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COMPARISON OF ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF RAW AND COOKED RICE OF IMPORTED AND NATIVE TO SRI LANKA

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ABSTRACT

*The emerging need for natural antioxidants has become a top priority in the field of research. With research articles emphasizing on the harmful side effects caused by synthetic antioxidants, scientists have focused on consumables which are used daily, in normal life and the importance of finding natural antioxidants in them. This study focuses on the evaluation of antioxidant and antimicrobial effects of five types of native Sri Lankan rice and five types of imported rice along with the cooking effect on rice. To assess the antioxidant capabilities of rice, total phenolic content (using Folin-Ciocalteu method), total flavonoid content (using Aluminium chloride method), total antioxidant capacity (using Ammonium molybdate), ABTS radical scavenging activity and Ferric Reducing Antioxidant Power (FRAP) methods were used. To assess the antimicrobial activity well diffusion technique was used. Significant differences were observed in total antioxidant capacity for both Sri Lankan (raw) against imported (raw) and Sri Lankan (cooked) against imported (cooked). ANOVA P values (raw rice - 0.027562 and cooked rice - 0.03931) were obtained showing the importance of traditional Sri Lankan rice. Furthermore traditional rice types like Heenati rice (HSR2) showed 100% inhibition of ABTS at 10 minutes and highest concentration of FRAP at 15 minutes (0.18381 mg/mL) showing exemplary results compared to other rice types. Antimicrobial activity of imported rice were lost after cooking and therefore a significant difference among native Sri Lankan cooked rice and imported rice was observed (P value - 0.049). Therefore with the obtained results in the study it can be concluded that Sri Lankan native rice had high antioxidant capacities and high antimicrobial activity when compared with the five imported rice types.**Keywords: Antioxidants, Imported rice, Sri Lankan rice, Cooking effect, Antimicrobial activity*

1.0 INTRODUCTION

A number of research is carried out to demonstrate the role of nutrients in diet, to control the risk and progression of chronic diseases like Cardiovascular Diseases (CVD) and cancer (Walter and Marchesan, 2011; Houston, 2005). Furthermore developing resistance against infections using natural components is of importance as well. Daily intake of vegetables, fruits and whole grains have shown reduced the risk of these diseases drastically. This factor could be attributed to the naturally present antioxidants found in these foods such as, phenolic compounds (polyphenols), carotenoids and tocopherols (Choi *et al.*, 2007). Antioxidants can be defined as organic molecules which act against Reactive Oxygen Species (ROS) and free radicals. By that to antioxidants protect the body from harmful metabolic effects exerted by these molecules to damage body cells (Goufo & Trindade, 2014).

Apart from the normal beneficial effects to the plants by phenolic compounds studies demonstrate the actions in antioxidant function in relation to human health. While free radicals are rapidly formed as a part of many metabolic actions in the human body, exogenous and endogenous sources expose the body to oxidants (Poljšak & Dahmane, 2012). An organism also includes antioxidants to balance these oxidants and free radicals. Under some situations the equilibrium between these two elements can be lost thus leading to oxidative stress. Oxidative stress causes damage to cells in many ways. Activating downstream signalling pathways, damaging cellular components and structures, changes to enzyme activity and gene expression, interruption of cell repair mechanisms and toxic material production are some the harmful mechanisms which are brought out by oxidative stress. Therefore scientists have linked oxidative stress to several chronic diseases like cancer, CVD, diabetes and ageing.

Action of phenolic compounds against the ROS or free radicals occur in different ways. Some acts as chain breaking antioxidants by scavenging reactive species like, superoxide, peroxyl and hydroxyl radicals. By recycling some antioxidants like α-tocopherol, lipid peroxidation can be supressed. Formation of free radicals by pro-oxidant metals like iron or copper is reduced by binding with these metals. Kept aside these direct effects, phenolics are capable to induce antioxidant protein synthesis and enhance antioxidant activity enzymes as well.

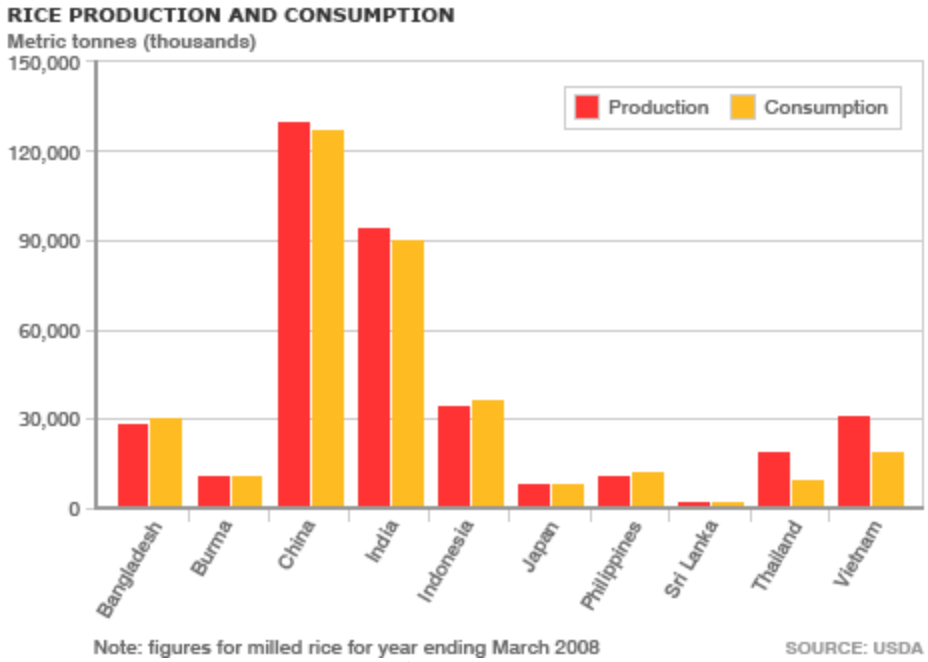


Figure 1 Consumption vs production of rice in different countries (BBC.com, 2008)

When considering rice consumption with some Asian countries, Sri Lanka rice consumption is towards the lower side. China and India have higher consumption considering and therefore it is shown that these countries have made this cereal there staple food. It also indicates that the nutritionals values of rice directly effects people worldwide as well. Therefore the importance of knowing what types of rice gives high yield of nutritional factors is a must.

