Evaluation and Comparison of Vitamin Profiles of Selected Traditional Rice and Yams Grown in Sri Lanka

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ABSTRACT: *The current study was designed to evaluate and compare the vitamin composition of selected traditional and improved rice varieties and four Dioscorea spp. commonly grown and consumed in Sri Lanka. Samples were collected from different locations representing all agro-climatic zones of Sri Lanka. The rice samples were cleaned, air-dried, husked, while the yam samples were peeled, freeze-dried, disintegrated and pooled to attain composite samples. These samples were analyzed for vitamins A, D, E, K1, B1 and B2 using HPLC method. All the samples analyzed were rich in vitamins B1 and B2. Vitamin B2 content in yams were higher than the vitamin B2 content of rice varieties studied. The studied rice varieties contained fat-soluble vitamins E and K only. Vitamin E was found only in traditional rice verities, Kaluheenati, Madathavalu and Suduheenati. Kaluheenati contained all the vitamins and the highest vitamins E (6.34±0.01 mg/100g), B1 (1.58±0.06 mg/100g) and B2 (2.11±0.03 mg/100g) contents. Yams contained fat-soluble vitamins A and K only. Vitamin A was found only in D. pentaphylla (Katuala). Only D. alata (both Rajala (0.74±0.00 µg/100g) and Angili ala (0.68±0.01 µg/100g) contained vitamin K. Vitamin K content of rice varieties was higher than that of yams studied. The highest vitamin K content was found in Pokkali (7.55±0.02 µg/100g) and Madathavalu (1.83±0.06 µg/100g) contained the lowest vitamin K content. The lowest vitamin K content found in rice varieties was higher than the highest vitamin K content available in yams. Traditional and improved rice varieties studied are richer in vitamin K compared to yams.*

Keywords*: HPLC, Sri Lanka, traditional rice, vitamin profiles, yams*

INTRODUCTION

Rice and yams play a vital role as staple foods in number of countries in the world (Elbehri *et al*., 2013). According to Sarwar *et al*., (2013) cereals grown in large extents across the world provide more calories than any other type of crops. Therefore, they are considered as staple food crops in many countries including in Sri Lanka. In their natural form or as in whole grain, they are a rich source of vitamins, minerals, carbohydrates, lipids and protein.

Rice (*Oryza sativa*) is the most significant cereal grown in Sri Lanka. It has been the staple food and the backbone of agricultural sector of Sri Lanka since antiquity. The long history of

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rice cultivation, the wide range of eco-edaphic conditions and various farming systems present in the country have resulted in a wide varietal diversity of rice. Over 3000 rice varieties have been reported in Sri Lanka (Helvetas, 2001).

Yams are the edible tubers of numerous species of the genus *Dioscorea* and are vital staple food of many tropical countries. Yam is composed mainly of starch, some proteins, lipids, vitamins and minerals (Polycarp *et al*., 2012). *Dioscorea* spp. is a popular minor element in Sri Lankan home gardens having the potential of generating large quantities of edible food material with minimal inputs. *Dioscorea* species are grown in many home gardens, especially in the wet and intermediate zones of Sri Lanka (Sangakkara and Frossard, 2014).

Vitamins are vital for the wellbeing of human and animals to sustain the life. Human body essentially requires small quantities of vitamins for optimal functioning. Insufficient intake of vitamins may lead to many health problems such as blindness, poor reproductive health, risk of anemia, slow growth and may even lead to other health problems such as development of certain cancers, diabetes, obesity and cardio vascular diseases (FAO and WHO, 2001). This is more prominent among children and elderly. Therefore, getting a balanced diet is essential to ensure the human health and nutrition (Hassan, 2012). This study was designed to evaluate and compare the vitamin A, D, E, K1, B1 and B2 contents in selected traditional rice and yams varieties of Sri Lanka.

METHODOLOGY

Materials

This study used the following HPLC grade standards and chemicals (Sigma Aldrich, USA) in analysis: vitamins A (retinol acetate), D (ergocalciferol), E (α-tocopherol) and K1 (phylloquinone) , vitamins B1 (thiamine) and B2 (riboflavin), Methanol, acetonitrile, ascorbic acid, ethyl alcohol, Potassium hydroxide, sodium chloride, glacial acetic acid, hexane sulphonic acid sodium salt, potassium dihydrogen phosphate and triethylamine, orthophosphoric acid.

