THE INFLUENCE OF COOKING ON THE ANTI-NUTRIENT CONTENTS OF TWO *Dioscorea* VARIETIES IN SRI LANKA

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The presence of anti-nutrients, which limit the food value of crops by causing toxicities and reducing the bioavailability of nutrients, is one of the major concerns for many valuable food crops. Local yams, one of the nutrient-rich but underutilized crops in Sri Lanka, with a significant content of anti-nutrient factors such as phenol, alkaloid, oxalate, phytate, tannin, saponin, which cause toxicity and bitterness, leads to the underutilization of these yams. In Sri Lanka, yams are commonly consumed after cooking in boiling water. Moreover, cooking is a scientifically proven traditional technique to reduce the amount of anti-nutrients in plant foods. However, the scientific studies on anti-nutrients in local yams were limited. Therefore, this study aimed to assess the alkaloids, saponins, and oxalate contents in two local yam varieties and investigate the effect of cooking on the anti-nutrient contents of these yams. Bulk samples of the two selected varieties, Pani ala (*Dioscorea alata*) and Kirikodol (*Dioscorea alata*) were collected from the Field Crop Research and Development Institute, Mahailluppallama, Sri Lanka. Yams were cooked in boiling water at a ratio of 1: 10 (W/V) for 30 minutes to determine the cooking effect. Alkaloids and saponin content were analyzed using gravimetric methods, and Oxalate content was analyzed using the Permanganate titration method. Recorded values were as the fresh weight basis. Data were analyzed using Analysis of Variance (ANOVA) with Minitab 19 statistical software. The highest alkaloid content was observed in uncooked Kirikodol (0.36±0.01%) and cooked Pani ala had the lowest (0.16±0.01%). Oxalate contents of tested samples ranged between 11.25±0.00 mg/100 g (cooked Pani ala) and 78.75±0.00 mg/100 g (uncooked Kirikodol). Saponin content ranged between 1.5±0.18% to 3.8±0.28%, the lowest for cooked Pani ala and the highest for uncooked Kirikodol. Uncooked Kirikodol showed the highest anti-nutrient content, while the lowest in cooked Pani ala among the tested samples. All the cooked samples showed reduced contents of three anti-nutrients than the uncooked samples. Thus, cooking is an effective way of minimizing the levels of alkaloids, saponin, and oxalate content in Pani ala and Kirikodol.

Keywords: Alkaloids, Anti-nutrients, *Dioscorea*, Oxalate, Saponin

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INTRODUCTION

Anti-nutrients are the chemical compounds commonly found in natural plant foods, which reduce the bioavailability of nutrients in the diet. In addition, they cause toxicities and affect the digestibility of proteins. The major anti-nutrient factors in plants include saponins, alkaloids, oxalates, tannins, and phytates. The presence of these problematic anti-nutrients is a major concern for many valuable food crops in Sri Lanka since they reduce the food value of crops. Soaking, de-hulling, cooking, and fermentation are important traditional methods to reduce anti-nutrients.

Yams, the edible tubers of the genus *Dioscorea*, are nutrient-rich but underutilized food crops in Sri Lanka, with the main component being carbohydrates and a significantly high amount of proteins. Compared to other crops, yams show higher crop adaptability, fewer pests and disease attacks, require low inputs and management practices, and produce high edible energy per hectare. However, the presence of anti-nutrients, such as phenol, alkaloid, oxalate, phytate, tannin, and saponin, which cause toxicity and bitterness, limited their food value and utilization. When considered separately, alkaloids cause gastro-intestinal upsets and neurological disorders, oxalate reduces the absorption of Calcium in the diet, and saponins cause inhibitory activities of digestive enzymes such as amylase, glucosidase, trypsin, chymotrypsin, and lipase, thereby cause indigestion-related health disorders (Samtiya *et al*., 2020). In Sri Lanka, yams are commonly consumed after cooking, a well-known traditional method used to reduce the number of anti-nutrients in plant foods.

