NUTRITIONAL PROPERTIES AND HYDROLYZING RATES OF RICE GROWN WITH BIOFILM BIO-FERTILIZER (BFBF)

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INTRODUCTION

Rice being the main staple food in Sri Lanka, annual per capita rice consumption is approximately 115 kg (Department of Census and Statistics, 2020). Due to the high demand for rice, farmers use excess amount of fertilizer to obtain an ample harvest. It has been reported that soil fertility, crop productivity, and natural microbial communities in agricultural ecosystems are adversely affected by excessive fertilizer usage (Bedano, 2006; Seneviratne, 2009). Eco-friendly Biofilm Biofertilizer (BFBF) has been introduced for rice farming to overcome the problems associated with Chemical Fertilizer (CF) and to promote sustainable agriculture in Sri Lanka (Seneviratne, 2009). A detailed analysis of the nutrient content of rice suggested that the nutrition value of rice varies based on the strain of rice, processing method and nutrient quantity of the soil in cultivation land (Rathna Priya *et al.*, 2019). Therefore, soil fertility in paddy land is directly accounted for changes in the nutritional composition of rice. BFBF may improve soil fertility by improving beneficial micro-organisms in the soil (Seneviratne, 2009). Thus, this study aims to evaluate the effect of BFBF on nutritional and hydrolizing rates of rice grown in paddy fields in Ampara, Sri Lanka.

METHODOLOGY

Sample collection and preparation

Rice samples were taken from the improved rice variety (Bg 94/1) grown with 6 fertilizer treatments (T_1−100% CF, T_2−80% CF, T_3−80% CF+BFBF, T_4−65% CF, T_5−65% CF+BFBF, and T_6−Control-soil) at Malwatte farm, Ampara, Sri Lanka. Collected rice samples were cleaned before the processing. Then samples were milled by using a milling machine and ground using a grinder. Samples were sieved using a 105 µm sieve.

Nutritional properties

Proximate composition

The moisture content, ash content, and crude fat content, on a dry weight basis were analyzed as per the protocols mentioned in AOAC (2005) with triplicates. Crude protein content was determined by using CHNS/O analyzer and used 6.25 as the nitrogen conversion factor. The micronutrient composition of rice samples was analyzed by ICP-OES (*Inductively coupled plasma-optical emission spectrometry*) after microwave digestion according to Tarantino *et al*., 2017. Moreover, Total dietary fiber content (TDF) and soluble dietary fiber content (SDF) in rice samples were analyzed according to the enzymatic gravimetric method as specified in the AOAC official method analysis. Finally, the available carbohydrate content was calculated using following formula. Carbohydrate (%) = 100% - (crude protein% + crude fat% + ash%+ TDF%).

Hydrolyzing rates

Hydrolysis rate with respect to α-amylase and amyloglucosidase were determined by the method described by Visvanathan *et al.*, 2016.

Statistical analysis

All data were presented as means (±standard deviation) of at least three independent experiments ($n \geq 3$), with each experiment having a minimum of three replicates of each sample. For comparisons between samples, data were analyzed by ANOVA. A probability of 5% or less was considered as statistically significant.

RESULTS AND DISCUSSION

Moisture content

Moisture content, which plays a significant role in determining the shelf-life (Juliano, 1985). As per the results it ranged from 10.2% (T_4) to 12% (T_1). Moisture content was not significantly different ($p>0.05$) among all treatments except T_2 and T_3 (Table 1). The results suggested that the moisture content of all samples found in the present study was laid within the recommended safety limit of 14% or less. (Klomklao *et al.,* 2017). Moreover, fibre content of the flour also influences the moisture content as higher fibre containing flour showed higher moisture content (Haruna *et al.*, 2011).

Ash content

The ash content plays a vital role in a food sample to reflect the minerals which are nutritionally valuable (Bhat and Sridhar, 2008). The ash content in rice samples were ranged from 2.1% (T_1) to 5.1% (T_4). There was no significant difference ($p>0.05$) of ash content in rice samples collected from T_3 and T_5 treatments. Whereas that was significantly different ($p>0.05$) in rice grown with other treatments. (Table 1).

Crude fat Content

Fat in rice is a good source of linoleic and other essential fatty acids but does not contain cholesterol (Eggum *et al*., 1982). Fat content in rice samples ranged between 1.0% (T_1) to 2.7% (T_4). Crude fat content in rice grown with different treatments was significantly different ($p>0.05$) among each other (Table 1).

