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Research Article

Assessment of Selected Proximate and Heavy Metals in Enset Products Collected from Tepi Market, Southwestern Ethiopia

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This study focused on determining selected proximate compositions, such as moisture content, ash content, crude fibre content, and crude fat content, of kocho and bulla enset products collected from Bechi, Fide, Yeki, Zinki, Addis Alem, and Korcha kebeles of Yeki woreda. Besides, heavy metals such as iron, copper, manganese, zinc, and lead have been analyzed. The result showed that all proximate compositions analyzed agree with other conducted researches, except moisture content because of heavy rainfall in Yeki woreda. On the other hand, the results obtained for iron and zinc were higher than the permissible limit set by international standards, while that of manganese was slightly higher than the standard limit. On the other hand, the results obtained for copper were below the permissible limit set, while lead was not detected in all samples. Therefore, the investigated kocho and bulla products can provide an appreciable amount of analyzed minerals.

1. Introduction

Enset (Ensete ventricosum) is a perennial, herbaceous monocot in the family Musaceae. It resembles the banana plant and is often referred to as "false banana." The plant is domesticated only in Ethiopia and is grown in the south and southwestern parts of the country [1]. It is one of the potential indigenous crops for food production and can be grown everywhere in Ethiopia [2]. Enset plants, which are traditionally grown in small plantations adjacent to homesteads, can grow to a height of six meters and provide valuable windbreaks and shade from direct sunlight. Because of its large leafy fronds, it is also a good plant to intercrop with coffee, potato, and other food crops, which benefit from shady growing conditions [3].

Ethiopia has long been known as a center of origin and diversity for large number of crop plants, of which enset (*Ensete ventricosum*) is the one having a great impact on local agriculture and food habit [4]. The edible part of enset is obtained from its pseudostem and corm. The major food product obtained from enset is kocho, bulla, and corm. Kocho is the bulk of fermented starch obtained from a mixture of decorticated leaf sheath and corm [5]. Bulla is an unfermented product that can be dehydrated to powder form and prepared into several recipes. Bulla can be prepared as a pancake, porridge, and dumpling [6]. Bulla is the sediment of insoluble starchy product separated from processed enset portion by squeezing and decanting the liquid [5]. The processed enset (kocho, bulla, and corm) are rich in carbohydrate and good sources of minerals [7]. Nutrient value of starchy foods depends mainly on their nutrient content, physicochemical properties of their starches, and the existence of antinutritional activities and toxic substances [8].

Enset has multipurpose in the livelihood of producing societies in Ethiopia. It contributes to indigenous ethnomedicinal values in traditional medicine, such as strengthening women after delivery and healing bone fractures in humans, and very highly fermented kocho is also used for stomach cramps and as feed for cows to facilitate placental discharge. Some varieties of enset also serve for birth control in humans [5]. Plant products include a carbohydrate-rich food source as well as fibre for making mats and rope [7].

Enset is the first important food source crop in Gurage, Kembata, Sidama, Gedio, Hadya, Jemjem, and Arero zones. It is the second important crop and a costaple food in Wolaita, Gamo-Gofa, and Kafa zones and Yem special woreda. It is considered the third most important food crop in Wollega, Illubabor, and in some parts of the southern region. In the second and third farming systems, cereals and other root crops take the primary and secondary importance [8].

The plant is perhaps the biggest vegetable of all and looks like a banana "tree." The food, however, comes mainly from the lower trunk, filled with starchy pith, which on the largest specimen can be a meter in diameter and three meters tall. A second food comes from underground, where a corm may be almost a meter long and a meter in diameter, packed with starch like some giant potato [9].

Enset food products are used as staple and/or costaple food for about 20% of the Ethiopian population, particularly in the southern, southwestern, and western parts of the country. The corm and pseudostem of enset plant are traditionally processed into primary food product, kocho and bulla. The process is an age-old technique in enset growing regions of the country and is still used without any scientific modification [10]. The present study was conducted to investigate selected proximate and heavy metal contents of kocho and bulla enset products widely consumed in Yeki woreda.

