

## Reduction in oxalate, acidity, phenolic content and antioxidant activity of *Amorphophallus paeoniifolius* var. Gajendra upon cooking

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### Abstract

The presence of oxalate and acidity has led to gross underutilization of *Amorphophallus paeoniifolius* (elephant foot yam- EFY), a salubrious tuber, consumed traditionally as a vegetable. Therefore, as a means to overcome oxalate and acidity problem, cooking in boiling water was studied. Boil-cooking of the EFY significantly decreased ( $p < 0.01$ ) the oxalate content, both soluble and insoluble. Boiling for 10 min caused 35.39% reduction in soluble oxalates and 44.76% in total oxalates. However, further increasing boiling duration did not cause any significant change in oxalates. Similar trends were noticed in the cooking-mediated reduction in sensorial acidity score. Alternately, boiling also reduced ( $p < 0.01$ ) phenolic content and antioxidant activity (DPPH) of EFY. Ten min boiling was found to be sufficient for reduction of oxalates to a reported safer level of 71 mg/ 100g. Therefore, proper cooking of the tuber can certainly enhance its consumption pattern without any possible deleterious effect (renal functioning and mineral bioavailability) on human population.

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### Keywords

Elephant foot yam

Oxalate

Acidity

Phenolic content

Boiling

### Introduction

Elephant foot yam (*Amorphophallus paeoniifolius* (Dennst.) Nicolson), an important but underutilized tuber of family Araceae (Aroids) is native to Asia. It is also called as King of Tuber Crops (Sengupta *et al.* 2008). The dry matter content in the tuber ranges from 17.50 to 24%, starch from 13.93 to 21.53%, sugar from 0.55 to 1.77%, protein from 0.84 to 2.60%, fat from 0.07 to 0.37% (Chattopadhyay *et al.* 2009). It is eaten in varied manners- boiled like potatoes and eaten with mustard, as curry, as pickle after boiling with tamarind leaves, as preserve after cooking in syrup. It can also be cooked with salt, chilly, tamarind and turmeric powder and is used as curry (Yesodharan and Sujana, 2007). In Assam (India), farmers consume a special dish made of EFY in the month of Bhadoh, which they perceive to be strength giving (Borah *et al.* 2008). Therapeutic uses of EFY include arsa (haemorrhoids), pliha (splenic disorders), gulma (tumor conditions), svasa (breathing disorders), kasa (cough) and asthila (prostate disorder) (Ayurveda Pharmacopeia of India, 2007). Various tuber extracts have been found to possess analgesic, cytotoxic activity, immune-modulatory activity, anthelmintic activity, anti-inflammatory, hepato-protective and anxiolytic activity (Shilpi *et al.* 2005; Angayarkanni *et al.* 2007; Tripathi *et al.* 2010; Ramalingam *et al.*

2010; De *et al.* 2010; Hurkadale *et al.* 2012; Saha *et al.* 2013). Recent work have shown that tuber extracts possess cytotoxic and apoptic activity against human colon carcinoma cell line HCT-15 (Ansil *et al.* 2014).

The major problem associated with the consumption of EFY is its acidity and/or oxalate content. These factors have averted the usage of elephant foot yam as a food crop. Acidity is experienced as irritative (itching-stinging-burning) sensation in the mouth and throat which may be followed by swelling. Its rubbing on external skin may also induce itching, thus pointing towards the intensity of irritation. The acidity is caused by needle like crystals of oxalate called raphides (Bradbury and Nixon, 1998; Lewu *et al.* 2010). Apart from acting as irritant, oxalate is considered to be anti-nutritional and toxic (Guil-Guerrero, 2014). Ingestion of higher amount of oxalate (2 g) can be fatal to humans (Libert and Franceschi, 1987). Oxalates can render minerals like iron, calcium, zinc, magnesium unavailable to body by chelating them. Therefore, consuming foods containing oxalate may cause deficiency of essential minerals in the body. The crystals of oxalates can also get deposited in kidney and cause renal stones, leading to renal failure. About 75% of kidney stones are composed of oxalates and consumption of foods containing oxalate increase urinary oxalate content to variable extent (Williams and Wandzilak, 1989;

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Holmes *et al.* 2001). Reducing oxalate in diet is necessary to prevent oxalate related maladies (Massey *et al.* 1993). For patients with kidney stone, dietary intake of oxalates should be restricted to 40–50 mg/ day (ADA, 2005).