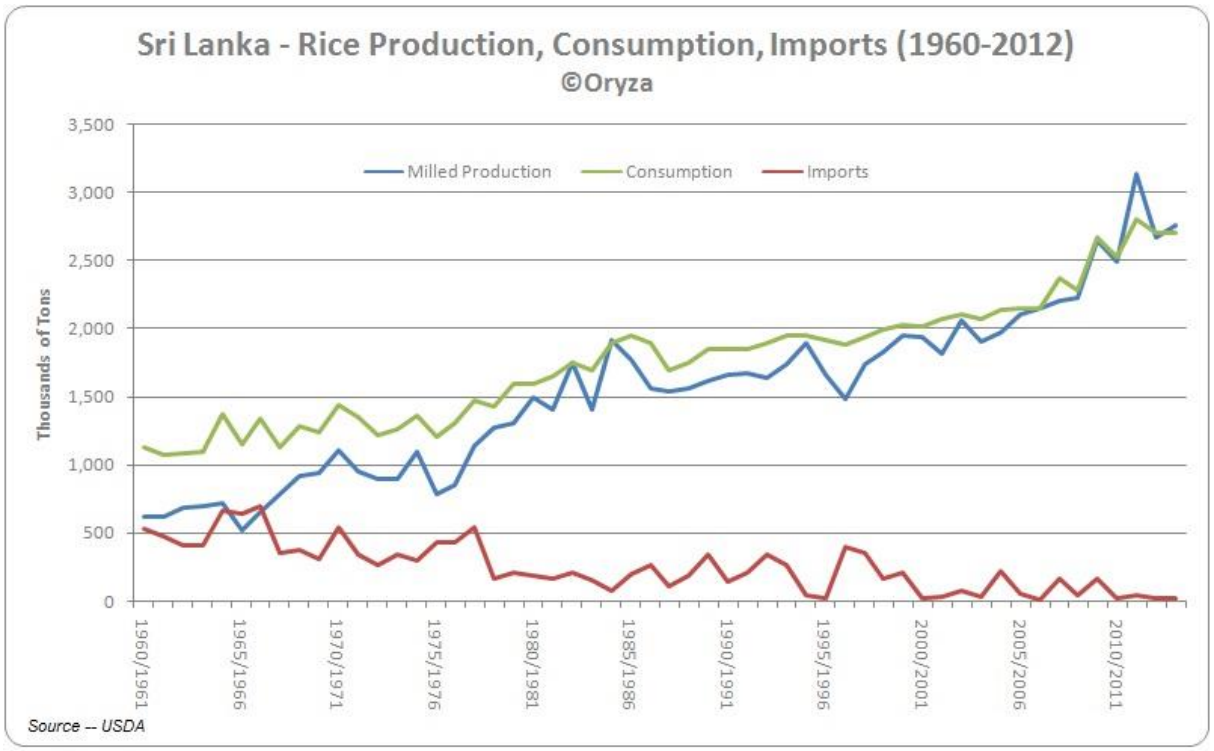


Figure 2 Sri Lankan rice production vs imports (Derpartment of Agriculture, 2013)

Sri Lanka from early days have been importing rice from other countries to compensate the consumption needs. However in the recent past production of rice has improved drastically and it is important to promote traditional Sri Lankan rice than the imported rice types. Though the production rate has gradually increased, a high number of people may be due to social status and financial income tempt to go for the more readily available imported rice in supermarkets than any traditional rice. It is important to make the public aware of the nutritional values which are high in these local products.

When considering phenolic compounds in rice, low molecular weight phenolic compounds are found in light brown pericarp colour grains while higher molecular weight compounds are found in black or red pericarp colour grains. It has been more than two decades since Dr. Ramarathnam and his colleagues found out that α-tocopherol, isovitexin and γ-oryzanol in rice exhibited similar antioxidant activity when compared with a common food preservative, butylated hydroxyanisole (Goufo & Trindade, 2014). Therefore up-to-date several phenolic compounds have been found. Light brown pericarp colour grains mainly include phenolic acids, ρcoumaric and ferulic acids. The main compounds in black or red pericarp grains are mainly anthocyanins, peonidin-3-O-β-D-glucoside and cyanidin-3-O-β-Dglucoside.

According to studies conducted the types of phenolic compounds present in rice is determined mainly by the pericarp colour. Furthermore the concentration of the phenolic compounds also show a good correlation with the pericarp colour. As an example red or black pericarp has higher concentrations of phenolic compounds than light brown grains. Not only the pericarp

colour, several downstream processes can affect the concentration of total phenolics present. Studies have shown that grain processing procedures like polishing and germination have direct effects on the concentration. Polishing without doubt reduces the content because phenolics mainly localize in the external layer of a grain. Tian *et al*. (2005) observed reduction of sinapoylsucrose and feruloylsucrose to sinapic and ferulic acids thus indicating germination caused phenolic metabolism which lead to hydrolysis. Surh & Koh, (2014) states that a significant decrease in anthocyanins and other phenolic compounds when rice was cooked. This may be possible due to the degradation of the compounds.

In respect to antimicrobial aspects, bacteria has the ability to develop resistance against the therapeutic agents by altering the genetic makeup. Therefore the emergence of a post antibiotic era is eminent. However with this situation in hand, it is required to evaluate possible natural sources with antimicrobial activities to develop antimicrobial drugs with novel mechanisms of action.

Sri Lanka, mainly being a country whose staple food is rice, is in the point of developing many rice varieties with good nutrient standards. However, over 300 different varieties of traditional rice are found all over the country. Therefore it is important to know the nutritional values which can prevent help many disease conditions. Let alone a comparison with imported rice types, evaluation studies of phenolics for these traditional rice types in Sri Lanka have been rarely conducted. This study is conducted in order to evaluate and compare the total antioxidant capacity, total flavonoid content, total phenolic content, Ferric Reducing Antioxidant Power (FRAP), ABTS scavenging activity and antimicrobial activity of ten different rice types native to Sri Lanka and imported rice.

2.0 MATERIALS AND METHODS

2.1 Materials

2.1.1 Rice types

Five different types of Sri Lankan native rice (Suwadal rice, Heenati rice, Rathu Nadhu rice, Pachchaperumal rice and Kurulu Thudha rice) and five different imported rice (Jasmine Thai rice, Ponni Indian rice, Sunrise Pakistani rice, Fortune Indian long grain rice and Premuim Pakistani Basmathi rice) were collected prior to the research.

2.1.2 Instrumentation

Fume hood (BIOBASE FH1000), Analytical weighing scale, micropipettes, hot air oven (meditry DHA-9053A), spectrophotometer (JENWAY 6305), weighing Scale and water bath (GEMMYCO YCW-010E).

2.1.3 Reagents

2, 2'-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) ($C_{18}H_{18}N_4O_6S_4$) (CAS-30931-67-0), ammonium molybdate ($[NH_4]_6Mo_7O_{24}.4H_2O$) (CAS-12054-85-2), aluminium chloride ($AlCl_3$) (CAS-7446-70-0), ascorbic acid ($C_6H_8O_6$) (CAS-50-81-7), ammonium persulphate ($(NH_4)_2S_2O_8$) (CAS-7727-54-0), Mueller-hinton agar powder, Folin-Ciocalteu phenol reagent, sulphuric acid (H_2SO_4) (CAS-7664-93-9), nutrient agar powder, gallic acid ($C_7H_6O_5$) (CAS-149-91-7), quercetin ($C_{15}H_{10}O_7.2H_2O$) (CAS-6151-25-3), methanol (CH_3OH) (CAS-67-56-1), saline, sodium sulphate

(Na2SO4) (CAS- 7757-82-6), McFarland solution and sodium carbonate (Na2CO3) (CAS-497-19-8).,

2.1.4 Glassware

Beakers (50 mL, 100 mL, 250mL and 5000 mL), conical flasks (250 mL and 500 mL), cuvettes, falcon tubes (15 mL and 50 mL), filter funnels, filter papers, spatulas, test tubes, volumetric flasks (150 mL) and watch glasses.

2.2 Methods

Short and long Control of Substances Hazardous to Health (COSHH) risk assessment forms were filled out prior to laboratory work and all the chemicals and reagents were evaluated for their risks and hazards (Refer Appendix). As such, necessary personal protective equipment and good laboratory practice were obliged upon to withhold the precautions and safety procedures throughout the entire course of work.

2.2.1 Preparation of rice types

100g of the ten different rice were collected and labelled accordingly (Table 1). Each sample was divided into two parts in order to have both raw and cooked samples. 50g of rice was cooked with a volume ratio of 1:2.5 with water for 15 minutes and dried in a hot air oven at 500C. The dried sample was then powdered. The remaining 50g were powdered to get the raw samples.

