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Prebiotic Potential of Resistant Starches and Dietary Fibers of Sri Lankan Traditional Rice Varieties and its Application in the Food Industry

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Abstract: This study was conducted to investigate the prebiotic potential of selected traditional rice varieties by inoculating with probiotic bacterium *Lactobacillus plantarum.* Five traditional rice varieties; *Suwandel, Pachchaperumal, Kaluheenati, Kuruluthuda* and *Madathawalu* as treatments and *BG-358* as control, were used in flour form. Dietary fiber (DF) and resistant starch (RS) content of selected varieties were measured according to AOAC method 2009.01, 2011.25 and 2002.02. Modified culture media was prepared by combining *MRS* agar with rice flour in 4:1 ratio and *L.plantarum* was inoculated while inhibiting other bacterial growth using *ciprofloxacin.* Colony forming units (CFU) and prebiotic activity score (PAS) was calculated by inoculating *L. plantarum* as the probiotic and *Escherichia coli* was used as enteric bacteria. Rice idli mix was developed by using two rice varieties with highest PAS value and the best product was determined by a sensory evaluation with twenty five semi-trained panelists. According to the results of DF, RS, CFU and PAS evaluation, all traditional varieties were significantly higher (p<0.05) compared to control variety. *Kaluheenati* possesses significantly (p<0.05) the highest value of 6.97±0.03% and 2.53 ± 0.02% for DF and RS respectively. CFU were varied from 1.87 ± 0.04x10^7 to 2.58 ± 0.05 x10^7 and highest CFUs were reported in *Kaluheenati*. PAS was varied from 1.23 ± 0.01 to 1.46±0.02 and *Kaluheenati* was reported significantly higher (p<0.05) score compared to other traditional varieties. Best idli mix was combination of *kaluheenati* and *suwandel* in 1:1 ratio. The results revealed that *kaluheenati* has the highest DF, RS and higher PAS reflects traditional varieties have higher prebiotic potential.

***Keywords*:** Dietary fiber, Prebiotics, Probiotics, Resistant starch, Traditional rice

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Introduction

In Sri Lanka, nearly 2000 traditional rice varieties were cultivated in past (Rambu kwella and Priyankara, 2016) and nowadays in the market, we can see some of main traditional rice varieties such as "*suwandel"*, "*kaluheenati" etc*. Today they have very high demand because of their organic cultivated nature, nutritive and medicinal value (Dharmasena, 2010). But cultivation is limited and also there is lack of investigation of their physio-chemical properties such as resistant starch content, the fermentation characteristics and the prebiotic potential of their dietary fiber fraction.

Resistant starch (RS) is the starch that escapes human small intestine digestion. It can be delivered to the lower gut for further microbial anaerobic fermentation (Topping *et al*., 2003).

Fermentation of RS and Dietary fiber (DF) by lower gut micro biota is one of the most important aspects to maintaining colon health. The human large intestine is heavily populated by numerous and diverse species of microorganism, which forming a complex micro flora community (Thursby and Juge, 2017). Colonic micro flora plays an important role in host health by maintaining the proper intestinal function, including development of immune system, inhibit the growth of pathogen and regulate metabolic pathway in the host (Mishra and Mishra, 2018).

Prebiotics are described as the indigestible carbohydrate which can improve a balanced intestinal micro flora, once administered orally as a food supplement. A prebiotic ingredient should resist towards the digestions in the upper gastrointestinal tract and be selectively fermented by intestinal micro flora associated with beneficial effects (Marko wiak and Slizewska, 2017). Traditional rice varieties can act as a vehicle to supply the fibers to human and there is a possibility of having a prebiotic potential of their fiber fraction. They are low in glycemic value and therefore can be recommend for the diabetic mellitus type 2 patients (Samaranayake *et al*., 2018).

The general aim of this study is to determine the prebiotic potential of resistant starches and dietary fibers of traditional rice varieties in Sri Lanka by calculating their prebiotic activity score.

Materials and Methods

In this research, used five traditional rice varieties; *Suwandel*, *Kaluheenati*, *Pachchaperumal*, *Kurulu thuda* and *Madathawalu*. Commercially cultivating *BG-358 samba* rice variety was selected as control. Each variety was purchased from Rice Research and Development Institute (RRDI) Sri Lanka.

