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Fermentation of White and Brown Rice Water Increases Plant Nutrients and Beneficial Microbes

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ABSTRACT

The wastewater after washing rice grains is known as washed rice water (WRW). WRW is often recommended for reuse as plant fertilizer, but little is known about the difference in the nutrient and microbial contents of WRW between white and brown rice. The study aims to answer this question and determine how much the nutrient contents in the WRW would change with fermentation and how fermentation would affect the phosphorus (P)- and potassium (K)-solubilization bacteria in the WRW. Medium-grained rice was washed at a volumetric rice-to-water ratio of 1:3 for 90 seconds at 0.357 × *g*. WRW was then fermented for 0 (fresh), 3, 6, and 9 days. The rice grains and WRW were analyzed for pH, electrical conductivity (EC), carbon (C), nitrogen (N), sulfur (S), ammonium (NH_4^+), nitrate (NO_3^-), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), zinc (Zn), and boron (B), as well as for total microbial population and P- and K-solubilizing bacteria. Brown rice grains had 26 to 324% higher P, K, Mg, and Zn than white rice. Nutrient contents in the WRW increased with increasing fermentation, except for C, which decreased with fermentation. At 9 days of fermentation, P, Ca, Mg, Cu, and B in the white rice water increased by 4 to 207%, which were also higher than in the brown rice water. The microbial population increased with fermentation for 3 days, then decreased after that, following the same C trend in the WRW from both rice types. P-and K-solubilization by bacteria in the WRW from both rice types increased with fermentation. The P solubilization was 25% higher in brown rice water, while the

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K solubilization was 67% higher in white rice water. Fermented rice water from white and brown rice was revealed to potentially improve plant growth and increase overall soil health due to their plant nutrient and microbial contents.

Keywords: Elements, fermentation, ratio, rice, rice water, wastewater

INTRODUCTION

Washed rice water (WRW) is obtained when milled rice grains are washed to remove the bran, dust, and dirt from the rice before the rice is cooked (Juliano, 1993). However, significant proportions of water-soluble nutrients are removed because of rice washing. Several studies have indicated that rice washing leads to the leaching of different proportions of nutrients from the rice grains into the WRW (Juliano, 1985; Nabayi, Sung, Zuan, Paing, & Akhir, 2021). For example, Nabayi, Sung, Zuan, Paing, and Akhir (2021) reviewed that WRW contains between 40 to 150 mg L^{-1} N, 43 to 16306 mg L^{-1} P, 51 to 200 mg L^{-1} K, 8 to 3574 mg L^{-1} Ca, 36 to 1425 mg L^{-1} Mg, and 27 to 212 mg L^{-1} S. It indicates that these leached nutrients have enriched the WRW, which potentially could be utilized as a liquid plant fertilizer, rather than WRW being discarded (Nabayi, Sung, Paing, & Zuan, 2021b). Other studies have reported that WRW can be used as a plant nutrient source (Bahar, 2016; Suryana et al., 2017; Wardiah & Rahmatan, 2014) due to the presence of carbohydrates, proteins, vitamins, and other minerals at various concentrations (Juliano, 1985; Purnami et al., 2014).

Recently, WRW was reused as part of communal programs, whereby WRW from different households is collected, pooled centrally, then used for fertilizing crops. For instance, in Lambangkuning Village, Indonesia, a WRW reuse program was established. This program comprised about 30 households, each producing about 5 L of WRW daily, totaling 150 L per day. The collected WRW was then pooled for reuse later to irrigate the crops in the village homes (Supraptiningsih et al., 2019). Similarly, Polo Geulis, a village in Central Bogor, Indonesia, practiced a centralized water-saving system, where WRW was collected from the residents and used to irrigate and fertilize the village herbs and vegetables (The Jakarta Post, 2017). Kalsum et al. (2011) reported that fermented WRW contains numerous nutrients essential to plant growth and development. Hapsoh et al. (2019) found higher vegetative growth of pepper due to the bacterial content of fermented WRW. Similarly, several studies have reported using WRW to increase the growth of other crops (Bahar, 2016; Hariyadi, 2020; Karlina et al., 2013; Yulianingsih, 2017).

