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Total Phenol Content and Antioxidant Potential of Traditional Breakfast Meals of Sri Lanka

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ABSTRACT

Introduction: According to folklore and Ayurveda, many tubers and flour made from various plant parts, traditionally used to made breakfast meals, are rich in nutrients and polyphenols. The objective of the study was to determine the total phenolics content (TPC) and antioxidant potential of some tubers and foods made with seeds and other plant parts. **Methods:** The TPC in the extracts was determined according to the Folin-Ciocalteu method (mg Gallic acid equivalents (GAE/100g FW) and antioxidant activity by 2, 2'–Azino–bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS)cation free radical decolouration assay (μmol/g Trolox equivalent antioxidant capacity; TEAC). **Results:** The TPC of raw and processed tubers ranged from 127-517 mg GAE/100g FW. Among the tuber varieties, *Dioscorea alata,* violet had the highest antioxidant potential. Among raw flour, *Vateriaco pallifera* had the highest phenolic content (1162) and lowest (79) was in *Caryota urens*. Antioxidant potential of raw and food prepared from indigenous flour ranged from 3-225μmol/g TEAC with *Vateriaco pallifera* raw flour having the highest antioxidant potential (225μmol/g). Processing decreased both the phenolics and antioxidant potential significantly ($P<0.05$). A significant correlation was observed between polyphenolic content and antioxidant capacity in raw flour ($r^2=0.993$). **Conclusion:** The traditional tubers and food prepared incorporating indigenous flour varieties are rich sources of phenols and antioxidants indicating their potential for utilisation in home food preparation and by the food industry.

Keywords: ABTS assay, antioxidants, indigenous flour, total phenolics content (TPC), tubers

INTRODUCTION

Elevation of free radicals in the biological tissues is closely associated with very common pathophysiological conditions such as inflammation, cancers, autoimmune diseases, neurodegenerative, cardiovascular and digestive system disorders and premature ageing (Halliwell, 2002). Long term oxidative stress may produce various

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complications such as risk of cardiovascular disease, diabetes and other metabolic diseases (Botero *et al*., 2009). The prevalence of obesity and diabetes among Sri Lankan men and women is 20.3%, 36.5% and 14.2% and 13.5% respectively (Wijewardene *et al.,* 2005). One in five adults in Sri Lanka is either pre-diabetic or diabetic (Katulanda *et al.,* 2008).

Antioxidants which are ingested with food play an important and supportive role in preventing damage to proteins, DNA and lipids (Halliwell, 2009) thus reducing cellular oxidative stress. Plant polyphenolics and phytochemicals are not only important as antioxidants but are also important as preventive agents of inflammation, have neuroprotective effects and regulatory effects on energy metabolism (Stevenson & Herst, 2007). Polyphenols also inhibit digestive enzymes such as α–amylases (Sharma & Pattabiraman, 1981). The naturally occurring antioxidants are also found to have potent iron chelating, neuroprotective and anti-inflammatory properties (Marzena & Mateusz, 2012). Common plant anti-oxidants are polyphenols, carotenoids, vitamin C and vitamin E (Weisburger, 1999).

More than five hundred polyphenols have been discovered so far and among the major classes are flavanols, flavones, flavan-3-ols, flavanones and anthocyanins (Manach *et al.*, 2004). Flavanoids are a major class of polyphenols that produce health benefits. The main dietary sources of polyphenols are fruits and beverages (fruit juice, wine, tea, coffee, chocolate and beer). Vegetables, dry legumes and cereals also contribute to some extent. Polyphenolics content and antioxidant potential of cereals, vegetables and medicinal plants have been extensively studied. However, little is reported on total phenolic content of traditionally consumed tubers or foods prepared with flour from indigenous plants, seeds and their antioxidant potential is available. Some of the tubers and foods made with indigenous flour from various plant parts (seeds, stem) have shown potential in reducing the glycaemic response (unpublished data). Thus the main objectives of this study were to determine the total phenolics content and antioxidant potential of raw flour and foods prepared from indigenous flour varieties and raw and boiled tubers consumed as breakfast foods and to observe the effect of processing on polyphenolics and antioxidant potential with an aim of increasing its utilisation. Raw *Caryota urens* (Sinhala; *kithul*) stem flour, *Cycas circinalis* seed flour, *Vateria copallifera* seed flour (*hal*), foods made using these flour *(Caryotaurens 'roti', Cycas circinalis 'roti', Cycas circinalis 'pittu' and Vateria copallifera 'pittu'*) and the raw and boiled tubers (*Canna indica* (*buthsarana*), *Maranta arudinacea* (*hulankeeriya*) *and Dioscorea alata* (*raja ala* violet and white) were the materials used.

