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Effects of Boiling Methods on Anti-nutritional Factors of Anchote (*Coccinia Abyssinica* (lam.) Cogn) tubers Grown in Western Ethiopia

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Abstract: the raw and boiled Anchote (Coccinia abyssinica) tubers were studied and compared for their anti-nutritional factors. The raw, boiled after peeling and boiled before peeling Anchote tubers had respective contents (mg/100g) of phytate 389.30, 333.63 and 334.74; for oxalate contents were 8.23, 4.23, and 4.66; for tannin contents were 173.55, 102.36 and 121.21; for cyanide contents were 12.67, 8.16 and 11.14.
Keywords: Anchote, boiled after peeling, boiled before peeling, anti-nutritional factors

1. Introduction

Anchote (*Coccinia abyssinica)* tubers are an endemic to the Western parts of Ethiopia (Amare, 1973), mainly in the Western region of Ethiopia highlands in Eestern Wollega, Western Wollega, Kelam Wollega, and Mattu (Westphal, 1974). The most widely used vernacular name is Anchote, spelt Ancootee in Oromo. It is also called: Ushushu (Welayita), Shushe (Dawuro), and Ajjo (Kafigna) (Demel *et al*., 2010). Like many other root, and tuber crops, Anchote is rarely eaten raw (Fufa, and Urga, 1997). Traditionally, boiled after peeling or boiled before peeling and/ or further cooking are applied prior to consumption. Anchote is found both cultivated and wild (Edwards, 1991). The total yield of Anchote is 150-180 quintals/hectare, which is in the range of the total yield of sweet potato, and potato (IAR, 1986). Anchote is Anchote is a valuable food source and according to local farmers, it helps in fast mending of broken/ fracture bones and displaced joints, as it contains high calcium, and proteins than other common and wide spread root and tuber crops (Endashaw, 2007). Traditionally, it is also believed that, Anchote makes lactating mothers healthier and stronger (Abera, 1995). Dawit and Estifanos (1991) reported that the juice prepared from tubers of Anchote has saponin as an active substance and is used to treat Gonorrhoea, Tuberculosis, and Tumor Cancer.

According to Aletor (1993), there are several antinutritional factors that are very significant in plants used for human foods. Anti-nutritional factors are known to reduce the maximum utilization of nutrients especially proteins, vitamins, and minerals (Ugwu and Oranye, 2006). Several anti-nutritional factors are present in root and tuber crops and are partially neutralized during ordinary cooking (Bhandari and Kawabata, 2004). Among Various antinutrients, and plant toxins, Phytate, Oxalate, Tannin, Cyanide and Trypsin inhibitors are found in root and tuber crops (Wanasundera and Ravindran, 1994), which may have adverse effects on health through inhibition of digestion, absorption, and growth.

Anchote, like many other root, and tuber crops, is rarely eaten raw. Traditionally, boiled after peeling or boiled before peeling and/or further cooking are applied before consumption. Presumed purpose of such processing is to make Anchote more palatable, digestible, to inactivate enzyme inhibitors, and other anti-nutritional factors to qualify it for human consumption. In the case of Anchote, however, no published information is available as to which traditional processing methods are optimal to reduce the effects of the inherent antinutritional factors. Therefore, it is imperative to investigate which traditional methods are decreases of its risk of human health. The main objective of this research was to determine the effect of traditional processing methods on anti-nutritional factors of Anchote (*Coccinia abyssinica* (Lam.) Cogn.) tubers grown in Western Ethiopia.

2. Materials and methods

2.1. Sample collection

A total of about 6 kilograms uninfected Anchote were collected from the 12 famers randomly selected (0.5 kilogram per house hold) of study site (Hara, Wayu kumba and Wayu kiltu kebeles) in Jima Arjo woreda, East Wollega Zone, Western Ethiopia. The samples were packed in polyethylene bags, kept in an ice box (to prevent moisture loss), and transported to Food Science and Bioprocess Technology Institute Research laboratory of Wollega University within three hours. Once in the laboratory, samples were mixed for composite analysis of the study variables and washed by clean water all together. The washed tuber was used for nutritional analysis.

