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Evaluation of Oxalate Content in Boyna and Taro Roots Grown in Areka (Ethiopia)

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Abstract

Oxalate is a product of protein metabolism and is one of the important nutrients in the human diet. Regular consumption of large amounts of food with high oxalate contents over a long period may result in nutrient deficiencies notably calcium and may contribute to kidney stone formation. The aim of this study was to investigate the physicochemical parameters level and oxalate contents of boyna and taro roots grown in Areka, Ethiopia using titration and UV-visible spectrophotometric methods. The moisture content of dry flour and fresh tuber of boyna was 78.33 ± 1.33 and 8.87 ± 0.12 respectively, and the moisture content of dry flour and fresh tuber of taro was 9.53 ± 0.12 and 68.47 ± 1.22 respectively. The ash content of the flour sample of boyna and taro was 4.53 ± 0.23 and 4.67 ± 0.23 respectively. The pH of the flour sample of boyna and taro was 6.07 ± 16 and 6.20 ± 16 respectively. From the statistical analysis using t-test, all physicochemical parameters were significantly different at $p < 0.05$ level. The oxalate level of samples using the titration method gives 211.77± 12.32 mg/100g and 140.45 ± 17.51 mg/100g for boyna and taro respectively. By the Uv-visible spectrophotometer, the oxalate content of boyna was 163.60 ± 1.67 mg/100g and the oxalate content of taro was 158.02 ± 0.57 mg/100g.

Keywords: Boyna, Oxalate, Physicochemical, Taro, Uv-visible spectrophotometer.

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Ethical: This study follows all ethical practices during writing.

Contents

Contribution of this paper to the literature
This study contributes to existing literature by determining some selected physicochemical parameters and oxalate content of boyna and taro tubers (roots) grown around areka, Ethiopia by using titration and Uv-visible spectroscopic methods.

1. Introduction

Root and tuber crops are widely cultivated in southern Ethiopia, and support a considerable portion of the country's population as a source of food. Prominent among these are: potato (*Solanum tuberosum* L.), sweet potato (*Ipomoea batatas* L.), enset (*Ensete ventricosum*), godere (taro) (*Colacasia esculanta* L.), yams (boyna) (*Dioscorea spp.*), Ethiopian dinch (*Coleus parviflorus*), koteharrie (*Diaspora bulbiferous*), and anchote (*Coccinia abyssinica*), cassava (*Mahinot esculenta*). Among these, enset, anchote, and some yams are endemic to Ethiopia [1].

Oxalic acid (or its dissociated form oxalate) is a product of protein metabolism and is one of the important nutrients in the human diet. Common dietary sources of oxalic acid include tuber crops like sweet potato (*Ipomoea batatas* L.), godere (taro) (*Colacasia esculanta* L.), yams (boyna) (*Dioscorea spp.*), cassava (*Mahinot esculenta*) and the others [2]. Regular consumption of large amounts of food with high oxalate contents over a long period may result in nutrient deficiencies notably calcium and may contribute to kidney stone formation. By consuming a food high in calcium together with foods containing oxalates, the insoluble calcium oxalate formed passes through the intestinal tract without absorption and thereby decreases the risk of kidney stone formation. Other studies have shown that fat and oils can bind oxalate and thereby make soluble oxalates unavailable for absorption [3].

Boyna *(Dioscorea abyssinica)*, belongs to the family *Dioscoreaceae* and the genus *Dioscorea*, is a climber plant twining to the right, with an herbaceous stem and a large tuber [4, 5]. It is cultivated during the raining season in the south, west and the south-west highlands of Ethiopia. Tubers of *D. abyssinica* have long been used for food, as they are rich in starch. Locally, *D. abyssinica* is commonly known by its vernacular name "Boyna" which is equivalent to the common English name "yam". The plant is still serving as a source of food within a restricted region of the country (Southern Nations Nationality Peoples Regional State) and few neighboring cities and rural areas to this region.

Taro or godare in amharic (*Colocasia esculenta*, *L*) belongs to the genus *Colocasia*, within the sub-family Colocasioideae of the monocotyledonous family Araceae. It is a vegetatively propagated, perennial tropical crop with a large peltate ("shield-shaped") or heart-shaped leaves [6]. Taro originates from humid tropical rainforest r**e**gions of Southeast Asia including India. Different researchers conclude that it is not possible to determine a single center of origin for taro [7]. Plants of the genus *Colocasia* are edible aroid with large leaves and one or more food storing in their underground corms [8]. Taro is one of the most nutritious and easily digested foods. Like many other root crops, taro corms are high in carbohydrate in the form of starch and low in fat and protein. Taro can grow in a wide range of soil from upland or dry land soils that are well-drained, non-flooded soils to soils that are in high rainfall areas or saturated for prolonged periods [9].