A limited number of studies are available on anti-nutrients in Sri Lankan yam varieties and their variations throughout the cooking process. Therefore, this study was conducted to assess the alkaloids, saponins, and oxalate contents in two *Dioscorea* varieties in Sri Lanka and investigate the effect of cooking on the anti-nutrient contents of these yam varieties.

MATERIALS AND METHODS

Sample Collection and Preparation

Bulk samples of the two selected varieties, Pani ala (*Dioscorea alata*) and Kirikodol (*Dioscorea alata*) were collected from the Field Crop Research and Development Institute, Mahailluppallama. Samples were peeled washed and cut into small pieces. For the preparation of cooked samples, peeled and washed yam samples were boiled in water at a ratio of 1: 10 (W/V) for half an hour. Both uncooked and cooked yam pieces were ground using a mortar and pestle.

Determination of Oxalate

Oxalate content was determined using the permanganate titration method described by Onwuka (2005) with modifications. For that, 10 g of the sample was boiled with 100 ml of distilled water and 5 ml of 6M HCl for an hour. Two drops of methyl red were added to the cooled filtrate and the pH was adjusted between 4-4.5. The mixture was heated to 90 ^{0}C in a water bath, cooled, and filtered. The filtrate was again heated at 90 ^{0}C and 10 ml of 5% $CaCl_2$ solution was added and mixed. The cooled mixture was kept in a refrigerator overnight. The mixture was centrifuged at 3000 rpm for 6 minutes. The precipitate was dissolved in 5 ml of

20% H_2S0_4. The solution was made up to 50ml with distilled water and titrated against 0.05 M $KMn0_4$ solution. Oxalate content was calculated using an equation [Oxalate content = 0.05×V×5×MW×100/10×2] where V= Title value (ml), MW= Molecular weight of anhydrous oxalic acid (90.034 g/mol) and expressed as milligrams of anhydrous oxalic acid in 100 g of yam (fresh weight).

Determination of alkaloids

Alkaloid content was determined using a gravimetric method described by Okwu and Ndu (2006) with modifications. In brief, alkaloids were extracted with 20 % glacial acetic acid in ethanol and precipitated using ammonia. The alkaloid precipitate was dried, weighed, and calculated on fresh weight basis.

Determination of saponin

Saponin content was determined using the method described by Senanayake *et al*. (2012). A sample of 2.5g was mixed with 25 ml of 20% ethanol. The suspension was heated using a hot water bath at 55oC for 4h with continuous stirring. The mixture was filtered and the residue was re-extracted twice with another 25ml of ethanol. The extracts were combined and reduced to one-quarter of the total volume by heating in a water bath at 900C. The aqueous portion of the concentrate was extracted thrice with 20 ml of diethyl ether by using a separating funnel. The extract was purified further with 40ml of n-butanol and washed thrice with 5% aqueous NaCl. The remaining solution will be evaporated at 95^0C over a water bath until obtaining a constant weight. The saponin content was calculated on a fresh weight basis as a percentage.

Statistical analysis

Data were analyzed using Analysis of Variance (ANOVA) with Minitab 19 statistical software. In all cases, statistical significance is quoted at the 5% significant level ($p<0.05$).

RESULTS AND DISCUSSION

The alkaloid contents of uncooked and cooked Kirikodol ala were recorded as 0.36±0.01 and 0.28±0.01 respectively, while uncooked and cooked Pani ala recorded their alkaloid contents as 0.21±0.01%, 0.16±0.01%. Similar results have been reported by Udensi et al. (2010). According to them, the low level of alkaloid found in *Dioscorea alata* is within the range of 0.12%–0.55%. A similar study was carried out by Ezeocha and Ojimelukwe in 2012 for water yam (*Dioscorea alata*) in Nigeria and obtained somewhat higher alkaloid contents (2.77%) compared to Pani ala and Kirikodol in Sri Lanka. The alkaloid levels vary depending on cultivated species and cultural practices. Higher levels of alkaloids are usually found in wild species, which cause a burning sensation in the mouth and throat, vomiting, and diarrhea. As an anti-nutrient, at lower levels, alkaloids only produce a bitter taste in the tuber tissues. However, a significant amount of alkaloids can be removed by boiling or soaking in water because of their water solubility (Abiodun and Akinoso, 2014). This may be the reason for the significant reduction of alkaloid contents during the cooking of Pani ala and Kirikodol.