2. Methodology

2.1. Study Area. This study was conducted in Yeki woreda that is one of the woredas of southwestern region of Ethiopia. The specific study area center is Tepi, which is located around 611 km southwest of Addis Ababa. It is situated at 7.2 degrees north latitude, 34.45 degree east longitude, and 1097 meters elevation above sea level (https://www.Tepi%20town%20information.com).

2.2. Sample Collection. The samples were randomly collected from 8 kebeles of Yeki woreda and transported to a chemistry laboratory for selected physicochemical and metal content analysis. From each kebele, three kocho samples and three bulla samples have been purchased and treated separately, and triplicate analysis was conducted for both samples from each kebele. The mean of the results were displayed with standard deviation. For this purpose, both bulla and kocho samples were purchased and composited to get representative sample. Then, it was dried by sunlight, after that it was ground using a mortar and pestle, and then, it was stored in plastic bags.

2.3. Chemicals and Reagents. All reagents used for this study were of analytical grade. Aqua-regia was used for sample digestion. The standard solutions of both Pb and Mn have been prepared by diluting their respective 1000 ppm stock solutions to 100 ppm. Then, working standards have been prepared by diluting these 100 ppm intermediate solutions to respective working standard. The working standards of Pb with 1 ppm, 2 ppm, 3 ppm, and 4 ppm have been prepared, while, for Mn, 0.5 ppm, 1.5 ppm, 2.5 ppm, and 3.5 ppm have been prepared for their AAS analysis. Both metals have been analyzed by FAAS equipped with deuterium arc background corrector and standard air-acetylene flame system using external calibration curve after the parameters optimized for maximum signal intensity of the instrument. The concentration of each mineral was determined from its calibration curve, and concentration was calculated by considering dilution factor and expressed for the dry basis. The reagents were selected to ensure that the contents of the elements to be determined or of possibly interfering elements were negligible in relation to the lowest element concentration to be determined. Distilled water was used throughout the experiment for sample preparation, dilution, and rinsing apparatus prior to analysis.

2.4. Materials. A mortar and pestle were used to crush the sample into the powder. Electronic balance (F2204G) was used to measure the mass of the samples, and Bunsen burner was used to carbonize the samples. Muffle furnace (F30420C) was used to ash the samples and the digital biobase oven anf determine the moisture content of the sample. AAS (BUCK Scientific MODEL 210VGP) was used to measure the absorbance of the sample. Besides the different laboratory materials such as Fume Hood (FH1800(X)), tongs, funnels, filter papers, flask, stove, measuring cylinder, and beaker were also used.

2.5. Proximate Composition Analysis

2.5.1. Moisture Content. Moisture content was determined by the method mentioned in [11], using the official method 930.15. The cleaned and dried crucibles were placed in desiccators and weighed (W_1). The sample around 40 g was accurately weighed (W_2) in a previously weighed crucible and put in desiccators every step. Then, the crucible with its content was put into an oven at 70°C for 42 h until constant mass was attained, and after cooling in desiccators to room temperature, they were weighted again (W_3). The moisture content was determined as follows:

$$\text{Moisture}\,(\%) = \frac{W_2 - W_3}{W_2 - W_1} \times 100. \quad (1)$$

2.5.2. Total Ash Content. Ash was determined by the method mentioned in [12]. Clean crucibles, dried at 100°C in an oven, were cooled in desiccators and weighed (W_1). Then, 3 g of the sample was weighed into a previously weighed crucible (W_2). Then, crucibles with their contents were burned in a muffle furnace set at 550°C for 2 h until a light gray ash resulted and then weighed (W_3) after cooling. The weight of the ash was expressed as a percentage of the initial weight of the sample as follows:

$$\text{Total Ash}\,(\%) = \left(\frac{W_2 - W_3}{W_2 - W_1}\right) \times 100. \quad (2)$$

