

# Sri Lanka Journal of Food and Agriculture (SLJFA)

ISSN: 2424-6913  
Journal homepage: [www.slicarp.lk](http://www.slicarp.lk)



## Research Paper

# Antioxidant and antimicrobial activities and starch digestion rate of the organically- and non-organically grown traditional rice varieties in Sri Lanka

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### Article History:

Received: 3 February 2017

Revised form received: 19 May 2017

Accepted: 30 June 2017

**Abstract:** The study was designed to assess the total phenolic content (TPC), free radical scavenging activity, antimicrobial property and *in vitro* starch digestion rate of three traditional rice varieties grown organically and non-organically in Sri Lanka. The 70% ethanol extractions of rice were subjected to TPC, DPPH - free radical scavenging assays to determine

antioxidant activity, and antimicrobial susceptible test against three bacteria, namely, *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus* to determine antimicrobial properties. Starch digestion of cooked rice samples were evaluated under *in vitro* conditions. Both TPC and free radical scavenging activity was significantly higher ( $P < 0.05$ ) in organically produced traditional rice while the non-organically produced traditional rice samples showed a significantly higher ( $P < 0.05$ ) antimicrobial activity against *S. typhi* and *S. aureus*. There was no significant difference ( $P > 0.05$ ) in the *in vitro* starch digestion rate between the two groups. Though the study only considered traditional rice varieties, the results suggested that organically produced traditional rice samples performed better than those non-organically produced, with regard to the antioxidant properties such as TPC and free radical scavenging activity. The *Madathawalu* cultivar performed superior role in total phenolic content, free radical scavenging activity and Glycemic Index compared to that of two other traditional rice varieties, namely, *Suwandel* and *Handiran*.

**Keywords:** Total phenolic content, free radical scavenging activity, Glycemic Index, antimicrobial property, traditional organic rice



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## Introduction

A dramatic increase of non-communicable disease related with food consumption, such as diabetes mellitus, cancers and food-borne illnesses have been reported in Sri Lanka in the recent years. Rice (*Oryza sativa* L) is the staple food of 17 countries in Asia and the Pacific, nine countries in

North and South America and eight countries in Africa (FAO, 2004). The source and the amount of agricultural inputs may influence directly the level of available nutrients and indirectly to the physiology and chemical composition of the plant (Arab *et al.*, 2015). Therefore, it can be suggested

that health benefits of rice grain may differ according to the agronomic practices. Therefore, the importance of studying difference between organically grown and non-organically grown rice varieties is emphasized. This research was thus conducted with the major objective of evaluating

## Materials and Methods

### Collection and preparation of rice samples

Paddy samples of three traditional rice varieties, namely, *Suwandel*, *Madathawalu* and *Handiran* were collected from five wholesale rice market places in Homagama, Thelijjavila, Labuduwa, Anuradhapura and Malabe in Sri Lanka. Ethanolic rice extractions were prepared by measuring 20 g of rice powder. The rice powder was obtained by separately milling 150 g of whole grains from each variety using a food processing grinder for four min. The powder was soaked in 100 ml of 70% ethanol with 8 hrs continuous shaking using a laboratory shaker at 150 rpm. Shaken rice solutions were kept for 16 hrs in dark at room temperature before filtering through cotton wool. Filtered solutions were evaporated by a rotary evaporator and extractions were stored at 4 °C. Cooked rice samples were prepared by cooking 100 g whole grains of milled rice in automatic rice cooker with 700 ml water for 45±5 min.

### Analysis of antimicrobial properties

The agar diffusion method proposed by Olayele and Opaleye (1999) was used to conduct antimicrobial susceptible test (AST). The three bacterial strains used in this study included *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus*. Twenty µl of test organisms were inoculated into sterile Petri dishes with the aid of a sterile loop. About 20 ml of sterile nutrient agar broth was poured into each dish. Petri dishes were kept in the laminar flow until nutrient agar solidified. Four wells were bored on each plate using 6 mm diameter sterilized cork borer. Rice extractions were dissolved in 2% DMSO solution, and 100µl of the solution was poured into properly labelled wells. They were allowed to stand for one hr for proper diffusion and then incubated at 37 °C for 24 hrs. The diameters of the inhibition zones were measured using a ruler, with an accuracy of 0.5 mm. Each inhibition zone

the traditional rice varieties produced in two different agricultural systems in Sri Lanka, for their medicinally important bio-actives; antioxidant, anti-diabetic and antimicrobial properties.

diameter was measured three times (three different plates) and the average was considered. The inhibition zone (IZ) was obtained as follows;

$$IZ (mm) = IZ \text{ dia. (mm)} - \text{Cork borer dia. (mm)}$$

where 'dia.' refers to the diameter.