Dietary fibers and resistant starches of traditional rice varieties were determined in the laboratory of National Institute of Post-Harvest Management (NIPHM) Sri

Lanka. Rice flour of the selected varieties was assessed for dietary fiber and resistant starch content. All samples were prepared in four replicates and the compositions were calculated as dry matter basis. Dietary fiber analysis was conducted according to the AOAC method 2009.01, 2011.25 and 2002.02 using the "*Megazyme* integrated total dietary fiber assay kit" (Bray, Ireland) and resistant starch analysis was conducted according to the AOAC method 2002.02 using the "*Megazyme* resistant starch assay kit" (Bray, Ireland).

The probiotic and prebiotic activities were conducted in the food microbiology laboratory of NIPHM. *Lactobacillus plantarum* was selected as the probiotic bacterium and use "*plantarum* probiotic culture" from "Green Living Australia" as the *L. plantarum* culture. Fermentation medium was prepared by combining De Man, Rogosa and Sharpe (MRS) agar with the flour of rice varieties. *Ciprofloxacin* antibiotic was used to selectively grow *L. plantarum* and inhibit the growth of other lactic acid bacteria based on the resistance of the *L. plantarum* to *ciprofloxacin* and its ability to produce acid from sorbitol (Bujalance *et al*., 2006). The modified medium was prepared by incorporating MRS agar and rice flour in 4:1 ratio. Weight of 60 g of MRS agar and 15 g of rice flour were dissolved in 1 liter of distilled water and added 5 mg of *ciprofloxacin*. Generally using non-prebiotic glucose media was used as the standard control to compare the prebiotic activity. The control medium was prepared using MRS agar and same amount of glucose instead of rice flour. The control and selected five varieties were used as the treatments of the experiment (Table 1).

Table 1: The experimental treatments of traditional rice varieties

Treatment	Fermentation Medium
T1	Standard medium (control)
T2	*Suwandel*
T3	*Kaluheenati*
T4	*Pachchaperumal*
T5	*Kurulu thuda*
T6	*Madathawalu*

Trial and error method was used to find out the exact ingredients of the fermentation medium. The developed mediums were inoculated with *L. plantarum* and incubated in an anaerobic culture jar (Anaerobic System Mark II) for 48 hours and microbial counts were calculated using standard plate count method.

Prebiotic Activity Score (PAS) was calculated by inoculating enteric bacteria, *Escherichia coli,* and compare the growth of *E. coli* with *L. plantarum*, according to the following equation (Fissore *et al*., 2015).

$$\left[\frac{(Probiotic\ log\ cfu/ml\ on\ prebiotic\ at\ 24\ hours - probiotic\ log\ cfu/ml\ on\ prebiotic\ at\ 0\ hours)}{(Probiotic\ log\ cfu/ml\ on\ glucose\ at\ 24\ hours - probiotic\ log\ cfu/ml\ on\ glucose\ at\ 0\ hours)}\right] - \left[\frac{(Enteric\ log\ cfu/ml\ on\ prebiotic\ at\ 24\ hours - enteric\ log\ cfu/ml\ on\ prebiotic\ at\ 0\ hours)}{(Enteric\ log\ cfu/ml\ on\ glucose\ at\ 24\ hours - enteric\ log\ cfu/ml\ on\ glucose\ at\ 0\ hours)}\right]$$

Rice *idli* mix from traditional rice was developed as the application of this study in food industry. The varieties with twohighest PAS values were used to develop rice *idli* mix. Best rice *idli* mix was selected by conducting a sensory evaluation was conducted in 5 point hedonic scale for 25 semi-trained panelists. Three recipes were developed as 100% of 1st variety, 100% of 2nd variety and 50% mix of both varieties. Appearance/ fluffiness, color, aroma, taste, sponginess, mouth feel and overall acceptability were used as the sensory parameters.

The best product selected from sensory analysis was analyzed for dietary fibers, resistant starches, CFUs of *L.plantarum* and PAS values. Steamed form of rice idli was used to analyze above parameters. Parametric data were analyzed using ANOVA in SPSS software and mean comparisons were performed using least significance difference test at *P<0.05* significance level. Sensory data were analyzed using Friedman non parametric data in SPSS software.

Results and Discussion

All selected rice varieties possess significantly higher amounts of DF and RS compared to control rice variety; *BG 358*. Among them, *Kaluheenati* rice variety possesses significantly ($p<0.05$) the highest value of 6.97±0.03% and 2.53±0.02% for DF and RS respectively (Table 2). Generally red rice varieties have higher DF values because of its remaining outer layers (Abeysekera *et al*., 2017; Savitha and Singh, 2011). In this research, all the selected varieties were red rice varieties except *Suwandel* which possess a low glycemic index (GI) value compared to *BG 358*due to its higher DF and RS values (Hettiarachchi *et al*., 2016).