2.5.3. Crude Fibre Content. Crude fibre content was determined by the method mentioned in [13]. 2 g of sample was transferred to 600 ml beaker and boiled with 200 ml 1.25%

sulphuric acid for 30 min. After digestion by 20 ml 28% NaOH for 30 min, the mixtures were filtered through a crucible filled with a layer of sea-sand using a vacuum pump, and the residues were washed with hot distilled water several times. The residue left was washed three times under vacuum, each time with 30 ml of 1% sulphuric acid solution and then distilled water, 1% sodium hydroxide solution and then distilled water and acetone, and dried by suction. It was then dried at 130°C for 2 h, cooled in desiccators, and weighed (W1). After incinerating the samples in a muffle furnace at 550°C for 2 h, they were cooled in desiccators and weighed again (W_2). The total crude fibre was expressed in percentage as follows:

$$\text{Crude Fibre} = \left(\frac{W_1 - W_2}{W_3}\right) \times 100, \tag{3}$$

where W_3 is the sample weight.

2.5.4. Crude Fat Content. Crude fat was determined based on the Soxhlet extraction method [14]. After aluminium cups with boiling chips were dried in a drying oven at $102 + 2$°C for at least 30 min, they were cooled in the desiccators to room temperature and weighed (W_1). 5 g of sample was weighed into thimbles and extracted with 70 ml diethyl ether. After finishing the extraction process, the cups were removed from the extractor and dried in 102°C oven for 30 min to evaporate the solvent. The extraction cups were cooled in desiccators for 30 min and weighed (W_2) immediately after taken out from the desiccators. The fat obtained was expressed as a percentage of the initial weight of the sample as follows:

$$\text{Crude Fat}\,(\%) = \left(\frac{W_2 - W_1}{W}\right) \times 100, \tag{4}$$

where W is the weight of sample.

2.6. Determination of Trace Metals

2.6.1. Digestion of Samples. For the analysis of Mn and content, flat-bottomed flasks (100 mL) fitted with reflux condenser were used in Kjeldahl apparatus hot plate to digest the enset samples. Buck Scientific Model 210 VGP (USA) atomic absorption spectrometer (AAS) equipped with deuterium arc lamp background corrector was used. The digestion optimization has been conducted to get clear and colorless solution to be injected to FAAS. For this purpose, the time and volume of reagents and mass of the samples have been optimized. Accordingly, different optimizations have been conducted and clear colorless solution was obtained by after digestion of 1 g of each samples by 14 mL of aqua-regia ($HCl : HNO_3$) at 250°C for 3 hrs. The optimum procedure was selected depending on minimum reagent volume consumption; digestion time; and obtaining clear solution. A reagent blank containing the same reagents subjected to the same digestion procedure was prepared for correcting effect of the blank. The reagent blank was run for each digested samples. Finally, the solutions have been filtered and diluted to 25 mL and then stored in refrigerator below 4°C.

2.6.2. Working Standard Solution Preparation. Determination of the concentration of Mn and Pb metals were carried out by an atomic absorption spectrophotometer. The standard solutions of both metals have been prepared by diluting their respective 1000 ppm stock solutions to 100 ppm. Then, working standards have been prepared by diluting these 100 ppm intermediate solutions to respective working standard. The working standards of Mn with 1 ppm, 2 ppm, 3 ppm, and 4 ppm have been prepared, while, for Pb, 0.5 ppm, 1.5 ppm, 2.5 ppm and 3.5 ppm have been prepared for their AAS analysis.