2.2. Sample preparation

The washed sample was grouped into three lots of two kilograms each. The first lot was used for analysis of raw Anchote tubers. The raw sample was sliced to uniform thickness 5 mm using a stainless steel knife. The second lot was used as boiled after peeling. The tuber was peeled and boiled for about three to three and half hours and

sliced to uniform thickness 5 mm using a stainless steel knife. The third lot was served as boiled before peeling. The washed tuber was boiled for about three to three and half hours, peeled and sliced to uniform thickness 5 mm using a stainless steel knife.

For anti-nutritional factors analyses, each of the three lot of samples were dried at a time in oven (Gallenkamp Hotbox Oven, size 2, Gallenkamp, UK) at 60°C for 72 hours. Each dried samples were milled into fine powder using electric grinder (NIMA-8300Burman, Germany) until to pass through 0.425 mm sieve mesh size, and finally packed into airtight polyethylene plastic bags to minimize heat build-up, kept in ice box and transported to Addis Ababa University, and stored in the desiccator until required for analysis.

2.4. Analysis of antinutritional factors

Determination of phytate content

The phytate was determined according to Latta andEskin (1980) and later modified by Vaintraub and Lapteva (1988). The absorbance of supernatant was measured at 500 nm using a UV-1600 spectrophotometer (UV-Visible spectrophotometer, Shimadzu, Japan). The phytate concentration was calculated from the difference between the control absorbance and that of the assayed sample. A series of standard solutions containing 5-40 mg/ml of phytic acid in distilled water was prepared and a standard curve was prepared (Latta & Eskin, 1980). The concentration of phytate was calculated from the standard curve, and results were expressed as phytic acid in mg per 100 g dry matter.

Determination of oxalate content

Oxalate was analyzed using the method originally employed by Ukpabi and Ejidoh (1989) in which the procedures involve three steps: digestion, precipitation, and permanganate titration. About 2.000 g of Anchote samples of each treatment in triplicates were suspended in 190 ml de-ionized water contained in a 250 ml volumetric flask; 10 ml of 6 M HCl was added and the suspension digested at the boiling point of water for 1 hr that followed by cooling. Then made up to 250 ml and filtered. Duplicate portion of 125 ml of filtrate were measured in to a beaker and four drops of methyl red indicator added, followed by the addition of concentrated NH_4OH solution drop wise until the test solution changes from salmon pink color to faint yellow color (pH 4-4.5). Each portion was then heated to 90 ^oC, cooled and filtered to remove precipitate containing ferrous ion. The filtrate was then again heated to 90 ^oC and 10 ml of 5 % $CaCl_2$ solution was then added while being stirred constantly. After heating it was cooled and left overnight in refrigerator. The solution was then centrifuged at a speed of 2500 rpm for 5 min the supernatant was decanted and the precipitate completely dissolved in 10 ml of 20 % (v/v) H_2SO_4 solution. At this point the total filtrate resulting from digestion of 2 g of flour was made up to 300 ml. aliquots of 125 ml of filtrate were heated until near boiling, and then titrated against 0.05 M standard $KMnO_4$ solution to a faint pink color which persists for 30 seconds.

Determination of condensed tannin content

Tannin content was determined by the method of Burns (1971) as modified by Maxson and Rooney (1972), using catechin as the tannin standard. About 2.0000g of Anchote samples of each treatment in triplicates were weighed in a screw cap test tube and extracted with 10ml of 1% HCl in methanol for 24 hours at room temperature with mechanical shaking. After 24 hours shaking, the solution was centrifuged at 1000rpm for 5 minutes. A 1ml of supernatant was taken and mixed with 5 ml of vanillin-HCl reagent (prepared by combining equal volume of 8% concentrated HCl in methanol and 4% Vanillin in methanol).

D-catechin was used as standard for condensed tannin determination. A 40mg of D- catechin was weighed and dissolved in 1000 ml of 1% HCl in methanol, which was used as stock solution. A 0, 12, 24, 36, 48 and 60 ml of stock solution was taken in test tube and the volume of each test tube was adjusted to 1ml with 1% HCl in methanol. A 5ml of vanillin-HCl reagent was added into each test tube. After 20 minutes, the absorbance of sample solutions and the standard solution were measured at 500nm by using water to zero the spectrophotometer, and the calibration curve was constructed from the series of standard solution. A standard curve was made from absorbance versus concentration and the slope and intercept were used for calculation.

