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Research paper

Improving the Nutritional Value and Shelf-life of Fermented Rice Water

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ABSTRACT

Consuming fermented rice water (FRW) is claimed to improve digestion and skin health, prevent diseases, and meet some of minerals dietary requirements. However, not enough studies have been conducted to prove such nutritional aspects of FRW. This investigation examined some major factors that can contribute to then utritional value and the shelf life of FRW. The study involved white and brown rice water collected after cooking the rice. Rice water was inoculated with *L. plantarum* (2%) and subject to 48 h of traditional fermentation at 37°C. Results showed that LAB counts in both brown inoculated (BI) and white inoculated (WI) samples were >7 log CF/mL after 48 h of fermentation. Hence, the counts in BI were consistently higher ($p < 0.05$) than those in white rice water (inoculated and uninoculated) during fermentation. Additionally, fermented and freeze-dried BI revealed significantly ($p < 0.05$) the highest niacin (308.59 ± 20.2 µg/g) and folic acid (718.36 ± 7.3 µg/g) contents. Furthermore, the highest ash (17.31%), Fe (4.47 ± 0.44 mg/g) and Phosphorous (17.04 ± 0.22 mg/g) were detected in freeze-dried FRW samples prepared using inoculated brown rice.

Keywords: Fermented rice water, *L. plantarum*, Niacin, Folic acid, minerals

Rice, also known as *Oryza sativa*, is a major cereal produce as well as a staple food in the human diet (Saleh *et al.* 2019). It is cultivated and produced in a wide range of locations with an annual global production of approximately 480 million tons (Muthayya *et al.* 2014). Nonetheless, over 90% of rice production takes place in the Asia-Pacific region (Papademetriou, 2000), with India ranking second as the world's largest rice producer after China (Khadge & Bajpai, 2018). In addition to the consumption of rice in various regular menus all over the world, fermented rice water (FRW) is a well-known food in India. FRW is not only an integral component in the Indians' cultural traditions, but also considered highly nutritious with good health benefits (Ray *et al.* 2016). Fermented rice water is traditionally prepared through spontaneous fermentation, by adding water to cooked rice, storing overnight at room temperature, and then straining the remaining fermented liquid, which is called fermented rice water. It has been reported that such FRW is a rich source of essential nutrients such as minerals, antioxidants, vitamins B and E (Khadge & Bajpai, 2018) and energy (Komalalakshmi & Kirthana, 2018). In addition, FRW aids in the treatment of gastrointestinal issues

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such as constipation, diarrhea and bloating, and acts as an effective hydrating electrolyte solution (Komalalakshmi & Kirthana, 2018). Despite these benefits of fermented rice water, there are some issues regarding this product including, the short shelf life and the insufficient scientific proof of its nutritional value. The short shelf life of this product seems to be a problem limiting the possible commercialization of FRW in the supermarkets. And the claimed nutritional values need to be confirmed and improved further to maximize the potential of FRWas a nutritious product. It is anticipated that increasing the shelf life of FRW can be achieved through the application of freeze drying and the nutritional value can be enhanced by adding some lactic acid bacteria (LAB) such as, *Lactobacillus plantarum.* Lactic acid bacteria are naturally-occurring microbes existing within fermented foods and beverages (Ray *et al.* 2016). LABs are probiotic in nature and hence, are generally regarded as safe (GRAS), with the most common species being *Lactobacillus* spp. and *Bifidobacterium* spp. Min *et al.* (2019). LABs can contribute significantly to various food fermentation processes and produce different flavour components and nutrients, such as short chain fatty acids and vitamins (Rezac *et al.* 2018). LABs are among the main probiotics that can enhance the nutritional properties in food and promote human health through the elimination of pathogenic microorganisms and improving the human microbiota (Satish Kumar *et al.* 2013). The probiotic technology has gained popularity and acceptance worldwide including Japan, USA, Australia and Europe. Products enriched with free and encapsulated probiotics are usually dairy-based, cereal-based and juice or vegetable-based products (Salmerón, 2017). According to Charalampopoulos *et al.* (2003), cereals are rich in nutrients that can be easily incorporated and used as prebiotics. They can therefore stimulate the growth of probiotic microorganism during fermentation.