METHODS

Plant materials

All the plant materials used in this study were authenticated at the National Herbarium, Royal Botanical Garden Peradeniya, Sri Lanka. *Caryota urens* flour was collected from Kurunegala district and *Cycas circinalis* seeds were collected from Matale, and *Vateria copallifera* fruits were collected from Kalutara district in Sri Lanka for analysis. Mature tubers were collected in bulk (~5kg) samples. *Dioscorea alata* (violet) *Dioscorea alata* (white), *Canna indica*, *Maranta arudinacea* were collected from several districts in Sri Lanka.

Food preparation

Caryota urens 'roti 'and *Cycas circinalis 'roti'* was prepared with *Caryota urens* flour (74 g), coconut scraping (26 g), water (25 mL), salt (1 g) and *Cycas* flour (37 g), wheat flour (37 g), coconut scraping (26 g), salt (1 g) and water (25 mL) respectively. Dough (50 g) was

prepared and flattened (radius - 10 cm, thickness-0.8 cm) and cooked under a medium flame for 10-15 min turning sides.

Pittu and *Vateria copallifera 'pittu'* were prepared with *Cycas* flour (37 g), rice flour (37 g), coconut scraping (26 g), salt (1 g) and water (40 mL) and *Vateria copallifera* flour (37 g), rice flour (37 g), coconut scraping (26 g), salt (1 g) and water (40 mL) respectively. The ingredients were hand mixed until flaky and packed into a bamboo column and steam cooked over a boiling water pan for 10-15 min.

Properly cleaned tubers were cut either into pieces (~25 g) or whole tubers were immersed in water (3:7 w / v) and boiled for 20-40 min until soft. In the case of *Canna indica* and two *Dioscorea alata* varieties, skin was removed before preparation. The whole tuber was immersed in water and cooked in *Maranta arudinacea* and *Canna indica* as done traditionally.

Reagents

2, 2'-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid), Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) and Potassium persulfate ($K_2S_2O_8$) were obtained from Sigma (Aldrich, Sweden). Folin-Ciocalteu's phenol reagent was purchased from BDH, England and gallic acid (3, 4, 5-trihydroxy benzoic acid) was obtained from Himedia Laboratories, India. All the chemicals aforementioned were of analytical grade.

Preparation of extracts

Freshly prepared food samples or raw tubers (*Dioscorea alata, Maranta arundinacea* and *Canna indica),* raw flour (*Caryota urens, Cycas circinalis* and *Vateria copallifera*) and *'roti'* and *'pittu'* made with these flours were dried in an oven at 40°C (REMI™ Laboratory oven) for 4-5 days and milled (IKA ® A11 basic) and sieved(20-50μm). The flour sample (0.5g) was dissolved in phosphate saline buffer (pH = 7.4) and mixed vigorously. The sample was then filtered using Whatman No.1 filter paper and used for analysis.

Folin-Ciocalteu assay for phenolics

TP content of prepared food and raw samples was quantified using Folin-Ciocalteu's method (Singleton *et al.,* 1999). Aliquots of flour extractions (1 mL) were added into 10 % (v / v) Folin-Ciocalteu (2 mL) and distilled water (10 mL). The mixture was incubated at room temperature for five min and Na_2CO_3 (2 mL) added, kept for 30 min and absorbance measured at 725 nm. Blanks were prepared by replacing the sample with water.