Sairi et al. (2018) found and biochemically identified the presence of beneficial microbes in WRW and suggested its use as a biofertilizer. Biofertilizer is recommended as part of sustainable agriculture, which aims to use fewer chemical fertilizers. The addition of beneficial microbes into the soil could increase plant nutrient availability, thereby reducing the demand for chemical fertilizers (Çakmakçi et al., 2007). N_2-fixing and P and K solubilizing bacteria are of great importance for plant nutrition because they can increase plants' N, P, and K uptake (Çakmakçi et al., 2006; Ekin, 2010). In addition, the direct application of plant growth-promoting (PGP) microbes can improve plant growth (Kumar et al., 2014). PGP bacteria and rhizobia are vital in supplying nutrients to plants, particularly in less fertile soils (Tan et al., 2014). However, these WRW studies, mostly done on white rice, lacked scientific details, such as whether the WRW use was fresh or fermented, and if fermented, for how long and how rigorously the rice grains were washed, and what were the nutrient contents in the WRW.

Rice is a vital source of carbohydrates, protein, and other essential nutrients for billions of people worldwide, particularly in developing nations (Huang et al., 2016). It is usual to wash rice to remove dirt and dust before cooking. Considering the rate at which rice is consumed, the practice of reusing WRW has the potential to save significant amounts of water and fertilizers. In addition, it will lead to less reliance on energy, particularly during the current environment of detrimental climate change.

Rice parboiling ensures the retention of some nutrients contained in the rice bran (Roy et al., 2011). However, removing the outer bran layers while milling the white rice renders it low in nutrients and bioactive chemicals (Saleh, Wang, Wang, S. Yang, et al., 2019). Compared with white rice, whole brown rice grain has higher mineral contents because of the remaining presence of the outer bran layer (Babu et al., 2009; de Simone Carlos Iglesias Pascual et al., 2013; Saleh, Wang, Wang, L. Yang, et al., 2019). Nabayi, Sung, Paing, and Zuan (2021b), as well as Srinuttrakul and Busamongkol (2014), have reported that the elemental concentrations in white rice and brown rice grains ranged from (in mg kg^{-1}) 1298 to 3830 P, 1109 to 1928 K, 72 to 437 Ca, 240 to 1284 Mg, 0.31 to 0.37 B, 4.96 to 5.31 Cu, 5.10 to 9.75 Fe, 22 to 44 Mn, and 23 to 33 Zn. However, there is no comparative study of rice water from white and brown rice grains in terms of their nutrient and microbial contents and how the nutrient and microbial contents change when the rice water is fermented. Nabayi, Sung, Zuan, and Paing (2021a) reported the presence of beneficial bacteria in white rice water, but there has been no similar study on brown rice water. In addition, there are limited studies on assessing P- and K-solubilizing bacteria in rice water, which could play a significant role in the higher plant growth of many crops, as reported by Nabayi, Sung, Zuan, Paing, and Akhir (2021). Therefore, the objectives of this paper were to (1) analyze the nutrient contents in white and brown rice grains and their respective rice water at different fermentation periods and (2) assess the P and K solubilization in the white and brown rice water at different fermentation periods.

MATERIALS AND METHODS

Materials

"Rambutan" (Padiberas Nasional Berhad, Malaysia) and "Eco-brown" (Serba Wangi Sdn. Bhd., Malaysia) brands of white rice and brown rice grains, respectively, were used in this study. A mixing machine (Bossman Kaden matte BK-100S, Japan) was used for washing the rice grains at a volumetric rice-to-water ratio of 1:3 for 90 seconds at a speed of 0.357 × *g* to ensure consistency, repeatability, and uniformity in rice washing. WRW from the white rice and brown rice grains were then subjected to a series of fermentation periods of 0 (fresh), 3, 6, and 9 days. The choice of fermentation periods followed Akib et al. (2015), who fermented rice water for 0, 2, 4, and 6 days, and they reported an increase in microbial count over time. Therefore, this study selected 0, 3, 6, and 9 days to observe the nutrient and microbial population before the fourth and after the sixth days. The fermentation was carried out without adding additives by covering the WRW container for 0, 3, 6, and 9 days. The containers were opened daily for about 20 minutes for aeration.

Elemental Analyses

Dry ashing of rice samples was carried out following Enders and Lehmann (2012). About 1 g of the oven-dried (at 105 °C) ground rice grain samples were placed in a muffle furnace and heated sequentially from 200 to 550 °C for 6 hours to obtain complete ash for additional tests. The rice samples' total C, N, and S content were determined using the CNS analyzer (LECO Corp., MI, USA). In contrast, P, K, Ca, Mg, Cu, Zn, and B were determined using Inductively Coupled Plasma Optical Emission Spectrophotometry (ICP-OES) (Thermo Fisher Scientific, iCAP 6000, Germany). The rice water samples at different fermentation periods were filtered using Whatman 1 filter paper (11 µm size). The total C, N, and S of the rice water were determined by a CNS analyzer (LECO Corp., MI, USA). At the same time, the P, K, Ca, Mg, Cu, Zn, and B were analyzed using an atomic absorption spectrophotometer (AAS) (PerkinElmer, PinAAcle, 900T, USA). The Kjeldahl procedure (Nelson & Sommers, 1983) was employed for ammonium and nitrate determination. The 827 pH and EC lab meters were used to determine the pH and EC, respectively (Metrohm AG, Switzerland) (McLean, 1983).