This study examined the possible improvement of fermented rice water nutritional values through fermentation in the presence of the probiotic *L. plantarum*.

MATERIALS AND METHODS

Materials

Medium grain (long white and medium brown rice) rice samples were purchased from local grocery stores in Melbourne, Vic. Australia. The freeze-dried *Lactobacillus plantarum* probiotic bacteria was kindly provided by Chr. Hansen (Bayswater, Vic, Australia). The selective media, trypticase soy agar (TSA) and de Man Rogosa Sharpe (MRS) agar and broth, and bactopeptone were purchased from Thermo Fisher Scientific (Scoresby, Vic, Australia). Niacin and folic acid standards (Pharmaceutical Secondary Standard), cyanogen bromide, ammonium hydroxide, nitric acid, bypyridyl and sulfanilic acid were purchased from Sigma-Aldrich (Castle Hill, NSW, Australia). Calcium hydroxide, sodium phosphate dibasic heptahydrate, hydrochloric acid, ammonium metavanadate, hydroquinone, ferrous ammonium sulfate hexahydrate, ammonium molybdate heptahydrate, ethanol, potassium dihydrogen phosphate, calcium hydroxide, ammonium sulfate, sodium carbonate, glycerol, sodium acetate anhydrous and glacial acetic acid and all disposable materials were purchased chem-store at the University of Melbourne specialist store (Bio21 Institute, Parkville, Vic, Australia).

Methods

Preparation of the probiotic culture

The freeze-dried probiotic (*Lactobacillus plantarum*) was resuscitated in de Man Rogosa Sharpe (MRS) broth through anaerobic incubation at 37°C for 48 hours. The final counts in the activated stock culture weredetermined using aspread plate technique on MRS agar following the procedures of Ajlouni (2020). The activated culture (at least 10^7 CFU/ml) was added to each rice water samples at 2% by volume.

Preparation of fermented rice water samples

Brown and white rice samples (200 g each) were rinsed with 600 mL tap water separately and drained. The rinsing water was kept for later usage. Each rinsed rice sample was cooked separately

for 30 minutes in a rice cooker recipe (Kambrook KRC150WHT, Kambrook Australia, Botany, NSW, Australia). The cooked rice was then divided into two equal sup-samples, and each sup-sample was re-soaked in 300 mL of the previously kept rinsing rice water using 500 mL Schott bottles as fermentation jars. One of these sup-samples was inoculated with 5 mL of the already activated *L. plantarum*, and the 2nd sup-sample was left as a control (uninoculated). The cooked and soaked rice samples were then fermented for 48 hours using an anaerobic incubator (Heraues BB16, Heraeus Instruments, Hanau, Germany) at 37°C. Aliquots (1.0 mL) were collected from each fermentation jarat 0, 24, and 48 h of fermentation and subjected to microbial counts. The fermented rice in water was strained and FRW was collected. About 50 ml of the fermented rice water (FRW) were stored refrigerated at 4°C for additional microbial analyses after 0, 24 and 48 hours of refrigerated storage,while the remaining FRW was quantitively distributed into 50 mL falcon tubes and frozen at -20°C overnight. The frozen FRW samples were then subjected to freeze drying using a benchtop freeze dryer (Dynavac FD3, Dynapumps, Seven Hills, NSW, Australia) following the standard manufacturer recommendations. This whole trial was repeated twice, and each analysis was performed in duplicate.

Enumeration of total and lactic acid bacteria counts

The microbial counts were conducted following the standard spread plate procedures (Ajlouni, 2020). On each day of measurement, serial dilutions of the samples were prepared using sterile 0.1% (w/v) bactopeptone. Aliquots (0.1 mL) from 10^{-4} to 10^{-6} dilutions were spread plated onto TSA and MRS plates in duplicates. These dilutions were selected based on preliminary testing procedures before starting the actual trials. Positive and negative control plates were prepared using the *L. plantarum* stock culture and sterile bactopeptone, respectively. Half of the inoculated plates were incubated aerobically and the 2nd half anaerobically at 37°C for 48 hours. The average bacteria count was reported as CFU/mL FRW.