Qualitative assays for polyphenols

Conc. HCl (5 mL) was added along the wall of the test tube containing an extract as prepared above. A few magnesium chips were added to the mixture and allowed to stand for 1-2 h. The colour of the froth and the solution were observed. The colours were compared with the colours for varying polyphenolsas reported in literature (Fong, Tin & Farnsworth, 1967).

Spectroscopic studies

Food extracts prepared according to the above mentioned procedure were subjected to spectroscopic studies by scanning in the range 250-600 nm using a UV / VIS double beam spectrophotometer against a phosphate saline buffer (pH 7.4). The spectral data obtained were compared against standard polyphenolic spectral data by Harborne (1973). The effects of changing pH by adding HCl or NaOH were observed especially for extracts containing anthocyanins. Further the presence of anthocyanins was confirmed by the spectral analysis and observing hipsochromic (523 nm) and bathochromic shifts (625 nm) after addition of acid and base respectively.

Antioxidant assay 2, 2'–Azino–bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt cation free radical (ABTS)

In vitro antioxidant free radical scavenging activity was determined by ABTS cation free radical decolouration assay. All reagents were prepared using deionised water.The ABTS (7 mmol) was dissolved in ethanol and phosphate saline buffer (pH = 7.4) and the stock solution (2.6 mL) was mixed with potassium persulfate ($K_2S_2O_8$- 2.45 mmol) (11.5 mL) and kept in the dark at room temperature for 12-16 h to facilitate free radical formation. Thus generated ABTS free radical cation ($ABTS^{+\cdot}$) solution was dark green in colour. The above stock solution was diluted with deionised water until the absorbance reached 0.700 (±0.02) at 734 nm. The prepared food sample extract (15 μL) was added to ABTS ($^{+\cdot}$) solution with a phosphate saline buffer until the total volume reached 3 mL. The reduction in absorbance was recorded for 6 min (Re *et al.,* 1999). The calibration curve was drawn using Trolox as standard. Antioxidant activity was expressed as (μmol/g) trolox equivalent antioxidant capacity (TEAC).

RESULTS AND DISCUSSION

Phenolics/polyphenolics and antioxidant potential of raw and boiled tubers

Total phenolics content and antioxidant potential in raw and processed tubers are stated in Table 1. The phenolics/ polyphenolics content of raw and processed tubers ranged from 127-517 mg GAE/100g FW. The variation of 22% -175% in the polyphenolic content in *Dioscorea alata* collected from different areas could be due to the different colour intensities which could be attributed to anthocyanin concentration in different samples observed in the edible part of the samples.

Trolox equivalent antioxidant capacities in raw and boiled tubers ranged from 12-147μmol/g TEAC. Among the tuber varieties, *Dioscorea alata* violet showed the highest antioxidant potential. However, the three *Dioscorea alata* samples collected from different locations had significantly ($P<0.05$)different antioxidant potentials. Both the TPC and antioxidant potential decreased on cooking.

The peel of the tubers had to be removed for food preparation. Kahkonen *et al.* (1999)

Table 1.Varietal and regional variation of TPC and antioxidant potentials of boiled tubers and their raw flour

	[1]*Total phenolic (mg GAE/100g FW)*	[1]*Trolox equivalent antioxidant capacity (μmol/g)*
Canna indica (District A-raw)	482±100	73±5
Canna indica (District A-boiled)	125±4	22±3
Maranta arundinacea (District A-raw)	127±10	14±4
Maranta arundinacea (District A-boiled)	51±2	26±2
Dioscorea alata (District A-raw)	517±12	147±4
Dioscorea alata (District A -boiled)	208±12	142±9
Dioscorea alata (District B-raw)	305±10	81±7
Dioscorea alata (District B- boiled)	105±5	69±5
Dioscorea alata (District C-raw)	188±12	27±3
Dioscorea alata (District C- boiled)	33±4	27±3
Dioscorea alata (District B-raw)	229±21	12±3
Dioscorea alata (District B-boiled)	98±7	75±8

