DOI: 10.47743/achi-2021-2-0013 *ACTA CHEMICA IASI*, **29_2**, 183-200 (2021)

ANTIOXIDANT ACTIVITY OF PHENOLIC COMPOUNDS IN *Dioscorea alata* L. (RAJA ALA) TUBER COOKING WATER

Ruwanthi Amarasekara and Suranga R. Wickramarachchi*

Department of Chemistry, Faculty of Science, University of Kelaniya, Sri Lanka

Abstract : Nutritional compounds in yams may leach out to the cooking water under most traditional domestic cooking methods. Finding alternative uses of these waste waters without discarding, can maximize the usage and importance of these yams. This study aimed to quantify the bioactive compounds and determine the antioxidant activity in the cooking water collected after boiling of *Dioscorea alata* L. tubers under two domestic cooking methods. Raja ala yams were boiled in water using conventional boiling (CB) and pressure cooking (PC) methods. The cooking water of both methods were collected and concentrated to obtain the solid crude product. Aqueous solutions of the crude product of CB and PC samples were prepared and subjected to qualitative phytochemical analysis. Further, they were assayed for total phenolic content (TPC), total flavonoid content (TFC), and total anthocyanin content (TAC). Antioxidant activity of each crude product was determined and a correlation between the antioxidant activity and TPC, TFC and TAC of the samples was developed using the Pearson's correlation method. Phytochemical screening of CB and PC samples showed the presence of alkaloids, flavonoids, phenols, saponin, tannins and coumarins and an absence of proteins. CB showed a significantly higher TPC than that of PC whereas the TFC of PC was higher than CB. Both methods however, showed no significant difference in TAC extraction. Antioxidant assays showed higher activity in CB over the PC sample. A strong correlation was observed between TPC versus DPPH activity (IC_{50} value) and TAA of the samples. It can thus be concluded that the wastewater of both cooking methods contain a significant amount of bioactive compounds, making it a nutritious source to be further investigated and to be made use for alternate purposes without discarding it.

***Keywords*:** Antioxidants, Boiling, *Dioscorea alata* L., Phenolics, Pressure cooking

* Suranga, Wickramarachchi, *email*: suranga@kln.ac.lk

Introduction

Root and tuber crops are stapled energy sources, second to cereals, and serves as a food source for over two billion people, especially to those living in the rural regions of Africa, Asia, and the Caribbean.[1] Although diverse varieties of these crops are grown worldwide, 99% of the total world production is accounted by only five major species namely potato (*Solanum tuberosum,* 46%), cassava (*Manihot esculenta*, 28%), sweet potato (*Ipomea batatas*, 18%), yams (*Dioscorea* spp., 6%) and taro (*Colocasia, Cytosperma, Xanthomonas* spp. 1%).[2] They are significant sources of vitamins, minerals, and other non-nutrient compounds that are responsible towards bioactive properties. These varieties are subjected to various cooking methods that adds pleasant sensory characteristics, improves digestibility, and makes the food microbiologically safer for consumption. However, various studies have shown that preparation methods such as boiling, steaming and baking[3,4] leads to a reduction of beneficial compounds that are originally present in raw food thus, minimizing the health benefits that are achieved upon consumption. Although the studies suggest that prolonged cooking periods are to be avoided when preparing food,[5] cooking of these crop varieties to some extent before consumption is however inevitable. Moreover, the excess water used in cooking is also drained out and discarded as waste while the tubers are consumed. At present day, as much scientific focus is drawn towards research based on green strategies, investigating this discarding cooking water can surely add value to the wastewater and maximize the usage potential and importance of root and tuber crop species.