Crude protein content

The high protein content of rice is of great nutritional advantage, and consumption will undoubtedly reduce protein malnutrition due to protein deficiency. Protein is the second major component next to starch. Crude protein in rice grown with different fertilizer treatments differed significantly from each other ($p>0.05$) and ranged from 5.6% (T_2) to 7.3% (T_4). (Table 1).

Available Carbohydrate

The most abundant nutrient content in rice is carbohydrate. The carbohydrate content in rice grown with all the treatments ranged from 68.3% (T_4) to 74.8% (T_1) and all the treatments are significantly different ($p>0.05$) from each other. (Table 1).

Micronutrient composition

Nutritionally important elements like Zinc and Manganese (Zubair *et al.,* 2012) contents were in the range of 2.2-9.9 µg/g and 0.7-1.5µg/g. A significantly higher ($p>0.05$) level of Zn was in rice grown with 65% CF+BFBF (T_5), and the rice grown with 80% CF+BFBF (T_3) had the highest level ($p>0.05$) of Mn. (Table 2).

Total dietary fibre (TDF) / Insoluble dietary fibre (IDF) and soluble dietary fibre (SDF) content

It was estimated that the amount of total fibre in rice grown in six different plots was 6.8 to 3.1 g/100 g belonging to 80% CF (T_2) treatment and 100% CF (T_1) treatment, respectively. (Figure 1).

Treatment	Sample	Moisture	Ash	Crude protein	Crude fat	Carbohydrates
T_1	100% CF	11.94±0.44[a]	2.13±0.01[e]	6.95±0.01[c]	1.06±0.08[f]	74.82±0.05[a]
T_2	80% CF	11.11±0.37[c]	4.08±0.11[c]	5.66±0.05[f]	2.29±0.01[d]	70.07±0.03[d]
T_3	80% CF+BFBF	11.11±0.27[c]	4.65±0.23[b]	6.93±0.11[d]	2.38±0.01[c]	71.27±0.02[c]
T_4	65% CF	10.22±0.46[e]	5.13±0.08[a]	7.31±0.13[a]	2.72±0.02[a]	68.30±0.02[f]
T_5	65% CF+BFBF	11.63±0.29[b]	4.65±0.15[b]	7.29±0.08[b]	1.96±0.01[e]	68.78±0.01[e]
T_6	Control (Normal soil)	11.03±0.16[d]	4.00±0.13[d]	6.03±0.02[e]	2.44±0.02[b]	71.56±0.05[b]

Hyrolizing rate

A primary main factor that directly affects the peak postprandial glucose responses is the *in-vitro* hydrolyzing rate. Inhibition the activity of α-amylase and amyloglucosidase by specific food or starch can retard the digestion of carbohydrates, thereby causing a decrease in the rate of glucose absorption into the blood. This can be considered as a preventive approach to diabetes (Kalita *et al.*, 2018; Warren *et al.*, 2015). Among the six treatments tested in the present analysis, maltose and glucose releasing rates were significantly different ($p<0.05$) and values ranged from 0.0063 $mmoldm^{-3}min^{-1}$ (T_4) to 0.0264 $mmoldm^{-3}min^{-1}$ (T_2) and 0.0017 $mmoldm^{-3}min^{-1}$ (T_4) to 0.0032 $mmoldm^{-3}min^{-1}$(T_6), respectively. (Table 3).

Table 1. Proximate composition

Values are mean ± SD for three samples of each variety, analyzed individually in triplicate

Small superscripts letters with in the same column indicate significant differences ($p<0.05$) of means among rice in different plots