Culture Media Preparation and Microbial Population Determination

Tryptic soy agar (TSA) and potato dextrose agar (PDA) were used to assess the bacteria and fungi population, respectively, in the different samples of the fermented rice water of the two rice grains, following Tan et al. (2014). The media were prepared by dissolving about 40 g each of TSA and PDA media and autoclaved at 121 psi for 20 minutes. The media were removed and poured into Petri dishes, then allowed to solidify under laminar flow. A serial dilution of up to 10^8 was performed on the different rice waters (from white and brown rice) at different fermentation periods (0-, 3-, 6-, and 9-day). After that, the serially diluted rice water was introduced onto the plates containing the TSA and PDA-prepared media for bacteria and fungi determination, respectively. The inoculated plates were incubated for 24 hours at 33 °C, and subsequently, the bacterial and fungi populations were counted for each plate (in triplicates). Plates having between 30 to 300 colony-forming units were selected to calculate the microbial population per mL of sample (Thomas et al., 2015).

Phosphorus and Potassium Solubilization Ability of the WRW Culture

The National Botanical Research Institute's Phosphate (NBRIP) broth was used to measure the amount of soluble phosphate in the culture supernatants using the vanado-molybdo-phosphoric acid method (Ribeiro & Cardoso, 2012). A combination of a stock solution containing phosphoric acid (KH_2PO_4) and 5 mL of concentrated sulfuric acid (H_2SO_4) was made up to 1 L using distilled water to form a standard curve. Each NBRIP broth was inoculated with 10 µL of each WRW culture suspension. A flask containing 100 mL of NBRIP culture medium with no inoculum was used as the control. Tan et al. (2014) described that the flasks were incubated at room temperature between 25 to 29 ^{o}C for 12 days, with a steady agitation at 100 rpm. The P solubilization of the cultures was assessed at various fermentation periods at 12 days of incubation. At each assessment, 25 mL of each culture media was transferred to 50 mL tubes and centrifuged at 8,000 × *g* for 20 minutes. Next, the 2.5 mL of the supernatant was transferred to a 50 mL beaker, then 20 mL of distilled water was added. A 2.5 mL of Barton's reagent was promptly added for the mixing reaction (Barton, 1948), and color development was permitted for 10 minutes. A spectrophotometer (Agilent Technologies 8453 7 Cuvette UV Vis Spectrophotometer, USA) set to 430 nm was used to determine the absorbance.

The bacterial ability to release potassium from the Aleksandrov broth was used to calculate the potassium solubilization rate quantitatively. One mL of the WRW culture was inoculated into 100 mL of the broth overnight. The amount of K released in the broth was quantified from three flask replicates after 12 days of incubation (Nabayi, Sung, Zuan, Paing, & Akhir, 2021). The inoculated broth cultures were centrifuged at 6,700 × *g* for 10 minutes to separate the supernatant from the broth and bacterial cells. One mL of the supernatant was transferred to a 50 mL volumetric flask, which was then marked up with distilled water and carefully mixed. AAS was used to determine the amount of available K in the supernatant (PerkinElmer, PinAAcle, 900T, USA).

Statistical Analysis

All data were subjected to analysis of variance (ANOVA) using Minitab software package version 20 (Pennsylvania State University, USA). The data were analyzed based on a randomized design in factorial arrangement with fermentation periods (0, 3, 6, and 9 days) and rice water types (white and brown rice water) as factors, and the Tukey's test procedure ($p < 0.05$) was used for the mean separations.

RESULTS AND DISCUSSION

Elemental Amount of Rice Grains

The highest element in white and brown rice grains was C which comprised about 40% of the total elements in the grains (Table 1). The inner endosperm of rice kernels is densely packed with starch, mainly carbohydrates (Saleh, Wang, Wang, L. Yang, et al., 2019; Zhou et al., 2002). The results showed that the nutrient contents are in the order: C > N > P > K > S > Mg > Ca > Zn > Cu > B for both rice types (Table 1). The elemental content of both rice grains was similar to the results obtained by Srinuttrakul and Busamongkol (2014), who also found that the nutrient contents of brown rice grains to be in the same order and ranged between (in mg kg^{-1}) 3024 to 3830 P, 140 to 1927 K, 980 to 1284 Mg, 72 to 128 Ca, and 23 to 33 Zn.