Determination of cyanide content

Cyanide content of Anchote samples were determined according to the official standard method of AOAC (1984), by Silver Nitrate titrimetric methods, in which the steps of distillation and titration was involved. About 10g of Anchote samples of each treatment in triplicates were weighed into a flask and soaked in 100ml of distilled water in separate 500 ml round bottom flask for 2hr. The Kjeldahl flask was adjusted before distilling the tip of delivery tube below surface of liquid and 100 ml distilled water were added. Thereafter, the mixtures in the flask were heated by steam distillation. The released cyanide was collected in a conical flask containing in 20 ml 0.01N $AgNO_3$ acidified with 1 ml concentrated HNO_3. When the gas has passed over, the distillate was filtered through sintered glass crucible and rinsed the test tube with little water. The distillate was then titrated against excess $AgNO_3$ with 0.02N KSCN, using ferric alum indicator. At the end point of titration, the color of the indicator changed from red to purple color. Using the relationship1 ml of 0.01 N $AgNO_3$ = 0.27 mg of cyanide.

2.6. Statistical analysis

Samples from each treatment were analyzed in triplicate. Data were subjected to analysis of variance (ANOVA) using SPSS version 15.0 for windows. Means were compared using Duncan's multiple range test.

3. Result and Discussion

3.2. Anti-nutritional factors content of raw and processed Anchote tubers

Some anti-nutritional factors (phytate, oxalate, tannin and cyanide) content of the raw and processed Anchote tuber is shown in Table 1.

Phytate

The raw Anchote tuber contained 389.30 mg/100g phytate. The phytate content of Anchote boiled after peeling and before peeling had 333.63 mg/100g and 334.74 mg/100g, respectively. The phytate content of Anchote boiled after peeling was significantly ($P<0.05$) lower than both boiled before peeling and raw Anchote tubers. Similarly, the mean phytate content of Anchote boiled before peeling was significantly ($P<0.05$) lower than raw Anchote tuber. The mean phytate content was reduced in boiled after peeling by 14.30% and in boiled before peeling by 14.01% compared to raw tubers. The evident reduction in phytate during cooking may be caused by leaching into the cooking medium, degeneration by heat or the formation of insoluble complexes between phytate and other components, such as phytate-protein and phytate-protein-mineral complexes (Sidhtraju and Becker, 2001). The reduction of phytate during processing Anchote tuber is expected to enhance the bioavailability of proteins and dietary minerals of the tubers and at the same time the lower level of phytate may have some health promotional activities. Currently there is evidence that dietary phytate at low level may have beneficial role as an antioxidant, anticarcinogens and likely play an important role in controlling hypercholesterolemia and atherosclerosis (Phillippy *et al.*, 2004). Because Anchote may provide a substantial portion of phytate, the nutritional consequences of phytate in Anchote should be investigated.

Oxalate

The raw Anchote tuber contained 8.26 mg/100g oxalate. The oxalate content of boiled after peeling and boiled before peeling of Anchote tuber had 4.23 mg/100g and 4.66 mg/100g, respectively. The oxalate content of Anchote boiled after peeling was significantly ($P<0.05$) lower than both boiled before peeling and raw Anchote tubers. Also the mean oxalate content of Anchote boiled before peeling was significantly ($P<0.05$) lower than raw Anchote tuber. The mean oxalate content was reduced in boiled after peeling by 48.79% and in boiled before peeling by 43.58% compared to raw Anchote tubers. The traditional processing methods were found effective methods to reduce the oxalate content in these tubers. Boiling may cause considerable cell rupture and facilitate the leakage of soluble oxalate into cooking water (Albihn and Savage, 2001), this may be the possible reason to observed high reduction in oxalate level upon boiling.

Table 1. Mean (± SE) anti-nutritional factors content of raw and processed Anchote

Treatment	Phytate (mg/100g)	Oxalate (mg/100g)	Tannin (mg/100g)	Cyanide (mg/100g)
RW	389.30 ± 0.39^{a}	8.23 ± 0.09^{a}	173.55 ± 0.35^{a}	12.67 ± 0.22^{a}
BAP	333.63 ± 0.29^{c}	4.23 ± 0.02^{c}	102.36 ± 0.46^{c}	8.16 ± 0.07^{c}
BBP	334.74 ± 0.42^{b}	4.66 ± 0.17^{b}	121.21 ± 0.11^{b}	11.14 ± 0.17^{b}

Means not followed by the same superscript letters in the same column are significantly different ($P<0.05$).
NB. RW stands for Raw Anchote, BAP: for Boiled after peeling and BBP: for Boiled before peeling.

