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Evaluation of bioavailability and sensory preference of processed Anchote (Coccinia Abyssinica) tubers in Eastern Wollega, Ethiopia

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Abstract: Purpose-The purpose of this study was to determine the bioavailability and Sensory preference of processed Anchote (Coccinia Abyssinica) tubers grown in Eastern Wollega, Ethiopia. Method-A total of about 6 kilograms uninfected Anchote were collected from 12 famers randomly selected the study site in Jima Arjo woreda, East Wollega Zone, Ethiopia. The samples were packed in polyethylene bags, kept in an ice box, and transported to Food Science research laboratory of Wollega University. Then,samples were mixed for composite analysis and washed by clean water all together. The washed tuber was grouped in to three sections of two kilograms for each section. The first section was used for anti-nutritional analysis, The second section was used for mineral content analysis whereas the third section was used for sensory analysis. The molar ratios for oxalate, calcium, zinc, Iron and phytate were calculated to evaluate the effects of elevated levels of oxalate and phytate in the bioavailability of dietary minerals. Result-The raw, boiled after peeling and boiled before peeling Anchote tubers had respective contents (mg/100g) of Ca 119.50, 115.70, and 118.20; for Fe contents were 5.49, 7.60, and 6.60; for Mg contents were 79.73, 73.50, and 76.47; for Zn contents were 2.23, 2.03, and 2.20; and for P contents were 34.61, 28.12, 25.45. 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. Discussion-In this study, Anchote tubers were found to contain low antinutritional factors, and except phytate. Moreover, there were further reductions of the antinutritional factors during processing. This implies, except phytate high in minerals, thereby improving the bioavailability of zinc and calcium. This study also revealed that, there was significant ($P<0.05$) taste preference of Anchote boiled before peeling and boiled after peeling, in which 66% of consumers gave priority taste for Anchote boiled before peeling. Therefore, traditional processing method of Anchote boiled before peeling is also effective technique. Conclusion-The raw Anchote tubers were found to contain low antinutritional factors, except phytate. Moreover, there were further reductions of the antinutritional factors during traditional processing. This implies, except phytate which might hinder iron bioavailability, traditional processing enables that the antinutritional factors in the Anchote couldn't hamper its nutritional value. Therefore, both methods of traditional preparation of Anchote were effective to reduce the levels of antinutritional factors, thereby improving the bioavailability of zinc and calcium. This study also indicated that consumer panels preferred the taste of Anchote boiled before peeling. Therefore traditional processing method of Anchote boiled before peeling is also effective technique and need to be encouraged in terms of consumers preference of Anchote taste.

Keywords: Anchote Tuber, Mineral Contents, Anti-Nutritional Factors, Bioavailability, Sensory Preference

1. Introduction

Anchote (*Coccinia abyssinica* (Lam.) Cogn) is a tuber crop, belongs to the order *Cucurbitales,* family *Cucurbitaceae* (Asfaw *et al.,* 1992), indigenous to Ethiopia (Addis, 2005). There are about 10 species of *Coccinia* in Ethiopia; however, only *Coccinia abyssinica* is cultivated for human consumption (Endashaw, 2007). 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). 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 endemic to the Western parts of Ethiopia highlands (Amare, 1973; Westphal, 1974). 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). The juice prepared from tubers of Anchote has saponin as an active substance and is used to treat Gonorrhoea, Tuberculosis, and Tumor Cancer (Dawit and Estifanos 1991).

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. Such processing can have both detrimental and beneficial effect to the nutrient content of food. Presumed purpose of such processing is to make Anchote more palatable, digestible, to inactivate enzyme inhibitors, and other anti-nutrient to qualify it for human consumption. In spite of the substantial level of bioavailability and sensory preference of Anchote tubers, there are no published studies. Therefore, the main objective of this research was to determine the bioavailability and sensory preference of processed Anchote (*Coccinia abyssinica*) tubers grown in East Wollega, 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. The first section was used for anti-nutritional analysis, The second section was used for mineral content analysis whereas the third section was used for sensory 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 *anti-nutritional factors* of anchote tubers. The raw sample was sliced to uniform thickness 5 mm using a stainless steel knife. The second lot was used for mineral content analysis. 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 used for sensory analysis. 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.

Then, each of the three lot of samples were dried at a time in oven (Gallenkamp Hotbox Oven, 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.