Seeds of six traditional red pericarp rice varieties, one traditional white pericarp rice variety, two improved white pericarp rice varieties and one improved red pericarp rice variety were collected for the analysis of vitamins. All the samples collected were harvested during *Maha* season of year 2015. Further, samples of four yam varieties were collected (Table 1).

Preparation of samples

A total of 500 g of healthy, undamaged edible parts (rice kernels and yams) of each sample were collected in polythene bags and transported to the laboratory immediately in temperature controlled containers. Air dried rice samples were husked and disintegrated. Yam samples were peeled, freeze dried (Labconco Lyph Lock 6, USA) and disintegrated. Collected samples were compared with germplasm samples provided by Plant Genetic Resource Center, Gannoruwa, Sri Lanka for authentication. After disintegration, equal amounts of samples received from each agro-climatic zone were pooled and homogenized to obtain the composite sample. The composite samples of ground rice samples were stored in suitable containers and stored under refrigerated at 5 ^{o}C. Yam samples were stored under frozen at -20 ^{o}C. The morphological details of yams and rice varieties are given in the Figure 1 and 2, respectively.

Table 1. Paddy and yam samples collected from different locations

Crop	Varieties	Pericarp Color	WL	WM	IL	IM	DL
Rice	*Suwandel*	White	✔	✔	✔	✔	✔
	Kaluheenati	Red	✔	✔	✔	✔	✔
	Kurulutuda	Red	✖	✔	✔	✔	✔
	Madathavalu	Red	✔	✔	✔	✔	✔
	Pachchaperumal	Red	✔	✔	✔	✔	✔
	Pokkali	Red	✔	✔	✔	✔	✔
	SuduHeenati	Red	✔	✔	✔	✔	✔
	Bg 360	White	✖	✖	✔	✖	✔
	Bg 352	White	✖	✖	✔	✖	✔
	AT 362	Red	✖	✖	✖	✖	✔
Local yams	*Katuala*		✔	✔	✔	✔	✔
	Agiliala		✔	✔	✔	✔	✔
	Rajala		✔	✔	✔	✔	✔
	kukulala		✔	✔	✔	✔	✔

Samples, ✔ : collected ✖ : not collected
WL- Wet Low country, WM- Wet Mid country, IL- Intermediate Low country, IM-Intermediate Mid country and DL- Dry Low country.

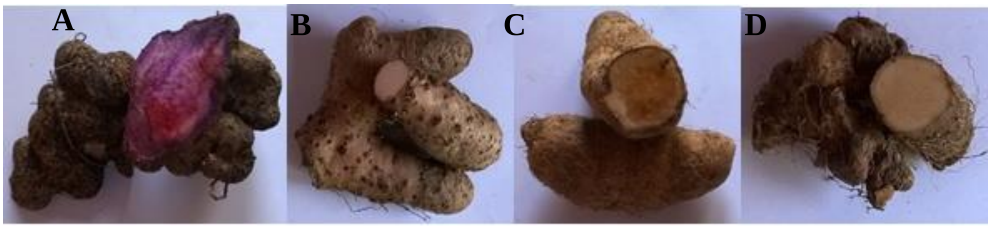

Figure 1. A- *Rajala* (*Dioscorea alata*), B-*Agiliala* (*Dioscorea alata*), C- *Kukulala* (*Dioscorea esculenta*), D- *Katuala* (*Dioscorea pentaphylla*)

Quantification of vitamins

Samples were analyzed for thaimine and riboflavin content by using HPLC method used by Aslam *et al*. (2008) and vitamin A, D, E and K_1 content by using HPLC method described by Aslam *et al*. (2012). All samples were analyzed in triplicate and if the coefficient of variance was over 15% the test/s were repeated.

Figure 2. A-*Suwandel*, B- *Kaluheenati*, C-*Kuruluthuda*, D-*Madathavalu*, E-*Pachchaperumal*, F-*Pokkali*, G-*Suduheenati*, H-BG 360, I-BG 352 and J-AT 362 varieties.

Analysis of vitamins A, D, E and K

The mobile phase was prepared by mixing methanol, acetonitrile and water at the ratio of 84:14:2 and filtering through 0.45 micron membrane using Millipore filtration unit. This was degassed using helium. A series of standards of vitamins A (retinol acetate), D (ergocalciferol), E (α-tocopherol) and K_1 (phylloquinone) was prepared in separate 10 mL volumetric flasks. The final volume (10 ml) was made up using methanol.