Colony forming units (CFU) of *L. plantarum* were calculated after 48 hours of anaerobic incubation period. The results of CFUs were indicated per 1 milliliter (ml) hence all rice varieties including the control medium, *BG 358* have higher CFU values more than the minimum therapeutic value of $1x10^6$ CFUs per ml (Ranadheera *et al*., 2012). The control medium, which has no rice flourwasn't consisting with DF or RS content. CFU of control medium was observed as $1.28 \times 10^{7*}$ per 1 ml which is significantly ($p<0.05$) lower than all the other modified mediums developed from rice flour.

Table 2: Dietary fiber, resistant starch, colony forming units and prebiotic activity score values of selected traditional rice varieties

Variety	DF (%)	RS (%)	CFU(10^7)	PAS
BG 358	4.24 ± 0.02^a	1.33 ± 0.03^a	1.49 ± 0.03^a	1.10 ± 0.02^a
Suwandel	6.32 ± 0.01^b	2.28 ± 0.01^b	2.05 ± 0.03^b	1.34 ± 0.01^b
Kaluheenati	6.97 ± 0.03^c	2.53 ± 0.02^c	2.58 ± 0.05^c	1.46 ± 0.02^c
Pachchaperumal	6.71 ± 0.01^d	2.24 ± 0.03^d	2.33 ± 0.04^d	1.32 ± 0.02^b
Kuruluthuda	6.63 ± 0.02^e	1.92 ± 0.01^e	1.87 ± 0.04^e	1.23 ± 0.01^e
Madathawalu	6.56 ± 0.02^f	2.11 ± 0.02^f	2.06 ± 0.05^f	1.24 ± 0.02^f

*Values in the same column with different superscripts differ significantly ($p<0.05$). Each value represents mean ± SD with four replicates.

According to above table (Table 2), all rice varieties possess satisfactory fermentation characteristics with probiotic bacteria *L. plantarum*. CFU values of traditional rice varieties were significantly higher than the control variety *BG 358* (1.49 ± 0.03%) and *Kaluheenati* possess the highest CFU value (2.58 ± 0.05%). This result revealed that traditional varieties possess *L. plantarum* growth enhancing factors more than control variety.

DF and RS values of traditional rice varieties were significantly higher than control variety and it can be the reason for the higher CFU values of traditional varieties compared to control variety (Abeysekera *et al*., 2017). The prebiotic potential should be evaluated to justify whether there is a relationship to increase the CFU values by DF and RS content. Prebiotic activity score (PAS) was calculated to check the prebiotic potential of traditional rice varieties (Zhang *et al*., 2012).

The results of PAS revealed that all varieties including control variety have PAS values more than 1.0 ranging from 1.18 ± 0.02to 1.46 ± 0.02. But traditional varieties have significantly higher PAS compared to control variety (1.18 ± 0.02) and *Kaluheenati* has the significantly highest PAS value (1.46 ± 0.02). The PAS value is exceeding one means all varieties have some extend of prebiotic potential and traditional varieties have significantly higher prebiotic potential compared to control. DF value of *Suwandel* variety was lower than all other traditional varieties, but has higher PAS value due to its high amount of RS. Although RS is a component of DF, buthas much more influence on prebiotic potential than DF.

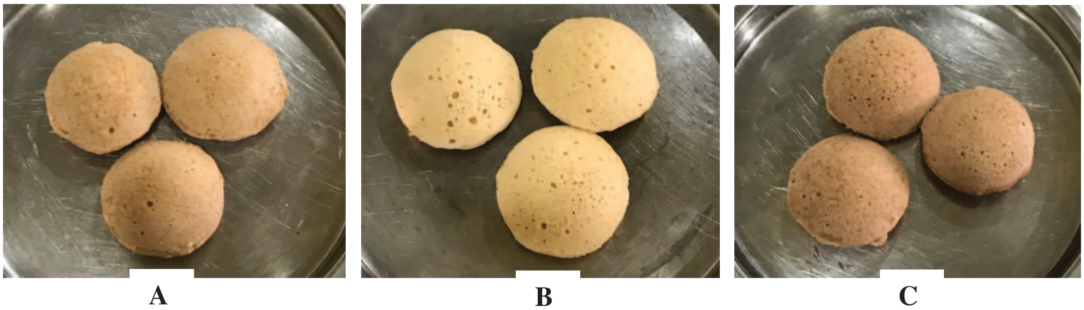

Plate 1: Rice *idli* made from traditional rice flour, A: 50% *Kaluheenati* and 50% *Suwandel,* B:100%*Suwandel,* C: 100% *Kaluheenati***.**

In rice *idli* development, use top two varie ties of PAS value. Highest value was for *kaluheenati* variety. *Suwandel* and *pach chaperumal* both together possess the second highest value of PAS. In here use *suwandel* variety with *kaluheenati* to develop rice *idli*, because *suwandel* is a white variety and higher consumer accepta bility compared to *pachchaperumal* (Plate 1).