3. Minerals Analysis

3.1. Determination of Calcium, Iron, Magnesium and Zinc

Calcium, iron, magnesium, and zinc were determined according to the standard method of AOAC (2000) using an Atomic Absorption Spectrophotometer (Varian Spectr AA. 20 plus). The washed silica dishes were placed in to Drying Oven at 90^{o}c for15 min. The dishes were then removed, and cooled down in desiccators for about 30 minutes, when cooled to room temperature weighed. About 2.000 g of Anchote samples of each treatment (Raw or control, boiled after peeling, and boiled before peeling) (in triplicate) were weighed in to each dish, then placed on a hot plate under a fume-hood in slowly increasing temperature until smoking ceases. When the samples become thoroughly charred, the dishes then placed in a Muffle Furnace, as near to centre as possible and ashed at 550 ^{0}C. The dishes were removed from a muffle furnace, cooled, seen to be clean, and white in appearance. Few drops of de-ionized water and concentrated nitric acid were added, dried, and return to a Muffle Furnace. Then checked until traces of carbon are fully ashed. Finally taken out of the muffle furnace placing immediately in a desiccators till cooled to room temperature.

The ash each sample was digested with 5 ml of 6 M HCl to wet it completely and carefully dried on a low temperature hotplate. 7 ml of 3 M HCl were added and the dish was heated on a hot plate until the solution just boils. Then it has been cooled, and filtered through a Whatman no.1 filter paper in to a 50 ml volumetric flask retaining as much of the solids as possible in the dish. Again 7 ml 3 M HCl was added to the dishes, and heat until the solution just boils. Then, cooled and filtered in to the volumetric flask. The dishes were then washed with water, and filtered in to the volumetric flask. The filter paper was washed thoroughly and collected in the flask. Since calcium is to be determined 2.5 ml of 10 % Lanthanum chloride solution

were added to the flask. Finally, diluted to the mark (50 ml) with freshly de-ionized water. The blank were prepared a blank by taking the same amount of reagents through all steps.

The stock standard solutions of minerals (zinc, calcium iron, and magnesium) were diluted with 0.3 N HCl to concentrations that fall within the working range (0.5, 1.0, 2.0, and 4.0 µg/ml for calcium analysis; 0.5, 1.0, 1.5, and 3.0 µg/ml for iron analysis; 0.25, 0.50, 0.75 and 1.50 µg/ml for magnesium analysis; 0.10, 0.20, 0.40, and 0.80 µg/ml for zinc analysis). The Atomic Absorption Spectrophotometer used for mineral determination was calibrated using standard solutions and the reagent blank solution was run with the sample.

The apparatus were set according to the instructions, and a calibration curve was prepared by plotting the absorption values against the metal concentration in µg/ml. Reading was taken from the graph, which depicted the metal concentrations that correspond to the absorption values of the samples, and the blank. The metal contents were calculated by using the following formula:

$$\text{Metal content (mg/100g)} = \frac{(A-B)xV}{10W}$$

Where:

W = wight of the sample (g)

V= volume of the extract (ml)

A = concentration (μg/ml) of sample solution

B = concentration (μg/ml) of blank solution

3.2. Determination of Phosphorus

Phosphorous was determined by the colorimetric method (Colorimeter SP 20, Bausch and Lamb) using Ammoniaum Molybdate (AOAC, 1984). About 1 ml of the clear extract solution was taken from the sample solution prepared from mineral analysis (determination of Ca, Fe, Mg and Zn) and diluted to 100 ml with deionized water in a 100 ml volumetric flask. A 5ml (triplicates) of the sample dilution was added into test tubes. A 0.5ml of molybdate and a 0.20ml aminonaphtholsulphonic acid was added into the test tube (sample solution) and mixed thoroughly step by step. A 0.20ml aminonaphtholsulphonic acid was added into the test tube repeatedly each time until the solution becomes clear. The solution was allowed to stand for 10 minute. The absorbance of the solution was measured at 660 nm against distilled water. Simultaneously with sample phosphorous, standard and blank analysis were carried out. Standard and blank solutions were prepared as above but 5 ml of working standard (reading A) and 5 ml of deionized water (reading B) in place of the sample dilution were used respectively. A standard curve was made from absorbance versus concentration. The phosphorus contents were calculated by using the following formula:

$$\text{Phosphorus (mg/100g)} = \frac{\text{A-B) x 50 x 100}}{\text{Slope x } W_f \text{ x 10}}$$

Where:

A = reading of the sample solution

B = reading of the blank solution

W_f = weight of sample.

The molybdate reagent was prepared by taking 75ml of 10N H_2SO_4 in a 250 ml volumetric flask and dissolving 6.25g of ammonium molybdate in a beaker in about 50ml of water and then transferring the beaker solution into volumetric flask. The aminonaphtholsulphonic acid reagent was prepared by dissolving 100mg of 1, 2, 4 aminonaphtholsulphonic acid in 39 ml of sodium bisulphite solution, and then adding 1 ml of sodium sulphite solution. A 15% sodium bisulphite and 20% sodium sulphite solution was prepared by dissolving 7.5g sodium bisulphate in 50ml of water and 2g sodium sulphite in 10ml of water respectively. The standard phosphrus solution was prepared by dissolving 438.8 mg of KH_2PO_4 (water free) in some water in a 100ml volumetric flask, adding 1 ml concentrated H_2SO_4 and diluting to the mark with water. The standard curve was made by taking 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0ml of the standard solution and diluting to 100ml.