Therefore, removal of acidity/ oxalate will benefit in better utilization of Araceae plants for food and feed purposes. Boiling as food processing operation could be effective in alleviating the oxalates. Osisioigu *et al.* (1974) found reduction in irritant effect of cocoyam after boiling for 15 min and complete disappearance after 1 h boiling. Wanasundera and Ravindran (1992) reported 40–50% loss of total oxalates in yam tubers (*Dioscorea alata* and *D. esculenta*) upon boiling. Iwuoha and Kalou (1995) also found boiling to cause significant reduction ( $p < 0.05$ ) in oxalate content of *Colocasia esculenta* and *Xanthosoma sagittifolium*. Bhandari and Kawabatta (2006) processed 4 different cultivars of wild yam tubers of Nepal by boiling and reported reduction of 31–53%. Catherwood *et al.* (2007) reported 64–77% reduction in total oxalate level of 4 different cultivar of Japanese taro by boiling.

Until now there is lack of information regarding reduction in oxalate and/ or acidity parameter of *Amorphophallus paeoniifolius* due to boiling. Thus, the study was undertaken to assess the reduction in oxalate content and acidity upon boiling, a commonly resorted method of cooking.

## Materials and Methods

### Sample preparation

Fully mature elephant foot yam (var. Gajendra) tuber was procured from Navsari Agriculture University, Navsari, Gujarat during March. The tubers were washed thoroughly, peeled, diced into 2 cm cubes and were immediately dipped in 0.1% potassium metabisulphite solution for 5 min to prevent browning. The cubes were then blanched in boiling water (cubes: water- 1: 6) for 10 min and cooled. The blanched cubes were allowed to surface dry for 15 min and packed in nylon pouches (Hitkari Industries Ltd., Parwanoo) and stored in deep freezer ( $-20 \pm 2^\circ\text{C}$ ) till further study.

### Cooking treatment

The frozen EFY cubes were thawed overnight in a refrigerator ( $5 \pm 1^\circ\text{C}$ ) and then cooked in excess boiling water (EFY: water- 1:6) for different time period i.e. 10, 20, 30 and 40 min. After boiling, EFY cubes and soak water was collected separately. EFY cubes were spread on blotting paper under air for cooling and then made into paste using mixer

(Maxie food processor, Inalsa Appliances, India) and stored at  $-20^\circ\text{C}$  till further analysis. The oxalate content (via hydrothermal degradation) as well as sensorial acidity was evaluated along with other characteristics viz. whiteness index, phenolic content and DPPH (2, 2 diphenyl-1-picryl hydrazyl) activity. The soak water was examined for the loss of yam solids.

### Physico-chemical analysis

Oxalate content was extracted and determined as per Okombo and Liebman (2010), with minor modifications. For total oxalate, 10 g sample was weighed into a 250 ml Erlenmeyer flask with 50 ml of 2 M hydrochloric acid. The flask was covered with aluminium foil and placed into water bath (Laboratory Glassware Co., Ambala Cantt., India) at  $75^\circ\text{C}$  for 30 min with intermittent swirling (5 min interval). The flask was then allowed to cool and 50 ml of deionised water was added and swirled. The mixture was then filled in plastic centrifuge tubes and centrifuged for 10 min at 3000 rpm. The supernatant was then filtered into plastic vials and refrigerated prior to oxalate analysis. For soluble oxalate, extraction was carried with deionized water. The extracted samples were then analyzed for oxalate using the oxalate kit (Trinity Biotech Co., Wicklow, Ireland).