Studies carried out on the cooking water after boiling potatoes (*Solanum tuberosum*) and sweet potatoes (*Ipomea batatas*) have been able

to identify alternate uses of the wastewater without having to discard it.[6,7] Domestic versatile uses of boiled potato water have been suggested for culinary purposes as a gluten-free substitute thickener to replace flour or cornstarch and to be added to any dish as it is a source of starch, proteins, vitamins B and C, potassium, fiber, and phytochemicals such as flavonoids and carotenoids that are beneficial for health. The water can also be used in home gardens to add nutrients to soil and as a fertilizer to nourish plants.[6] As means of identifying possible ways to reuse the discarding water after processing sweet potatoes, Ishiguro et al. (2016) conducted animal experiments where mice were fed with sweet potato peptide (SPP) prepared using the wastewater.[7] SPP inhibited body weight gain and visceral fat gain and lowered the cholesterol levels and levels of triglyceride in the tested mice. Hence it could be expected that further research would enable the use of sweet potato peptide as a food material for people with metabolic disorders. Drinking the leftover water could also help as a weight loss method and as a slimming aid.[8] So far, only a handful of research has been carried out concerning the discarding cooking water of root and tuber crop varieties. Hence, the current study was performed using Dioscorea yams (Raja ala) as another example to identify and prove the potential of wastewater to be a source of bioactive compounds which can be used for alternate purposes.

The underground edible tubers of Raja ala belonging to the *Dioscorea alata* L. species is commonly referred by many names such as Greater yam, Purple yam, Water yam, and Winged yam.[9] This traditional yam had been a main dietary constituent of the past Sri Lankan community and is even consumed at present day. Mature tubers of the yam are mainly consumed boiled, as a breakfast meal, although it can also be eaten roasted

or fried, as porridge or even as a curry making a good substitute for potatoes.[10] Two of the most common domestic cooking methods employed for boiling yams are conventional boiling using a closed utensil or pressure cooking. The utilized culinary method can cause significant changes in the structure and composition of phenolic compounds and influence the nutritive value of the yam.[5] At the same time, cooking softens the cellular tissues and matrix of the yam and facilitates the release of compounds into the cooking water. Therefore, the possibility of leaching out of beneficial constituents from the yam makes it essential to investigate the cooking water for phenolic compounds and bioactive properties.

In the recent years, much interest is drawn towards phenolic compounds, mainly due to the perceived health benefits achieved from the bioactive properties possessed in them. Reactive oxygen species (ROS) generated within the human body as a result of essential metabolic processes are imperative for redox homeostasis.[11] However, the loss of balance between the production of free radicals and the functioning of defense mechanisms in the body results in oxidative stress which acts as a contributing factor towards many chronic diseases such as diabetes, cardiovascular diseases, cancer, neurodegenerative diseases, and much more, which predominate among the leading causes of death.[12] Scientific studies show that oxidative stress and its related diseases can be prevented or retarded by the dietary intake of antioxidants.[11] Phenolic compounds can act as antioxidants, neutralizing excess reactive free radicals in the body preventing cellular damage. Thus, research on the antioxidant activity have become an important study area, undertaken as an attempt to manage chronic diseases. Under such circumstances, identifying the antioxidant potential in cooking water of boiled yams will create novel applications of wastewater without having to discard it. Moreover, the possibility of

extraction of these natural antioxidants from the wastewater may also provide an alternative to the use of synthetic antioxidants such as BHT (butylated hydroxytoluene) and BHA (butylated hydroxyanisole) which have been linked to toxicity through carcinogenic effects.[13]

Therefore, this study aimed at quantifying the total phenolic content (TPC), total flavonoid content (TFC), total anthocyanin content (TAC) and screening the antioxidant activity in the cooking water of *D. alata* tubers using conventional boiling (CB) and pressure cooking (PC) methods.

Experimental

Materials

Dioscorea alata L. tubers (Raja Ala) were purchased from a fixed vendor at the local market. All the chemicals and reagents used in this study were of analytical grade.

Preparation of tubers

Raja ala tubers were washed with water to remove soil and dirt. The grayish brown skin was peeled off and tubers were diced into small cubes of approximately 1 × 1 cm.

Cooking methods of yam samples

The raw yam cubes (850 g) were boiled in distilled water (2 L) under conventional boiling (CB) in a closed stainless-steel pot for a period of 45 minutes and using pressure cooking (PC) in a pressure cooker type autoclave under pressurized steam of 15-20 lb./in^2 for an overall time period of 30 minutes. After cooling, the cooking water of both methods was drained out and collected separately, filtered, and centrifuged to obtain a clear supernatant of the extract. Extracts were concentrated by evaporating at 70°C to obtain a dry solid crude product. Aqueous solutions of the crude extracts (30.0 mg/mL) were prepared as CB and PC samples.

