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

T.L.H.S. Hemanthi¹, D.A.C.K. Dalukdeniya², C.P. Rupasinghe¹ and R.M.U.S.K. Rathnayake²*

¹Department of Agric. Engineering, Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya, Sri Lanka
²Department of Food Science and Technology, Faculty of Applied Sciences, Sabaragamuwa University of Sri Lanka, Belihuloya, Sri Lanka
*Corresponding Author: udayarathnayaka@gmail.com

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

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 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.

**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 Olaye and Opaley (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 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;

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

**Antioxidant properties: determination of 2, 20-diphenyl-1-picyrylhydrazyl (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,
The percentage of radical-scavenging ability (RSA) was calculated by using the formula:

\[
% \text{RSA} = \frac{[\text{Absorbance of control } \times 515\text{nm} - \text{Absorbance of sample } \times 515\text{nm}]}{\text{Absorbance of control } \times 515\text{nm}} \times 100
\]

**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. Cottrill (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.

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

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

**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).

| Sample   | *Escherichia coli* | *Salmonella typhi* | *Staphylococcus aureus* |
|----------|--------------------|--------------------|-------------------------|
| SN*      | 0.0                | 4.7±0.2            | 5.80±0.3                |
| SO       | 0.0                | 4.2±0.3            | 5.56±0.3                |
| HN       | 0.0                | 3.2±0.2            | 6.20±0.4                |
| HO       | 0.0                | 2.7±0.3            | 6.06±0.2                |
| MN       | 0.0                | 3.8±0.3            | 5.73±0.2                |
| MO       | 0.0                | 3.4±0.3            | 5.34±0.3                |

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. typhii* was shown by the *Suwendel* sample compared to other two traditional rice cultivars, while recording the 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 Nattanej (2013).

**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 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*.

**References**

Arab, A., Zamani, G.R., Sayyari, M.H. and Asili, J. (2015): Effects of chemical and biological fertilizers on morpho-physiological traits of marigold (*Calendula officinalis* L.). *European J. Medicinal Plants*, 8(1): 60-68.

Asami, D.K., Hong, Y.J., Barrett, D.M. and Mitchell, A.E. (2003): Comparison of the total phenolic and ascorbic acid content of freeze-dried and air-dried marionberry, strawberry, and corn grown using conventional, organic, and sustainable agricultural practices. *J. Agric. Food Chem.*, 51(5): 1237-1241.

Brand-Williams, W., Cuvelier, M.E. and Berset, C.L.W.T. (1995): Use of a free radical method to evaluate antioxidant activity. *LWT-Food Sci. Technol.*, 28(1): 25-30.

Ccottrill, R.A., McKinley, R.S., Kaak, G., Dutil, J.D., Reid, K.B. and McGrath, K.J. (2001): Plasma non-esterified fatty acid profiles and 17β-oestradiol levels of juvenile immature and maturing adult American eels in the St Lawrence River. *J. Fish Biol.*, 59(2): 364-379.

FAO (2004): Rice and human situation. In: Rice and Human Nutrition (p. 2). Food and Nutrition Division, Viale delle Terme di Carcalla, Rome, Italy.
Rocio, M.C. and Rion, J.L. (1982): A review of some antimicrobial substances isolated from medicinal plants reported in the literature: Review of phytochemical analysis on garlic 1978 – 1972. Phytotherapy Rev., 3: 117-125.
Singleton, V.L., Orthofer, R. and Lamuela-Raventos, R.M. (1999): Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods in Enzymol., 299: 152-178.
Sompong, R., Siebenhandl-Ehn, S., Linsberger-Martin, G. and Berghofer, E. (2011): Physicochemical and antioxidative properties of red and black rice varieties from Thailand, China and Sri Lanka. Food Chem., 124(1): 132-140.
Tamer, D.M. (2009): Antimicrobial activity of certain Indian medicinal plants used in Folkloric Medicine. J. Ethnopharmacol., 174: 217-220.
Thompson, L.U., Button, C.L. and Jenkins, D.J. (1987): Phytic acid and calcium affect the in vitro rate of navy bean starch digestion and blood glucose response in humans. The Amer. J. Clinical Nutri., 46(3): 467-473.