Oxalates can have a harmful effect on human nutrition and health, especially by reducing calcium absorption and aiding the formation of kidney stones (Noonan and Savage, 1999). High-oxalate diets can increase the risk of renal calcium oxalate formation in certain groups of people (Libert and Franceschi, 1987). The majority of urinary stones formed in humans are calcium oxalate stones (Hodgkinson, 1977). Currently, patients are advised to limit their intake of foods with a total intake of oxalate not exceeding 50–60 mg per day (Massey *et al.*, 2001). The traditionally processed Anchote tubers analyzed in this study are low compared to the recommendations for patients with calcium oxalate kidney stones. Under these guidelines, processed Anchote tubers analyzed could be recommended not only for normal healthy people but also consumption for patients with a history of calcium oxalate kidney stones, assume about 1 kg of Anchote would be necessary for consumption per day. Therefore, the reduced oxalate content resulting from traditionally processed Anchote tubers could have a positive impact on the health of consumers to enhance the bioavailability of essential dietary minerals of the tubers, as well as reduce the risk of kidney stones occurring among consumers. Hence, boiling the tuber would reduce the nutritional problems that the high levels of oxa1ates could cause.

Tannin

The tannin content of raw Anchote tuber was 173.55 mg/100g. The tannin content of boiled after peeling and boiled before peeling of Anchote tuber had 102.36 mg/100g and 121.21 mg/100g, respectively. The tannin content of Anchote boiled after peeling was significantly ($P<0.05$) lower than both boiled before peeling and raw

Anchote tubers. Similarly, the mean tannin content of Anchote boiled before peeling was significantly ($P<0.05$) lower than raw Anchote tubers. The mean tannin content was reduced in boiled after peeling by 41.87% and in boiled before peeling by 30.12% compared to raw tubers. The reduction in the levels of tannin during heat treatment might be due to thermal degradation and denaturation of the antinutrients as well as the formation of insoluble complexes (Kataria *et al*., 1989). The toxicity effects of the tannin may not be significant since the total acceptable tannic acid daily intake for a man is 560 mg (Anonymous, 1973). Since the tannin content of raw Anchote tuber is very low compared to its critical toxicity effect and further reduced during traditional processing, its anti-nutritional effect may be insignificant in both raw and processed tuber.

Cyanide

The results of the present study showed that cyanide in raw, boiled after peeling and boiled before peeling Anchote tuber were 12.67 mg/100g, 8.16 mg/100g, and 11.14 mg/100g, respectively. The cyanide content of Anchote boiled after peeling was significantly ($P<0.05$) lower than both boiled before peeling and raw Anchote tubers. The mean cyanide content of Anchote boiled before peeling was also significantly ($P<0.05$) lower compared to mean raw.

The mean cyanide content was reduced in boiled after peeling by 35.59% and in boiled before peeling by 12.08% compared to raw tubers. It has been reported that higher intake of cyanides could result in the development of neurological disease in humans (Montgomery, 1980). The amounts of cyanide produced, only plants that accumulate more than 50 to 200 mg are considered to be dangerous (Kingsbury, 1964). However, smaller amount of cyanides could have several long-term adverse effects on human health (Bhandari and Kawabata, 2004). The results obtained showed that the processed tuber could be considered safe with regard to cyanide poisoning due to the fact that the cyanide levels were far below the detrimental levels of 50 to 200 mg (Kingsbury, 1964). However, the amount remaining cyanide content might be slightly toxic to people who consume high quantities of Anchote tubers and need to be further study.

4. Conclusion

The present finding uncovered information on antinutritional factors (Phytate, Oxalate, Tannin and Cyanide) of raw and boiled Anchote tubers collected from western Ethiopia. The results of this study showed that raw Anchote contains low levels of antinutrients (Oxalate, tannin, and cynide) except phytate, when compared to other reported raw roots and tubers. Moreover, there were further reductions of the antinutritional factors during boiling process.

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