EFY paste was evaluated for sensory acidity by a panel of judges selected from the Division using a 5 point semi-structured acidity evaluation card. The panel members (Age group: 23–28 years; Sex: male) who were trained to judge the EFY acidity by applying the yam paste on the soft part (inside) of the forearm for 3–4 min for feeling the itchy sensation if any, were asked to make a “vertical mark” giving the relevant sample number along a 5-point semi-structured linear scale to indicate the intensity of acidity (0-no acidity, 4- extreme acidity) sensation (Kumar *et al.* 2013).

Hunter  $L^*$ ,  $a^*$ ,  $b^*$  values of EFY paste were measured employing spectro-colorimeter, Colorflex® Model No. 45/0 (Hunter lab, Reston, USA) and converted to whiteness index (WI) from formula as shown;

$$WI = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}}$$

Solid loss in soak/ cook water was determined by the gravimetric method as described in AOAC (2000).

Total phenolic content and antioxidant activity was evaluated in hydro-methanolic extract. 10 g of paste was weighed with 50 ml of 80% methanol and shaken for 1 h. It was then centrifuged at 4000

Table 1. Changes in oxalate, acidity and other physico-chemical parameters upon boiling of elephant foot yam

Boiling time (in min)	Soluble oxalate (mg/ 100g)	Total oxalate (mg/ 100g)	Acridity score	Whiteness index	Solids loss (%)	Total phenol (mg GAE/ g)	DPPH activity ( $\mu\text{mol TE/ g}$ )
10	8.38 $\pm$ 0.16 <sup>b</sup>	39.99 $\pm$ 0.76 <sup>b</sup>	0.16 $\pm$ 0.01 <sup>b</sup>	55.91 $\pm$ 0.43	0.67 $\pm$ 0.05 <sup>a</sup>	7.01 $\pm$ 0.10 <sup>c</sup>	6.46 $\pm$ 0.10 <sup>c</sup>
20	8.15 $\pm$ 0.07 <sup>ab</sup>	38.02 $\pm$ 0.26 <sup>a</sup>	0.12 $\pm$ 0.01 <sup>a</sup>	55.82 $\pm$ 0.45	1.27 $\pm$ 0.07 <sup>b</sup>	5.98 $\pm$ 0.12 <sup>b</sup>	5.43 $\pm$ 0.25 <sup>bc</sup>
30	7.89 $\pm$ 0.12 <sup>ab</sup>	37.66 $\pm$ 0.15 <sup>a</sup>	0.10 $\pm$ 0.01 <sup>a</sup>	55.16 $\pm$ 0.24	1.62 $\pm$ 0.06 <sup>c</sup>	4.74 $\pm$ 0.06 <sup>a</sup>	4.35 $\pm$ 0.42 <sup>b</sup>
40	7.66 $\pm$ 0.11 <sup>a</sup>	37.14 $\pm$ 0.27 <sup>a</sup>	0.10 $\pm$ 0.01 <sup>a</sup>	54.94 $\pm$ 0.87	1.95 $\pm$ 0.07 <sup>d</sup>	4.37 $\pm$ 0.10 <sup>a</sup>	2.74 $\pm$ 0.31 <sup>a</sup>
F value	6.81 <sup>**</sup>	20.19 <sup>**</sup>	11.05 <sup>**</sup>	0.77	71.48 <sup>**</sup>	149.82 <sup>**</sup>	29.55 <sup>**</sup>

Values are mean  $\pm$  standard error; n= 4; means with different alphabetic superscript in different rows are significantly different as determined by Tukey's test; \*\* p < 0.01, \* p < 0.05

rpm for 30 min. The supernatant was collected and extraction was repeated. The supernatant was pooled and stored at -20°C till analysis. The total phenolic content was determined as per Cordenunsi *et al.* (2004). Standard curve was prepared using gallic acid. Based on absorbance, the total phenolic content of sample was calculated using standard curve equation,  $y=0.0025x+0.0209$ ;  $R^2=0.99$ ; where, y is absorbance and x is concentration of gallic acid (GA) and results were expressed as  $\mu\text{g GA per g}$  of sample. Antioxidant capacity based on DPPH (2, 2 diphenyl-1-picryl hydrazyl) radical was analyzed following the method given by Brand-Williams *et al.* (1995). Based on percent inhibition of absorbance of sample, trolox equivalent was determined using following standard curve equation:  $y=531.51x-0.22$ ;  $R^2=0.99$ ; where, y is the percent inhibition and x is the concentration of trolox ( $\mu\text{mol}$ ). The results were expressed as  $\mu\text{mol trolox equivalent (TE) per g}$  sample.