Table 1 Labelling of different rice types

No	Sample Name	Common Label	RAW/ COOKED
1	Suwandal rice (Sri Lankan)	SS	RAW - SSR_1 COOKED – SSC_1
2	Heenati rice (Sri Lankan)	HS	RAW - HSR_2 COOKED - HSC_2
3	Rathu Nadhu rice (Sri Lankan)	RS	RAW - RSR_3 COOKED - RSC_3
4	Pachchaperumal rice (Sri Lankan)	PS	RAW- PSR_4 COOKED - PSC_4
5	Kurulu Thudha rice (Sri Lankan)	KS	RAW - KSR_5 COOKED - KSC_5
6	Jasmine Thai Basmathi rice (Imported)	JI	RAW - JIR_6

			COOKED - JIC_6
7	Ponni Indian rice (Imported)	PI	RAW - PIR_7 COOKED - PIC_7
8	Sunrise Pakistani rice (Imported)	SI	RAW - SIR_8 COOKED - SIC_8
9	Fortune Indian long grain rice (Imported)	FI	RAW - FIR_9 COOKED - FIC_9
10	Premium Pakistani Basmathi rice (Imported)	PPI	RAW - $PPIR_{10}$ COOKED - $PPIC_{10}$

2.2.2 Extraction of rice

From the grounded powder 1g of each sample was homogenized with 20 mL of 80% methanol. Samples were the agitated for 1 hour at room temperature on a roller mixer. Next the samples were centrifuged for 10 minutes at 3000rpm and the supernatant was collected. The residue was then again dissolved in 20 mL of 80% methanol and the above procedure was repeated twice. The three supernatants were mixed and collected in falcon tubes as the extract (Walter *et al.*, 2013).

2.2.3 Assessment of Total Phenolic Content (TPC)

To assess the total phenols 80 µL sample of was mixed with 200 µL of 0.25N Folin-Ciocalteu phenol reagent and 2 mL of distilled water. The mixture was incubated at room temperature for 3 minutes. 1 mL of Sodium carbonate (7.5g/100mL) was added. The mixture was then incubated at room temperature for 2 hours. Absorbance was measured at 765nm. Concentrations of TPC were calculated using the gallic acid standard curve, and the results were given as a gallic acid equivalent per 100g grain (Walter *et al.*, 2013)..

2.2.4 Assessment of Total Flavonoid Content (TFC)

To assess the total flavonoids 0.5 mL of extract was mixed with 1.5 mL 75% ethanol, 0.1 mL of 10% aluminium chloride, 0.1 mL of 0.1M Potassium acetate and 2.8mL of distilled water. Absorbance was measured at 415nm. The concentration was calculated for the equivalence of quercetin.

2.2.5 Assessment of Ferric Reducing Antioxidant Power (FRAP)

The FRAP reagent was prepared using 25 mL of 300 mM acetate buffer (pH 3.6), 2.5 ml of 30mM ferric chloride and 2.5 ml of 10 mM TPTZ solution (which was dissolved in 40 mM HCl). 1.5 mL of FRAP reagent was mixed with 1 mL of distilled and 100 µl of extracted sample. The sample mixture was incubated at room temperature for 4 minutes and absorbance was measured at 593 nm. Using an ascorbic acid

standard curve the concentrations were calculated (Benzie & Strain, 1996).

2.2.6 Assessment of Total Antioxidant Capacity (TAC)

0.6 M sulfuric acid, 4 mM ammonium molybdate and 28 mM sodium sulfate was prepared and mixed in the ratio of 1:1:1. 1ml of this TAC reagent was added to 3ml of the sample. The mixture was then incubated at 90 ^{0}C for 90 minutes in a hot air oven. The spectrophotometer was blanked using 3ml of methanol and the absorbance was measured at 695nm. According to the ascorbic acid standard curve the concentration values were calculated. (Ahmed, Khan & Saeed, 2015).

2.2.7 Assessment of ABTS radical scavenging activity

The ABTS stock solution was prepared using 5 ml of 0.4M ammonium persulfate and 5 ml of 2 M ABTS solution. This solution was then kept in a dark place for 16 hours to incubate and after the incubation period 3 mL of the solution was diluted with 100 mL of absolute methanol. Following the dilution initial concentration of the diluted working solution was noted down. 2850 µl of ABTS working solution was mixed with 150 µL of sample. The absorbance were measured at 734nm.

3.0 RESULTS

3.1 Total Antioxidant Capacity (TAC)

2.2.8 Antimicrobial activity of rice extracts (Well diffusion technique)

Bacterial subcultures were prepared on nutrient agar plates and using them, microbes (E.coli and Staphylococcus) were inoculated on Muller-Hinton agar plates (Appendix). The plates were labeled accordingly, divided into 4 equal parts and using pipette tips three wells were punched. 25 µL of sample were added to opposite wells while and equal amount of 1% DMSO was added as the negative control. To the remaining quadrant a Gentamicin commercially available antibiotic disk was placed as the positive control. In an incubator at 37^{0}C the plates were incubated overnight and the inhibition zones were measured.

2.2.9 Statistical Analysis

Mean absorbances were calculated from the triplicates of sample that were taken in each experiment. Single factor ANOVA (Analysis of Variance) and Pearson's correlation analysis in IBM SPSS statistics 21 was used, where for ANOVA the 95% interval or a significance of less than 0.05 was considered and in correlation a P value greater than 0.01 was considered.

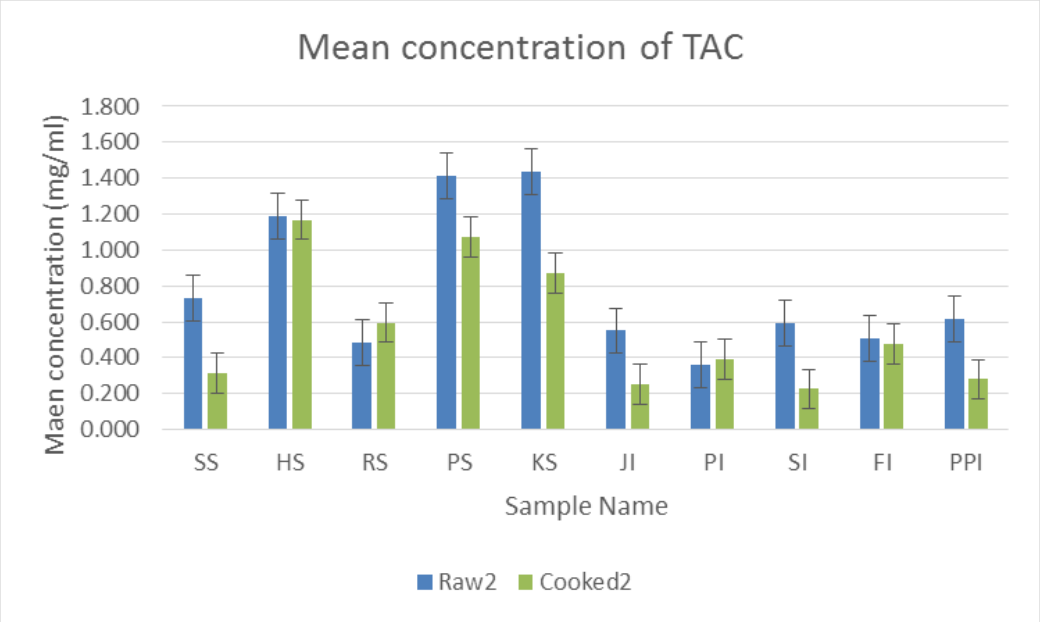


Figure 3 Mean concentration values of Total antioxidant capacity, expressed as ascorbic acid equivalents (AAE)

Table 2 Anova: Single Factor for Total antioxidant capacities of raw Sri Lankan rice (Rs) vs raw imported rice (Ri)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
TAC (Rs)	5	175172.7	35034.55	2.02E+08
TAC (Ri)	5	87445.45	17489.09	11014645

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	7.7E+08	1	7.7E+08	7.227852	0.027562	5.317655
Within Groups	8.52E+08	8	1.06E+08			

Total	1.62E+09	9

Table 3 Anova: Single Factor for Total antioxidant capacities of cooked Sri Lankan rice (Cs) vs cooked imported rice (Ci)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
TAC (Cs)	5	134009.1	26801.82	1.37E+08
TAC (Ci)	5	65372.73	13074.55	18677587