Nitrogen was the second highest element in both the rice grains, which agreed with Saleh, Wang, Wang, L. Yang, et al. (2019), who reported that protein, a source of N, is the second highest element in rice grains after C. Furthermore, Saleh, Wang, Wang, S. Yang, et al. (2019) reported that brown rice grains have greater N content than white rice grains because of their outer bran layer, which is the main source of N in the rice, which corroborated the results obtained in this study as brown rice grains had 2.4% higher N content than in white rice grains. The nutrient contents of the white and brown rice grains were generally similar to each

Table 1
Mean (± SE) nutrient content of the white and brown rice grains

Parameters	White rice grains	Brown rice grains
Ash content (%)	0.95 ± 0.04	1.76 ± 0.05
Moisture content (%)	14.39 ± 0.21	17.42 ± 0.18
Organic carbon (%)	30.30 ± 0.02	30.68 ± 0.06
C (%)	40.30 ± 0.01	40.80 ± 0.01
N (%)	1.25 ± 0.01	1.28 ± 0.01
C: N ratio	32.24 ± 0.01	31.87 ± 0.01
S (%)	0.10 ± 0.004	0.12 ± 0.03
NH_4^+ (mg kg^{-1})	215.45 ± 4.41	174.08 ± 4.13
NO_3^- (mg kg^{-1})	100.82 ± 8.53	131.76 ± 9.10
P (mg kg^{-1})	1320.83 ± 34.04	3284.62 ± 43.67
K (mg kg^{-1})	1130.83 ± 22.64	2081.17 ± 25.40
Ca (mg kg^{-1})	427.08 ± 5.72	163.32 ± 3.92
Mg (mg kg^{-1})	244.93 ± 10.26	759.50 ± 21.52
B (mg kg^{-1})	0.33 ± 0.09	1.40 ± 0.02
Cu (mg kg^{-1})	5.25 ± 0.12	5.15 ± 0.10
Zn (mg kg^{-1})	5.01 ± 0.09	6.30 ± 0.06

other for C, N, S, and Cu, but not for P, K, Ca, Mg, Zn, and B. Brown rice grains had 26 to 324% higher in P, K, Mg, and Zn, but lower in Ca by 162% than the white rice grains. The greater amount of Ca in the white rice grains could be due to rice varietal differences. Nutrient content in rice can vary between genotypes and environmental conditions (Huang et al., 2016). However, the higher P, K, Mg, Zn, and B in the brown rice grains were because of the intact bran layer in the brown rice, as compared with its absence in white rice grains. When the outer bran layers are removed during polishing or milling, the polished grain has fewer nutrients and bioactive chemicals because these elements are concentrated in the rice grain's outer bran layers (Saleh, Wang, Wang, S. Yang, et al., 2019; Sharif et al., 2014). The study also revealed a higher ash percentage (85%) in brown rice than in the white rice grains, indicating higher amounts of inorganic elements in brown rice (Table 1). Overall, the chemical analyses of the rice grains agreed with several studies that also reported greater nutrient content in brown rice as compared to white rice (Babu et al., 2009; de Simone Carlos Iglesias Pascual et al., 2013; Saleh, Wang, Wang, L. Yang, et al., 2019).

Effect of Fermentation on the Nutrient Content of WRW from White Rice and Brown Rice Grains

Nutrient analyses of white and brown rice water showed increasing levels of EC, (NH_4^+), N, S, P, K, Ca, Mg, Cu, and B with increasing fermentation periods. In contrast, increasing fermentation decreased pH, C, NO_3^-, and Zn levels (Table 2). Brown rice water was found to have significantly ($p < 0.01$) higher NH_4^+, N, S, and K contents on day 9 of the fermentation period at 4, 5, 7, and 122%, respectively, than the white rice water at the same fermentation period. However, white rice water recorded significantly ($p < 0.01$) higher contents in P, Ca, Mg, Cu, and B by 47, 4, 49, 77, and 207%, respectively, on day 9 of the fermentation period than on the brown rice water. The higher Ca, and Cu content in the white rice water could be due to the higher content of Ca and Cu in the white rice grains (Table 1), as reported too by other studies (Nabayi, Sung, Paing, & Zuan, 2021b; Saleh, Wang, Wang, L. Yang, et al., 2019). However, the higher P, Mg, and B in white rice water could be because of the mineralization of WRW by the microbes, which would lead to an increase in these elements due to the utilization of the highly available C as an energy source by the microbes. These results agreed with several studies that reported that fermentation of cereals had increased the concentrations of P, Ca, Mg, and Zn, mainly due to the loss of dry matter, as microbes mineralized the carbohydrate and protein contents in the cereals, leading to the availability of these elements (Blandino et al., 2003; Pranoto et al., 2013). Similarly, Nabayi, Sung, Paing, and Zuan (2021b) reported an increase in the nutrient content of white rice water with an increase in the fermentation period due to the mineralization by the microbes present in the fermented WRW. The greater P in the white rice water, despite its lower content in the rice grains, could be attributed to the P solubilization by the beneficial microbes in the white rice water. Earlier studies had isolated and identified the presence of *Bacillus velezensis* and *Enterobacter mori* species in fermented white rice water (Nabayi, Sung, Zuan, & Paing, 2021a; Sairi et al., 2018), which were reported as plant growth-promoting bacteria that could solubilize P and K (Bhattacharyya & Jha, 2012; Deepa et al., 2008). The presence of nutrients and microbes in the rice water and their increase with fermentation could be the reasons for the higher growth of various plants, as found by several studies (Bahar, 2016; Hariyadi, 2020; Karlina et al., 2013; Yulianingsih, 2017). Iskarlia (2018) opined that rice water could be used as an organic fertilizer for plant use, while Supraptiningsih et al. (2019) stated that rice water could enhance soil fertility.