Five grams of each sample was weighed, transferred into 100 ml conical flask and the contents were mixed with 0.5 g ascorbic acid and 25 mL ethyl alcohol and shaken for 5 min. Next, 5 mL of 12 N KOH was added and heated under reflux for 30–40 min at 80 ºC, cooled down over 30 min. Then sample was separately extracted with 50 mL, 30 mL and 20 mL diethyl ether, respectively. For quick separation, 2–3 drops of sodium chloride were added. Final solution was concentrated to obtain 1 mL of sample. Resulted 1 mL of sample was dissolved in 5 mL HPLC grade methanol. Extracted samples were filtered through 0.45 µm syringe filters. HPLC analysis was performed using C18 column (4.6 × 250 mm 5 µm) with a linear gradient of methanol, acetonitrile and water (84:14:2) at a constant flow rate of 1 mL/min, 30 min of run time and injection volume of 20 µL. A UV detector was engaged for the detection of peaks, using two channels simultaneously at a wavelength of 295 nm, a bandwidth of 4 nm and another wavelength of 280 nm. All the analyses were performed in triplicates. Vitamins were quantified using regression equation of calibration curve of each standard.

Analysis of vitamins B1 and B2

The mobile phase was prepared by mixing 50 mL of acetonitrile and 10 mL of glacial acetic acid and volume was made up to 1000 mL using double distilled water. The final mixture was filtered through 0.45 micron membrane using Millipore filtration unit. Buffer was prepared by dissolving 1.08 g of hexane sulphonic acid sodium salt and 1.36 g of potassium dihydrogen phosphate in 940 mL of double distilled water and 5 mL of triethylamine was added to the solution. The final pH was adjusted to 3.0 with orthophosphoric acid. Buffer and methanol were mixed at a ratio of 96:4 and filtered through 0.45 µm membrane filter and degassed using helium. Standards of vitamins B1 (thiamine) and B2 (riboflavin) were prepared in separate 10 mL volumetric flasks. The final volume (10 ml) was made up using double distilled filtered water.

Ten grams of each sample was weighed and transferred into conical flasks and 25 ml of extraction solution was added, kept on a shaking water bath at 70 ºC for 40 min. Next, the sample was cooled down, filtered and finally the volume was made up to 50 mL with extraction solution. Extracted samples were filtered through 0.45 µm syringe filters. HPLC analysis was performed using a C18 column (4.6 x 250 mm 5 m) with a linear gradient of Buffer: methanol (96:4) at a constant flow rate of 1 ml/min, 25 minutes of run time and 60 µL of injection volume. A UV detector was engaged in the detection of peaks, using 3 channels simultaneously at a wavelength of 210 nm, 270 nm and 280 nm with a bandwidth of 5 nm. All the analyses were performed with 3 replicates. The vitamin yield was quantified using regression equation of calibration curve of each standard.

Determination of recovery and precision of estimation

The accuracy of the method was tested by a recovery study. Pre-analyzed samples were spiked with 50%, 100% and 150% excess of the standard vitamins and re-analyzed following the same method. The experiment was repeated 3 times, average recovered vitamins content was quantified using regression equation and the percent recovery was calculated accordingly. The precision of the used method was calculated by determining inter and intra-day variations in the three replicates of vitamins at two concentration levels (200–400 ng per spot) and the Percent Standard Relative Deviation (RSD) was calculated.

Statistical analysis

One-way analysis of variance was carried out on the experimental results. The significance of differences between means was compared by Tukey's Multiple Comparisons Procedure. All calculations were performed using SAS 9 statistical software**.**

RESULTS AND DISCUSSION

Vitamin composition of traditional rice varieties

Vitamin content of traditional rice varieties is shown in Table 2. Cereals are a good source of vitamins from the vitamin B group. In industrial countries, they provide nearly 50–60% of the daily requirement of B vitamins. The most predominant fat-soluble vitamin is tocopherol, which exists in concentrations above 20 mg/kg. Vitamins are concentrated in the outer layers of the grains, especially in the aleurone layer as well as in the germ. Therefore, milling of rice grains into white will eliminate most of the vitamins. Thus, the consumption of whole grain will be of nutritional benefit for the consumer (Gobbetti and Gänzle, 2013). Gobbetti and Gänzle (2013) reported that same traditional rice varieties tested in this study were rich in Vitamins B1 and B2 as well as Vitamin E out of fat-soluble vitamins. In addition to vitamin E, vitamin K was found in the samples tested. Vitamins A and D were not detected during this analysis in the same way reported by Longvah *et al.*, (2017). Only *Kaluheenati*, *Madathavalu* and *Suduheenati* rice varieties contained vitamin E while the highest vitamin E content found in *Kaluheenati*.