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	4.71E+08	1	4.71E+08	6.051807	0.039316	5.317655
Within Groups	6.23E+08	8	77843702			
Total	1.09E+09	9				

3.2 Total Phenolic Content (TPC)

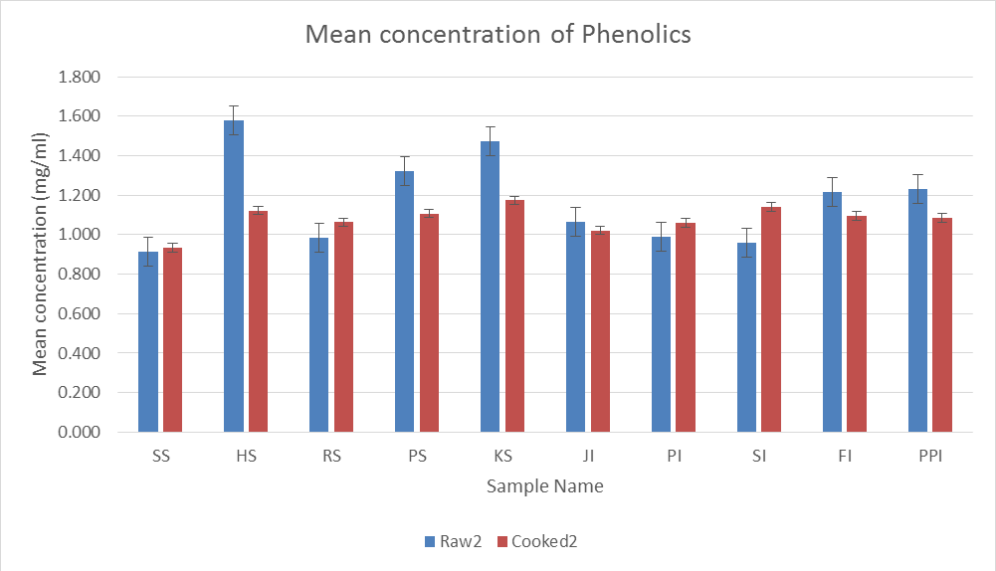


Figure 4 Mean concentrations of TPC, expressed as gallic acid equivalents (GAE)

Table 4 Anova: Single Factor for Total Phenolic Contest of raw Sri Lankan rice (Rs) vs raw imported rice (Ri)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
TPC (Rs)	5	83371.43	16674.29	96482286
TPC (Ri)	5	56228.57	11245.71	17602286

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	73673469.4	1	73673469	1.291559	0.288656	5.317655
Within Groups	456338286	8	57042286			

Total	530011755	9

Table 5 Anova: Single Factor for Total Phenolic Contest of cooked Sri Lankan rice (Cs) vs cooked imported rice (Ci)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
TPC (Cs)	5	54114.29	10822.86	9152000
TPC (Ci)	5	44914.29	8982.857	7319837

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	8464000	1	8464000	1.027694	0.340376	5.317655
Within Groups	65887347	8	8235918			
Total	74351347	9				

3.3 Total Flavonoid Content

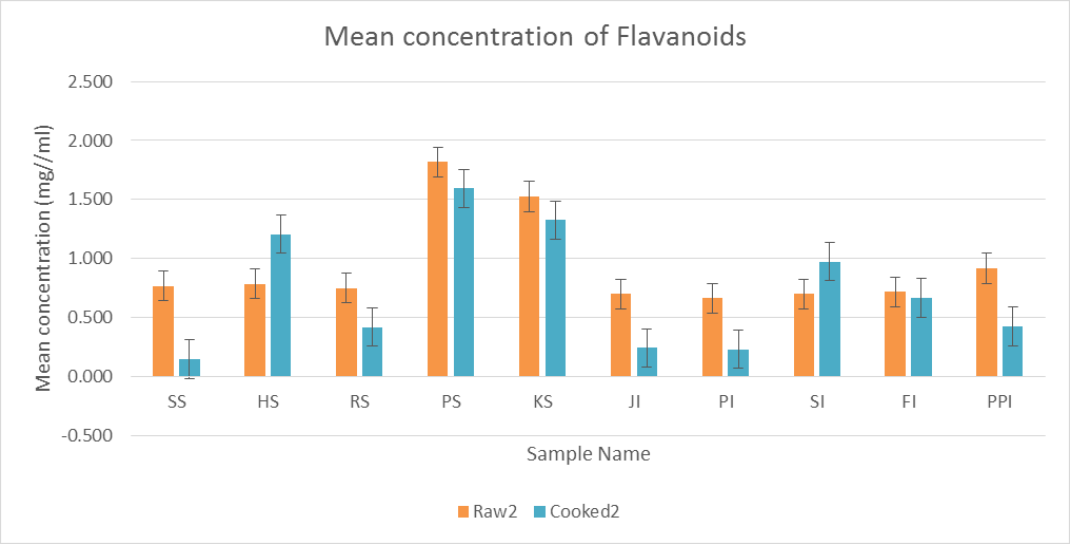


Figure 5 Mean concentrations of Total Flavonoid Content, expressed as quercetin equivalents (QE)

Table 6 Anova: Single Factor for Total Flavonoid Contest of raw Sri Lankan rice (Rs) vs raw imported rice (Ri)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
TFC (Rs)	5	80208.33	16041.67	2.84E+08
TFC (Ri)	5	15208.33	3041.667	11558160

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	4.23E+08	1	4.23E+08	2.858435	0.129362	5.317655
Within Groups	1.18E+09	8	1.48E+08			

Total	1.6E+09	9

Table 7 Anova: Single Factor for Total Flavonoid Contest of cooked Sri Lankan rice (Cs) vs cooked imported rice (Ci)

SUMMARY

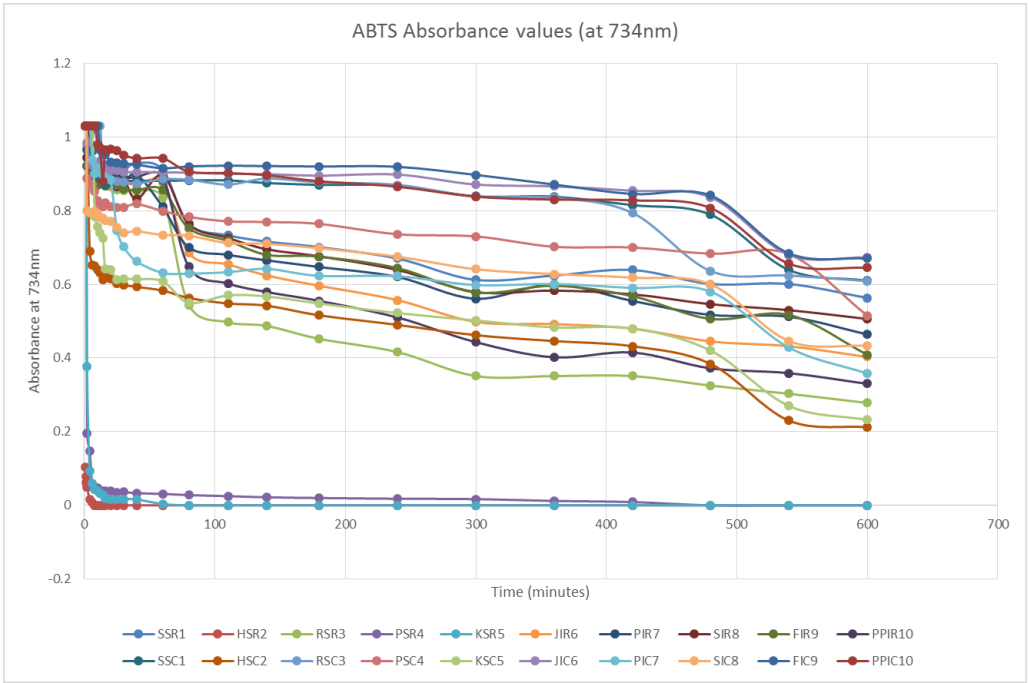
Groups	*Count*	*Sum*	*Average*	*Variance*
3	5	55625	11125	2.21E+08
4	5	14375	2875	20282118