Qualitative analysis of phytochemical constituents

CB and PC samples were subjected to preliminary phytochemical screening as follows:

Alkaloids : Mayer's test

Aqueous HCl solution (1%, 5.0 mL) was added to the sample (1.0 mL) and heated using a water bath for 10 minutes. While hot, the sample was treated with a few drops of Mayer's reagent (0.3 mL). The formation of a cream colour precipitate indicates the presence of alkaloids.[17]

Flavonoids : Alkaline reagent test

The sample (3.0 mL) was mixed with NaOH (2%, 2.0 mL) and resulted in the formation of a concentrated yellow colour solution. Dilute H_2SO_4 solution (0.1 mL) was then added to the mixture. Disappearance of the concentrated yellow colour on the addition of dilute acid indicates the presence of flavonoids.[18]

Phenols : Ferric chloride test

Ferric chloride solution (10%, 0.25 mL) was added to the sample (5.0 mL). The development of blue or green colour indicates the presence of phenols.[19]

Saponin : Froth test

The sample (2.5 mL) was diluted to 10.0 mL using distilled water and was shaken vigorously for 15 minutes. The formation of about 1 cm layer of stable persistent froth indicates the presence of saponin.[19]

Tannins : Lead acetate test

Lead acetate solution (1%, 1.0 mL) was added to the extract (5.0 mL). Formation of a flocculent white precipitate indicates the presence of tannins.[19]

Coumarins

NaOH (10%, 3.0 mL) was added to the extract (3.0 mL) and mixed. The formation of yellow colour indicates the presence of coumarins.[20]

Proteins : Xanthoproteic test

Extract (2.0 mL) was treated with few drops (1.0 mL) of concentrated HNO_3, cooled under tap water and heated for 2 minutes. The formation of a yellow-coloured solution which turns orange on the addition of a few drops of NaOH solution (40% w/w) indicates the presence of proteins.[21]

Quantitative analysis of phenolic compounds

Total phenolic content (TPC)

The total phenolic content in the samples were determined using the Folin-Ciocalteu method [22] with slight modifications and using gallic acid as the standard. In brief, the sample or standard (0.20 mL) was added to distilled water (0.80 mL) followed by the addition of Folin-Ciocalteau reagent diluted ten times with distilled water (5.0 mL). Samples were incubated for 60 seconds and saturated Na_2CO_3 solution (7.5% w/v, 4.0 mL) was added. The solutions were mixed well and incubated for 30 minutes in the dark at room temperature. Absorbance was measured against

a blank at 765 nm wavelength using the UV-Visible spectrophotometer (Agilent Technologies, Cary 60, UV-Vis). Quantification was based on the standard calibration curve that was plotted using concentrations of 100.0, 150.0, 200.0, 250.0, 300.0, 350.0 μg/mL of gallic acid. Total phenolic content was expressed in terms of mg of gallic acid equivalent per gram of fresh weight of Raja ala (mg GAE/g FW)

Total flavonoid content (TFC)

Total flavonoid content of the tuber extracts were determined using the aluminum chloride colorimetric assay [23] with modifications using catechin as the standard. The extract or standard (25.0 μL), distilled water (100.0 μL) and $NaNO_2$ (10.0 μL) were added into the 96 well microplate using a micropipette (100.0 μL). It was allowed to stand for 5 minutes and

$AlCl_3$ in methanol (10% w/v, 15.0 μL) was added. After 6 minutes, NaOH (50.0 μL) and distilled water (50.0 μL) were added and allowed to stand for 30 seconds. Absorbance was measured at 510 nm wavelength using the microplate reader (Multiskan FC, 1.00.94). Quantification was based on the standard calibration curve that was plotted using concentrations of 100.0, 200.0, 300.0, 400.0, 500.0 μg/mL of catechin. Total flavonoid content was expressed in terms of mg of catechin equivalent per gram of fresh weight of Raja ala (mg CE/ g FW).