Table 2. ICP-OES analysis of minerals in different soil treatment

	Sample	Macroelements				Trace elements					
		Mg (mg/g)	Ca (mg/g)	K (mg/g)	Na (mg/g)	Zn (μg/g)	Mn (μg/g)	Cu (μg/g)	Al (μg/g)	Cr (μg/g)	Pb (μg/g)
T_1	100% CF	0.24±0.88[e]	0.03±0.64[b]	0.07±1.01[d]	0.004±0.07[b]	2.21±0.15[f]	0.74±0.92[f]	0.20±0.02[d]	2.60±1.78[d]	0.02±0.22[b]	0.03±0.22[b]
T_2	80% CF	0.49±0.00[d]	ND	0.15±0.01[c]	0.004±0.13[b]	5.79±0.30[b]	1.11±1.22[e]	0.22±0.37[b]	2.51±0.09[e]	0.03±0.36[a]	0.36±0.36[a]
T_3	80%CF+BFBF	0.56±1.64[c]	0.04±0.75[a]	0.16±1.85[b]	0.006±0.07[a]	2.48±0.11[e]	1.52±1.89[a]	0.25±0.48[a]	6.46±3.72[a]	0.02±0.13[b]	0.01±0.13[c]
T_4	65% CF	0.62±1.50[b]	0.04±0.53[a]	0.16±1.84[b]	0.004±0.04[b]	3.03±0.09[d]	1.18±0.68[c]	0.18±0.10[e]	2.76±1.56[c]	0.01±0.09[c]	0.03±0.09[b]
T_5	65%CF+BFBF	0.66±1.69[a]	ND	0.16±2.01[b]	0.004±0.06[b]	9.85±0.14[a]	1.24±1.53[b]	0.21±0.21[c]	2.91±4.05[b]	0.02±0.06[b]	0.02±0.06[b]
T_6	Control (Normal soil)	0.50±0.00[d]	0.03±0.01[b]	0.17±0.01[a]	0.003±0.10[c]	3.13±0.02[c]	1.16±0.10[d]	0.15±0.24[f]	1.77±0.83[f]	0.02±0.27[b]	0.02±0.27[b]

Values are mean ± SD for three samples of each variety, analyzed individually in triplicate

Small superscripts letters with in the same column indicate significant differences ($p<0.05$) of means among rice in different plots

Table 3. Maltose and Glucose releasing rates

Treatment	Sample	Maltose releasing rate ($mmoldm^{-3}min^{-1}$)	Glucose releasing rate($mmoldm^{-3}min^{-1}$)
T_1	100% CF	$0.0135^{d} \pm 0.0001$	$0.0025^{d} \pm 0.0000$
T_2	80% CF	$0.0264^{a} \pm 0.0004$	$0.0029^{b} \pm 0.0000$
T_3	80% CF+BFBF	$0.0075^{e} \pm 0.0000$	$0.0028^{c} \pm 0.0001$
T_4	65% CF	$0.0063^{f} \pm 0.0003$	$0.0018^{e} \pm 0.0000$
T_5	65% CF+BFBF	$0.0137^{c} \pm 0.0005$	$0.0017^{f} \pm 0.0001$
T_6	Control (Normal soil)	$0.0156^{b} \pm 0.0001$	$0.0032^{a} \pm 0.0001$

Values are mean ± SD for three samples of each variety, analyzed individually in triplicate

Small superscripts letters with in the same column indicate significant differences ($p<0.05$) of means among rice in different plots.