Saponins are identified as an anti-nutrient compound, because of their bitter taste and throat-irritating activity (Gemede & Ratta, 2014). Saponins, in high concentrations, impart a bitter taste and astringency in dietary plants. The bitter taste of saponin is the major factor that limits its use. In addition, saponins bind with minerals such as Zinc and reduce their bio-availability. However, there are some beneficial types of saponins, which inhibit the growth of cancer cells and lower blood cholesterol levels (Ogbuagu, 2008). Further, Saponin compounds serve as natural antibiotics, which help the body to fight infections and microbial invasion (Sopido *et al*., 2000). Uncooked Pani ala and Kirikodol recorded their saponin contents as 2.2±0.3% and 3.8±0.3% respectively while cooking has a significant effect on the

saponin contents by reducing the value to 1.5±0.2% for Pani ala and 3.4±0.3% for Kirikodol. Ezeocha and Ojimelukwe (2012) carried out a similar study for water yam in Nigeria and observed similar findings where boiling reduced the saponin content from 2.71% (raw) to 1.37% (boiled). Further, similar saponin content of 3.36% was obtained by Ezeocha and Onwuka (2012) for raw *Dioscorea dumetorum* (Trifoliate yam) in Nigeria.

Oxalic acid and oxalates occur naturally in plants but they have little or no useful effect on human health as high levels in diets lead to irritation of the tissues; the digestive system, particularly the stomach and kidney (Hodkinson, 1977). Furthermore, the anti-nutrient oxalic acid has been shown to impair the absorption of magnesium, zinc, iron, and calcium in the intestine by complexation (Bohn *et al*., 2004). Calcium oxalate can have a deleterious effect on human nutrition and health by accumulating kidney stones. Therefore, according to previous studies the consumption of a high amount of oxalate could be fatal, because of oxalosis or the formation of calcium oxalate deposits in vital tissues or organs of the body (Sanz & Reig, 1992). The minimum dose of oxalate required to cause death in an adult is 4 - 5 g. However, there are some reports recording that 10–15 g is the usual amount required to cause fatalities (Noonan & Savage, 1999). The oxalate intake should be less than 40–50 mg per day recommended by the American Dietetic Association, (2005) for patients with kidney stone problems (Ertop & Bektas, 2018). Oxalate contents of tested samples ranged between 11.25±0.00 mg/100 g (cooked Pani ala) and 78.75±0.00 mg/100 g (uncooked Kirikodol). Cooked yam samples recorded significantly lower oxalate contents compared to uncooked yams. According to the above safety standards, reported by various scientists, at least around 5 kg of yams are required to ingest the lethal dose of oxalates. However, consumption of Pani ala or Kirikodol may not be safe for persons with kidney stones.

Further, uncooked Kirikodol recorded the highest values for all the tested anti-nutrients and the lowest values were recorded by cooked Pani ala (Table 1). Cooking made a significant effect on all three tested anti-nutrients by reducing the amount.

Table 1. Alkaloids, saponin and oxalate contents of uncooked and cooked yams

	Pani ala			**Kirikodol**		
	Uncooked	**Cooked**	**Reduction (%)**	**Uncooked**	**Cooked**	**Reduction (%)**
Alkaloid (%)	0.21±0.01	0.16±0.01	23.8	0.36±0.01	0.28±0.01	22.2
Saponin (%)	2.2±0.3	1.5±0.2	31.8	3.8±0.3	3.4±0.3	10.5
Oxalate (mg/100 g)	33.75±0.00	11.25±0.00	66.7	78.75±0.00	56.25±0.00	28.6

CONCLUSION

Alkaloids and saponin contents of Pani ala and Kirikodol were at a low level. According to the previously reported safety standards, both uncooked and cooked Pani ala and Kirikodol consisted of a safe level of oxalate for consumption as food. Further, cooking is an effective way of reducing the alkaloids, saponin, and oxalate contents in Pani ala and Kirikodol.

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