Total anthocyanin content (TAC)

The total anthocyanin content was estimated spectrophotometrically using pH differential method.[24] Initially, the dilution factor was determined by mixing the extract (1.00 mL) with different volumes of KCl buffer (pH 1) such that the absorbance obtained at 520 nm was within the range of 0.2–1.4 AU. Thereafter, two dilutions of each sample extract were prepared by adding KCl buffer (0.025 M, pH 1) (2.0 mL) to the extract (1.00 mL) and by adding sodium acetate buffer (0.4 M, pH 4.5) (2.0 mL) to the extract (1.00 mL) separately. The two dilutions were allowed to equilibrate for 15 minutes, and the absorbance of each dilution was measured at both wavelengths of 520 nm and 700 nm against a blank of distilled water using the UV-Visible spectrophotometer (Agilent Technologies, Cary 60, UV-Vis). Monomeric anthocyanin concentration was calculated and expressed as mg of cyanidin-3-glucoside equivalent per gram of fresh weight of Raja ala (c-3-gE mg/g FW).

In-vitro antioxidant activity assays and correlation studies

DPPH free radical scavenging assay

The antioxidant activity was measured in terms of hydrogen donating or radical scavenging ability using the stable DPPH (2,2-diphenyl-1-picrylhydrazyl) method with several modifications.[25] A stock solution of the extract or the BHT standard (1.00 mg/mL) was prepared in distilled

water, and it was further diluted using methanol to obtain a concentration series of 500.00, 250.00, 125.00, 62.50, 31.25, 15.63, 7.81 μg/mL. Sample or standard from each concentration (160 μL) was transferred to the 96 well microtiter plate using a micropipette (1000 μL). Methanolic DPPH solution (0.25 mM, 40.0 μL) was added into each well using a micropipette (100.0 μL), the reagents were mixed and incubated for 20 minutes in the dark at room temperature. The absorbance of each sample well and the control was measured at 520 nm wavelength using the microplate reader (Multiskan FC, 1.00.94). The percentage inhibition was calculated and plotted against the respective sample concentrations. The antiradical activity of the sample was expressed as the IC_{50} value which is the concentration of the extract providing 50% discoloration of DPPH solution.

Total antioxidant activity (TAA)

The total antioxidant activity was determined using the phosphomolybdenum method with minor modifications using gallic acid as the standard.[26] A concentration series of 1.25, 2.50, 3.75, 5.00, 6.25, 7.50 mg/mL was prepared using the sample extract or standard. Sample or standard of each concentration (0.30 mL) was mixed with reagent solution (sulfuric acid (0.6 M): sodium phosphate (28 mM): ammonium molybdate (4 mM) in 1:1:1 volume ratio) (3.0 mL). The reaction mixture was incubated at 95°C for 90 minutes in a water bath. The tubes were allowed to cool to room temperature and absorbance of all sample mixtures were measured at 695 nm wavelength against a blank using the UV-Visible spectrophotometer (Agilent Technologies, Cary 60, UV-Vis). Quantification was based on the standard calibration curve plotted using gallic acid. The antioxidant capacity was expressed as mg gallic acid equivalent per mg of fresh weight of Raja ala (mg GA/mg FW).

Statistical analysis

All the samples were analyzed in triplicate and expressed as mean ± standard deviation. Microsoft Excel 2010 was used in reporting means,

standard deviations and plotting graphs. Data were analyzed statistically using GraphPad Prism 9.1.0 software. One-way analysis of variance (ANOVA) was applied followed by Tukey's multiple comparison test with 95% confidence level. Significant differences in means were shown at $P \leq 0.05$. Correlation studies between antioxidant activities vs. TPC, TFC and TAC of the samples were performed using the Pearson's correlation method.

Results and discussion

The results of the preliminary phytochemical screening analysis presented in Table 1 indicates that alkaloids, flavonoids, phenols, saponin, tannins and coumarins are present in the water obtained from both CB and PC cooking methods. The absence of aromatic amino acids in the proteins that leached out into the cooking water may have given a negative result in protein screening using the xanthoproteic test.

Table 1. Preliminary phytochemical screening results of CB and PC sample extracts.