### In vitro starch digestion rate

Two grams of available carbohydrate portions of each of the cooked rice samples (whole grains) were placed in dialysis bags (12,000 molecular weight cut-off), 10 mL of pooled human saliva was added and stirred for 10 sec. and the volume was adjusted to 35 mL with distilled water. Then the samples were dialyzed against 800 mL of distilled water at 37 °C for 3 hrs (n = 4). For 3 hours, 1 ml dialysate samples were taken out at hourly intervals and analyzed for total sugars by 3,5-dinitrosalicylic acid method (Thompson *et al.*, 1987). *In vitro* starch digestion rate (IVSDR) was calculated using white bread as the reference sample, as follows;

$$IVSDR = [100/\text{total sugars in the dialysis of white bread, mg per ml}] \times \text{total sugars in the dialysate of rice sample, mg per mL}$$

### Antioxidant properties: determination of 2, 20-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging ability

The DPPH radical-scavenging ability of rice extracts was evaluated according to the procedure reported by Brand-Williams *et al.* (1995). The reaction mixture contained 1.5 ml DPPH working solution (4.73 mg of DPPH in 100 ml ethanol HPLC-grade) and 300 µl of rice extract. The mixture was shaken and incubated for 40 min in the dark at room temperature. The absorbance was read at 515 nm relative to the control (as 100%) using a spectrophotometer (U-1100,

Hitachi, Japan). The percentage of radical-scavenging ability (RSA) was calculated by using the formula:

$$\% \text{ RSA} = [(Absorbance \text{ of control}_{515nm} - Absorbance \text{ of sample}_{515nm}) / Absorbance \text{ of control}_{515nm}] \times 100$$

### Determination of total phenolic content (TPC)

The TPC of extracts was determined using the Folin–Ciocalteu’s reagent (Singleton *et al.*, 1999). A 0.5 ml aliquot of rice methanolic extract was

added to 2.5 ml of freshly diluted 10% Folin–Ciocalteu’s reagent. After 10 min of reaction time, 2.5 ml of 7.5 % sodium carbonate solution was added to the mixture. The sample was incubated thereafter in thermostat at 45 °C for 45 min. The absorbance of the resulting blue colour was measured at 760 nm against a blank. Gallic acid was used as the standard and TPC was expressed as mg gallic acid equivalent per 100 g rice.

## Results and Discussion

### Antioxidant Properties of Rice Samples

#### DPPH value and total phenolic contents (TPC)

The DPPH radical scavenging activity of the samples ranged from 16.89±0.78 to 4.55±1.85 mg ascorbic acid equivalent in 1 g of rice extraction (Table 1). The highest mean DPPH activity was observed for MO while the lowest for SN rice sample. Ccotrill (2001) claimed that accumulation of ascorbic acid (vitamin C), which is known as a powerful antioxidant, is induced by plants subject

to oxidative stress such as full sunlight, droughts, and low nitrogen content. Our previous study on physiochemical properties of raw rice (data not shown) showed that organically-produced rice has low protein content than the non-organically produced rice. Although the agronomic practices carried out during crop growth of the traditional rice varieties collected in the present study were not available, the stress induced by low nitrogen in organic systems may have increased the antioxidant capacity of the rice samples compared to those grown in conventional systems.

Table 1. DPPH scavenging activity and total phenolic acid (TPC) content of rice samples.

Sample	DPPH mg ascorbic acid equivalent /g of rice extract	TPC mg gallic acid equivalents/g of rice
SO*	7.39±1.82 <sup>b</sup>	1.84±0.00 <sup>c</sup>
SN	4.55±1.85 <sup>a</sup>	1.24±0.00 <sup>e</sup>
HO	16.44±0.98 <sup>e</sup>	1.99±0.03 <sup>b</sup>
HN	8.43±0.94 <sup>c</sup>	0.95±0.00 <sup>f</sup>
MO	16.89±0.78 <sup>e</sup>	2.22±0.03 <sup>a</sup>
MN	9.40±0.25 <sup>d</sup>	1.42±0.01 <sup>d</sup>

Data represented as mean ± SE (n=3). Within a column, means followed by same letter are not significantly different at P=0.05. \* SO = *Suwandel* organic, SN = *Suwandel* non-organic, HO = *Handiran* organic, HN = *Handiran* non organic, MO = *Madathawalu* organic, MN = *Madathawalu* non-organic

The TPC of the samples ranged from 2.22±0.03 to 0.95±0.00 mg gallic acid equivalents/g of rice. The highest mean TPC was observed for organically-produced *Madathawalu* (MO) while the lowest for non-organically produced *Handiran* (HN) rice sample. Asami *et al.* (2003) reported that organically produced rice samples show superior TPC results than the non-organically produced

samples. Asami *et al.* (2003) also reported 52% more ascorbic acid and significantly higher polyphenol contents in organically grown corn than conventionally grown corn. Among the organically-produced rice varieties, the two red pericarp rice varieties used in this study (*Madathawalu* – MO, and *Handiran* - HO) showed

higher antioxidant values than the white pericarp rice variety *Suwandel*.

Sompong *et al.* (2011) reported that coloured rice shows higher antioxidant capacity than white rice. Results of this study showed that there is high potential of coloured rice to be used in organic systems for prevention and management of oxidative stress associated diseases.

