alternative source of these bioactive compounds is important. However, future research should answer the challenges concerning the extraction and testing of the compounds as there is a time-dependent metabolite production during the fermentation process (Kim *et al.*, 2010). In addition, the profile of other microbes which construct the compounds should be delineated to obtain a comprehensive overview. Adding the effectiveness of the bioactive compounds to certain pathogenic microbes in vitro might enrich the data of the potential of local rice wine as a source of compounds with health benefits .

CONCLUSION

A traditional rice wine originated from West Nusa Tenggara Province is sold without distillation process. It still contains live yeast species namely *Saccharomyces cerevisiae* which is responsible for a fermentation process. Furthermore, it contains at least six bioactive compounds which have been reportedly related to antimicrobial, antiviral, antioxidant, and other biological activities that might have health benefits.

ACKNOWLEDGEMENT

The authors would like to express our gratitude to Mr. Muhammad Ilyas and Mrs. Indri Ramadhani for their assistants during the sample collection in Lombok, Nusa Tenggara Barat. This study was also funded by the Budget Implementation List (DIPA) of Research Center for Biology FY 2019 Ref. No. 3400.001.051.F

CONFLICT OF INTEREST

The authors whose names are listed declare there is no conflict of interests in the article being submitted for publication.

REFERENCES

Ahsan, T., Chen, J., Zhao, X., Irfan, M., & Wu, Y. (2017). Extraction and identification of bioactive compounds (eicosane and dibutyl phthalate) produced by *Streptomyces* strain KX852460 for the biological control of *Rhizoctonia solani* AG-3 strain KX852461 to control target spot disease in tobacco leaf. *AMB Express*, *7*(1), 1–9. https://doi.org/10.1186/s13568-017-0351-z

Alvim, R. P. R., de Cássia Oliveira Gomes, F., Garcia, C. F., de Lourdes Almeida Vieira, M., & de Resende Machado, A. M. (2017). Identification of volatile organic compounds extracted by headspace solid-phase microextraction in specialty beers produced in Brazil: Identification of volatile compounds in specialty beers. *Journal of the Institute of Brewing*, *123*(2), 219–225. https://doi.org/10.1002/jib.416

Bartowsky, E. J. & Henschke, P. A. (2008). Acetic acid bacteria spoilage of bottled red wine -A review. *International Journal of Food Microbiology. 125*(1), 60-70. https://doi.org/10.1016/j.ijfoodmicro.2007.10.016

Caballero, B., Trugo, L. C., & Finglas, P. M. (2003). *Encyclopedia of food sciences and nutrition*. Available at http://agris.fao.org/agris-search/search.do?recordID=US201300082710 (retrieved on 15 August 2020)

Cai, H., Zhang, Q., Shen, L., Luo, J., Zhu, R., Mao, J., Zhao, M., & Cai, C. (2019). Phenolic profile and antioxidant activity of Chinese rice wine fermented with different rice materials and starters. *LWT*, *111*, 226–234. https://doi.org/10.1016/j.lwt.2019.05.003

Canelas, A. B., ten Pierick, A., Ras, C., Seifar, R. M., van Dam, J. C., van Gulik, W. M., & Heijnen, J. J. (2009). Quantitative evaluation of intracellular metabolite extraction techniques for yeast metabolomics. *Analytical Chemistry*, *81*(17), 7379–7389. https://doi.org/10.1021/ac900999t

Capece A., Romano P. (2019) Yeasts and Their Metabolic Impact on Wine Flavour. In: Romano P., Ciani M., Fleet G. (eds) Yeasts in the Production of Wine. *Springer*, New York, NY. https://doi.org/10.1007/978-1-4939-9782-4_2

Daglia, M., Papetti, A., Grisoli, P., Aceti, C., Dacarro, C., & Gazzani, G. (2007). Antibacterial Activity of Red and White Wine against Oral Streptococci. *J. Agric. Food Chem.*, *55*(13), 5038–5042. https://doi.org/https://pubs.acs.org/doi/abs/10.1021/jf070352q

de la Fuente-Blanco, A., Sáenz-Navajas, M. P., & Ferreira, V. (2016). On the effects of higher alcohols on red wine aroma. *Food Chemistry*, *210*, 107–114. https://doi.org/10.1016/j.foodchem.2016.04.021

De Vuyst, L., Harth, H., Van Kerrebroeck, S., & Leroy, F. (2016). Yeast diversity of sourdoughs and associated metabolic properties and functionalities. *International Journal of Food Microbiology*, *239*, 26–34. https://doi.org/10.1016/j.ijfoodmicro.2016.07.018

Gehr, R., Chen, D., & Moreau, M. (2009). Performic acid (PFA): tests on an advanced primary effluent show promising disinfection performance. *Water Science and Technology: A Journal of the International Association on Water Pollution Research*, *59*(1), 89–96. https://doi.org/10.2166/wst.2009.761