Niacin determination

Niacin contents in the freeze-dried fermented rice water powder (FRWP) samples were quantified calorimetrically (AOAC, 2005). In this method, a niacin stock solution (100 µg/mL) was prepared in 25% alcohol using a volumetric flask and diluted further to 10 µg/mL as a standard working solution. Different volumes (0, 2.5, 5, 7.5, 10 and 12.5 mL) from the working solution were transferred to a series of Erlenmeyer flasks containing 0.75 g of calcium hydroxide each and mixed with 40 mL of Milli-Q water.

Sample solutions were prepared by dissolving 0.1 g of each FWRP sample and 0.75 g calcium hydroxide in 40 mL of Milli-Q water. All solutions (standards and samples) were then autoclaved (121°C and 15 psi) for 2 hours and cooled to room temperature. Each autoclaved solution (sample and standard) was transferred to volumetric flasks and diluted to 50 mL with Milli-Q water. Aliquots (10 mL) from each solution were transferred to tubes containing 4 g ammonium sulfate and 1 mL of phosphate buffer solution (pH 8). All tubes were then warmed to 60°C and centrifuged for 5 minutes (5000×g, 4 °C) using a bench top centrifuge (ALLEGRA X-12R, Beckman Coulter, Australia), and filtered through a Whatman No. 2V filter paper. The filtrates were collected in test tubes and allowed to stand for 30 minutes in a nice bath. Approximately 0.5 mL of 55% sulfanilic acid solution and 5.0 mL of 10% cyanogen bromide solution were added to each tube and allowed to stand for 12 to 15 minutes at room temperature. The absorbance of niacin standards and sample solutions were measured at 470 nm using a UV-Vis spectrophotometer (GENESYS™ 180, Thermo Fisher Scientific, Scoresby, Vic, Australia).Concentrations of niacin was calculated using the linear equation and expressed as µg niacin/g FWRP.

Folic acid determination

Folic acid contents in FRWP samples were quantified using the colorimetric method reported by Modupe *et al.* (2020). Folic acid standard stock solution (100 µg/mL) was prepared using 0.1 M sodium carbonate

solution and diluted further to prepare 5 different standard concentrations. For sample preparation, approximately 0.1 g of each FWRP sample was weighed into a test tube and mixed with 5 mL of 0.1M sodium carbonate. The mixture was then centrifuged for 10 minutes using a bench top centrifuge at 5000×g and 4 °C, and the supernatant was quantitively collected in a volumetric flask (10 mL). The residue was washed again with another 3 mL sodium carbonate. The combined supernatants were brought to a finals volume of 10 mL, and then filtered using a Whatman No. 2V filter paper. The absorbance of standard and sample extract solutions was measured at 285 nm using a UV-Vis spectrophotometer. The folic acid contents were calculated and reported as μg folic acid/g FWRP.

Ash and minerals analyses

Approximately 1.0 g of each FRWP sample was placed in a pre-weighed clean crucible and charred in a fume hood using a Bunsen burner. The crucibles were then placed a muffle furnace at 550°C till a white-grey ash was formed (Ajlouni, 2019). The weight of the ash was calculated, before mixing with 5 mL concentrated hydrochloric acid and boiling for 5 minutes in the fume hood. The hydrolysed ash was carefully transferred into a beaker, mixed with 40 mL Milli-Q water, and boiled for 10 minutes. The solution was then cooled and filtered through a glass wool, diluted to 100 mL in a volumetric flask using Milli-Q water and designated as ash extract.