Phytochemical constituent	CB sample	PC sample
Alkaloids	+	+
Flavanoids	+	+
Phenols	+	+
Saponin	+	+
Tannins	+	+
Coumarins	+	+
Proteins	-	-

The results obtained in the quantitative analysis of phenolic compounds are presented in Table 2.

Table 2. Total phenolic content (TPC), total flavonoid content (TFC) and total anthocyanin content (TAC) of sample extracts.

Sample extract	TPC (mg GAE/g FW)	TFC (mg CE/g FW)	TAC (mg c-3-gE/ g FW)
CB	14.713 ± 1.039 [a]	11.501 ± 0.154 [a]	0.075 ± 0.034 [a]
PC	9.848 ± 0.548 [b]	12.962 ± 0.955 [b]	0.049 ± 0.020 [a]

Results are expressed as mean ± standard deviation (n = 3). Different letters in superscript in each column are significantly different from each other (ANOVA, Tukey's test, $P \leq 0.05$).

The TPC values and TFC values of both samples showed a significant difference ($P \leq 0.05$) when the two cooking methods were compared. Hence, the recovery of phenolic compounds including flavonoids differed greatly and was affected by the cooking methods that were employed. The highest phenolic contents were observed in the CB sample showing that conventional boiling would extract more phenolics into the cooking water whereas a significantly lower value was observed in the PC sample which shows a lower leaching out of phenolics under pressure cooking method. The loss of flavonoids was significantly higher in the PC method than the CB method.

The results show that both CB and PC cooking methods led to a loss of anthocyanins from the yam. However, when comparing the two cooking methods, TAC values of both samples exhibit no significant difference ($P > 0.05$), showing that the leaching out of these constituents are nearly equal when Raja ala is prepared using either of the two cooking methods. Thus, the loss of anthocyanins have not been affected by the cooking method. The observed TAC values were very low when compared to TPC and TFC contents of samples under both cooking methods (Table 2). Even though anthocyanins are water soluble, they occur in plant materials as

structures with conjugated double bonds carrying a positive charge (flavylium cation form) as shown in Figure 1.

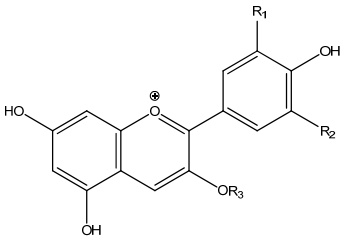

Figure 1. Flavylium cation form of anthocyanins.

The protonated form of anthocyanin is only stable under acidic environments thus requiring mild acidic water for the extraction of anthocyanins.[14] Hence, both cooking methods showed a preservation of anthocyanins in the yam itself to a greater extent during cooking.

Tamaroh et al. (2018) reported the results of a study on acidified solvent extraction of *Dioscorea alata* L. yam flour.[27] The highest and the lowest TAC values were present in the methanolic HCl extract (2.47 mg c-3-gE/g FW) and the ethanolic tartaric acid extract (0.18 mg c-3-gE/ g FW) respectively. Both the values were higher than the results obtained in the current study (Table 2), thus confirming that the use of acidified solvents leads to a better extraction of anthocyanin compounds. Moreover, Fang et al. (2011) reported the TAC of fresh purple yam tubers to be 0.31 mg c-3-gE/g FW.[22] The anthocyanin contents detected in the cooking water of CB and PC samples of the current study (0.075 and 0.049 mg c-3-gE/ g FW respectively) were lower than the value stated in literature, thus suggesting that most of the anthocyanins may have retained in the yam when the yams were boiled.

The DPPH radical scavenging activity and the TAA of the samples were analyzed graphically and are presented by Figure 2 and Figure 3 respectively.

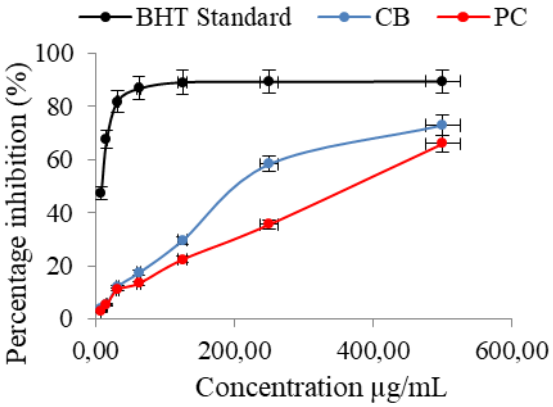

Figure 2. DPPH radical scavenging activity.