2.7. Statistical Data Analysis. The data obtained from the analysis of all parameters were analyzed using Excel.

3. Results and Discussion

3.1. Parameters Analyzed. From Table 1, the results showed that moisture content of kocho sample was 90% to 95%, which is higher for the sample collected from Korcha kebele, while the lowest was found for the sample collected from Yeki kebele. On the other hand, the moisture content for bulla was 84% to 89% that was higher for the sample collected from Korcha kebele, while the lowest was for the sample collected from Bechi kebele. These results have been higher than that reported by [2]. This higher moisture content may be resulted from the environmental factor that Yeki woreda is one of the woredas of southwestern region getting high amount of rain fall annually, and the area is always wet throughout the year.

Total ash content of kocho sample was 2% to 2.6%, and it was higher in the sample collected from Zinki kebele, while it was lower in the sample collected from Bechi and Addis Alem kebeles. On the other hand, total ash content for bulla was 1% to 1.4%, and it was higher in the sample collected from Yeki and Korcha kebeles, while the lower moisture content was found for the sample collected from Bechi kebele. These results slightly agree with the result obtained by [7]. This may be because of similarity of enset verities under investigation, from which the products were prepared and the process of preparation of products.

Crude fibre content of kocho samples were 16% to 16.5%, and it was higher for the sample collected from Bechi, while the lowest was for the sample collected from Addis Alem kebele. On the other hand, the crude fibre content of bulla samples was 2% to 2.4%, and it was higher for the sample collected from Korcha kebele, while the lowest was for the sample collected from Bechi kebele. These results agree with the result obtained in [15]. This also may be because of similarity of varieties of enset, from which the products have been prepared and the process of preparation of products.

Crude fat content of kocho samples obtained was the highest for the sample collected from Zinki kebele, while the lowest was for the sample collected from Yeki kebele.

Table 1: Analyzed parameters (dry base).

Kebele	Samples	Moisture content (%)	Ash content (%)	Crude fibre content (%)	Crude fat content (%)
Bechi	Kocho	93 ± 0.13	2 ± 0.21	16.5 ± 0.22	0.8 ± 0.23
	Bulla	84 ± 0.12	1 ± 0.25	2 ± 0.31	0.3 ± 0.13
Fide	Kocho	92 ± 0.15	2.5 ± 0.11	16.4 ± 0.21	0.72 ± 0.21
	Bulla	86 ± 0.16	1.2 ± 0.21	2.2 ± 0.25	0.29 ± 0.16
Yeki	Kocho	90 ± 0.12	2.3 ± 0.22	16.3 ± 0.18	0.5 ± 0.24
	Bulla	88 ± 0.15	1.4 ± 0.22	2.3 ± 0.22	0.34 ± 0.15
Zink	Kocho	94 ± 0.15	2.6 ± 0.24	16.2 ± 0.19	0.9 ± 0.18
	Bulla	87 ± 0.11	1.3 ± 0.24	2.1 ± 0.32	0.32 ± 0.12
Addis alem	Kocho	92 ± 0.14	2 ± 0.25	16 ± 0.23	0.7 ± 0.22
	Bulla	85 ± 0.13	1.1 ± 0.18	2.2 ± 0.21	0.33 ± 0.13
Korcha	Kocho	95 ± 0.17	2.2 ± 0.26	16.1 ± 0.25	0.6 ± 0.25
	Bulla	89 ± 0.11	1.4 ± 0.18	2.4 ± 0.24	0.31 ± 0.14

Besides, the crude fat content of bulla samples was 0.29% to 0.34%, and it was highest for the sample collected from Yeki kebele, while the lowest was for the sample collected from Fide kebele. The results obtained for this parameter agree with that obtained in [16]. This also may be because of similarity of varieties of enset, from which the products have been prepared, and the process of preparation of products.

3.2. Optimization of Sample Digestion. For each kocho and bulla sample, different digestion procedures using the mixture HCl and HNO_3 were conducted by varying volume of the mixture, digestion temperature, and digestion time. Therefore, the digestion conducted using 1 gm sample with 14 ml of aqua regia at 250° for 180 min was selected because of the solution obtained was colorless according to Table 2.