4. Analysis of Antinutritional Factors

4.1. Determination of Phytate Content

Phytate was determined by the method of Latta and Eskin (1980) and later modified by Vantraub and Lapteva (1988). About 0.1000g of Anchote samples of each treatment (Raw or control, boiled after peeling, and boiled before peeling) were extracted with 10ml 2.4% HCl in a mechanical shaker (Eberbach) for 1hour at an ambient temperature and centrifuged at 3000rpm for 30 minute. The clear supernatant was used for phytate estimation. A 2ml of wade reagent (containing 0.03% solution of $FeC_{l3}.6H_2O$ and 0.3% of sulfosalicilic acid in water) was added to 3mL of the sample solution (supernatant) and the mixture was mixed on a Vortex (Maxi Maxi II) for 5 seconds. The absorbance of the sample solutions were measured at 500 nm using UV- VIS spectrophotometer (Beckman DU-64-spectrophotometer, USA).

A series of standard solution were prepared containing 0, 4.5, 9, 18, 27 and 36 µg/ml of phytic acid (analytical grade sodium phytate) in 0.2N HCl. A 3ml of standard was added into 15ml of centrifuge tubes with 3mL of water which were used as a blank. A 1mL of the Wade reagent was added to each test tube and the solution was mixed on a Vortex mixer for 5 seconds. The mixtures were centrifuged for 10 minutes and the absorbances of the solutions (both the sample and standard) were measured at 500nm by using de ionized water as a blank. A standard curve was made from absorbance versus concentration (Appendex VII) and

the slope and intercept were used for calculation. The phytate content was calculated by using the following formula:

Phytic acid (µg/100g) = [(absorbance - intercept)/ (slope * density * weight of sample)] * {10/3}

4.2. Determination of Phytate Phosphorus and Non-Phytate Phosphorus Content

Phytate and phosphorous were determined by the above methods. Phytate phosphorus was calculated by assuming 28.18% of phytate ($C_6P_6O_{24}H_{18}$) is phosphorus. The non-phytate phosphorus was determined from the difference between phytate phosphorus and total phosphorus, whereas, the proportion of phosphorous as phytate was calculated by phytate phosphorus devided by total phosphorus (Khetarpaul and Sharma, 1997). The phytate phosphorus, non-phytate phosphorus, and phosphorous as phytate content was calculated by using the following formula:

Phytate phosphorus (mg/100g) = phytate content (mg/100g) x 28.18%

Non-phytate phosphorus (mg/100g) = Phytate phosphorus (mg/100g) - Total phosphorus (mg/100g)

Phosphorous as phytate (%) = Phytate phosphorus (mg/100g)/ Total phosphorus (mg/100g)

4.3. 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, oxalate precipitation, and permanganate titration. About 2.000 g of Anchote samples of each treatment (Raw or control, boiled after peeling, and boiled before peeling) was 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 pointof water for 1 h 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. The oxalate content was calculated by using the following formula:

Oxalate (mg/100g) = $[(T * V_1 * D_f * 10^5)] / (V_2) * W]$

Where: T = Normality of Potassium permanganate; V_1= Volume of Potassium permanganate (titrant); D_f = the dilution factor which is 26.8; V_2 = Volume of extract oxalate (titrand) and W = Weight of sample in gram.

After analysis of phytate and oxalate the molar ratio of phytate and oxalate to calcium, zinc and iron were calculated to evaluate the effect of elevated levels of phytate and oxalate in the bioavailability of dietary minerals. As the ratios are the better indicators of the bioavailability than the amounts of the mineral and the phytic acid in the diet (Omoruyi *et al.*, 2007).

4.4. Determination of Condensed Tannin Content

Tannin content was determined by the method of Burns (1971) as modified by Maxson and Rooney (1972). About 2.0000g of Anchote samples of each treatment (Raw or control, boiled after peeling, and boiled before peeling) 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 (Appendex VIII) and intercept were used for calculation. The condensed tannin content was calculated by using the following formula:

$$\text{Condensed Tannin (mg/100g)} = \frac{(A_s - A_b) - \text{Intercept}}{\text{Slope} \times d \times W}$$

Where:

A_s = sample absorbance

A_b = blank absorbance

d = Density of solution (0.791g/ml)

W = Weight of sample in gram

4.5. 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 (Raw or control, boiled after peeling, and boiled before peeling) 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. The cyanide content was calculated by using the following formula:

$$\text{Cyanide (mg/100g)} = \frac{V_{AgNO3}*0.27}{W} \text{ X } 100$$

Where:

V_{AgNO3} = Volume of silver nitrate = (20-(2*V_{KSCN}))

V_{KSCN} = Volume of potassium thiocyanate consumed

W= weight of sample.