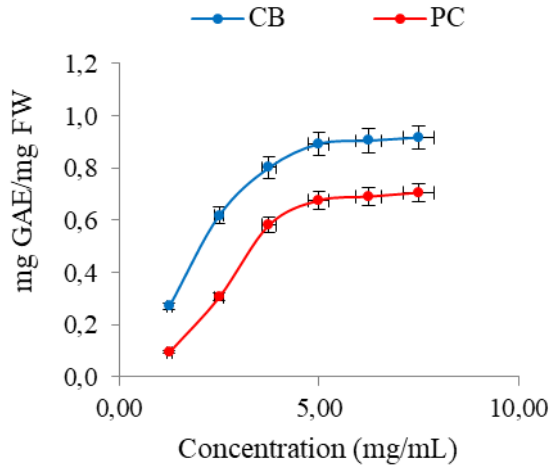

Figure 3. Total antioxidant assay of cooking water of *D. alata* tubers.

When comparing the two cooking methods, both graphs show that the antioxidant activity of CB water is significantly higher ($P \leq 0.05$) than that of PC water at all tested sample concentrations. IC_{50} values of DPPH assay in CB and PC samples were 209.014 ± 10.428 and 371.615 ± 8.982 µg/mL respectively. CB exhibited a significantly higher antioxidant activity

($P \leq 0.05$) than PC sample, indicating that CB method extracts more compounds rich in antioxidant activity relative to PC method. Both samples showed moderate activity implying that both the cooking methods have resulted in leaching out of considerable amounts of antioxidants into the cooking water. Although studies have not been carried out on the antioxidant activity of the cooking water after boiling Raja ala tubers, the antioxidant activity present in the yam before and after boiling the tubers have been reported.[28]. DPPH - IC_{50} value of the raw yam (18.62 ± 3.82 mg FW/mL) was less than that of the boiled yam (24.91 ± 2.91mg FW/mL) showing that the antioxidant activity present in the yam has been reduced when the yams are boiled. This could have been due to the removal of water-soluble antioxidants into the discarding water. Hence, these results also explain the moderate antioxidant activity detected in the cooking water of the present study.

Table 3. Pearson's correlation coefficients (r) of DPPH and TAA *vs* TPC, TFC and TAC in the extracts.

Antioxidant activity	TPC (mg GAE/g FW)	TFC (mg CE/g FW)	TAC (mg c-3-gE/ g FW)
DPPH activity (IC_{50} value)	-0.856	0.712	-0.926
TAA (mg GAE / mg FW)	0.811	-0.754	0.967

The correlation between antioxidant activities and TPC, TFC, TAC are given by Table 3.

A high TPC, TFC, and TAC leads to a high antioxidant activity given by a low DPPH - IC_{50} value and a high TAA value. Thus, a significantly negative correlation between DPPH activity and phenolics[15] and a significantly positive correlation between TAA and phenolics is taken as a good correlation. A strong negative correlation between DPPH activity

vs. TPC and TAC and a strong positive correlation between TAA vs. TPC and TAC suggests that phenolic compounds including anthocyanins that are available in the cooking water contributes towards the antioxidant activity in the wastewater. A weak correlation was observed between the antioxidant activities and TFC showing an inconsiderable contribution of flavonoids in the water to the antioxidant activity. Although TFC assay results showed that the discarding water of both cooking methods had a significant amount of flavonoids, the aluminium chloride colorimetric assay shows positive results towards compounds having an aromatic ring bearing a catechol moiety.[16] The presence of catecholic groups on non-flavonoid compounds without antioxidant activity may have resulted in a poor correlation between antioxidant activities and TFC in the samples.