[1]n=6; GAE-Gallic acid equivalent; FW- fresh weight;

reported that carrot, beet root and potato peel contained considerable amounts of phenolics ranging from 4.3-6.3 mg GAE/g DW. Therefore if the tubers are processed with the peel, the total phenolic content could be higher. In case of *Dioscorea alata* (white) and *Maranta arundinacea* variety, the antioxidant capacity increased on cooking and could be due to thermal processing enhancing antioxidant activity(Veronica *et al.,* 2002).

Phenolic/polyphenolic and antioxidant potential in raw and foods prepared from indigenous flour

TPC in raw indigenous flour and food prepared incorporating indigenous flour ranged from 79-1162 mg GAE/100g fresh weight. Among the raw flour *Vateria copallifera* had the highest phenolic content and lowest was observed in the *Caryota urens* raw flour. Antioxidant potential of raw and food prepared from indigenous flour ranged from 3-225 μmol/g TEAC with *Vateria copallifera* raw flour having the highest antioxidant potential. All the foods were prepared by incorporating coconut and contribution from the coconut was 125 mg GAE/100g on fresh weight basis.

Caryota urens flour was prepared from the partially matured trunk of *Caryota urens*. The matrix of the trunk was crushed with mortar and pestle and washed with water several times. Starches in the matrix were separated after decanting the water. This starch was dried under sunlight for 4-6 days and used in food preparation. The low phenolic/polyphenolic content in *Caryota urens* flour could be due to the loss during preparation as washing was done at several stages. Even during preparation, the open pith surface turned brown, when exposed to air due to oxidative browning. This is an indication of the presence of high amounts of phenolics in *Caryota urens* pith. The decanted water which was light brown in colour indicated that some soluble polyphenolics leached out during processing. Consequently *Caryota urens* flour and *roti* made incorporating *Caryota urens* flour had the lowest antioxidant capacity. The nutritional quality of the flour could be preserved by modifying the method of flour preparation so as to preserve the polyphenols in the flour.

As indicated in Table 2, processing showed a decline in the both TPC and antioxidant potential compared to raw materials.*Vateria copallifera pittu* showed the highest reduction due to processing which corresponded to 70%. The reduction percentages in *Cycas circinalis roti* and *pittu* were 34% and 15 % respectively. However, antioxidant potentials in cooked foods were below 11 μmol/g TEAC except in *Vateria copallifera pittu*. Coconut flesh incorporated in food preparation had 58 μmol/g TEAC. A significant correlation was observed

Table 2. TPC and antioxidant potentials of indigenous raw flour/foods prepared with raw flour

	[1]*Total phenolic (mg GAE/100g FW)*	[1]*Trolox equivalent antioxidant capacity (μmol/g)*
Caryota urens raw flour	79±3	3±2
Caryota urens roti	145±13	5±1
Cycas circinalis raw flour	179±17	10±1
Cycas circinalis pittu (*madu* flour: rice flour-1:1)	117±6	11±4
Cycas circinalis roti (*madu* flour: wheat flour-1:1)	153±12	11±2
Vateria copallifera raw flour	1162±26	225±7
Vateria copallifera pittu (*hal* flour: rice flour-1:1)	347±14	47±2

[1]n=6; GAE-Gallic acid equivalent; FW- fresh weight;

between polyphenolics and antioxidant capacity in raw flour (r^2=0.993). The reported phenolics in rye (*Secale cereale*) flour and wheat (*Triticuma estivum*) grain were 0.5 and 0.2 mg GAE / g DW (Kahkonen *et al.*, 1999). However, the above reported values are lower than that of *Caryota urens* flour. This shows that all the above traditional flour varieties are better sources of phenolics. The highest percentage reduction in *Vateria copallifera 'pittu'* (70%) could be attributed to the degradation of phenolics due to increased temperature during cooking, exposure to oxygen and change of pH which could lead to degradation of the phenolics. However in *Caryota urens 'roti'* there was an increase in TPC compared to raw flour.