4.6. Sensory Analysis

Sensory evaluation was conducted in Food Science and Bioprocess Technology Institute research laboratory of Wollega University, Ethiopia. Boiled Anchote after peeling and boiled Anchote before peeling was evaluated by fifty consumers of 29(58%) males and 21(42%) females participants and 49 (98%) of the consumers were between the ages of 16-35 years. The consumers were recruited from staff and students of Food Science and Bioprocess Technology Institute at Wollega University, Ethiopia. The general demographic questions and frequency of consumption of consumers were completed before sensory evaluation of the products. They were selected if they indicated that they consumed Anchote boiled before and after peeling at least once per month. Additional criteria used to screen consumers were: no food allergies and/or no frequent illness, nonsmoker, willing to evaluate Anchote and available to participate during the scheduled testing dates.

In the sensory evaluation session, the consumers were seated in an open well illuminated laboratory and about 20 grams of each two samples were presented to each consumer on a tray at ambient temperature (≈25°C) within 2 hrs after boiling. The consumers were asked to indicate which of the two coded samples taste is preferred on the score card. The non-directional paired comparison test, exactly a two-sided preference (a version of paired comparison test) according to the "forced choice" technique (ISO, 1983), with the question: "Of these two samples, which one do you prefer?" was carried out with fifty consumers. The samples were served with identical container coded with 3-digit random numbers, half of the consumers were asked to taste one sample first, the others to taste it second. Necessary precautions were taken to prevent bias of tasting by ensuring that consumers rinsed their mouth with water before and after each tasting of sensory evaluation. Consumers expressed their preference taste of the boiled after peeling and boiled before peeling Anchote tubers using paired comparison test.

4.7. Statistical Analysis

Mineral and ant-nutritional analyses were followed one way analysis of variance (ANOVA). Means were compared using Duncan's multiple range test (Duncan, 1955). All the statistical analyses were performed on the results obtained using SPSS version 15.0 for windows. Also non-directional paired comparison t-test was used to analyze the responses of the consumers with regard to their preference taste for the sample (Roessler, *et al*., 1978). Significance difference was accepted at the 0.05 ($P<0.05$) level of probability (Steel and Torrie, 1980).

5. Result and Discussion

5.1. Mineral Content of Raw and Processed Anchote

Minerals in the diet are responsible for several existing problems relating to human health (Milton, 2003). The human body requires more than twenty two mineral elements that can be supplied by an appropriate diet in varying amounts for proper growth, health maintenance, and general well-being (WHO/FAO, 1998). Deficiency diseases could be prevented by sufficient intake of specific nutrients/minerals that are involved in many biochemical processes. Root and tuber crops are one of important sources of minerals that are linked to prevent deficiency diseases such as Anemia and Rickets and daily consumption of these foods is being encouraged (Leterme, 2002).

5.2. Calcium

Calcium is the major component of bone and assists in teeth development. Calcium concentrations are also necessary for blood coagulation and for the integrity of intracellular cement substances (Okaka and Okaka, 2001). The calcium content of the raw Anchote was 119.5mg/100g. The mean calcium contents of boiled after peeling and boiled before peeling of Anchote tuber was 115.70 mg/100g and 118.20 mg/100g, respectively. The mean calcium content of Anchote boiled after peeling was significantly ($P<0.05$) lower than both boiled before peeling and raw Anchote tubers. The mean calcium content of Anchote boiled before peeling was non significant ($P>0.05$) compared to mean raw Anchote. The calcium content was decreased in boiled after peeling by 3.18% and in boiled before peeling by 1.65% compared to raw tubers. The loss of calcium from boiling is not as such pronounced

and this little reduction may be due to less leaching of the calcium to the boiling water (Brody, 1994).

5.3. Iron

The mean iron content of the raw, boiled after peeling and boiled before peeling Anchote were 5.49 mg/100g, 7.60mg/100g and 6.60g/100g, respectively. The mean iron content of Anchote boiled after peeling was significantly ($P<0.05$) higher than both boiled before peeling and raw Anchote tubers. The mean iron content of Anchote boiled before peeling was non significant ($P>0.05$) compared to mean raw Anchote. The iron content was increased in boiled after peeling by 38.43% and in boiled before peeling by 20.22% compared to raw tubers. Increase in the iron content may be due to contamination of iron from the cooking utensils (Omoruyi *et al.*, 2007). In addition, the increment could be due to peeling has been done in a knife made of stainless steel then leaching from its skin or cooking utensils (Akin-Idowu *et al.*, 2009).