*In-vitro* starch digestion rate

The mean *in vitro* starch digestion rate of the tested traditional rice samples varied from 62.54±1.53 to 43.58±2.3 compared to white bread as the reference (Figure 1). Results showed no significant difference (P>0.5) in starch digestion rate between organically- and non-organically produced traditional rice samples, though *Madathawalu* recorded the lowest while *Handiran* showed the highest digestion rates.

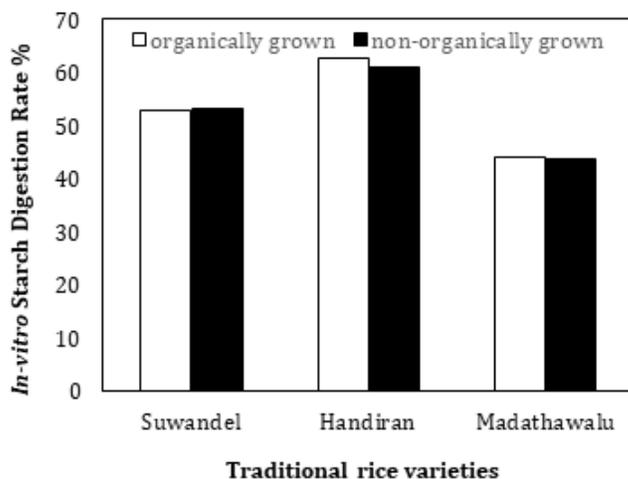


Figure 1. *In vitro* starch digestion rate of organically and non-organically grown traditional rice varieties

Jenkins *et al.* (1981) reported that carbohydrate foods are digested and absorbed at different rates. The glycemic index (GI) is classification of the blood glucose raising potential of carbohydrate foods. Lower GI foods show lower starch digestion and absorption and are believed to have beneficial effects in management of diabetes. *Madathawalu* showed the highest beneficial effects in management of diabetes among the traditional rice varieties tested in this experiment.

**Antimicrobial Properties of Rice**

Murakami *et al.* (2005) reported that rice has become an interesting candidate as a natural antibacterial agent. The present study was conducted to identify effect of agronomic practices on antimicrobial activity of rice. The results of the present study revealed a significant difference between organically produced and non-organically produced rice in their antimicrobial activity against *S. typhi* (Table 2).

Table 2. Zone of inhibition (mm) of rice extraction on tested organisms

Sample	<i>Escherichia coli</i>	<i>Salmonella typhi</i>	<i>Staphylococcus aureus</i>
SN*	0.0	4.7±0.2 <sup>a</sup>	5.80±0.3 <sup>bc</sup>
SO	0.0	4.2±0.3 <sup>b</sup>	5.56±0.3 <sup>cd</sup>
HN	0.0	3.2±0.2 <sup>d</sup>	6.20±0.4 <sup>a</sup>
HO	0.0	2.7±0.3 <sup>e</sup>	6.06±0.2 <sup>ba</sup>
MN	0.0	3.8±0.3 <sup>c</sup>	5.73±0.2 <sup>bc</sup>
MO	0.0	3.4±0.3 <sup>d</sup>	5.34±0.3 <sup>d</sup>

Data represented as mean ± SE (n=3). Within a column means followed by the same letter are not significantly different at P=0.05. \* SO = *Suwandel* organic, SN = *Suwandel* non-organic, HO = *Handiran* organic, HN = *Handiran* non organic, MO = *Madathawalu* organic, MN = *Madathawalu* non-organic

Available literature (Rocio and Rion, 1982; Mayeux, *et al.*, 1997; Tamer, 2009) suggests that plant extracts with high radical scavenging activity are effective in the treatment of oxidative stress-related disorders such as diseases borne by pathogenic microorganisms. Further, Rocio and Rion (1982) reported that the difference in the antimicrobial activity of different plant extracts could be due to the phytochemical content and radical scavenging property. However, according to the results of the present study, the highest inhibition activity against *S. typhi* was shown by the *Suwendel* sample compared to other two traditional rice cultivars, while recording the

## Conclusion

Organically grown seed samples of three traditional rice varieties showed high antioxidant activity compared to respective non-organically produced rice samples. The non-organically grown traditional rice varieties showed high antimicrobial activity compared to those grown in

lowest antioxidant activity. Therefore, the antibacterial activity observed could be due to other chemical compounds exist, but antioxidants.

The highest inhibition activity against *S. aureus* was shown by *Handiran* rice sample. In parallel, all three non-organically produced traditional rice cultivars showed higher antimicrobial activity than those produced organically for both *S. typhi* and *S. aureus*. The antibacterial effect reported against *S. aureus* was higher than that of other two tested bacteria. These results are in agreement with those of a similar study carried out by Pornpan and Natthanej (2013).

organic systems. Though the three traditional tested showed no significant difference in starch digestion rate between two categories, the lowest starch digestion rate was observed in the variety *Madathawalu*.

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