3.3. Heavy Metals Analysis by Atomic Absorption Spectrometer. Both metals have been analyzed by FAAS equipped with deuterium arc background corrector and standard air-acetylene flame system using external calibration curve after the parameters optimized for maximum signal intensity of the instrument. The concentration of each mineral was determined from its calibration curve, and the concentration was calculated by considering dilution factor and expressed for the dry basis.

From Table 3, iron is required for blood formation and it is important for normal functioning of the central nervous system and its deficiency resulted in anemia and affects the brain functioning in infants [17, 18]. The recommended dietary allowance for iron in adults and children is 10 mg/day and for female adults, it is 15 mg/day. The result showed that iron content was 53.6 mg/kg to 55.82 mg/kg for kocho samples, and the highest was iron content obtained for the sample collected from Zinki kebele, while the lowest was in the sample collected from Addis Alem kebele. On the other hand, the iron content was 50.05 mg/kg to 52.92 mg/kg for bulla samples, and the highest iron content was obtained for the sample collected from Yeki kebele, while the lowest was in the sample collected from Fide kebele. This indicates that the concentration of iron in all samples was above the recommended value by WHO. Hence, all samples would be rich in iron content, but care should be taken to minimize the health risk related to high iron concentration.

Copper is an essential micronutrient required in the growth of both plants and animals and leads to a decrease in iron content in some tissues and can cause anemia, liver and kidney damage, and stomach and intestinal irritation [17, 18]. The recommended dietary allowance is 3 mg/day for adults and 2 mg/day for children. The result showed that copper was 0.53 m/kg to 0.58 mg/kg for kocho samples, and the highest copper content was in the samples collected from Addis Alem kebele, while the lowest was in the sample collected from Korcha kebele. Besides, the result showed that copper was 0.50 mg/kg to 0.53 mg/kg for bulla samples, and the highest iron content was in the sample collected from Addis Alem kebele, while the lowest was in the sample collected from Fide kebele. The result indicates that the concentration of copper in all samples was below the recommended value by WHO. Hence, the samples are free from the fear of health problem related to high copper concentration.

Manganese plays an important role in redox processes and needed in small amounts. It is required for the proper metabolism and activations of several important enzyme systems [18]. The recommended dietary allowance (RDA) for manganese is 4.5 mg/day. The result indicated that manganese was 6.57 mg/kg to 6.93 mg/kg for kocho samples, and the highest manganese content was in the sample collected from Yeki kebele, while the lowest was in the samples collected from Korcha kebele. On the other hand, the manganese content was 5.50 mg/kg to 5.54 mg/kg for bulla samples, and the highest manganese content was in the samples collected from Zinki kebele, while the lowest was in the sample collected from Bechi kebele. The result indicates that the concentrations of manganese in all samples were found to be above the recommended value. Hence, all samples would be rich in manganese. Besides, even though the manganese contents obtained were higher than the recommended value, they are close to it, and the samples are free from fear of health problems related to high content.

Zinc is essential for human growth and development. Its deficiency causes poor growth and retarded development [18]. Zinc is an essential micronutrient associated with

Table 2: Digestion optimization.

Trial	Sample (g)	Volume of HCl (ml)	Volume of HNO_3 (ml)	Volume of aqua regia (ml)	Digestion temp (^{0}c)	Digestion time (min)	Result
1	1	3	1	6	200	160	Yellowish solution
2	**1**	**3**	**1**	**14**	**250**	**180**	**Clear colorless**
3	1	3	1	10	300	200	Yellow solution

Table 3: Metals concentration.