Higher levels of N, S, and K were found in the brown rice water because of their relatively higher concentrations in the brown rice grains (Table 2). Most of these elements are higher in the outer layer of the brown rice grains and washing the rice would lead to the leaching of these elements into the water. Protein, for instance, is concentrated in the outer bran layer of rice grains; hence, brown rice has a higher protein concentration than milled rice (Saleh, Wang, Wang, L. Yang, et al., 2019). The NO_3^- content in WRW increased with fermentation for the white and brown rice for 3 days, then declined after that. However, significantly higher ($p < 0.01$) NO_3^- was recorded in brown rice water at 3 days of fermentation, which was 20% more than in white rice water during the same fermentation period. The decline in NO_3^- after 3 days of fermentation could be due to denitrification during fermentation. The higher concentration of NO_3^- at 3 days was attributed to the higher bacterial population, which could indicate the presence of beneficial bacteria, such as N-fixing bacteria. Nabayi, Sung, Zuan, and Paing (2021a) found higher N-fixation bacteria in white rice water fermented for 3 days compared to other fermentation periods. Similarly, the increase in N and NH_4^+ with the fermentation period indicating the presence of N-fixation bacteria.

C content decreased with increasing fermentation by 71% and 62% in white and brown rice water, respectively, throughout the fermentation period. The higher C in the white rice water at day 0 of the fermentation period was because of the lack of the outer layer, which exposed the endosperm of the white rice grains and led to the higher leaching of C during the rice washing (unlike in the brown rice grains that still had their outer layer). The inner endosperm of rice kernels is densely packed with starch (Zhou et al., 2002). The decrease in C with fermentation was because C is an energy source for microbes. Significantly higher ($p < 0.05$) C at the lower fermentation period could indicate the limited activity of microbes, which agreed with the microbial population results (Figure 1).

Generally, the increase in the concentrations of the elements with increasing fermentation could be explained

Table 2

Mean (± SE) of the interaction between fermentation period and WRW types on pH, EC, N, C, S, NH_4^+, NO_3^-, P, K, Ca, Mg, Cu, Zn, and B