5.4. Magnesium

The mean magnesium content of the raw, boiled after peeling and boiled before peeling of Anchote tubers were 79.83 mg/100g, 73.50 mg/100g and 76.47 mg/100g, respectively. The magnisium content of Anchote boiled after peeling was significantly ($P<0.05$) lower than both boiled before peeling and raw Anchote tubers. Similarly, the mean magnisium content of Anchote boiled before peeling was significantly ($P<0.05$) different compared to mean raw Anchote. The magnesium content was reduced in boiled after peeling by 7.93% and in boiled before peeling by 4.21% compared to raw tubers. The reduction of magnesium from boiling might be due to the fact that magnesium oxalate is less soluble than the potassium and sodium salts (Poeydomenge *et al.,* 2007), this may be the possible reason to observed reduction in magnesium level upon boiling.

Table 1. *Mineral content of raw and processed Anchote samples*

Treatment	Calcium (mg/100g)	Iron (mg/100g)	Magnesium (mg/100g)	Zinc (mg/100g)	Phosphorus (mg/100g)
RW	119.50±0.36^{a}	5.49±0.39^{a}	79.73±0.85^{a}	2.23±0.12^{a}	34.61±0.70^{a}
BAP	115.70±0.21^{b}	7.60±0.19^{b}	73.50±0.92^{c}	2.03±0.06^{b}	28.12±0.08^{b}
BBP	118.20±1.49^{a}	6.60±0.32^{a}	76.47±0.61^{b}	2.20±0.10^{ab}	25.45±0.25^{c}

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.

5.5. Zinc

The zinc content of raw Anchote tuber with a mean value was 2.23 mg/100g. The mean zinc content of boiled after peeling and boiled before peeling of Anchote tuber was 2.03 mg/100g and 2.20 mg/100g, respectively. The zinc content of Anchote boiled after peeling was significantly ($P<0.05$) lower than raw Anchote tubers. The zinc content of Anchote boiled before peeling was non significant ($P>0.05$) compared to both mean boiled after peeling and raw Anchote. The mean zinc content was reduced in boiled after peeling by 8.97% and in boiled before peeling by 1.35% compared to raw tubers.

5.6. Phosphorus

The phosphorus content of the raw Anchote was 34.61 mg/100gm. The phosphorus content of boiled after peeling and boiled before peeling of Anchote tuber was 28.12 mg/100g and 25.45 mg/100g, respectively. The phosphorus content of Anchote boiled before peeling was significantly ($P<0.05$) lower than both boiled after peeling and raw Anchote tubers. In the same way, the mean phosphorus content of Anchote boiled after peeling was significantly ($P<0.05$) different compared to mean raw Anchote tubers. The mean phosphorus content was reduced in boiled after peeling by 18.75% and in boiled before peeling by 26.47% compared to raw tubers. The losses of phosphors content in tuber due to leaching on boiling might occur up 25% (True *et al*., 1979), this may be the possible reason to observed reduction in magnesium level in this study.

5.7. Anti-nutritional factors content of raw and processed Anchote

Anti-nutrients are known to reduce the maximum utilization of nutrients especially proteins, vitamins, and minerals (Ugwu, and Oranye, 2006). So that, the levels of anti-nutritional factors in the Anchote tubers are important in the assessment of its nutritional status.

5.8. 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 (Siddhtrraju 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.

5.9. 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 2. *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 & 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.

5.10. 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 decrease 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), this may be the possible reason observed in this study. Tannin content of most food is usually reduced by processing and this has been reported to enhance the bioavailability of iron. The toxicity effects of the tannin may not be significant since the total acceptable tannic acid daily intake for a man is 560 mg/100g (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 antinutritional effect may be insignificant in both raw and processed tuber.

5.11. Cyanide

Cyanide, either in synthetic inorganic forms as in KCN or NaCN, or organic forms as in cyanogenic glucosides, is a potent specific inhibitor of several enzyme-catalyzed

processes (Aletor, 1993). 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 Anchote tuber. 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/100 g 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/100g (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.

5.12. Phytate Phosphorus and Non-Phytate Phosphorus

The phytate phosphorus and non-phytate phosphorus content of raw and processed Anchote is shown in Table 4.4. The phytate phosphorus content of raw, boiled after peeling and boiled before peeling Anchote tubers were 109.71 mg/100g, 94.02 mg/100g, and 94.22 mg/100g, respectively. The phytate phosphorus content of Anchote boiled after peeling and boiled before peeling was significantly ($P<0.05$) lower than raw Anchote tubers. The mean phytate phosphorus content of Anchote boiled before peeling was non significant ($P>0.05$) compared to mean boiled after peeling Anchote tuber. The mean phytate phosphorus content was reduced in boiled after peeling by 14.31% and in boiled before peeling by 14.12% compared to raw tubers.