Haleyur, N., Shahsavari, E., Mansura, A. A., Koshlaf, E., Morrison, P. D., Osborn, A. M., & Balla, A. S. (2016). Comparison of rapid solvent extraction systems for the GC–MS/MS characterization of polycyclic aromatic hydrocarbons in aged, contaminated soil. *MethodsX*, *3*, 364–370. https://doi.org/10.1016/j.mex.2016.04.007

Hong, X., Chen, J., Liu, L., Wu, H., Tan, H., Xie, G., Xu, Q., Zou, H., Yu, W., Wang, L., & Qin, N. (2016). Metagenomic sequencing reveals the relationship between microbiota composition and quality of Chinese Rice Wine. *Scientific Reports*, *6*(1), 1–11. https://doi.org/10.1038/srep26621

Jamir, L., Kumar, V., Gat, Y., Kumar, A., & Kaur, S. (2019). Wine: a potential source of antimicrobial compounds. *Journal of Wine Research*, *30*(3), 220–237. https://doi.org/10.1080/09571264.2019.1652151

Karabay-Yavasoglu, N. U., Sukatar, A., Ozdemir, G., & Horzum, Z. (2007). Antimicrobial activity of volatile components and various extracts of the red alga Jania rubens. *Phytotherapy Research: PTR*, *21*(2), 153–156. https://doi.org/10.1002/ptr.2045

Karpova, T., Pekonen, P., Gramstad, R., Öjstedt, U., Laborda, S., Heinonen-Tanski, H., Chávez, A., & Jiménez, B. (2013). Performic acid for advanced wastewater disinfection. *Water Science and Technology*, *68*(9), 2090–2096. https://doi.org/10.2166/wst.2013.468

Kawarai, T., Furukawa, S., Ogihara, H., & Yamasaki, M. (2007). Mixed-species biofilm formation by lactic acid bacteria and rice wine yeasts. *Applied and Environmental Microbiology*, *73*(14), 4673–4676. https://doi.org/10.1128/AEM.02891-06

Khoomrung, S., Martinez, J. L., Tippmann, S., Jansa-Ard, S., Buffing, M. F., Nicastro, R., & Nielsen, J. (2015). Expanded metabolite coverage of *Saccharomyces cerevisiae* extract through improved chloroform/methanol extraction and tert-butyldimethylsilyl derivatization. *Analytical Chemistry Research*, *6*, 9–16. https://doi.org/10.1016/j.ancr.2015.10.001

Kim, A. J., Choi, J. N., Kim, J., Park, S. B., Yeo, S. H., Choi, J. H., & Lee, C. H. (2010). GC-MS based metabolite profiling of rice Koji fermentation by various fungi. *Bioscience,* *Biotechnology, and Biochemistry*, *74*(11), 2267–2272. https://doi.org/10.1271/bbb.100488

Kumar, V. (2011). Antibacterial activity of crude extracts of *Spirulina platensis* and its structural elucidation of bioactive compounds. *Journal of Medicinal Plants Research*, *5*(32). https://doi.org/10.5897/JMPR11.1175

Kwon, D.Y., Nyakudya, E., Jeong, Y.S. (2014). Fermentation: Food Products. In: Neal K. Van Alfen, Encyclopedia of Agriculture and Food Systems. *Academic Press*. The USA https://doi.org/10.1016/B978-0-444-52512-3.00155-8

Lee, J., Lee, Y., Ha, J., Yoo, M., & Jang, H. W. (2018). Simultaneous determination of four bioactive compounds in Korean rice wine (makgeolli) by solvent extraction coupled with gas chromatography-mass spectrometry. *International Journal of Food Properties*, *21*(1), 139–146. https://doi.org/10.1080/10942912.2017.1414841

Manivannan, P., Muralitharan, G., & Balaji, N. P. (2017). Prediction aided in vitro analysis of octa-decanoic acid from *Cyanobacterium Lyngbya* sp. as a proapoptotic factor in eliciting anti-inflammatory properties. *Bioinformation*, *13*(9), 301–306. https://doi.org/10.6026/97320630013301

Mazzoli, R., Riedel, K., & Pessione, E. (2017). Editorial: Bioactive compounds from microbes. *Frontiers in Microbiology*, *8*. https://doi.org/10.3389/fmicb.2017.00392

NCBI. (2020). Pubchem Compound Summary for Performic acid, Ascorbic acid, Octadecanoic acid, Tetratriaconate, Eicosane. Retrived from https://pubchem.ncbi.nlm.nih.gov/ (16 Desember 2020).