Parameters	Fermentation period (days)							
	0	3	6	9	0	3	6	9
	White rice water				Brown rice water			
pH	6.5 ± 0.20a	5.2 ± 0.12b	4.3 ± 0.10c	3.7 ± 0.11d	6.7 ± 0.21a	5.8 ± 0.13b	4.7 ± 0.10c	4.1 ± 0.08d
EC (µS cm^{-1})	563.7 ± 21.42a	613.3 ± 19.54b	677.0 ± 23.64b	783.4 ± 27.12c	291.1 ± 13.32c	332.0 ± 12.5c	382.7 ± 13.76d	412.7 ± 16.04d
C (%)	0.34 ± 0.021b	0.22 ± 0.016c	0.19 ± 0.017d	0.10 ± 0.010fg	0.21 ± 0.018a	0.14 ± 0.010e	0.11 ± 0.010ef	0.08 ± 0.008g
N (%)	0.015 ± 0.001c	0.017 ± 0.001bc	0.021 ± 0.002a	0.021 ± 0.002a	0.017 ± 0.001bc	0.018 ± 0.001b	0.021 ± 0.002a	0.022 ± 0.002a
S (%)	0.012 ± 0.001abc	0.011 ± 0.001bc	0.012 ± 0.001abc	0.014 ± 0.002a	0.010 ± 0.001c	0.013 ± 0.001ab	0.014 ± 0.002ab	0.015 ± 0.002a
NH_4^+ (mg L^{-1})	10.5 ± 0.71c	11.8 ± 0.67bc	13.9 ± 0.84a	13.9 ± 0.82a	11.4 ± 0.69bc	12.3 ± 0.82b	14.0 ± 0.91a	14.4 ± 0.92a
NO_3^- (mg L^{-1})	4.33 ± 0.39b	6.86 ± 0.54d	5.56 ± 0.48c	5.06 ± 0.51cd	5.30 ± 0.47c	8.20 ± 0.83a	4.30 ± 0.45d	5.17 ± 0.42cd
P (mg L^{-1})	90.8 ± 8.60e	209.7 ± 14.20b	264.1 ± 13.26a	264.9 ± 12.87a	84.0 ± 8.10e	106.7 ± 9.69e	153.7 ± 14.30d	180.7 ± 16.02c
K (mg L^{-1})	118.1 ± 10.90e	135.7 ± 11.41de	162.4 ± 12.10d	164.3 ± 13.54d	264.0 ± 14.90c	283.2 ± 18.93c	321.6 ± 18.39b	364.5 ± 17.92a
Ca (mg L^{-1})	8.48 ± 0.94b	13.53 ± 0.98a	13.69 ± 1.01a	14.61 ± 1.03a	8.06 ± 0.85b	13.08 ± 0.99a	14.03 ± 1.02a	14.01 ± 1.06a
Mg (mg L^{-1})	27.9 ± 1.16e	66.8 ± 3.20b	81.9 ± 5.32a	87.0 ± 5.95a	20.8 ± 0.87e	45.1 ± 3.24d	54.7 ± 4.11c	58.4 ± 4.63c
Cu (mg L^{-1})	0.08 ± 0.006a	0.104 ± 0.011b	0.112 ± 0.010c	0.115 ± 0.011a	0.026 ± 0.001c	0.031 ± 0.002c	0.040 ± 0.003c	0.065 ± 0.004c
Zn (mg L^{-1})	0.201 ± 0.012e	0.124 ± 0.011d	0.099 ± 0.006cd	0.032 ± 0.002e	0.585 ± 0.042a	0.436 ± 0.031b	0.333 ± 0.021c	0.311 ± 0.021c
B (mg L^{-1})	0.100 ± 0.072c	0.191 ± 0.081c	0.201 ± 0.013b	0.319 ± 0.021a	0.075 ± 0.004c	0.095 ± 0.004c	0.171 ± 0.083b	0.104 ± 0.067c

Note. Means that different letter(s) within the same rows are statistically different ($p < 0.05$) according to Tukey's test in two ways. First, the solubility of these elements in water could have played a role in their higher availability, as some elements have a higher solubility in water than others (Petrucci et al., 2011). Second, the presence of these elements in the water led to the colonization of the WRW by microbes. In the process, they break down the C content and fix and solubilize additional elements, such as N, P, and K. Therefore, the increase in the elements with fermentation was because of the beneficial microbes in the fermented rice water, which corroborated with other studies that reported the presence of beneficial microorganisms in fermented white rice water (Nabayi, Sung, Zuan, & Paing, 2021a, Nabayi, Sung, Paing, & Zuan, 2021b; Sairi et al., 2018).

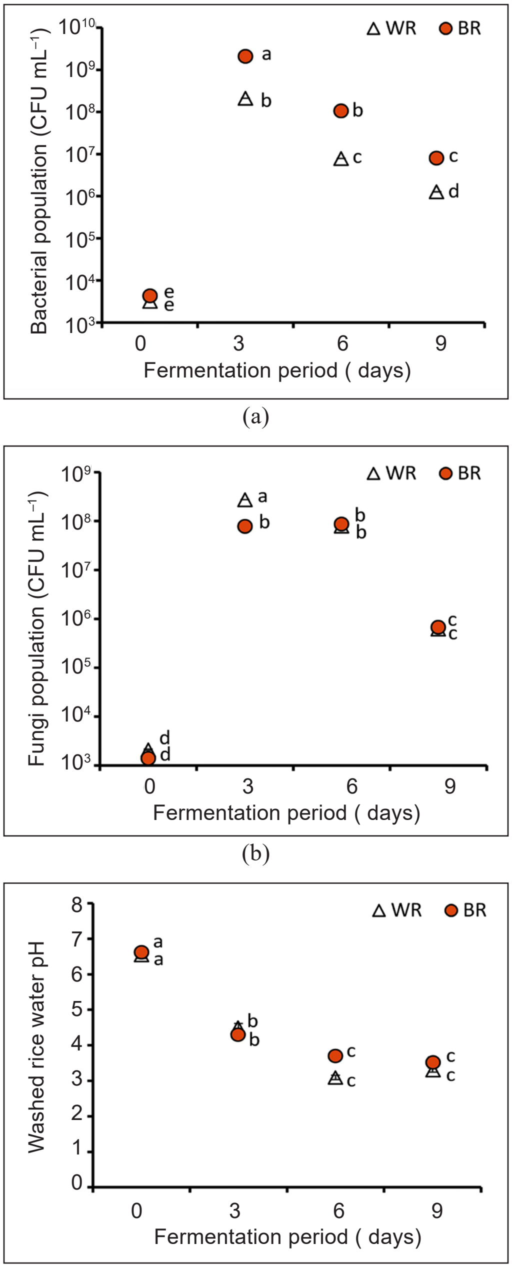
Effect of Fermentation Period on the Microbial Population in WRW from White Rice and Brown Rice