Table 3. *Phytate phosphorus and non-phytate phosphorus Contents of raw and processed Anchote samples*

Treatment	[1]Phytate phosphorus (mg/100g)	[2]Non-phytate phosphorus(mg/100g)	[3]Proportion of phosphorous as phytate (%)
RW	109.71±0.12[a]	75.10±0.82[a]	3.17±0.07[c]
BAP	94.02±0.08[b]	65.89±0.01[c]	3.34±0.01[b]
BBP	94.22±0.29[b]	68.77±0.09[b]	3.71±0.03[a]

Means not followed by the same superscript letters in the same column are significantly different ($P<0.05$).
[1] Phytate phosphorus was calculated by phytate times 28.18%.
[2] Non-phytate phosphorus was the difference between phytate phosphorus and total phosphorus.
[3]Proportion of phosphorous as phytate was calculated by phytate phosphorus devided by total phosphorus.
NB. RW stands for Raw Anchote, BAP: for Boiled after peeling and BBP: for Boiled before peeling.

The non phytate phosphorus content of raw, boiled after peeling and boiled before peeling Anchote were 75.10 mg/100g, 65.89 mg/100g, and 68.77 mg/100g, respectively. The mean non phytate phosphorus content of Anchote boiled after peeling was significantly ($P<0.05$) lower than both boiled before peeling and raw Anchote tubers. Similarly, the mean non phytate phosphorus content of Anchote boiled before peeling was significantly ($P<0.05$) lower compared to mean raw Anchote tubers. The proportion of phosphros as phytate content of raw, boiled after peeling and boiled before peeling Anchote tubers were 3.17%, 3.34%, and 3.71%, respectively. The percentage of phytate phosphorus to total phosphorus is very important since the phytate phosphorus cannot be utilized by human beings. The effect of phytate on phosphrus absorption in the presence of high phytate intakes has led to the suggestion that the proportion of phosphorus as phytate may be a better index of phosphrus bioavailability (Melaku *et al*., 2005), in which the diets with proportion of phosphorus as phytate (%) ≤ 50 % in foods from roots and tubers are regarded as being adequate in bioavailable phosphate. The values in this study were lower than the reported critical proportion of phosphors as phytate, which implies the Anchote tubers are adequately bioavailable the phosphors element. Therefore, consumptions of Anchote tuber may help to ameliorate prevalent mineral deficiencies caused by their limited bioavailability and may lead to better mineral status.

5.13. Molar ratios of Ca: Phy, Ox: Ca, Phy: Zn, Phy: Fe and [Ca] [Phy]/[Zn]

The molar ratios for oxalate, calcium, zinc, Iron and phytate were calculated to evaluate the effects of elevated levels of oxalate and phytate in the bioavailability of dietary minerals. Bioavailability is the ability of the body to digest and absorb the mineral in the food consumed. The calculated values are also compared with the reported critical toxicity values for these ratios.

5.14. [Calcium]/ [Phytate] Molar ratios

The molar ratios of Ca:Phy in Raw, Boiled after peeled and boiled before peeled Anchote were 5.05, 5.71, and 5.78, respectively. The mean Ca:Phy molar ratio of Anchote boiled before peeling and boiled after peeling was significantly ($P<0.05$) higher than raw Anchote tubers. The mean Ca:Phy molar ratio of Anchote boiled after peeling

was significantly (P<0.05) lower than boiled before peeling Anchote tubers. Phytic acids markedly decrease Ca bioavailability and the Ca:Phy molar ratio has been proposed as an indicator of Ca bioavailability. The Ca: Phy molar ratios >6, indicative of poor calcium bioavailability (Oladimeji et al., 2000). The values in the present study were lower than the reported critical molar ratio of Ca:Phy, indicating that absorption of calcium not adversely affected by phytate in these tubers.

5.15. [Oxalate]/ [Ca] Molar ratios

The molar ratios of Ox:Ca obtained in raw, boiled after peeled and boiled before peeled Anchote were 0.03, 0.02, and 0.02, respectively. The Ox:Ca molar ratios of Anchote boiled after peeling and boiled before peeling was significantly (P<0.05) lower than raw Anchote tubers. The Ox:Ca molar ratios of Anchote boiled before peeling was non significant (P>0.05) compared to compared to mean boiled after peeling Anchote tuber. The importance of oxalate contents of an individual plant product in limiting total dietary Ca availability is of significance only when the ratio of Ox:Ca is greater than one (Frontela *et al*., 2009). Under this circumstance, the oxalate has potential to complex, not only the Ca contained in the plant, but also that derived from other food sources (Davis, 1979). Consumption of oxalates may result in kidney disease and a high ratio of Ox:Ca in the diet also may cause chronic calcium deficiency (Hassan *et al*., 2007). From the result, it was observed that, Anchote tubers had Ox:Ca values are lower than the reported critical value (1.0), which implies that a low level of oxalate could have no adverse effects on bioavailability of dietary calcium in these tubers.