#### Statistical analysis

The experiments were conducted in quadruplicates and data obtained were analyzed by one way analysis of variance using IBM SPSS Statistics version 20. Significant differences between means were determined using Tukey's test.

## Results and Discussion

#### Effect of boiling on oxalate content (soluble and total) of EFY

The soluble oxalate (water soluble) exists in plants as crystals of sodium, potassium and ammonium salts (Holloway *et al.* 1989). Oxalic acid chelates the metal ions and exists as crystals of water insoluble salt of calcium, iron or magnesium (Noonan and Savage, 1999). Apart from acting as an irritant, oxalates have their own repercussions on human body, the major effects being deposition of calcium oxalate

crystals in the kidneys (renal stones) and reduction in bioavailability of minerals. The total oxalate content in various species of Dioscorea varied from 67-104 mg /100g while soluble oxalate content varied from 37-85 mg/ 100g (Bhandari and Kawabatta, 2004). The soluble oxalate content in various varieties of EFY have been found to vary from 2.91 to 18.50 mg/ 100g (Chhattopadhyay *et al.* 2009). Boiling is one of the best means employed by humans for making foods more palatable and less toxic. A significant destructive effect of boiling upon oxalate level was observed. The soluble oxalate level declined from 12.97 mg/ 100g (0 min boiling) to 7.66 mg/ 100g (40 min boiling) i.e. by 40.9%, whilst the total oxalate content (soluble and insoluble together) decreased from 72.39 mg/ 100g (0 min boiling) to 37.14 mg/ 100g (40 min boiling) i.e. by 48.7%. Table 1 further shows that both oxalates declined increasingly with increasing boiling duration, but the greatest reduction occurred during initial 10 min of boiling; the oxalate content in yam cooked for longer duration (20, 30 and 40 min) did not differ significantly from that obtained after 10 min boiling. Boiling has been reported to be effective in reducing the oxalates in various root crops i.e. colocasia, wild yam, Japanese taro and trifoliate yam tuber (Iwuoha and Kalu, 1995; Bhandari and Kawabatta, 2006; Catherwood *et al.* 2007; Abiodun *et al.* 2014). Shimi and Haron (2014) suggested optimal cooking for reducing the oxalate content. Thermal degradation/ decomposition at higher temperature and leaching of oxalates in cook water) might have caused the reduction (Sefa-Dedeh and Agyir-Sackey, 2004; Judprasong *et al.* 2006). Leaching of oxalate is facilitated by skin softening during boiling.

#### Effect of boiling on acidity of EFY

Acidity is an unpleasant phytochemical quality found in plants of Araceae (Aroid) family. The corms

and leaves of most cultivars of the edible aroids are acrid i.e. if eaten raw, they may cause soreness and swelling of the lips, mouth and throat. Acrid sensation is perceived as a severe itching, stinging or burning sensation in the mouth and throat, followed by swelling. There might be less severe irritation or itching of external skin, on hands and arms. Calcium oxalate crystals are thought to be causing itching sensation. When in sufficient quantity, calcium oxalate crystals cause mechanical abrasion of the mucous membranes and causes irritation and burning sensation in the mouth and throat. Acridity i.e. itchiness felt upon consumption of EFY was determined by hand sensing technique as carried out by Paull *et al.* (1999). The sensory acidity scores varied from 2.12 (0 min boiling) to 0.10 (40 min boiling) (Table 1). The EFY not subjected to boiling was perceived to be definitely acrid by the judges as the application of paste on the forearm caused a definite itching sensation. Cooking by boiling for 10 min or more significantly ( $p < 0.01$ ) decreased the acidity, although the decrease was slight and non-significant in the yam cooked beyond 10 min. The EFY was perceived to be only minutely acrid after cooking in boiling water.