All the traditional rice varieties possessed vitamin K while only Bg 360 contained vitamin K out of improved rice varieties tested. Remarkably, the highest vitamin K content was found in *Pokkali* while the lowest vitamin K level of 1.83±0.06 µg/100 g was found in *Madathavalu*. The highest vitamin B1 (thiamine) content was found in *Kaluheenati* and the second highest vitamin B1 content was found in *Suduheenati*. Vitamin B1 contents of *Kuruluthuda*, *Pachchaperumal, Pokkali*, Bg 352 and At 362 were not significantly different (p<0.05). Meanwhile, they contained the lowest levels of thiamine compared to other rice varieties analyzed. The vitamin B2 (riboflavin) content of all tested rice varieties was higher than the thiamine levels. The riboflavin content ranged from 0.39±0.00 mg/100 g to 2.11±0.03 mg/100 g. *Kaluheenati* possessed the highest riboflavin content compared to other rice samples analyzed. *Pachchaperumal* and *Pokkali* contained the exactly similar amounts of vitamin B2 (Table 2).

Table 2. Vitamin content of selected rice varieties*

Rice variety	Vitamin E (mg/100 g)	Vitamin K (µg/100 g)	Vitamin B1 (mg/100 g)	Vitamin B2 (mg/100 g)
Suwandel	N/D	4.29±0.01[d]	0.73±0.00[d]	1.26±0.00[c]
Kaluheenati	6.34±0.01[a]	3.64±0.04[e]	1.58±0.06[a]	2.11±0.03[a]
Kuruluthuda	N/D	5.03±0.01[c]	0.44±0.00[e]	0.17±0.00[g]
Madathavalu	2.07±0.00[b]	1.83±0.06[g]	0.70±0.01[d]	0.56±0.01[b]
Pachchaperumal	N/D	6.93±0.01[b]	0.43±0.00[e]	0.94±0.00[d]
Pokkali	N/D	7.55±0.02[a]	0.38±0.01[e]	0.94±0.00[d]
Suduheenati	1.67±0.00[c]	4.27±0.01[d]	1.04±0.01[b]	0.39±0.00[f]
Bg 360	N/D	2.08±0.00[f]	0.93±0.00[c]	1.62±0.01[b]
Bg 352	N/D	N/D	0.38±0.00[e]	0.86±0.02[e]
At 362	N/D	N/D	0.36±0.02[e]	0.80±0.01[e]
p Value	0.000	0.000	0.000	0.000

*Data represent the mean of three replicates fresh weight basis. Values followed by the different superscript in each column are significantly different (P<0.05)

Considering vitamin profiles of all the rice varieties analyzed, traditional rice varieties found to be rich in vitamins compared to improved rice varieties tested. According to International Union for Conservation of Nature (2016) traditional varieties were contain higher concentrations of vitamins and richer in fiber while possess a lower glycemic index compared to improved rice varieties.

Vitamin composition of selected traditional yams

Table 3 shows the vitamins found in traditional yams. Only vitamins A, K, B1 and B2 were found in yams analyzed. Vitamins D and E were not detected in yams tested. It was observed that the water soluble vitamin content was higher than that of the fat soluble content in yams tested. Vitamin A was found only in *Katuala (D. pentaphylla)* at 26.25±1.09 mg/100 g level and it was the only fat-soluble vitamin found in *Katuala (D. pentaphylla).* Vitamin K or Phylloquinon was only found in *Angiliala* and *Rajala* which comes under the same species *D. alata*. *Rajala (D. alata)* possessed 0.74±0.00 mg/100 g and *Angili ala (D. alata)* contained 0.68±0.01 mg/100 g which was significantly different from *Rajala (D. alata).*

The highest thiamine or vitamin B1 content was found in *Katuala (D. pentaphylla)* while the second highest thiamine level was detected in *Rajala (D. alata)*. *Kukulala (D. esculenta)* consisted with 0.66±0.02 mg/100 g of thiamine while the lowest vitamin B_1 content of 0.34±0.02 mg/100 g was found in *Angili ala (D. alata).*