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.7E+08	1	1.7E+08	1.411994	0.268802	5.317655
Within Groups	9.64E+08	8	1.21E+08			
Total	1.13E+09	9				

3.4 ABTS assay

Figure 5 ABTS absorbance value graph



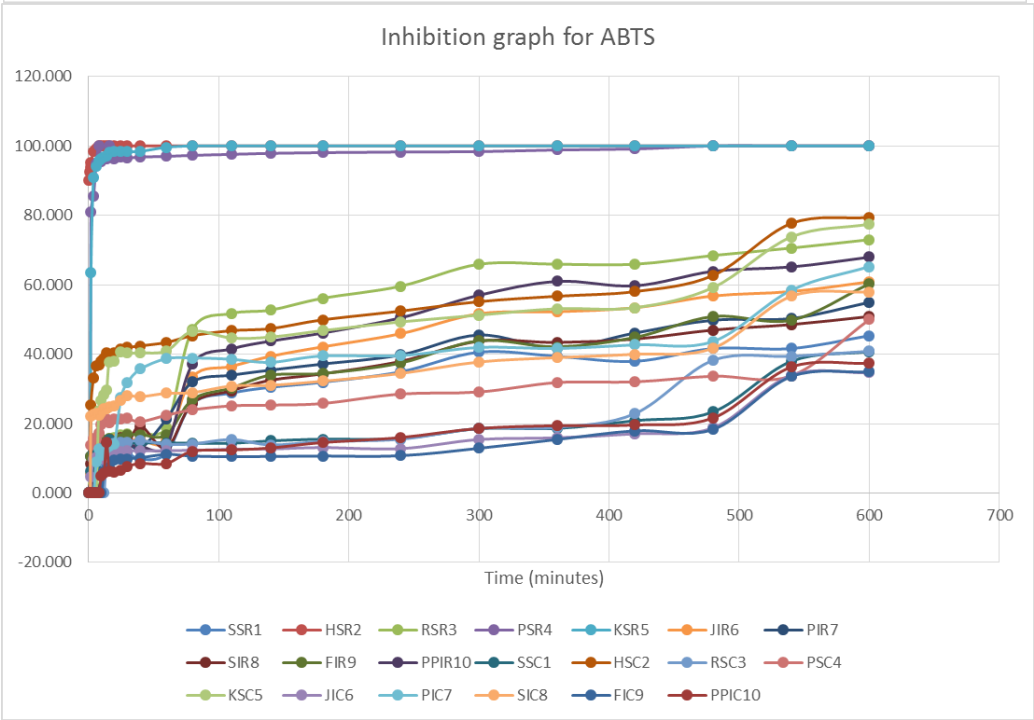


Figure 7 ABTS inhibition activity graph

3.5 FRAP assay

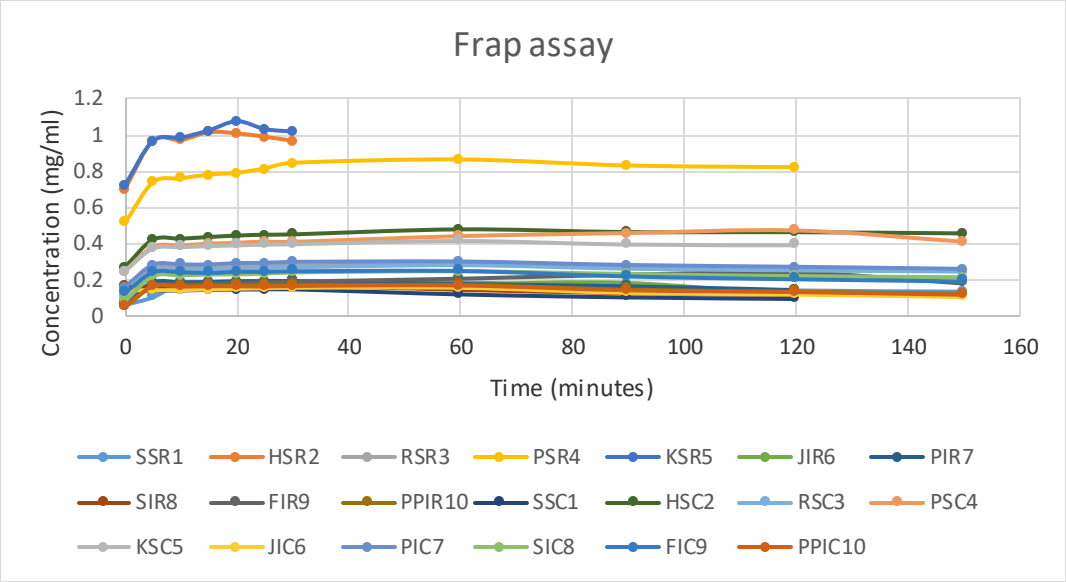


Figure 8 FRAP assay

3.6 Antimicrobial assay

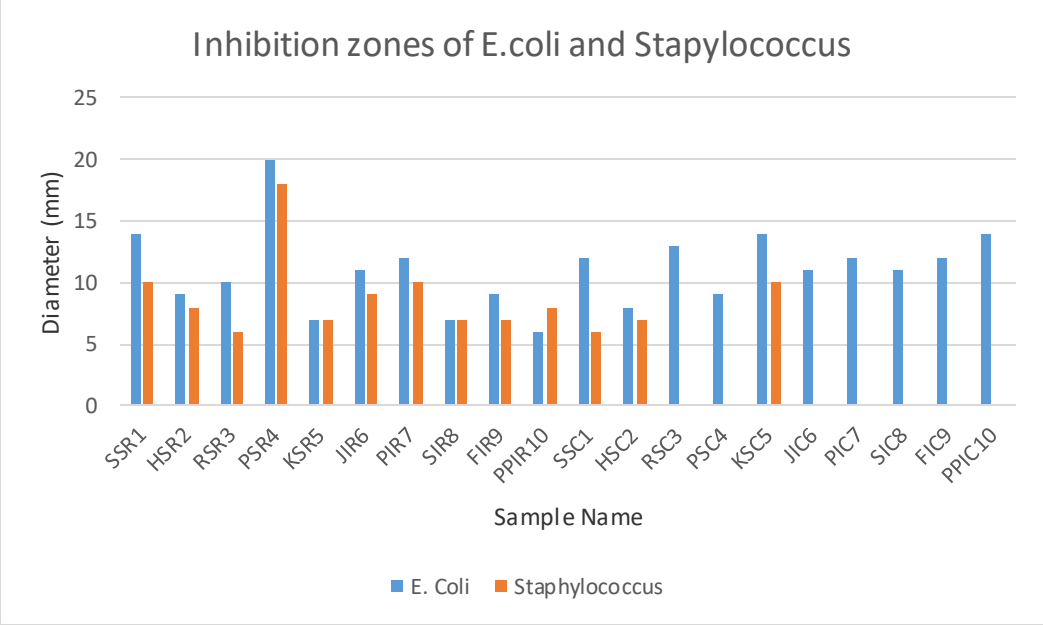


Figure 9 Inhibition zones seen in Muller-Hinton agar plates

Table 8 Anova : Single factor for Staphylococcus

SUMMAR
Y

Groups	*Count*	*Sum*	*Average*	*Variance*

Sample name	20	210	10.5	35
Diameter (mm)	20	113	5.65	24.3447368 4

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	235.225	1	235.225	7.92740898 4	0.00767632 5	4.09817 2
Within Groups	1127.55	38	29.6723684 2			
Total	1362.775	39				