Conclusion

Bioactive compounds such as phenols, alkaloids, flavonoids, saponin, tannins and coumarins leach out into the water when yams are boiled using both cooking methods. The total phenolic content (14.713 ± 1.039 mg GAE/g FW) is significantly higher in the CB cooking water than that of PC (9.848 ± 0.548 mg GAE/g FW) sample and the total flavonoid content is higher in the PC water (12.962 ± 0.955 mg CE/g FW) than the CB sample (11.501 ± 0.154 mg CE/g FW), suggesting that the leaching out of phenolic compounds including flavonoids are significantly affected by the cooking method. The loss of anthocyanins from the yam remain more or less unaffected by the cooking method. IC_{50} values of CB (209.014 ± 10.428 µg/mL) and PC (371.615 ± 8.982 µg/mL) samples showed moderate activity in the cooking water. TAA of CB and PC samples were 0.917 ± 0.016 and 0.707 ± 0.002 mg GAE/mg FW respectively.

Antioxidant assays showed higher activity in CB samples over PC, suggesting that a higher amount of compounds with antioxidant activity are present in the cooking water when the yams are boiled using the conventional method rather than pressure cooked. Correlation studies emphasized that phenolic compounds including anthocyanins present in the water contributed to the antioxidant activity in the samples. The study shows that the cooking water obtained after conventional boiling of yams contain a higher phenolic content and higher antioxidant activity than that obtained by pressure cooking. However, the overall results show that the cooking water of both methods is a good source of bioactive compounds having antioxidant activity. Therefore, further investigation on these bioactive compounds will allow the discovery of alternate uses of the waste cooking water without having to discard it.

References

1. Lebot, V. *Tropical root and tuber crops: cassava, sweet potato, yams and aroids*, 2nd ed.; CABI: Wallingford, UK, 2009.
2. Jayakody, L.; Hoover, R.; Liu, Q.; Donner, E. Studies on Tuber Starches. II. Molecular structure, composition and physicochemical properties of yam (*Dioscorea Sp.*) starches grown in Sri Lanka. *Carbohydr. Polym.* **2007**, *69*(1), 148-163.
3. Ezeocha, V. C.; Ojimelukwe, P. C. The impact of cooking on the proximate composition and anti-nutritional factors of water yam (*Dioscorea alata*). *J. Stored Prod. Postharvest Res.* **2012**, *3*(13), 172-176.
4. Wanasundera, J. P. D.; Ravindran, G. Effects of cooking on the nutrient and antinutrient contents of yam tubers (*Dioscorea alata* and *Dioscorea esculenta*). *Food Chem.* **1992**, *45*(4), 247-250.
5. Kamrunnaher, M.; Rashid, M.; Sayem, A. S. M.; Islam, M. Z. Effect of boiling on bioactive compounds and radical scavenging activity of anthocyanin-rich vegetables: red amaranth and red skin potato. *GSC biol. pharm. sci.* **2019**, *7*(2), 127-137.
6. *https://www.cookist.com/heres-why-you-should-always-have-some-potato-water-on-hand/* (assessed on: 04.11.2021)