Table 2. Vitamin content of selected traditional yams*

Yam variety	**Vitamin A (µg/100 g)**	**Vitamin K1 (µg/100 g)**	**Vitamin B1 (mg/100 g)**	**Vitamin B2 (mg/100 g)**
Katuala	26.25±1.09	N/D	1.28±0.02[a]	2.93±0.02[a]
Angiliala	N/D	0.68±0.01[b]	0.34±0.02[d]	2.61±0.03[b]
Rajala	N/D	0.74±0.00[a]	1.04±0.00[b]	0.76±0.00[d]
Kukulala	N/D	N/D	0.66±0.02[c]	2.49±0.01[c]
p Value		0.00	0.00	0.00

*Data represent the mean of three replicates fresh weight basis. Values followed by the different superscript in each column are significantly different (P<0.05)

Vitamin B2 or riboflavin levels detected in selected yams were higher than the thiamine levels detected. As same as the thiamine content *Katuala (D. pentaphylla)* contained the highest riboflavin content. Although *Angili ala (D. alata)* contained the lowest amount of thiamine it contained second highest riboflavin content of 2.61±0.03mg/100 g. *Rajala (D. alata)* consisted with the lowest vitamin B_2 content of 0.76±0.00 mg/100 g while *Kukulala (D. esculenta)* contained 2.49±0.01mg/100 g of riboflavin level.

Of all the yams tested, *Katuala (D. pentaphylla)* was rich in vitamins compared to other yams. Poornima & Ravishankar (2009) reported that *Dioscorea* tubers contained approximately 1.67 mg/100 g of ascorbic acid, 0.70 mg/100 g of thiamin and 0.43 mg/100 g of riboflavin. Okwu & Ndu (2006) have shown that vitamin C content of *Dioscorea* yams varied from 0.44 mg/100 g to 1.93 mg/100 g while riboflavin level ranged between 0.001 mg/100 g and 0.009 mg/100 g and thiamine level varies from 0.007 mg/100 g to 0.01 mg/100 g. USDA National Nutrient Database (2008) reports that approximate ascorbic acid, thiamine and riboflavin contents of *Dioscorea* yams as 17.1 mg/100 g, 0.11 mg/100 g and 0.03 mg/100 g respectively. According to Longvah *et al*., (2017) potatoes (*Solanum tuberosum*) which is commonly consumed tuber crop worldwide contains 0.06±0.004 mg/100 g of vitamin B1, 0.01±0.001 mg/100 g of vitamin B2, 1.26±0.31 mg/100 g of vitamin D, 0.01±0.01 mg/100 g vitamin E and 3.00±0.34 mg/100 g vitamin K. It shows *Dioscorea* spp studies were richer in water soluble vitamins compared to potatoes. There are considerable variations among the values reported by different authors in different studies. These differences can be due to the different environmental, soil and climatic conditions which analyzed yams grown. Composition of deposits of a plant is highly affected by the environment. The samples obtained in this study represent the whole Sri Lanka and they were obtained from different agro-climatic zones. Even the studies mentioned above also have conducted in different parts of the world. Therefore, this environmental variation is reflected by the different compositional valued obtained by different analysts.

CONCLUSIONS

All traditional rice and yam samples analyzed were rich in vitamin B1 and B2. Vitamin B2 content in yams studied was higher than the vitamin B2 content of traditional rice varieties studied. However, rice varieties contained only vitamin E and K out of the fat-soluble vitamins. Vitamin E was found only in *Kaluheenati*, *Madathavalu* and *Suduheenati,* which were red pericarp rice varieties. Vitamin K was available in all rice varieties analyzed except in Bg 352 and At 362, which were two improved rice varieties. *Kaluheenati* contained all these vitamins while holding the highest vitamin E, B1 and B2 amounts.

Yams analyzed were contained only vitamin A and K out of the fat-soluble vitamins. Vitamin A was only found in *D.pentaphylla* (*Katuala*). Only *D. alata* (both *rajala* and *Angili ala*) contained vitamin K. However, vitamin K contents of rice varieties were higher than the vitamin K contents of yams studied. The highest vitamin K content was found in *Pokkali* and *Madathavalu* contained the lowest vitamin K content, which was already higher than the vitamin K content available in yams. Therefore, rice varieties studied were richer in vitamin K compared to yams studied.

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