Higher bacteria and fungi populations were found on day 3 of the fermentation in the white and brown rice water, while the least was recorded on day 0 (Figure 1). However, brown rice water had a greater bacterial population at all levels of fermentation than white rice water. For instance, on day 3 of the fermentation period, the brown rice water had an 89% increase in bacterial population than the white rice water. The higher bacterial population in brown rice water could be attributed to the brown rice grains' higher P, K, Mg, B, and Zn elements (Table 1). In addition, Brown rice grains have higher mineral elements because of the outer bran layer, which contains significant proportions of elements compared to white rice grains (Saleh, Wang, Wang, L. Yang, et al., 2019).

In contrast, the white rice water had a 71% higher fungi population than the brown rice water on day 3 of the fermentation period, which decreased with increasing fermentation regardless of the rice type. The decrease in microbial population (bacteria and fungi) with increasing fermentation was because of microbial competition, as the C was the only energy source for the microbes. Therefore, this indicated that the rice water could not sustain the proliferation of the microbes beyond 3 days. These results agreed with studies that reported a decrease in the microbial population in white rice water with increasing fermentation due to the decrease in the C content of the white rice water (Akib et al., 2015; Nabayi, Sung, Zuan, & Paing, 2021a, Nabayi, Sung, Paing, & Zuan, 2021b). For the different fermentation periods (except for day 0) and rice water types, the bacterial populations were within the range expected for microbial colonization (10^6 colony-forming units per mL), as stated by Thomas et al. (2015). The pH of the WRW decreased with increasing fermentation, irrespective of the rice type. The decrease in pH of the rice water agreed with Nabayi, Sung, Zuan, and Paing (2021a) as well as Rousk et al. (2009), who reported a lower pH (acidic) with fermentation due to the microbial activity that produced organic acids and thereby lowering the pH of the culture.

Effect of Fermentation Period on Phosphorus and Potassium Solubilization of WRW from White Rice and Brown Rice Grains

After each fermentation period, i.e., 0-, 3-, 6-, and 9-day, the WRW was incubated for 12 days and tested for P and K solubilization (Figure 2). The highest P solubilization was found at 6-and 9-day fermentation periods for brown rice and white rice water. On average, brown rice water had 22% higher P solubilization across all fermentation periods than white rice water. However, K solubilization was highest on 3-day fermentation and decreased with increasing fermentation for rice water. The white rice water had 67% higher K solubilization than the brown rice water, despite having a lower K grain content. This increase could be explained by P and K solubilizing microbes, as indicated by the microbial population results (Figure 1). The general decrease in the microbial population after 3 days of fermentation could indicate the decrease in the K solubilization bacteria in the rice water. This result agreed with other studies that reported a decrease in microbial population because of the decrease in the C content of white rice water (Akib et al., 2015; Nabayi, Sung, Zuan, & Paing, 2021a, Nabayi, Sung, Paing, & Zuan, 2021b). The P and K solubilization of rice water by microorganisms is sustained by the C content of the rice water, which the microbes use as energy. He et al. (2016) stated that WRW could be used as a source of C for microorganisms. In addition, Adugna (2016) stated that the effectiveness and availability of microorganisms depend on the availability of C.