Phosphorus determination

Phosphorus content in the ash extract of each FRWP sample was determined using the vanadium phosphomolybdate colorimetric method (James, 1995) with minor modifications. A stock phosphate solution (0.1 mg P/mL) was prepared using potassium dihydrogen phosphate in Milli-Q waterand diluted further to prepare 5 points standard curve. Vanadate-molybdate reagent was prepared by dissolving 10 g of ammonium molybdate in 200 mL of water at about 50°C and cooled to room temperature. Approximately 0.5 g of ammonium vanadate was dissolved in 150 mL of boiling water, cooled, and mixed with 70 mL of concentrated nitric acid. Molybdate solution was then gradually added to vanadate solution and diluted to 500 mL with Milli-Q water.

Specific volume from the phosphorus stock solution (0.1 mg P/mL) was transferred to a series of 50 mL volumetric flasks, mixed with 12.5 mL Vanadate-molybdate reagent, and brought to the final volume (50 mL) with Milli-Q water. The final phosphorus concentrations in these flasks were 0, 2.5, 5.0, 7.5 and 10 μg/mL. Similar procedures were applied to the FRW ash extracts, where 5 mL of the ash extract was mixed with 12.5 mL Vanadate-molybdate reagent in a 50 mL volumetric flask.

The absorbance of standard and samples were measured at 420 nm using a UV-V is spectrophotometer and results were reported as mg P/g FWRP.

Iron determination

Iron contents in each FRWP samples were determined following the procedures of James (1995). In this method, the reagents 2,2'-Bypyridyl (0.1%) and hydroquinone (2.5%) solutions were prepared in Milli-Q water. Acetate buffer was prepared by dissolving 8.3 g of dried anhydrous sodium acetate in water, mixed with 12 mL glacial acetic acid and dilute to 100 mL with Milli-Q water.

Iron stock solution (250 mL) was prepared at concentration of 0.01 mg/mL using ferrous ammonium sulfate hexahydrate. The iron stock solution was acidified with 1 drop of 5M hydrochloric acid and diluted to the final volume in a volumetric flask. Iron standard solutions were prepared by transferring 0, 0.5, 1.0, 2, and 3.0 mL from the iron stock solution into five 10 mL volumetric flasks and diluting to 10 mL using Milli-Q water. The 10 mL of each Fe standard was then transferred into a conical flask, mixed with 3 mL buffer, 2 mL hydroquinone and 2 mL bipyridyl solutions. These final solutions were mixed well before measuring the absorbance at 520 nm using a UV-V is spectrophotometer.

Iron content in the sample ash extract was determined following the same procedures. The ash extract (5

mL) was diluted to 10 ml with Milli-Q water in a 10 mL volumetric flask, followed by transferring the content into a conical flask and adding the reagents as in the standard.

The standard curve absorbance was plotted against iron concentration. Iron concentration in the sample was determined using the linear equation and expressed as μg iron/g FRWP.

STATISTICAL ANALYSIS

The generated data were analysed for significant differences between treatments and sample means using a one-way analysis of variance (ANOVA) and Fisher's least significant difference (LSD) *post hoc* test (α = 0.05), respectively. All statistical analyses were conducted using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA). Results were reported as means ± standard deviations.

RESULTS AND DISCUSSION

Effect of inoculation with *L. plantarum* on the microbial counts during rice water fermentation