Kebele	Samples	Mean concentration (mg/kg) ± SD Fe	Cu	Mn	Zn	Pb
Bechi	Kocho	53.48 ± 0.03	0.57 ± 0.06	6.58 ± 0.01	24.58 ± 0.18	BDL
	Bulla	52.40 ± 0.11	0.51 ± 0.07	5.50 ± 0.11	22.58 ± 0.21	BDL
Fide	Kocho	54.05 ± 0.07	0.55 ± 0.05	6.84 ± 0.02	23.66 ± 0.17	BDL
	Bulla	50.05 ± 0.12	0.50 ± 0.01	5.48 ± 0.13	20.66 ± 0.23	BDL
Yeki	Kocho	53.92 ± 0.05	0.54 ± 0.08	6.93 ± 0.04	24.53 ± 0.19	BDL
	Bulla	52.92 ± 0.14	0.52 ± 0.04	5.53 ± 0.15	22.53 ± 0.21	BDL
Zinki	Kocho	55.82 ± 0.04	0.56 ± 0.05	6.64 ± 0.03	24.83 ± 0.15	BDL
	Bulla	51.82 ± 0.13	0.51 ± 0.05	5.54 ± 0.14	21.83 ± 0.22	BDL
Addis Alem	Kocho	53.60 ± 0.02	0.58 ± 0.04	6.59 ± 0.05	25.04 ± 0.13	BDL
	Bulla	51.60 ± 0.05	0.53 ± 0.03	5.51 ± 0.17	21.04 ± 0.23	BDL
Korcha	Kocho	54.43 ± 0.02	0.53 ± 0.09	6.57 ± 0.06	23.63 ± 0.19	BDL
	Bulla	52.50 ± 0.15	0.51 ± 0.02	5.52 ± 0.16	22.63 ± 0.24	BDL
WHO/FAO		10–15 mg/day	3 mg/day	4.5 mg/day	10–15 mg/day	0.1 mg/kg

number of enzymes, especially those associated with synthesis of ribonucleic acid. Zinc deficiency limits the rate of recovery for protein energy in malnourished children [19]. WHO recommended standard for zinc in adults and children is 15 mg/day and 10 mg/day, respectively. The result showed that zinc was 23.63 mg/kg to 25.04 mg/kg for kocho samples, and the highest zinc content was in the sample collected from Addis Alem kebele, while the lowest was in the sample collected from Korcha kebele. On the other hand, zinc content was 20.66 mg/kg to 22.63 mg/kg for bulla samples, and the highest zinc content was in the sample collected from Korcha kebele, while the lowest was in the sample collected from Fide kebele. The result indicates that the concentration of zinc was above the recommended value according to WHO for children. Hence, all samples would be rich in Zn content for children. In addition to this, the result indicates that all samples were above the recommended value, and hence, these samples would be rich in Zn content. But care should be given to minimize the health risk related to high zinc content in the consumption of these food products.

The results showed that lead was below detection limit for both kocho and bulla products. This agrees with the permissible limit set by WHO/FAO. Therefore, consumers of these products can use them without any fear of any health problem related to high content of lead.

4. Conclusion and Recommendation

The sample of kocho and bulla were collected and analyzed for selected proximate and heavy metals content analysis from Bechi, Fide, Yeki, Zinki, Addis Alem, and Korcha kebeles. Proximate compositions such as moisture content, ash content, crude fibre content, and crude fat content as well as heavy metals like iron, copper, manganese, zinc, and lead have been analyzed for both kocho and bulla samples. From proximate compositions, moisture content was slightly higher than other studies. This may be because Yeki woreda is the area that got rainfall throughout the year. On the other hand, all other results of proximate compositions analyzed agree with other conducted researches. In terms of heavy metals, the results obtained for iron and zinc were higher than the permissible limit set by international standards, while that of manganese was slightly higher than the standards. On the other hand, the results obtained for copper were below the permissible limit set, while lead was not detected in all samples. Therefore, kocho and bulla products investigated can provide an appreciable amount of analyzed minerals. Finally, the current study result showed that the high amount of iron in the products should be considered to minimize any health problem related to high iron concentration in the food.

Data Availability

Data are available and can be offered where necessary.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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