7. Ishiguro, K.; Kurata, R.; Shimada, Y.; Sameshima, Y.; Kume, T. Effects of a sweetpotato protein digest on lipid metabolism in mice administered a high-fat diet. *Heliyon* **2016**, *2*(12), e00201.
8. *https://www.dailymail.co.uk/health/article-4009502/Want-lose-weight-Try-drinking-waste-water-cooking-sweet-potatoes-scientists-say.html* (assessed on: 04.11.2021)
9. O'Sullivan, J. N. In *Yam nutrition nutrient disorders and soil fertility management*; ACIAR: Canberra, 2010, pp 11–15.
10. Rajapaksha, U. *Traditional food plants in Sri Lanka*; Hector Kobbekaduwa Agrarian Research and Training Institute: Colombo, 1998.
11. Lobo, V.; Patil, A.; Phatak, A.; Chandra, N. Free radicals, antioxidants and functional foods: impact on human health. *Pharmacogn Rev*. **2010**, *4* (8), 118-126.
12. Phaniendra, A.; Jestadi, D. B.; Periyasamy, L. Free radicals: properties, sources, targets, and their implication in various diseases. *Indian J. Clin. Biochem*. **2014**, *30*(1), 11–26.
13. Branen, A. L. Toxicology and biochemistry of butylated hydroxyanisole and butylated hydroxytoluene. *J. Am. Oil Chem. Soc.* **1975**, *52*(2), 59-63.
14. Martín, J., Navas, M. J., Jiménez-Moreno, A. M., & Asuero, A. G. Anthocyanin pigments: importance, sample preparation and extraction. *Phenolic Compounds - Natural Sources, Importance and Applications*. (2017) Published.
15. Fidrianny, I., Suhendy, H., & Insanu, M. Correlation of phytochemical content with antioxidant potential of various sweet potato (*Ipomoea batatas*) in West Java, (2018) Ind
16. Pękal, A.; Pyrzynska, K. Evaluation of aluminium complexation reaction for flavonoid content assay. *Food Anal. Methods* **2014**, *7*(9), 1776–1782.
17. Ajiboye, B. O.; Ibukun, E. O.; Edobor, G.; Ojo, A. O.; Onikanni, S. A. Qualitative and quantitative analysis of phytochemicals in senecio biafrae leaf. *Int. J. Pharm. Sci.* **2013**, *1*(5), 428-432.
18. Khalid, S.; Shahzad, A.; Basharat, N.; Abubakar, M.; Anwar, P. Phytochemical screening and analysis of selected medicinal plants in Gujrat. *J. Phytochem. Biochem.* **2018**, *2* (1), 108.
19. Viji, M. O.; Wilson, N.; Veena, V. V. Phytochemical profiling of tuber extracts of *Dioscorea alata*. *VISTAS* **2016**, *5*(1), 133-137.

20. Roghini, R.; Vijayalakshmi, K. Phytochemical Screening, Quantitative analysis of flavonoids and minerals in ethanolic extract of *Citrus paradisi*. *Int. J. Pharm. Sci. Res.* **2018**, *9*(11), 4859-4864.

21. Narasimhan, R.; Sathiyavani. Phytochemical screening and evaluation of protein content in the seed extracts of *Cucurbita maxima*. *Int. J. Pharm. Life Sci.* **2014**, *5*(7), 3637-3642.

22. Fang, Z.; Wu, D.; Yü, D.; Ye, X.; Liu, D.; Chen, J. Phenolic compounds in chinese purple yam and changes during vacuum frying. *Food Chem.* **2011**, *128*(4), 943-948.

23. Ukom, A. N.; Ojimelukwe, P. C.; Ezeama, C. F.; Ortiz, D. O.; Aragon, I. J. Phenolic content and antioxidant activity of some under-utilized nigerian yam (Dioscorea Spp.) and Cocoyam (Xanthosomamaffa (Scoth)) Tubers. *IOSR J. Environ Sci. Toxicol. Food Technol.* **2014**, *8*(7), 104-111.

24. Giusti, M. M.; Wrolstad, R. E. Characterization and measurement of anthocyanins by uv-visible spectroscopy. *Curr. Protoc. Food Anal. Chem.* **2001**, *00*(1), F1.2.1-F1.2.13.

25. Chatatikun, M.; Chiabchalard, A. Phytochemical screening and free radical scavenging activities of orange baby carrot and carrot (*Daucus carota* Linn.) root crude extracts. *J. Chem. Pharm. Res.* **2013**, *5*(4), 97-102.

26. Noumi, E.; Snoussi, M.; Noumi, I.; Valentin, E.; Aouni, M.; Al-sieni, A. Comparative study on the antifungal and antioxidant properties of natural and colored *Juglans regia* L. Barks: a high activity against vaginal candida strains. *Life Sci. J.* **2014**, *11*(8), 327-335.

27. Tamaroh, S.; Raharjo, S.; Murdiati, A.; Anggrahini, S. Total phenolic content and antioxidant activity of anthocyanin extract from purple yam (*Dioscorea alata* L.) flour using different solvents. *Pak. J. Nutr.* **2018**, *17*(6), 260-267.

28. Abeynayake, R.; Sivakanesan, R. Effect of boiling on the antioxidant capacity of *Dioscorea alata* (raja ala) grown in Sri Lanka. *Trop. Agric. Res.* **2015**, *26*(1), 109-119.