The average initial microbial count of the activated *Lactobacillus plantarum* used to inoculate the samples was determined to be 10^7 log CFU/mL. Microbial counts in white and brown fermented rice water (FRW) samples were examined during the 48 hours of fermentation. The detected number of bacteria showed significant (P<0.05) increase in the viable counts in most FRW samples after 48 h of fermentation (Table 1). Furthermore, the counts of probiotic, and total anaerobic in FRW generated from brown(BI) and white (WI) inoculated rice samples were significantly ($p < 0.05$) higher than those in uninoculated rice samples(BU and WU).The greatest recorded probiotic counts were observed in BI and WI after 48 h of fermentation (9.38 ± 0.02 and 9.12 ± 0.16 log CFU/ml, respectively). The smallest probiotic counts after 48 h of fermentation were detected in WU rice sample (8.23 ± 0.23). Nonetheless, results showed that the viable microbial counts in FRW samples were significantly ($p < 0.05$) affected by the addition of *L. plantarum* (Table 1). These reported counts after 48 h of fermentation were evidently above the minimum therapeutic dose (10^7 CFU/mL) required for a probiotic product (Giri *et al.* 2018; Ignat *et al.* 2020). These results demonstrated that inoculating rice water with *L. plantarum* could improve the final LAB counts significantly ($p < 0.05$) after 48 h of fermentation. Furthermore, it could be concluded that *L. plantarum* has the capacity to thrive in both brown and white rice water. Similar findings were reported in the literature in other fermented food products. Ogodo *et al.* (2019) found that inoculating sorghum flour with *L. plantarum* increased the total LAB counts from 6.74 log CFU/g to 8.90 log CFU/g after 48 h of fermentation. The same authors reported in 2017 (Olaoye *et al.*) that inoculating tigernut milk "*kunnu-aya*" samples with *L. plantarum* increased the LAB counts from 4.9 CFU/mL to 6.74 log CFU/mL) after 48 hours of fermentation. The study by Olaoye *et al.* (2017) also reported higher total bacteria counts in the uninoculated compared to inoculated samples, which was attributed to the possible antimicrobial effect of *L. plantarum* on some microorganisms and the creation of a better environment (lower pH) for other bacteria to grow.

Table 1: Changes in the bacterial counts (Log CFU/ml) in rice water during fermentation at 37°C

Agar Medium and incubation type	Fermentation time (h)	Log CFU/mL			
		*WU	WI	BU	BI
TSA (aerobic	24	6.63 ± 0.14^c	7.90 ± 0.56^b	7.13 ± 0.04^c	8.87 ± 0.51^a
	48	8.27 ± 0.07^d	8.78 ± 0.09^c	9.19 ± 0.05^b	9.34 ± 0.03^a
TSA (Anaerobic)	24	6.72 ± 0.01^d	7.94 ± 0.01^b	7.28 ± 0.19^c	8.20 ± 0.04^a
	48	8.36 ± 0.01^c	9.05 ± 0.25^b	9.05 ± 0.07^b	9.37 ± 0.04^a
MRS (probiotic)	24	5.97 ± 0.87^d	8.25 ± 0.11^a	7.34 ± 0.46^c	8.58 ± 0.03^{ab}
	48	8.23 ± 0.23^c	9.12 ± 0.16^b	9.31 ± 0.04^b	9.38 ± 0.02^b

Values represent means ± standard deviation (n = 4). Means followed with different superscript letters in a column within each agar medium corresponds to significant differences (p < 0.05).
** FRW from: WU = white uninoculated rice; WI = white inoculated; BU = brown uninoculated; BI = brown inoculated.*

Bacterial populations in fermented brown rice water (BI and BU) samples were consistently higher ($p < 0.05$) compared to fermented white rice water samples (WI and WU) (Table 1). Similar results were obtained by Kim *et al.* (2012) working on quantifying the microbiological profiles of rice, where overall microbial counts for retail brown rice were higher than that of white rice. Such observations could be attributed to the fact that brown rice still has its hull intact as opposed to white rice, which is not only dehulled, but also polished (Panneerselvam *et al.* 2017). According to Chavan *et al.* (1989), majority of the microorganisms involved in the natural fermentation process of cereals are found on the surface of the seed. This is because the surface of the rice seed which consists of the bran and embryo, contain high amounts of fibers and vitamins and thus, are capable of hosting microbial associations to a greater extent (Ravichanthiran *et al.* 2018). The microbial population decreases as rice is subjected to further processes such as de-hulling, milling, and polishing. Moreover, factors such as sugar concentration, lactic acid concentration and pH are previously reported to affect the survivability of probiotics during fermentation and storage (Charalampopoulos & Pandiella, 2010; Charalampopoulos *et al.* 2003; Olaoye *et al.* 2017).

High probiotic counts in fermented foods, including FRW, can decrease the pH, hydrolyse major food components, and improve the organoleptic properties of the final product. Additionally, a high probiotic count (>10^7 CFU/mL) providesbeneficial effects on human gut microbiota (Giri *et al.* 2018). This is because LABs are capable of producing bacteriocin and bacteriocins-like metabolites as a result of fermentation, which are known to inhibit pathogens (Camargo Prado *et al.* 2015).