Table 9 Anova : Single Factor for Staphylococcus inhibition zones (Cooked Sri Lanka vs Cooked imported)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Cooked Srilankan	5	23	4.6	19.8
Cooked imported	5	0	0	0

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*

Between Groups	52.9	1	52.9	5.343434	0.049566	5.317655
Within Groups	79.2	8	9.9			
Total	132.1	9				

3.7 Correlation graphs

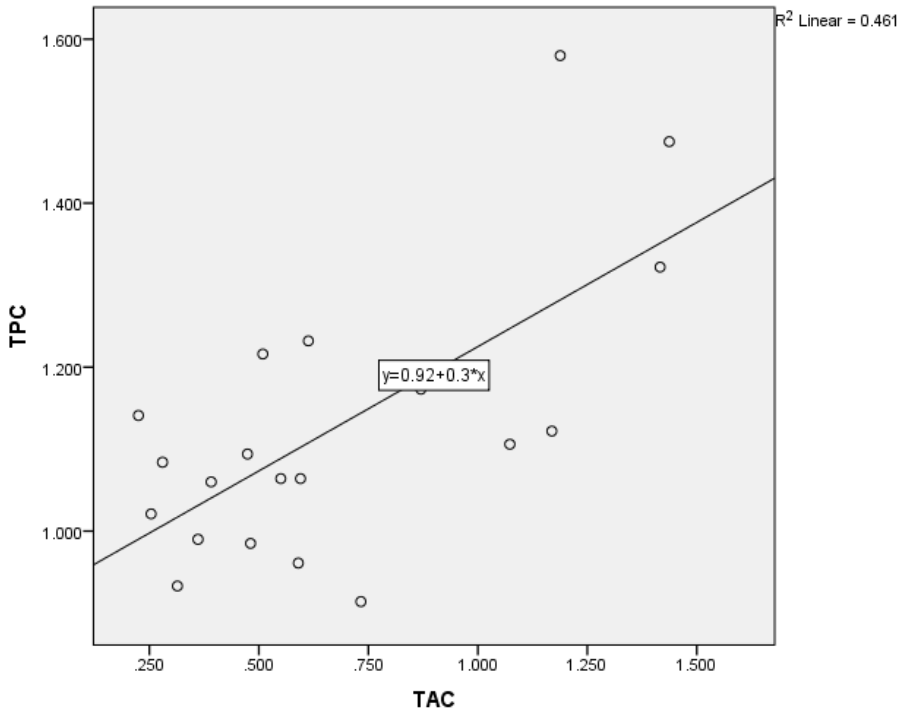


Figure 10 Correlation between TAC and TPC

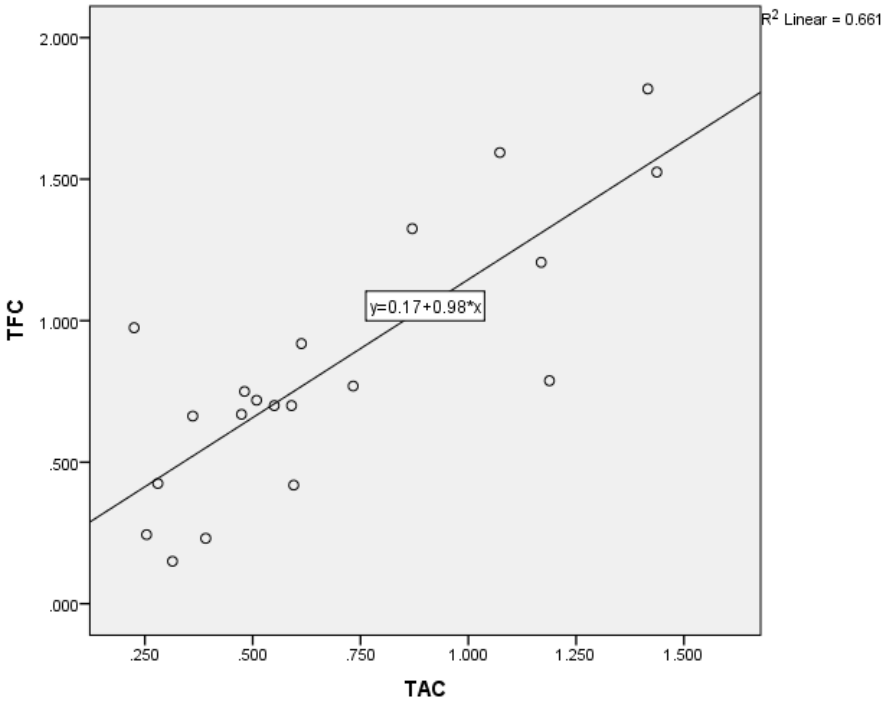


Figure 11 Correlation between TAC and TFC

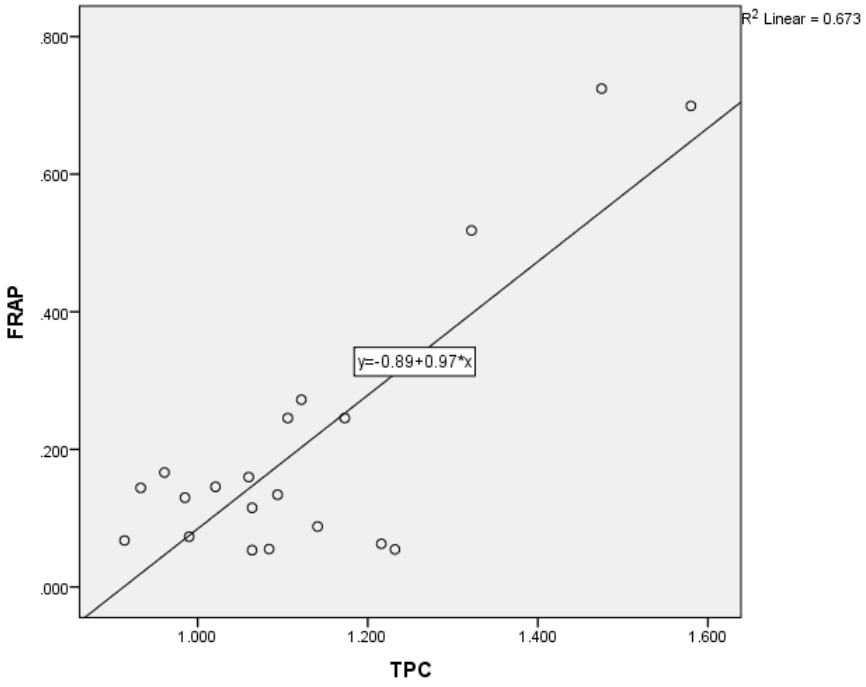


Figure 12 Correlation between TPC and FRAP

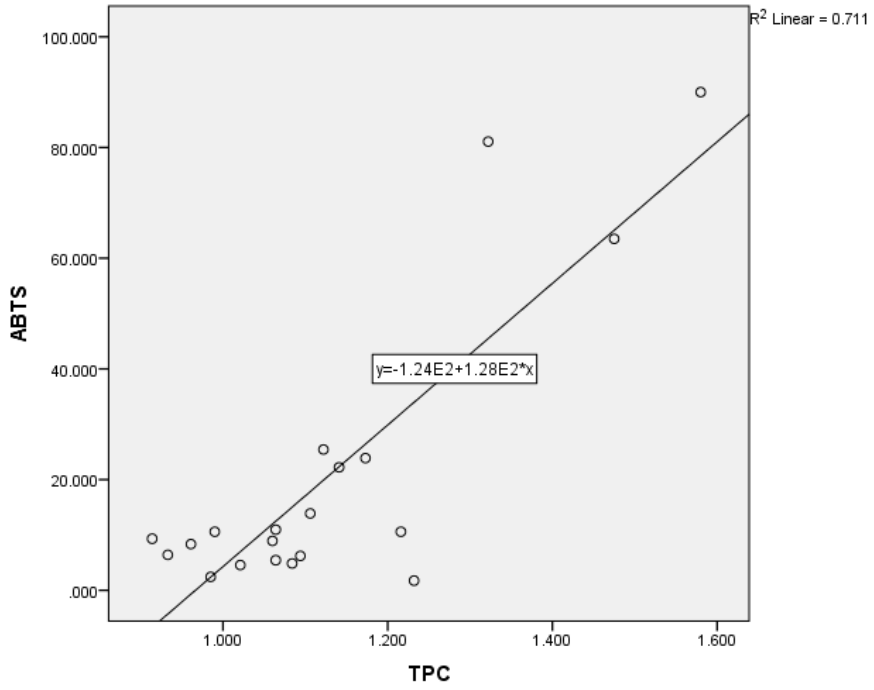


Figure 13 Correlation between TPC and ABTS

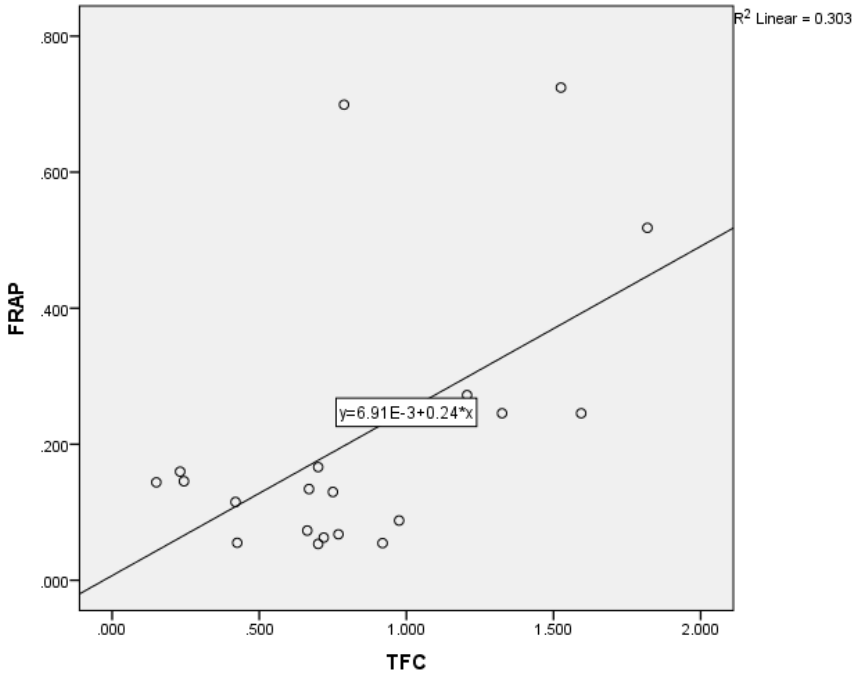


Figure 14 Correlation between TFC and FRAP

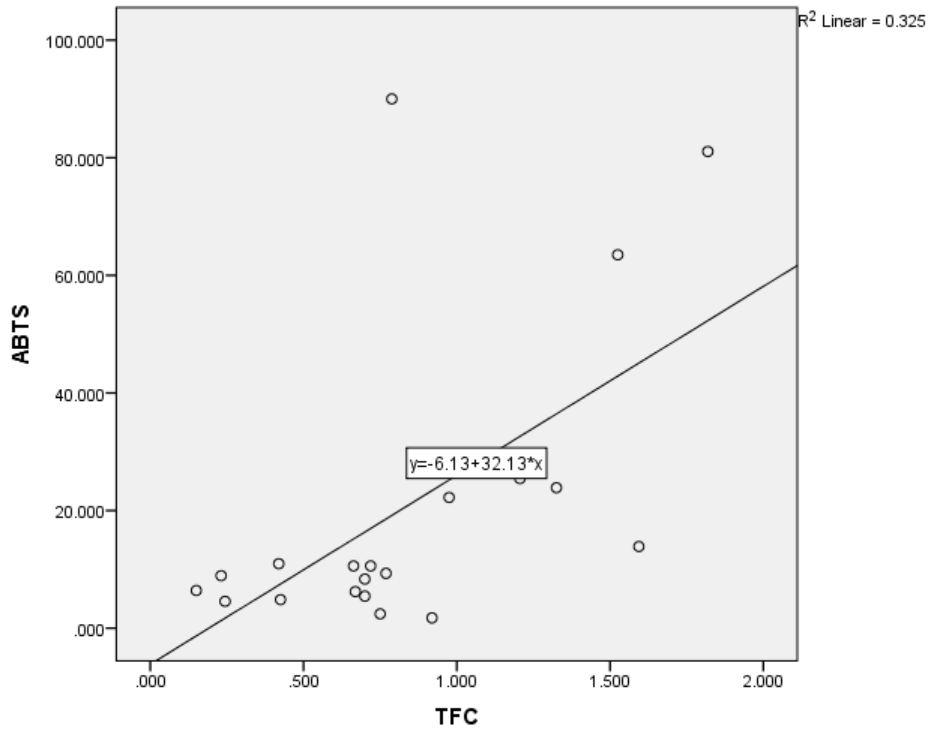


Figure 15 Correlation between TFC and ABTS

Table 10 Pearson's Correlation data

	TAC	TPC	TFC	ABTS	FRAP
TAC		0.679	0.813	0.783	0.829
TPC			0.517	0.843	0.820
TFC				0.57	0.55
ABTS					0.925
FRAP					

4.0 DISCUSSION AND CONCLUSION

Rice is the staple food in Sri Lanka and nearly over 50% of the world population's staple food as well. However in Asia this value ranges up to 90% in rice consumption and production. Therefore most of the calorie in-take these parts of the world is through this staple food, rice. Importance is as such research conducted on this important food is not that visible in Sri Lanka. On a recent study by Gunaratne *et*

al., (2011) it was shown that the traditional rice had 68-86% higher TPC content than Sri Lanka's new varieties. Furthermore 86 to 90% higher TAC activity was also observed. This research therefore indicate that traditional rice in Sri Lanka has good antioxidant properties therefore in this study the comparison between imported rice and Sri Lankan rise was conducted.