The aim of this study is to analyze some physicochemical parameters level and the oxalate content of boyna and taro grown in Areka, Ethiopia using titration and UV-visible spectrophotometric methods.

2. Materials and Methods

2.1. Sample Collection and Preparation

The research samples were collected purposively from Areka Agricultural Research center farmland. One local boyna and One variety of taro (root) from released varieties namely **boloso-1** were purposively collected from Areka Agricultural Research center farmland. The sampling area was located at the latitude of 7.064100^0 N, longitude 37.687007^0 E and elevation of 1775.20m asl. After collection, the samples were taken to Wondo genet Agricultural Research Center, Natural Products Laboratory for preparation for further analysis. The tuber samples were peeled and washed properly with distilled water before cutting into small pieces. Then air-dried for 5 days before being grinded (chopped) into powder form using mortar and pestle (electric grinder). The powdered samples were sieved to obtain fine powder.

2.2. Physicochemical Parameters Analysis

2.2.1. Moisture Content

The dishes used for the moisture determination were dried at 130 ^{0}C for 1 hr in a drying oven. The mass of each dish was measured (M_1) using the digital electronic balance and about 5 g of the samples were weighed into each of the dishes (M_2). The sample was then mixed thoroughly and dried at 100 ^{0}C for 6 hr. After drying is completed, the mass was measured (M_3). The moisture content was calculated from the equation:

$$\text{Moisture (\%)} = \frac{(M2-M3)}{(M2-M1)} \text{X } 100$$

M_1: mass of the dish, M_2: mass of the dish and the sample before drying, and M_3: mass of the dish and the sample after drying.

2.3. Ash Content

The crucibles used for the analysis were cleaned by drying at 120 ^{0}C in a drying oven and ignited at 550 ^{0}C in the furnace for 3 hr. Then the crucibles were removed from the furnace and cooled in desiccators. The mass of each of the crucible was measured by digital analytical balance (M_1) and about 2.5 g of tuber crops flour was weighed into each crucible (M_2). The crucibles were dried at 120 ^{0}C for one hour in a drying oven. The crucibles were then placed in a furnace at about 550 ^{0}C for 1 hr. After one hour the crucibles were removed from the furnace, cooled, 5 drops of distilled water were added to each of the crucible and placed in the furnace at 550 ^{0}C for 30 min. After that, crucibles were removed from the furnace, allowed to cool and 5 drops of distilled water and nitric acid were added

to each of the crucibles. Then the crucibles once again were inserted into the furnace until they become free from carbon and the residue appears grayish white. Then, the crucibles were removed from the furnace and placed in desiccators. Finally, the mass each crucible was weighed as (M_3).

The total ash was calculated from the equation:

$$\text{Ash (\%)} = \frac{(M3-M1)}{(M2-M1)} \text{ X } 100$$

Where M_1: mass of the dried crucible, M_2: mass of the crucible and the sample before inserting furnace, M_3: mass of the crucible and the sample after taking out from the furnace.

pH

Calibration of the pH meter was done using a buffer solution of pH 4 and pH 7. A 5 g of each flour sample was dispersed in 25 mL of distilled water and allowed to stand for 30 minutes with constant stirring. The electrode of the pH meter was dipped into the dispersion with constant shaking until the stable reading was obtained. At equilibrium, the values were recorded with the aid of pH meter. Triplicate measurements (determinations) were made in all cases and the result was the average of the triplicate measurements.

2.4. Oxalate Content Analysis

2.4.1. Titration Method

The oxalate content was determined using the method originally employed by Iwuoha and Kalu [10]. The procedure involves three steps: digestion, oxalate precipitation and permanganate titration.