Impact of inoculating rice water with *L. plantarum* on selected vitamin contents after fermentation

The contents of selected vitamins in freeze-dried fermented rice water (FDFRW) obtained from rice water inoculated with *L. plantarum* are shown in Table 2. Results revealed that niacin (vitamin B3) contents in BI, BU and WI were significantly ($p < 0.05$) different and ranged from 308.59 ± 20 to 73.93 ± 6.5μg/g in BI and WI, respectively. Similarly, folic acid (vitamin B9) contents varied significantly ($p < 0.05$) among all treatments (Table 2). The detected amounts of folic acid in FDFRW ranged from 718.36 ± 7.3in BI to 178.93 ± 9.9 μg/g in WU. These quantities were also smaller than those in WI. Furthermore, the niacin (73.93 ± 6.5 μg/g) and folic acid (257.29 ± 37.5 μg/g) contents in WI samples were significantly ($p < 0.05$) lower than those in BU (196.01 ± 77.9 μg/g; 480.77 ± 5.7 μg/g, respectively).

Table 2: Vitamin contents in fermented rice water powder (FDFRW)

Sample	Vitamin content (μg/g FDFRW)	
	Niacin	**Folic acid**
WU	23.20 ± 16.1^{cd}	178.93 ± 9.9^{d}
WI	73.93 ± 6.5^{c}	257.29 ± 37.5^{c}
BU	196.01 ± 77.9^{b}	480.77 ± 5.7^{b}
BI	308.59 ± 20.2^{a}	718.36 ± 7.3^{a}

Values represent the means ± standard deviation (n = 3). Means followed with different superscript letters in a column correspond to a significant difference (p<0.05).

These results confirmed that the niacin and folic acid contents in FDFRW samples inoculated with *L. plantarum* samples were significantly higher ($p < 0.05$) than those of the un-inoculated FDFRW samples in all treatments, except WU. Additionally, results in Table 2 showed that FDFRW samples from inoculated and un-inoculated brown rice had higher niacin and folic acid contents than those prepared from white rice. The FDFRW samples from brown rice inoculated with *L. plantarum* had the highest niacin content (308.59 ± 20.2 μg/g) and folic acid content (718.36 ± 7.3 μg/g) as compared to the rest of the samples. These observations were in agreement with the previous findings of Panneerselvam *et al.* (2017), who reported that brown rice is known to be more nutritious than white rice, due to the presence of the embryo and bran which contains a myriad of bioactive and nutritional compounds including, but not limited to that of vitamins.

Several investigations had reported the increment of some vitamins due to the presence of probiotic bacteria during fermentation. Enujiugha & Badejo (2017) reported that the presence of LABs is usually associated with substantial productions of B vitamins, particularly folic acid, during fermentation. Ghosh *et al.* (2015) mentioned that folic acid content increased from 0.28± 0.01 to 2.42 ± 0.04 µg/g) in a rice-based fermented beverage "Haria" using white glutinous rice inoculated with *L. fermentum* at the 24th hour of fermentation. Thompson *et al.* (2020) demonstrated that *L. plantarum* significantly ($p < 0.05$) increased the folate content (0.49 to 0.59 µg/g fresh weight) in vegetable-cereal based fermented products. Furthermore, Sanni *et al.* (1999) reported an increase in niacin content from 42.0 to 53.2 µg/g after the fermentation of cereal-soybean based blends inoculated with *L. plantarum* and *Saccharomyces cerevisiae.*

It should be noted here that the amounts of folic acids and niacin reported by these authors were significantly smaller than the quantities detected in the current study (Table 2). These discrepancies could be attributed to variations in tested samples,methods of analysis, and the fact that freeze dried powder was in our current investigation. Although niacin can be synthesized by tryptophan in the human body, the generated quantity is regarded to be insufficient in meeting nutritional requirements (Fukuwatari & Shibata, 2013). Thus, consumption of FRW may contribute to the daily recommended vitamin intake. Finally, our results indicated that using brown rice water inoculated with *L. plantarum* is a recommended option for producing a rice-based fermented beverage rich in these vitamins.