The antioxidant capacities of studied raw and prepared food samples ranged from 3.0- 225 μmol / g TEAC and 5.0-142 μmol / g TEAC respectively. The highest antioxidant capacity was in raw *Vateria copallifera* flour (225 μmol / g TEAC) and lowest in the raw *Caryota urens* flour (3 μmol / g TEAC). In cooked food, *Caryota urens 'roti'* had the lowest antioxidant capacity (5 μmol / g TEAC) whereas *Dioscorea alata* (violet-District A) had the highest (142 μmol / g TEAC) activity. As with TPC, on cooking the antioxidant capacity also decreased. However, such a reduction was not found in *Dioscorea alata* (violet) collected from Monaragala, Sri Lanka and could be attributed to a heat stable bioactive compound having antioxidant properties. Antioxidant capacities of the tubers were higher than that of potato (7.1 μmol / g TEAC) (Cook *et al.*, 1998).

The antioxidant capacities of some of the studied foods were higher than that of reported breakfast cereals (Miller *et al.,* 2000). Thus consumption of traditional breakfast foods is beneficial as antioxidants are reported to lower oxidative stress under a hyperglycaemic condition which in turn lowers the serum antioxidants (Rao & Agrawal, 1999; Ceriello *et al*., 1998). Most of the foods that are frequently consumed by Sri Lankans contribute a high glycaemic load but are sometimes low in antioxidants. In this context some of our traditional breakfast foods could be used to fulfill our antioxidant requirements. In the case of some foods in this study, the processing could be modified to increase the antioxidant potential.

Qualitative characterisation of polyphenolics

The colour obtained when the food items were subjected to ortho-cyanidine test which indicated the presence of anthocyanins, flavones, flavonols, isoflavanone and leucoanthocyanin respectively as shown in Table 3. According to available spectral data, the anthocyanin of *Dioscorea alata* violet appears to be a cyanidin derivative.The anthocyanin derivative is comparable with that reported in the literature (523 nm) (Imbert & Seaforth, 1968; Rasper & Coursey, 1967). The violet variety of *Dioscorea alata* tubers studied were found to be either light or deeply violet or faintly pink in colour depending on area of collection. The faint pink coloured tubers turned purple on

Table 3. Polyphenolics in foods (qualitative) as indicated by the O-cyanidine test

*Food items**	*Colour*	*Polyphenol group*
Dioscorea alata (violet)	reddish pink	Anthocyanin
Caryota urens roti	reddish pink	Flavones
Canna indica	reddish magenta	Flavanoles
Vateria pittu, *Cycas pittu*	yellow	Isoflavanone
Dioscorea alata (white)	pink	Leucoanthocyanin
Maranta arundinacea	white	ND

*n=6; ND- not detected.

boiling. This could be attributed to conversion of leucoanthocyanins to anthocyanins due to heat treatment and oxygen. In the white variety the whole tuber was white. Orthocyanidin test results and spectral data further confirmed the presence of leucoanthocyanins. However, in the case of the white variety, when the tuber was cut open, the surface became light brown. This may be due to the action of polyphenolglycosidases and oxidases on polyphenols (enzymatic browning). Further, on boiling, an increase in brown colour was observed. This could be due to a stable polyphenol oxidase or non-enzymatic browning.

It can be said that tubers and food prepared from indigenous flour varieties are rich sources of polyphenolics and antioxidants compared to breakfast cereals. However, in most cases processing decreased both phenolic and antioxidant capacities except in *Maranta arundinacea* and *Dioscorea alata* (white).The potential of some of these tubers and foods made with different flour types in decreasing the post-prandial glucose (unpublished data) and the high antioxidant potential compared to many commonly consumed foods indicate a high possibility for these to be utilised as food or as ingredients in food preparations.

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