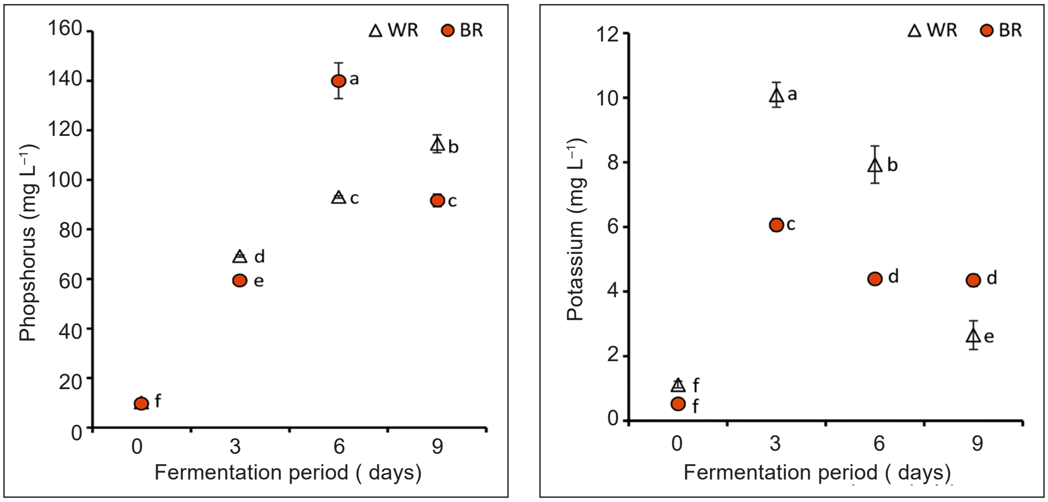
(a)

(b)

(c)

Figure 1. Mean (±SE) of (a) total bacteria population, (b) fungi population, and (c) pH in white rice water (WR) and brown rice water (BR) at different fermentation periods. For the same chart, means with different letters are significantly ($p < 0.05$) different from each other using Tukey's test

Note. BR = Brown rice water; WR = White rice water; CFU = Colony forming unit



(a) (b)

Figure 2. Mean (±SE) of quantitative solubilization of (a) phosphorus and (b) potassium of white rice water (WR) and brown rice water (BR) fermented at different periods. For the same chart, means with different letters are significantly ($p < 0.05$) different from each other using Tukey's test
Note. WR = White rice water; BR = Brown rice water; CFU = Colony forming unit

Therefore, the increase in P and K in the rice water was because of the presence of P and K solubilizing bacteria that colonized the rice water due to fermentation. Nabayi, Sung, Zuan, and Paing (2021a) reported the presence of *Bacillus* spp. and *Enterobacter* spp. in white rice water, and studies have identified the *Bacillus* and *Enterobacter* species as plant growth-promoting bacteria that could solubilize P and K (Bhattacharyya & Jha, 2012; Deepa et al., 2008). The P solubilization rates of both rice water found in this study were higher than the bacterial strains used by Sugumaran and Janarthanum (2007) as well as Tan et al. (2014), which solubilized a maximum of 14.15 mg L^{-1} and 4.29 mg L^{-1}, respectively, after 5 and 4 days of incubation. The use of microbes as biofertilizer components to improve soil health and plant productivity is considered an alternative to chemical fertilizers (Suhaimee et al., 2021). Park and DuPonte (2008) reported that beneficial microbes provide their benefits to crops through root colonization to stimulate growth and development. Bacteria that can solubilize nutrients like P and K are essential because they can convert insoluble P and K in soils into soluble forms. P and K solubilization could significantly benefit the plants because phosphorus is relatively immobile, making soils and plants deficient in P since this nutrient is unavailable for plant uptake. The enhanced P and K release by bacteria is linked to the bacteria synthesizing acids, alkalis, or chelates (Tan et al., 2014). The use of fermented rice water could reduce the need for inorganic fertilizers through the beneficial roles of the microbes present, such as P and K solubilizing microbes found in this study and through catalase and indole acetic acid production and N-fixation ability of the microbes as reported by other studies (Akib et al., 2015; Nabayi, Sung, Zuan, & Paing, 2021a; Sairi et al., 2018).

CONCLUSION

White rice water had 5 to 208% higher nutrients than brown rice water, despite the higher nutrient content of the brown rice grains. The elements in both rice water increased with increasing fermentation. The microbial population in rice water was the highest on day 3 and, after that, decreased. Both rice waters showed microbial P- and K-solubilizing activities throughout the fermentation period. Other essential microbial analyses of the rice water, such as N-fixation, catalase, and phytohormone production potentials due to the presence of the microbes in the WRW, need to be further carried out to prove the worth of using WRW as a source of plant nutrients. Therefore, the results of this study suggest that continuous application of WRW into the soil as a nutrient source would positively impact plant growth and soil fertility. The presence of beneficial microorganisms in the fermented WRW, common wastewater in households, makes it suitable for sustainable agriculture as these microbes would impact plant growth and soil fertility without endangering the environment, as is the case with chemical fertilizers.

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DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported herein.

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