Ash and mineral contents in FDFRW inoculated with *L. plantarum*

The highest ash contents in freeze dried fermented rice water (FDFRW) were detected in samples prepared using inoculated brown rice (BI) (17.31%) followed by brown uninoculated (BU) (9.71%). However, both inoculated and uninoculated white rice samples(WI and WU) had the lowest quantities (2.88% and 1.98%, respectively) (Table 3).

Table 3: Mineral content of fermented rice water powder (FRWP) samples

Sample	Ash Content (%)	Mineral content (mg/g FRWP)	
		Phosphorus	Iron$^{(NS)}$
WU	1.98^{a}	1.41 ± 0.08^{cd}	3.53 ± 0.23^{a}
WI	2.88^{a}	1.66 ± 0.08^{c}	4.19 ± 0.99^{a}
BU	9.71^{b}	4.90 ± 0.27^{b}	3.57 ± 0.02^{a}
BI	17.31^{c}	17.04 ± 0.22^{a}	4.47 ± 0.44^{a}

Values represent the means ± standard deviation (n = 3). Means within a column followed by the same superscript letters were not significant different (p > 0.05).

The ash content in FDFRW from brown rice (inoculated and uninoculated) were greater than those from fermented white rice water. This could be attributed to the presence of hull on brown rice compared to de-hulled, milled and polished white rice as discussed previously and explained by Panneerselvam *et al.* (2017). In addition, ash content in FDFRW prepared from inoculated BI was greater than their un-inoculated counterpart (BU). The enhanced ash contents in FDFRW samples inoculated with *L. plantarum* corroborate with findings from some similar previous studies. Olaoye *et al.* (2017) found increases in ash content in fermented tigernut milk "*kunnu-aya*"inoculated with *L. plantarum* as compared to their uninoculated counterparts. Ntuli *et al.* (2013) mentioned that ash content could be an indicator of the mineral composition levels in a given sample. As such, it can be deduced that a higher ash content could be associated with higher levels of mineral composition.

The determined minerals in FDFRW samples showed larger quantities of phosphorus (17.04 ± 0.22 mg/g) and iron (4.47 ± 0.44 mg/g) in BI. Furthermore, similar to the observations on ash contents, the smallest amounts of phosphorous (1.41 ± 0.08 mg/g) and iron (3.53 ± 0.23 mg/g) were recorded in WU treatment (Table 3).The same data revealed also insignificant ($p > 0.05$) differences in iron quantities among all treatments. Nonetheless, samples BI had the highest iron content (4.47 ± 0.44 mg/g) while WU had the lowest iron content (3.53 ± 0.23 mg/g). As reflected by the higher ash content, similar trends were observed

in phosphorus content in FDFRW samples. Significant differences ($p < 0.05$) in phosphorus contents were detected among all samples, with brown FDFRW sample inoculated with *L. plantarum* containing the highest amount (17.04 ± 0.22 mg/g). Based on the results obtained, brown FDFRW samples were significantly ($p < 0.05$) higher in phosphorus content than in white FDFRW samples. These findings supported the facts that phosphorous in grains is usually stored in the form of phytate and majority of ricephytates are present on the aleurone layers. Hence, milling and subsequent polishing results in substantial decreases of phytates in white rice (Baek *et al.* 2014). Furthermore, it can be observed that the inoculated samples had significantly ($p < 0.05$) higher phosphorus content as opposed to their uninoculated counterparts. In this study, the enhanced phosphorus contents detected in *L. plantarum* inoculated FDFRW samples could be attributed to the function of the enzyme phytase release in the presence of add *L. plantarum*. Previous studies by Bergillos-Meca *et al.* (2013); Nkhata *et al.* (2018); and Sokrab *et al.* (2014) explained that the increases in phosphorus content in the presence of LAB might be attributed the hydrolysis of anti-nutritional phytic acid by phytases as a result of fermentation. Phytic acid, also known as phytates, is present naturally in rice and are known to form insoluble complexes with minerals resultingin a strong metal ion chelation and hence, reducing the bioavailability of these minerals (Giri *et al.* 2018; Greffeuille *et al.* 2011). Natural fermentation was previously reported to substantially decrease the amount of phytic acids (Lopez *et al.* 1983) through the action of the natural grain enzyme and the extracellular phytases from LAB which in turn lowers the binding capacity between phosphorus and other minerals (Ghosh *et al.* 2015; Giri *et al.* 2018). LABs have been reported to release the enzyme phytase which can utilization phytic acids as substrates (Damayanti *et al.* 2017; Ghosh *et al.* 2015). Consequently, it can be deduced that the adding *L. plantarum* would contribute to the degradation of phytic acid in BI samples through its phytase activity and hence, increases the amount of phosphorus in FDFRW from brown rice by several folds.