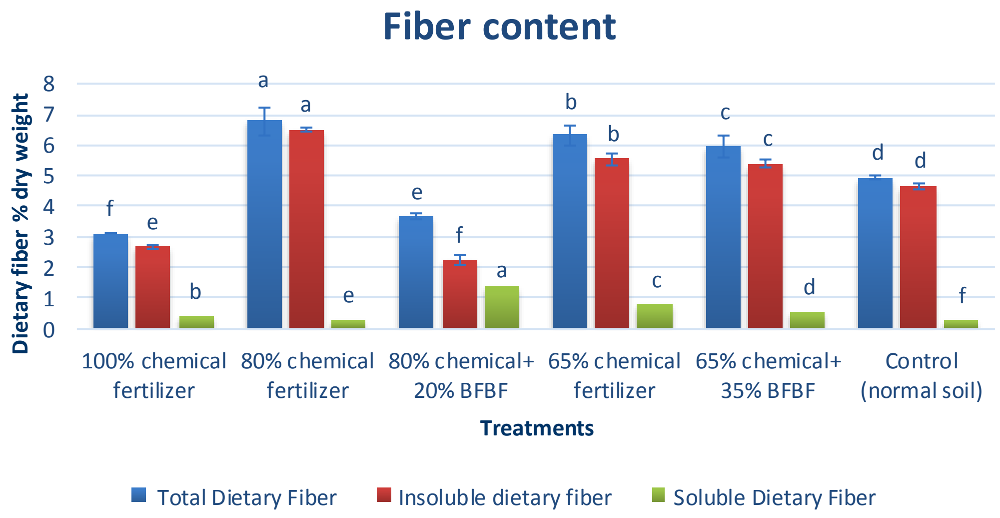

Figure 1. Graph of Fibre content

CONCLUSION

The results of this research show that the protein, total dietary fiber and zinc content in rice grown with 65% CF+BFBF treatment were higher than those in the rice grown with 100% CF and the control sample. Accordingly, the use of BFBF confirms that it has improved the nutritional efficiency of rice. There was a significant difference in hydrolyzing rates in rice grown with different fertilizer treatments and rice grown with 65% CF showed the lowest hydrolyzing rate. Protein and micronutrient malnutrition are some health concerns in all age groups of the population in Sri Lanka. Considering the results, higher protein and higher Zn content in rice grown with 65% CF+BFBF may be a promising outcome for protein and Zn malnutrition. Therefore, it could be concluded that the 65% CF+BFBF is an eco-friendly and economically viable recommendation for rice farming. Popularizing BFBF among farming communities will substantially impact our local rice production in terms of cost-cutting and nutritional enhancement. However, further investigations should be continued covering all agro-ecological zones and seasons where paddy is grown before confirming this fertilizer recommendation.

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REFERENCES

AOAC (2005). Official methods of analysis of AOAC International, Gaithersburg, MD, USA: AOAC

Bedano, J. C., Cantú, M. P. and Doucet, M. E. (2006). Influence of three different land management practices on soil mite (Arachnida: Acari) densities in relation to a natural soil', Applied Soil Ecology, 32(3), 293–304.

Bhat, R. and Sridhar, K. R. (2008). Nutritional quality evaluation of electron beam-irradiated lotus (*Nelumbo nucifera*) seeds. Food Chemistry, 107(1), 174–184.

Doa.gov.lk. 2020. Rice cultivation. [online] Available at: <https://doa.gov.lk/rrdi/index.php?option > [Accessed 12 November 2020].

Eggum, B.O. (1979). The nutritional value of rice in comparison with other cereals. In: Proceedings, Workshop on Chemical Aspects of Rice Grain Quality, IRRI. Los Banos, Laguna, The Philippines; 91–111.

Haruna, M., Udobi, C. and Ndife, J. (2011). Effect of added brewers dry grain on the physico-chemical, microbial and sensory quality of wheat bread, American Journal of Food and Nutrition, 1(1), 39–43.

Juliano, B.O. (1985b). Rice: Chemistry and Technology. 2nd ed. St. Paul, MN: Am. Assoc. Cereal Chem, 774.

Kalita, D. Holm D.G., LaBarbera D. V., Petrash, J. M., Jayanty, S.S. (2018) 'Inhibition of α-glucosidase, α-amylase, and aldose reductase by potato polyphenolic compounds', Plos One, 13(1).

Khan, M. S., Zaidi, A. and Musarrat, J. (2009). Microbial strategies for crop improvement', in Microbial Strategies for Crop Improvement, 1–358.

Klomklao, P., Kuntinugunetanon, S. and Wongkokua, W. (2017). Moisture content measurement in paddy. Journal of Physics: Conference Series, 901(1), 1–5.

Rathna Priya, T., Eliazer Nelson, A.R.L., Ravichandran, K. (2019). Nutritional and functional properties of coloured rice varieties of South India: a review. J. Ethn. Food 6, 11

statistics.gov.lk. 2020. [online] Available at: <http://www.statistics.gov.lk/> [Accessed 13 November 2020].

Seneviratne G., Thilakaratne R., Jayasekara A., Seneviratne K., Padmathilake K., De Silva M. (2009). Developing Beneficial Microbial Biofilms on Roots of Non legumes: A Novel Biofertilizing Technique. Microbial Strategies for Crop Improvement. Springer, Berlin, Heidelberg.

Tarantino, T. B. (2017). Microwave-Assisted Digestion Using Diluted Nitric Acid for Multi-Element Determination in Rice by ICP OES and ICP-MS. Food Analytical Methods. Food Analytical Methods, 10(4), 1007–1015.

Warren, F. J., Zhang, B., Waltzer, G., Gidley, M.J., Dhital, S. (2015). 'The interplay of α-amylase and amyloglucosidase activities on the digestion of starch in in vitro enzymatic systems', Carbohydrate Polymers. Elsevier Ltd., 117, pp. 185–191.

Visvanathan R., Jayathilake, C., Liyanage, R. (2016). A simple microplate-based method for the determination of α-amylase activity using the glucose assay kit (GOD method), Food Chemistry, 211, 853-85.