Table 4. *Calculated Ca:Phy, Ox:Ca, Phy:Zn, Phy:Fe and [Ca][Phy]/[Zn] molar ratios of raw and processed Anchote samples.*

Treatment	Ca:Phy (molar ratio)[1]	Ox:Ca (molar ratio)[2]	Phy:Zn (molar ratio)[3]	Phy:Fe (molar ratio)[4]	[Ca][phy]/[Zn](mol/kg)[5]
RW	5.05±0.02[c]	0.03±0.001[a]	17.35±0.91[a]	5.58±0.75[a]	0.52±0.02[a]
BAP	5.66±0.01[b]	0.02±0.001[b]	14.82±0.54[b]	3.92±0.19[b]	0.47±0.01[b]
BBP	5.78±0.07[a]	0.02±0.001[b]	14.83±0.23[b]	4.28±0.15[b]	0.44±0.02[b]

Means not followed by the same superscript letters in the same column are significantly different (P<0.05)
[1] mg of Calcium/molecular weight of Calcium: mg of phytate/molecular weight of phytate.
[2]mg of oxalate/molecular weight of oxalate: mg of calcium /molecular weight of calcium.
[3]mg of phytate/molecular weight of phytate: mg of zink/molecular weight of zink.
[4]mg of phytate/molecular weight of phytate: mg of iron/molecular weight of iron.
[5](mg of Calcium/molecular weight of Calcium) (mg of phytate/molecular weight of phytate)/ (mg of zink/molecular weight of zink) devided by 100.
NB. RW stands for Raw Anchote, BAP: for Boiled after peeling and BBP: for Boiled before peeling.

5.16. [Phytate]/ [Zink] Molar ratios

The Phy:Zn molar ratios of Raw, boiled after peeled and boiled before peeled Anchote were 17.35, 14.86 and 14.98, respectively. The Phy:Zn molar ratios of Anchote boiled after peeling and boiled before peeling was significantly (P<0.05) lower than raw Anchote tubers. The Phy:Zn molar ratios of Anchote boiled before peeling was non significant (P>0.05) compared to compared to mean boiled after peeling Anchote tuber. The importance of foodstuffs as a source of dietary zinc depends on both the total zinc content and the level of other constituents in the diet that affect zinc bioavailability. Phytate may reduce the bioavailabity of dietary zinc by forming insoluble mineral chelates at a physiological pH (Oberleas, 1983). Zinc deficiency has been shown to be the cause of dwarfism and hypogonadism among adolescents (Prasad, 1984). Zinc has been described as the essential mineral most adversely affected by phytate and the phytate-to-zinc molar ratio has been proposed as an indicator of zinc bioavailability (Sirkka, 1997). Phytate: zinc molar ratios >15, indicative of poor zinc bioavailability (Morris and Ellis, 1989). The values of boiled after peeling and boiled before peeling Anchote were lower than the critical molar ratios of Phy:Zn, which indicates the availability of zinc good due to traditional processed Anchote tuber.

5.17. [Phytate]/[Iron] Molar Ratios

The phytate:iron molar ratios of raw, boiled after peeling and boiled before peeling Anchote tuber was 5.58, 3.92 and 4.28, respectively. The phytate:iron molar ratios of Anchote boiled after peeling and boiled before peeling was significantly (P<0.05) lower than raw Anchote tubers. The phytate:iron molar ratios of Anchote boiled before peeling was non significant (P>0.05) compared to compared to mean boiled after peeling Anchote tuber. Phytate begins to lose its inhibitory effect on iron absorption when phytate:iron molar ratios are less than 1.0, although even ratios as low as 0.2 exert some negative effect (Hurrell, *et al*., 2003). The phytate:iron molar ratios greater than 0.15 regarded as indicative of poor iron bioavailability (Siegenberg *et al*., 1991). This result indicated that, the phytate:iron molar ratios raw and processed tubers are greater than the critical value, which implies the absorption of iron from raw and processed Anchote inhibited by phytate and as a result the bioavailability of iron is poor.