In the Total antioxidant capacity assay (TAC), a green coloured complex was formed due to the rice extracts ability to reduce Mo (VI) to Mo (V). This coloured compound was measured at 695nm. When considering the Sri Lankan rice and imported rice, Sri Lankan rice has the highest TAC activity where both raw and cooked samples gave significant differences against the imported samples (Table 2 & 3) and among the group of Sri Lankan rice, KS>PS>HS>SS>RS (1.437>1.416>1.188>0.733>0.481 mg/mL) for the raw rice while HS>PS>KS>RS>SS (1.169>1.073>0.870>0.595>0.314 mg/mL) was for cooked rice. Non overlapping error bars of Sri Lankan rice with imported rice in the concentration graph shows that is a significant difference among these groups while this fact is further confirmed by the ANOVA P values (raw rice - 0.027562 and cooked rice - 0.03931) at a significance of 0.05 and F values being greater than F crit values in both occasions (F raw - 7.227852 > F raw crit - 5.317655 and F cooked - 6.051807 > F cooked crit - 5.317655). Furthermore TAC showed positive correlations with both TPC and TFC while TFC showed a higher correlation. (R^2 TFC – 0.661> R^2 TPC – 0.461). The cooking effect on any Sri Lankan or imported does not show a significant difference when considering the TAC assay, however in all 10 samples cooked or raw a decrease in concentration is visible indicating that the cooking effect may have reduced the total antioxidant capacity. These values are consisted with the above mentioned study by Gunaratne *et al.*, (2011) because though that study compared with Sri Lankan new varieties the traditional rice proportion was more or less the same and they had a high number (90%) significant improvement in TAC values. Therefore the significant difference observed in this study can be justified as well. In Sompong *et al.*, (2011) showed that when Chinese, Thailand and Sri Lankan rice were compared, Sri Lankan rice type had poor antioxidant capacities. Therefore this study is not consistent with the results but in that study, not traditional rice were chosen therefore it is hard to compare the results with this study.