- **Digestion:** At this step, 2 g of flour was suspended in 190 mL of distilled water contained in a 250-mL volumetric flask; 10 mL of 6M HCl was added and the suspension digested at 100 ^{0}C for 1 h, followed by cooling, and then made up to 250 mL before filtration.
- **Oxalate precipitation:** 125 mL of the filtrate were measured into a beaker and four drops of methyl red indicator were added, followed by the addition of concentrated NH_4OH solution (dropwise) until the test solution changed from its salmon pink color to a faint yellow color (pH 4-4.5). The content was then heated to 90 ^{0}C, cooled and filtered to remove precipitate containing ferrous ion. The filtrate was again heated to 90 ^{0}C and 10 mL of 5% $CaCl_2$ solution was added while being stirred constantly. After heating, it was cooled and left overnight at 5 ^{0}C. The solution was then centrifuged at a speed of 2500 rev/min for 5 min. The supernatant was decanted and the precipitate completely dissolved in 10 mL of 20% (v/v) H_2SO_4, solution.
- **Permanganate titration:** The total filtrate resulting from the digestion of 2 g of flour was made up to 300 mL. Aliquots of 125 mL of the filtrate were heated until near boiling, and then titrated against 0.05 M standardized $KMnO_4$, the solution to a faint pink color which persisted for 30 s. The calcium oxalate content was calculated using the formula.

$$\text{Oxalate content} = \frac{\text{T x Vme x DF}}{\text{ME x MF}} \text{ X } 10^5$$

Where: T = Titer value of $KMNO_4$ (ml), Vme= volume- mass equivalent (that is, 1 ml of 0.05 M $KMNO_4$, = 0.00228 g of anhydrous oxalic acid), Df= dilute factor (Vt/A that is, total volume of titrate/ Aliquot used = 2.4), Mf= mass of sample used, ME= molar equivalence of $KMNO_4$ in oxalate concentration in g/dm^3 = 5.

2.5. UV-Visible Spectrophotometric Method

The UV-Visible spectroscopic method was adopted from the literature [11]. Total oxalate was measured by weighing 1.0 g sample of dried tubers in the beaker and boiled in 150 mL water containing 27.5 mL 6 M HCl plus two drops of caprylic alcohol (octanol) for 25 min. The mixtures were cooled, transferred to a 250 mL volumetric flask and made up to mark. The mixtures were then filtered through Whatman 541 filter paper. The first 80 mL filtrate was discarded and the rest was retained for analysis. A volume of 10 mL of this filtrate was evaporated to near dryness at 40-45 ^{0}C in a vacuum oven, and re-dissolved in 10 mL of 0.01 M H_2SO_4. The total oxalate in the sample was analyzed using a UV-Visible spectrophotometer.

2.6. Data Analysis

Significance differences in physicochemical parameters level of two tuber crops were subjected to t-test using Microsoft Excel software.

3. Results and Discussion

3.1. Physicochemical Parameters

The results of selected physicochemical parameters analyzed for boyna and taro were given in Table 1.

Table-1. Physicochemical parameters of boyna and taro (n = 3).

Sample code	[a] Moisture content (fresh tuber (%)	[a] Moisture content (dry flour (%)	[a] Ash content (%)	[a] pH
LB	78.33 ± 1.33	8.87 ± 0.12	4.53 ± 0.23	6.07± 0.16
B-1	68.47 ± 1.22	9.53 ± 0.12	4.67 ± 0.23	6.20 ± 0.16

Note: Where [a] is Values mean ± SD of triplicate flour and fresh tuber samples.
LB represents Local boyna (yam) variety.
B-1 represents Boloso-1 one variety of taro (godare) from released varieties.

3.2. Moisture Content

The moisture content of boyna and taro was determined (tested) for two different sample conditions. The first one was moisture content of the fresh tuber sample which was carried out immediately after sample collection. The second one was moisture content of the dry flour which was carried out after the samples were

dried, grinded and changed to flour. From Table 1 the moisture content of fresh tuber of local boyna and taro was 78.33 ± 1.33 and 68.47 ± 1.22 respectively. Whereas the moisture content of dry flour was 8.87 ± 0.12 and 9.53 ± 0.12 for local boyna and taro respectively. According to the finding of Huang, et al. [12]; Azene and Molla [13] research done on three local taro cultivar grown in Taiwan the moisture content of fresh taro tuber ranges from 60 - 83%. Therefore, the value of moisture content of this study i.e. 68.47 ± 1.22 was within the range of the above findings. From the report of Koteswara, et al. [14] the moisture content of different collection of boyna (yam) in Orissa, India was in the range between 66.67% and 75.09%. But in the present study, the moisture content of local boyna variety was out of this range (78.33 ± 13). The difference may be arising from the difference in the variety of two samples, the difference in environmental conditions including difference in climatic condition and soil type.