5.18. [Calcium][Phytate]/[Zink] Molar Ratios

The values of [Ca][phytate]/[Zn] millimolar ratio for raw, boiled after peeling and boiled before peeling Anchote had 0.52 mol/kg, 0.47 mol/kg, and 0.35 mol/kg, respectively. The [Ca][phytate]/[Zn] millimolar ratios of Anchote boiled after peeling and boiled before peeling was significantly ($P<0.05$) lower than raw Anchote tubers. The [Ca][phytate]/[Zn] millimolar ratios of Anchote boiled before peeling was non significant ($P>0.05$) compared to compared to mean boiled after peeling Anchote tuber. The potentiating effect of calcium on zinc absorption in the presence of high phytate intakes has led to the suggestion that the [Phy][Ca]/[Zn] millimolar ratio may be a better index of zinc bioavailability than the [Phy]/[Zn] molar ratio alone (Obah and Amusan, 2009). High calcium levels in foods can promote the phytate-induced decrease in zinc bioavailability when the [Ca][phytate]/[Zn] millimolar ratio exceeds 0.5 mol/kg (Gibson, 1994). In this study, except the raw tuber, the values of [Ca][Phy]/ [Zn] millimolar ratios of both processed tubers were found less than the critical level. Therefore, traditional processing methods would appear that the possible contribution to increase zinc availability.

5.19. Sensory Preference of Traditional Processed Anchote

Anchote tubers boiled after peeling and boiled before peeling were presented to fifty consumers panels to preference taste. Among them 33 (66%) consumers preferred tubers boiled before peeling whereas 17 (34%) consumers preferred Anchote boiled after peeling Anchote. The results were evaluated according to statical t-test table of paired compareson test, at $p<0.05$ level of significance; one sample must be selected at least 33 times out of fifty consumers to be a significantly different. The Anchote boiled before peeling was selected 33 times out of fifty consumers, which meets the critical value of the table to be a significantly difference. As a result, there is a significant ($P<0.05$) preference of taste of Anchote boiled before peeling to Anchote tubers boiled after peeling.

6. Conclusion

The present finding uncovered information on the mineral contents (Zinc, Iron, Calcium, Sodium, Magnesium and Phosphorus) and antinutritional factors (Phytate, Oxalate, Tannin and Cyanide) of raw and processed Anchote tubers from Eastern Wollega, Ethiopia. Sensory preference taste of Anchote boiled after peeling and boiled before peeling was also reported. In addition, the relative bioavailability of the minerals was assessed by calculating molar ratios of antinutrient to the contained minerals.

The results of this study showed that raw Anchote contains appreciable quantity of calcium, magnesium, iron and low levels of antinutrients (Oxalate, tannin, and cynide) except phytate, when compared to values reported in the literature. As shown in this study the traditional processing methods of Anchote were very important because that improved the bioavailability of zinc contained in the Anchote tubers. This study also indicated that traditional processing methods decreased the calcium, iron, zinc content of the tubers. Among the traditionl processing methods, boiled before peeling proved to be better in some mineral contents considered in this investigation.

The levels of anti-nutritional factors in Anchote are important in the assessment of its nutritional status. In this study, the raw Anchote tubers were found to contain low antinutritional factors, except phytate. Moreover, there were further reductions of the antinutritional factors during traditional processing. This implies, except phytate which might hinder iron bioavailability, traditional processing enables that the antinutritional factors in the Anchote couldn't hamper its nutritional value. Therefore, both methods of traditional preparation of Anchote were effective to significantly reduce the levels of antinutritional factors, thereby improving the bioavailability of minerals such as zinc and calcium known to be affected by these anti-nutrients. This study also indicated that consumer panels preferred the taste of Anchote boiled before peeling. Therefore traditional processing method of Anchote boiled before peeling is also effective technique and need to be encouraged in terms of consumers preference of Anchote taste.

Recommendation

The following recommendations are made based on the above findings.

- ✓ Increased cultivation of Anchote need to be enouraged in all part of the western part of the country, because of its high nutrient contents like protein, crude fiber, Ca, Zn, Mg and low antinutritional factors.
- ✓ Both methods of food preparation were effective in reducing the levels of anti-nutritional factors and increase zinc bioavailability. However, boiling of Anchote before peeling is recommended for Anchote preparation in households and restaurants not only due to minimal processing losses in some nutrient compositions but also in terms of consumer preference taste.
- ✓ The iron content in the Anchote tuber increased during traditional processing. On the other hand, the absorption of iron from processed Anchote tubers were significantly inhibited by phytate. Consequently, the bioavailability of iron is presumed to be poor. Therefore, Anchote tubers should either be supplemented with other iron reach foods or fortified with iron elements to combat Iron deficiency.
- ✓ Future works need to focus on identifcation and quantification of other nutrients, and unaddressed antinutrients in Anchote tubers.

- ✓ Studies should be undertaken for new product development using Anchote tubers.
- ✓ Moreover, the effect of boiling periods, optimal boiling temperature, and storage times, soil types, varieties, and maturity time on the minerals and antinutritional factors of Anchote should be studied.

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