#### *Effect of boiling on loss of solids in cook water*

Solids loss in cooking water is an important parameter affecting the viability of boiling in water as cooking method. It reflects the hydrothermal degradation occurring in food on cooking and thereby serves as an indicator of lethality of boiling as cooking process. The loss of solids during cooking even during steeping, is a normal phenomenon which can lead to decreased nutritional and economic value of the food, but also can help in effacing anti-nutritional elements present in the food system. The boiling time appeared to have a significant direct effect on the solids loss occurring in cook water (Table 1). The solids loss ranged from 0.67 to 1.95%. The significant increase ( $p < 0.01$ ) in losses with cooking time is invariably a result of softening arising from the rise in temperature and cooking duration (Chiang and Luo, 2007). Vegetable softening during cooking occurs due to loss in turgor and changes occurring in cell wall matrix polysaccharides which causes loss of water and cellular solutes (Lewicki, 1998; Gliszczynska-Swigło *et al.* 2006; Xu *et al.* 2014). Boiling has also been known to cause skin rupture of the tuber (Albihn and Savage, 2001). The thermal softening behavior of cassava has been well illustrated by Sajeev *et al.* (2008) and Sajeev *et al.* (2010).

#### *Effect of boiling on whiteness value of EFY*

The whiteness value of EFY paste obtained after cooking in boiling water for different periods are presented in Table 1. Cooking in boiling water did not alter the whiteness value of the EFY paste significantly (Table 1), although a very minute decline (non-significant) in whiteness was observed with increasing cooking time. Thus there was no perceivable browning (enzymatic and non-enzymatic) during the plain-water boiling process. Polyphenol oxidases, the main causatives of enzymatic browning are heat labile in nature and are destroyed at 80°C (Vamos-Vigyazo, 1981).

#### *Effect of boiling on phenolic content and antioxidant activity of EFY*

The total phenolic content (in terms of gallic acid) as determined by the Folin-Ciocalteu Reagent assay, declined from 7.01 mg/ g (10 min boiling) to 4.37 mg/ g (40 min boiling) and, at the same time DPPH scavenging activity (in terms of trolox) dropped from 6.46  $\mu\text{mol/g}$  (10 min boiling) to 2.74  $\mu\text{mol/g}$  (40 min boiling). The total phenolic content in wild yam tubers ranged from 13 to 166 mg/ 100g (Bhandari and Kawabata, 2004). Both the phenolics and DPPH activity showed a high degree of correlation ( $r=0.93$ ). Referral to Table 1 indicates that an increased boiling time caused a significant decrease ( $p < 0.01$ ) in both the anti-oxidative capacity determining parameters. This, in turn, points toward the loss of phytochemicals (antioxidants) during the cooking process. Leaching of phenolic antioxidant constituents in cook water upon boil-cooking, has been reported to be the main factor causing reduction in anti-oxidative parameters (Xu and Chang, 2008). Further, boiling itself is high thermal lethality process. The breakdown of phenolics during boiling treatments may also have contributed toward the declining trend in the total phenolic content and DPPH activity (Scalzo *et al.* 2007). The other possibilities favouring the reduction of antioxidants as a result of cooking of the yam could be chemical transformation, decomposition of phenolics, protein-polyphenol interaction under thermal conditions (Makris and Rossiter, 2001; Murakami *et al.* 2004; Perla *et al.* 2012) etc. Various workers have experimented upon numerous vegetables to discover cooking, boiling in particular, to be negatively impacting the anti-oxidative nature.

#### **Conclusion**

Boiling, a commonly utilized cooking process was examined for its effect on oxalate and acidity problem found in *Amorphophallus paeoniifolius*.

Boiling caused an appreciable reduction in oxalate content (both soluble and total) as well as sensorial acidity score. Simultaneously, reduction in total phenolic content as well as DPPH activity and increment in solids loss in cook water was also noticed. Boiling elephant foot yam for ten minutes was found to be sufficient in reducing the oxalates way below the reported safer level of 71 mg/ 100g (Kumoro *et al.* 2014).

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