3.3. Ash content

From Table 1 the ash content of local boyna was 4.53 ± 0.23 and that of taro was 4.67 ± 0.23. According to the finding of Njoku and Ohia [15]; Mbofung, et al. [16] the ash content of taro (godare) ranged from 3.54%-7.78%. The ash content of taro (godare) in this study (4.67 ± 0.23) was found in this range. Ash content of the B-1 obtained was 4.67% ± 0.23 which was higher than 0.6-1.3% finding of Bradbury and Holloway [17] and 3.92% finding of Azene and Molla [13]. The observed difference in the ash contents may be attributed to climatic factors, the soil type, varietal and, cultivar difference. From the report of Koteswara, et al. [14] the ash content of different collection of boyna (yam) in Orissa, India was in the range between 1.89% -7.08%. The value of ash content of the present study was 4.53 ± 0.23 which was within the range of the above finding.

3.4. pH

From Table 1 the pH of local boyna and taro was 6.07± 0.16 and 6.20 ± 0.16 respectively. According to the report of Adewale, et al. [18] the pH of flour from two varieties of yam (boyna) from Nigeria was 6.40± 0.01. The pH of local boyna variety in the present study was found to be 6.07± 0.16. This indicates the flour of this study was slightly acidic than the mentioned report. The observed slight difference in the pH may be attributed to climatic factors, the soil type, varietal, and cultivar difference. According to the finding of the Alcantara [19] the pH of taro corm was found to be 7.81. The value of the pH of taro (boloso-1) of the present study was 6.20 ± 0.16. The result reveals that the flour of the present study was acidic whereas the flour of the research finding was slightly basic. The difference may be because of the climatic factors, the soil type, cultivar, and varietal difference.

3.5. Oxalate Content

The Oxalate content of flour of local boyna and taro was determined by using the titration method and UV-visible spectroscopic method. The results were given in Table 2.

Table-2. Oxalate content of local boyna and taro by titration and UV-visible spectroscopic method (n = 3).

Sample code	[a]Oxalate (mg/100g) (db)	
	titration method	UV-vis method
LB	211.77 ± 12.32	163.60 ± 1.67
B-1	140.45 ± 17.51	158.02 ± 0.57

Note: Where [a]is Values mean ± SD of triplicate flour samples.
db is dry basis
LB represents Local boyna (yam) variety.
B-1 represents Boloso-1 one variety of taro (godare) from released varieties.

From Table 2 oxalate content of local boyna and taro by titration method was 211.77 ± 12.32 and 140.45 ± 17.51 mg/100g respectively. The oxalate level of taro (boloso-1) in this study was less than the oxalate level reported by Azene and Molla [13]; Alcantara [19]; Jirarat, et al. [20]. The difference in oxalate level of two reports maybe because of the varietal difference between two samples, agro ecological-difference like temperature, climate and soil type. Again the oxalate level of local boyna in this study was less than the oxalate level reported by Shajeela, et al. [21] which was ranged between 260 ± 0.01 and 780 ± 0.01mg/100g. The difference in oxalate level of two reports maybe because of the varietal difference between two samples, agro ecological-difference like temperature, climate, soil type, the difference in performance of the analytes (chemicals) used while performing tests and even the difference in analyst who performed the tests.

From Table 2 by uv-visible spectrophotometric method the oxalate content of local boyna was 163.60 ± 1.67 mg/100g and oxalate content of taro was 158.02 ± 0.57 mg/100g. The level of oxalate in this study for both samples was greater than the report of Durowoju and Popoola [22] the oxalate content of Nigerian tubers which was found in the range of (0.46- 2.56 mg/100g FW). The difference may be due to the difference in sample location, agro-ecological difference, and climatic condition including soil type. The main difference in the results may arise from sample condition in reporting the results. The present study was reported the oxalate level of samples in the dry basis whereas in the report of Durowoju and Popoola [22] the oxalate level of the samples was reported in a fresh basis.

4. Conclusion

In this study selected physicochemical parameters and Oxalate level of local boyna and taro grown in Areka, Ethiopia was analyzed by titration and Uv-Visible spectrophotometric methods. The results indicated that all of the physicochemical parameters of both tuber crops were within the range of different research findings done on the same samples.

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