According to the sensory results, best rice *idli* mix was the combination of *Kaluheenati* and *Suwandel* in 1:1 ratio. Color, taste, sponginess and overall acceptability sensory parameters were significantly different while appearance, aroma and mouth feel parameters were significantly not different.

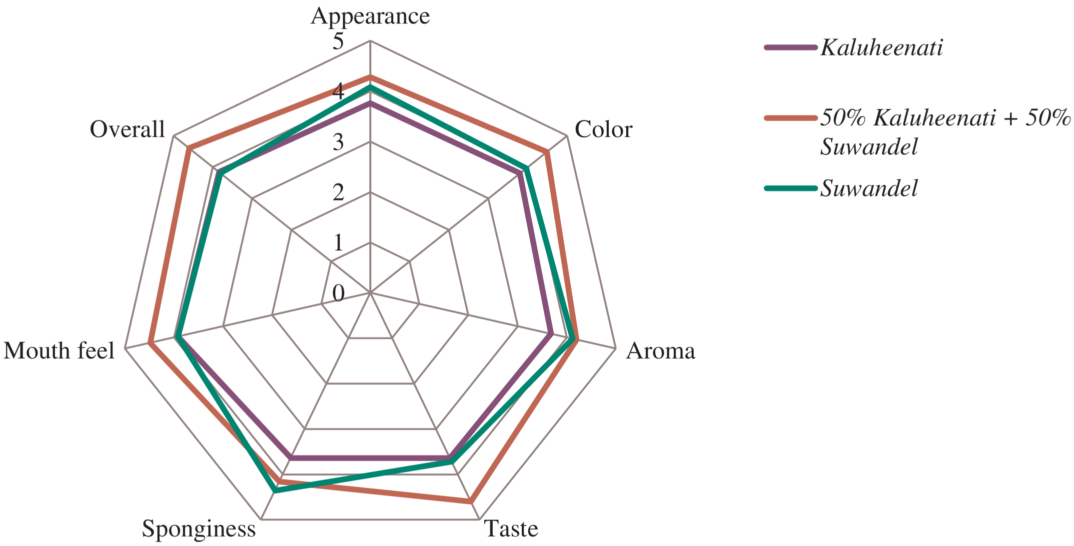

Figure 2: Sensory properties of rice idli made from traditional rice varieties.

The DF, RS, CFUs and PAS values of best rice *idli* mix after cooking were 6.73±0.04, 2.86±0.05, $3.02x10^7$ and 1.58±0.03 respectively. There was no any huge difference in DF content of cooked *idli* compared to raw rice flour. RS value drastically increased after cooking due to increase RS type 3 and type 5 (Ordonio and Matsuoka, 2016). Therefore CFU and PAS values also increased in cooked form compared to raw rice flour. Consuming rice *idli* mix after cooking provide considerable amounts of DF and RS and it provide good prebiotic source for the healthy gut micro flora in the host. Prebiotics enhance the growth and development of probiotics and it make a hostile environment to the pathogenic bacteria in the host's gut(Markowiak and Slizewska, 2017). Also consumption of higher DF and RS good for diabetes mellitus type 2 patients (Samaranayake *et al*., 2018).

Conclusion

According to the results of the study, the selected traditional varieties possess significantly higher amounts of DF and RS compared to newly improved and commercially cultivated varieties such as *BG 358*. High amount of DF and RS is good for diabetes mellitus type two patients due to its low GI value. The prebiotic activity score also significantly higher in traditional varieties compared to commercially cultivated varieties. Higher PAS reflects higher prebiotic potential in traditional varieties. RS has more influence on prebiotic potential compared to DF and higher RS containing varieties indicate higher prebiotic potential. *Kaluheenati* is the best traditional variety among other varietiesbecause of its highest DF, RS and PAS. Therefore, consuming traditional rice varieties gives health benefits towards consumer by providing prebiotics which enhance the growth and development of beneficial bacterial flora such as probiotics living in the human gut. Further *in vivo* study is required to evaluate extend of prebiotics in traditional rice to stimulate the growth of probiotic micro flora living in human gut ecosystem. The health benefits of traditional rice could be provided to the consumer by applying developed food products to the food industry.

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