Unsimilar to the significant increments in phosphorous contents after fermentation of rice water, no significant differences ($p > 0.05$) in iron contents were detected among all FDFRW samples. However, data showed minor increases in iron content (1 mg/g) in fermented samples. Such insignificant Fe increases in FDFRW could be attributed to various factors. Among these major factors are, (1) the significantly smaller initial iron content in rice (0.008 mg/g) in comparison to 3.2 mg/g phosphorous (Majumder *et al.* 2019), (2) enzymatic hydrolysis of phytate during fermentation in the presence of LAB releases phosphorus, but no iron, (3) using only a single species of LAB, and rice during the preparation of FRW, since mixed cereal and starter cultures could contribute to the increase in Fe during fermentation. Agarry *et al.* (2010) reported that the increase in Fe, Ca, Mg and K infermented Nigerian drink was affected by the type of the mixed cereals used in the fermentation. A mixture of millet + malted rice yielded 48.38 ± 0.23 mg iron/100 mL. However, replacing malted rice with wheat under the same conditions increased the amount of iron in the fermented drink to 71.83 ± 0.09 mg/100 mL (Agarry *et al.* 2010).

It should be mentioned also that the amount of Fe reported in this current study (3.53 to 4.47 mg/g) was significantly larger than the values (0.008 mg/g = 8.8 ppm) reported in brown rice (Majumder *et al.* 2019) and (0.00026 mg/g = 0.26 ppm) in rice based fermented beverage (Giri *et al.* 2018). These significant variations could be attributed mainly to differences in the method of analyses. As the current study used a spectrophotometric method, it is recommended that more accurate instrumental methods such as atomic absorption spectroscopy (AAS) or Inductively Coupled Plasma (ICP) should be used for comparison in the future studies.

CONCLUSIONANDRECOMMENDATIONS

Fermented rice water (FRW) is considered a common food among a large proportion in south Asia, especially, Indian and Sri Lankan. FRW is a rich source of essential nutrients such as minerals, antioxidants, and some vitamins. This study amid to

improve the nutritional value and to extend shelf life of fermented rice water (FRW) *via* enrichment with *Lactobacillus plantarum* and freeze drying. Inoculating rice water with *L. plantarum* before fermentation increased the LAB counts in fermented rice water and caused significant increments in some vitamins and mineral contents. Furthermore, using rice water from brown rice was more productive and more nutritious than white rice. The applied freeze during technique could maintain these nutrients during the long-term storage, since the water activity in such final freeze-dried fermented rice water (FDFRW) powder will be <3%. Inoculation of rice water with probiotic bacteria before fermentation and freeze drying improved its nutritional value. The FDFRW has been proven to be a potential to be a future healthy drink for daily consumption. The FDFRW powder can be easily dissolved in water. FDFRW from brown rice variety was found to be a better substrate than white rice variety as reflected by the higher viable LAB counts and subsequently, higher folic acid, niacin, and phosphorus contents. Based on these preliminary findings, it is recommended that follow-up investigations using mixed cereals and mixed probiotics together a long with a long-term shelf-life and sensory tasing are necessary before such healthy and cheap products (FDFRW) can be made commercially available.

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