Packeiser, H., Lim, C., Balagurunathan, B., Wu, J., Zhao, H. (2013). An extremely simple and effective colony PCR procedure for bacteria, yeasts, and microalgae. *Appl. Biochem. Biotechnol., 169*(2), 695–700. https://doi.org/10.1007/s12010-012-0043-8

Pires, E. J., Teixeira, J. A., Brányik, T., & Vicente, A. A. (2014). Yeast: the soul of beer's aroma-a review of flavour-active esters and higher alcohols produced by the brewing yeast. *Applied Microbiology and Biotechnology*, *98*(5), 1937–1949. https://doi.org/10.1007/s00253-013-5470-0

Ramya, B., Malarvili, T., & Velavan, S. (2015). GC-MS analysis of bioactive compounds in Bryonopsis laciniosa fruit extract. *IJPSR*, *29*, 3375–3379. https://doi.org/10.13040/IJPSR.0975-8232.6(8).3375-79

Ray, M., Ghosh, K., Singh, S., & Mondal, K. C. (2016). Folk to functional: An explorative overview of rice-based fermented foods and beverages in India. *Journal of Ethnic Foods*, *3*(1), 5–18. https://doi.org/10.1016/j.jef.2016.02.002

Rhee, S. J., Lee, J.-E., & Lee, C.-H. (2011). Importance of lactic acid bacteria in Asian fermented foods. *Microbial Cell Factories*, *10*(1), 1–13. https://doi.org/10.1186/1475-2859-10-S1-S5

Rodríguez-Vaquero, M. J., Vallejo, C. V., & Aredes-Fernández, P. A. (2020). Antibacterial, antioxidant and antihypertensive properties of polyphenols from argentinean red wines varieties. *Open Journal of Pharmacology and Pharmacotherapeutics*, *5*(1), 001–006. https://doi.org/10.17352/ojpp.000010

Sánchez, M. C., Ribeiro-Vidal, H., Esteban-Fernández, A., Bartolomé, B., Figuero, E., Moreno-Arribas, M. V., Sanz, M., & Herrera, D. (2019). Antimicrobial activity of red wine and oenological extracts against periodontal pathogens in a validated oral biofilm model. *BMC Complementary and Alternative Medicine*, *19*. https://doi.org/10.1186/s12906-019-2533-5

Snopek, L., Mlcek, J., Sochorova, L., Baron, M., Hlavacova, I., Jurikova, T., Kizek, R., Sedlackova, E., & Sochor, J. (2018). Contribution of red wine consumption to human health protection. *Molecules* , *23*(7), 1684. https://doi.org/10.3390/molecules23071684

Stucky, B. J. (2012). SeqTrace: a graphical tool for rapidly processing DNA sequencing chromatograms. *Journal of Biomolecular Techniques: JBT*, *23*(3). https://doi.org/10.7171/jbt.12-2303-004

Sujaya, I. N., Antara, N. S., Sone, T., Tamura, Y., Aryanta, W. R., Yokota, A., Asano, K., & Tomita, F. (2004). Identification and characterization of yeasts in brem , a traditional Balinese rice wine. *World Journal of Microbiology & Biotechnology*, *20*(2), 143–150. https://doi.org/10.1023/B:WIBI.0000021727.69508.19

Sujaya, I. N., Mikumo, D., Orikasa, Y., Urashima, T., & Yuji, O. D. A. (2011). Baking properties of *Saccharomyces cerevisiae* strains derived from brem, a traditional rice wine from Bali. *Food Science and Technology Research*, *17*(4), 369–373. https://doi.org/10.3136/fstr.17.369

Sumerta, I. N. & Kanti, A. (2018). Taxonomic approach for species diversity of yeasts and yeasts-like fungi through D1/D2 region of large subunit ribosomal DNA sequences. *Biosaintifika*, *10*(1), 72–78. https://doi.org/10.15294/biosaintifika.v10i1.11588

Swamy, M. K., Arumugam, G., Kaur, R., Ghasemzadeh, A., Yusoff, M. M., & Sinniah, U. R. (2017). GC-MS based metabolite profiling, antioxidant and antimicrobial properties of different solvent extracts of Malaysian *Plectranthus amboinicus* leaves. *Evidence-Based Complementary and Alternative Medicine: eCAM*, *2017*. https://doi.org/10.1155/2017/1517683

Vamvakas, S. S., & Kapolos, J. (2020). Factors affecting yeast ethanol tolerance and fermentation efficiency. *World Journal of Microbiology & Biotechnology*, *36*(8), 1–8. https://doi.org/10.1007/s11274-020-02881-8

Walker, G. M. & Walker, R. S. K. (2018). Enhancing yeast alcoholic fermentations. *Advances in Applied Microbiology*, *105*, 87–129. Academic Press. https://doi.org/10.1016/bs.aambs.2018.05.003