For the quantification of phenolic compounds literature has often referred to the Folin-Ciocalteu reagent method where with phenolic concentration (Not only phenolic but all reducing substances) is proportional to the blue colour intensity of the complex. According to the data obtained the phenolic content of Sri Lankan and imported does show similarities in values however, HS, KS and PS (HS being the highest - 1.580 mg/mL) in the Sri Lankan raw rice category show the highest phenolic content. When ANOVA was conducted to for both Sri Lankan and imported cooked and raw rice, the P values (raw - 0.288656 and cooked -0.340376) were not within the significance range of 0.05 (Table 4 & 5). F crit values were higher than F values further confirming the lack of a significant difference (F raw - 1.291559 < F raw crit - 5.317655 and F cooked -1.027694 < 5.317655). Just as in TAC values a decrease in the concentrations of phenolic content is also

present after cooking however a significant difference was not present.

Aluminium chloride colorimetric method was used to estimate the Total Flavonoid Content (TFC). Aluminium chloride has the ability to form stable complexed with C-5 or C-3 hydroxyl group of flavonols or flavones. The formed yellow coloured compound can be measured at 415nm. Out of the ten samples PS and KS show high flavonoid content (1.819 and 1.525 mg/mL) However just as in TPC, no significant difference was observed among the samples. PS and KS do not have overlapping error bars however as a whole the P values were high than the desired level (raw - 0.129362 and cooked - 0.268802) (Table 6 & 7). Consistent with all three results TFC too showed reduction in number of concentration after cooking. Relatively high correlation to TAC is seen with Flavonoids than TPC and therefore it can said that TFC compounds may play higher role in antioxidant action than TPC (Figure 11).

ABTS assay can be defined as a screening method for antioxidant activity in hydrophilic or lipophilic antioxidants which includes carotenoids, flavonoids, plasma antioxidants and hydroxycinnamates. 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) which is pre-formed by the reaction between potassium persulphate and ABTS. This radical is then reduced when exposed to hydrogen donating antioxidants. Duration for the radical cation to get inhibited and the antioxidant concentration are both taken into consideration when interpreting the antioxidant activity. The highest inhibition was present in the samples HSR_2>KSR_5>PSR_4 (Inhibition completes at 100% in 10mins, 80 mins and 480mins respectively). All these samples come under the category Sri Lanka raw samples. HSR_2, should be further investigated due to its high antioxidant capabilities being able reduce the ABTS radicals in 10 mins.

Redox linked colorimetric method is used in Ferric Reducing Antioxidant Power (FRAP) assay. The intense blue colour of the complex form of ferrous ferric tripyridyl triazine (Fe III TPTZ) is reduced by the antioxidants present and this activity is measured against time (Bordbar *et al.*, 2013). Several studies indicate the correlation between FRAP assay against TPC, TFC, TAC and ABTS (Palombini *et al.*, 2013). The highest reducing activity was achieved at 15 mins (0.18381 mg/mL) for HSR_2 and that result is further certified with the result of ABTS obtained. Therefore high antioxidant activity can be observed in this Sri Lankan rice, HSR_2. Furthermore KSR_5 activity was obtained at 20mins. In both FRAP and ABTS the cooked samples showed slow activity in reducing the agents present in both of these reagents.

When considering correlation data among the 5 assays, it is evident that all have good positive correlations among each other. Out of both TPC and TFC, TFC has a higher correlation towards the activity of TAC. (TFC correlation – 0.813 > TPC correlation – 0.679 and R^2 TFC – 0.661> R^2 TPC – 0.461) (Table 10). However, out of the TPC and TFC, TPC shows a higher correlation towards ABTS and FRAP than TFC. TPC correlation values of 0.843, 0.820 for ABTS and FRAP are greater than TFC correlation values of 0.57, 0.55. Furthermore R^2 values are greater for TPC as well (R^2 for TPC against FRAP and ABTS – 0.673, 0.711 and R^2 values for

TFC against FRAP and ABTS – 0.303, 0.325). Therefore it can be concluded that the reducing power for the antioxidant activity is through TPC.

Antibacterial effects of rice was measured through an antimicrobial susceptibility testing method which is well diffusion. E. coli and staphylococcus were used as the test organisms. All samples showed inhibition for E coli while samples RSC_3, PSC_4, JIC_6, PIC_7, SIC_8, FIC_9 and $PPIC_{10}$ did not show any inhibition for staphylococcus. Mainly may be due to degradation of antimicrobial components and the strength of staphylococcus may be high than E. coli therefore higher concentrations of sample may be needed. However, all the raw samples showed inhibition for both microbes. Single factor ANOVA was conducted for the group staphylococcus and a significant different was observed in these groups. A P value of 0.007676 which is < 0.05 significant level with F value which is 7.9274 > than F crit 4.098172. This significance was further investigated with an ANOVA for the Sri Lanka cooked rice and imported cooked rice. This gave a clear idea on the reason for the earlier difference by giving a significance difference among the populations Sri Lankan cooked and imported cooked. P value of 0.049 ($P<0.05$) indicated that after cooking the antimicrobial qualities of the imported rice were lost yet the Sri Lankan retained them causing the inhibition. There are no studies to support this area therefore more studies need to be conducted to confirm these findings.

Considering all the above results in conclusion a significant difference was observed among TAC values in the raw proportion and cooked proportion of Sri Lanka vs imported rice comparison. However no significant differences were observed in TPC or TFC values. FRAP and ABTS showed that rice varieties like HSR_2, KSR_5 and PSR_4 had high reducing capabilities. Among these three kinds, Heeneti rice (HSR_2) showed great results in ABTS and FRAP both and therefore more research should be conducted to exploit the properties of this rice. Another important finding is that the significant difference which was observed in the staphylococcus antimicrobial population where a significant difference was observed. Furthermore it was found out that this difference was mainly caused by the high difference between cooked proportions of Sri Lankan and imported rice. After cooking imported rice had lost the ability to inhibit the microbe, staphylococcus. These findings clearly indicate that traditional Sri Lankan rice has high antioxidant and antimicrobial abilities over imported rice and it is important to make public aware of this factor and promote the consumption of more traditional rice in the mere future.

5.0 FURTHER WORK

Sri Lanka has around 300 different varieties of traditional rice up to date. Therefore the research can be further taken in order to test for many of these different varieties and find out whether the antioxidant and antimicrobial activities show significant differences to existing rice varieties.

The study was based on a methonalic extraction method. However different solvents can be used to determine the different extraction yields and thereby compare the extraction potentials of different solvents. As for as example for solvents acetone water, acetone, water accompanied with assays such as 1,1-Diphenyl-2-picryhydrazyl (DPPH) in many

research articles. Therefore with addition of another assay comparison between assays is made more efficient and justification of results becomes much easier.

Investigations can be further expanded in order to even check for antioxidant properties like, β-carotene (Vitamin C) levels and tannin levels or even different nutrient levels like nitrogen or phosphate levels to give a proper nutritional profile on the desired plant.

Primitive screening methods were used in the study for the identification of antioxidant and antimicrobial identification. However, methods like high performance liquid chromatography (HPLC) can be used to isolate components which cause the action that we require. Therefore more complex research can be carried out to give conclusive reasoning to the data obtained by assays.

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