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RESEARCH ARTICLE

EFFECT OF INDIGENOUS FERMENTATION PROCESS AND ENSETE VARIETY ON MINERAL COMPOSITION OF KOCHO, AN ETHIOPIAN TRADITIONAL FERMENTED FOOD

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ABSTRACT

Ensete ventricosum (Welw Chessman), is a perennial, herbaceous, monocarpic and monocotyledonous crop in the family Musaceae (Westphal 1975). The experiment was carried out using different fermentation time (0, 30, 60 and 90 days) and two Enset varieties to check out the effects of fermentation time and enset variety on the nutritional composition of kocho. In this study, kocho samples had been prepared from yedbereye and Lemat enset variety, which are processed by using indigenous Gurage kocho processing methods. The outcomes revealed that, mineral composition of kocho were affected by fermentation time and Enset ventricosum variety. The outcomes of this study showed that the content of Ca, Mg, K, and Zn content was found higher in the unfermented kocho prepared from yedebreye (190.11 mg/100 g, 117.47 mg/100 g, 860.99 mg/100 g, and 3.87 mg/100 g respectively) than the content in unfermented prepared from Lemat (183.89 mg/100g, 112.68 mg/100g, 857.06 mg/100g and 2.17 mg/100g respectively). On the other hand, the content of Na, Fe, Cu and Mn was found higher in the unfermented kocho prepared from Lemat (27.94 mg/100 g, 3.58 mg/100 g, 3.30 mg/100 g and 2.95 mg/100 g respectively) than the content in unfermented kocho prepared from yedebreye (22.54 mg/100g, 2.66 mg/100g, 2.41 mg/100g and 1.56 mg/100g respectively). The Ca, Mg, Na and Fe content differ significantly ($P < 0.05$), whereas, the K, Cu, Zn and Mn content does not differ significantly ($P > 0.05$) among the two verities. As fermentation time increased, Ca, Mg, K, Na and Cu contents increased however Na, Fe and Zn contents decreased. The Fermentation time significantly ($p<0.05$) affects the calcium, iron, magnesium, manganese and zinc content in Kocho prepared from Lemat and Yedebreye enset variety while the potassium and sodium content was significantly ($p<0.05$) affected in kocho sample prepared from Yedebreye variety.

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INTRODUCTION

Enset (Ensete ventricosum (Welw.) Chessman, is a perennial, herbaceous, monocarpic and monocotyledonous crop in the family Musaceae (solomun *et al*., 2008). The corm and pseudostem of enset yield a highly carbohydrate-rich staple or co-staple food, for the quarter of the Ethiopian population that inhabits the south and south-western part of Ethiopia. Enset is a crop that tolerates prolonged drought periods, flooding and many diseases. Due to its drought tolerance, it is regarded as a priority crop in Ethiopia, where it makes a major contribution to the food security of the country (Tobiaw and Bekele, 2011). Regions where enset is used as staple food are usually less affected by the recurrent drought periods that occur in Ethiopia (Chakoro, and Mekuria, 2015).

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Enset can easily be stored without the need for refrigeration and is available throughout the whole year. It can be accessed at any time when there is food shortage and other crops fail as a result of drought, diseases or other factors. However, enset has been harvested so intensively during droughts that some important clones have become extinct, thereby reducing the genetic diversity of the crop (Brandt *et al*., 1997). Enset represents 65% of the total crop production in the southern regions of Ethiopia. Productivity is very high compared to other crops but varies depending on edaphic factors, altitude, cultural practices and varietal differences (Genet Birmeta, 2004).Enset is usually harvested at onset of flowering, 5 to 8 years after planting, and is grown with generations of plants mixed, thus being a reliable food source over time. The vegetative growth habit of enset is similar to banana plants, but enset is not grown for the fruits; enset fruits contain mostly large and very hard. For humans, edible parts of enset are the pseudostem (squeezing and fermentation gives the main food source from enset, a

product called "kocho") and the corm (the underground stem) that can be cooked like an enormous potato, weighing upto 70 kg (Brandt *et al*., 1997). It is a multipurpose plant with a range of utilities including food, feed, construction and medicinal uses. Moreover, enset cultivation improves soil by permanent soil tillage due to its high demands to soil fertility and soil structure (Mohammed*et al*., 2013).Fermentation is widely applied in the processing of enset for the preparation of a wide variety of foods in Ethiopia and it contributes to the development of acceptable texture, flavour and improves the nutritional quality, digestibility and safety of foods. Fermentation has also been identified to significantly improve the nutritional value (protein quality) of enset based foods (Hiwot Bekele, 2015).

Enset products (Kocho and bulla) are the foods consumed fermented. Since Bulla and Kocho are one of the main energy sources and serve as the staple and co-staple food for many people in Ethiopia, knowledge of the fermentation process is of particular interest for proper utilization of the crop (Hiwot Bekele, 2015). The fermented kocho is often stored in pits that are lined with Enset leaves. The kocho must be left in a storage pit for a minimum of a month, but it can be stored for many months and even for several years *(Melese, 2012,* Kelbessa *et al*. 1997). Traditionally, enset is fermented prior to consumption. Such processing can have both detrimental and beneficial effects of the nutrient content of the food. Apart from some studies carried out to determine the chemical composition of some ensetvarieties (solomun *et al*., 2008); there are no comprehensive studies to evaluate the impact of the indigenous food processing on the mineral nutritional quality of the foodproduct (kocho) obtained by indigenous fermentation. Indeed (HiwotBekele, 2015) investigated the effects of fermentation time, variety and processingmethods on physicochemical qualities, chemical composition, microbial quality and sensory acceptance of Bula. Therefore, the main objective of this study is to evaluate the effects of enset variety and indigenous processing (fermentation) method of kocho on its mineral nutritional composition.

MATERIALS AND METHODS

Site description: The samples for the experiment were collected from Guraghe Zone in the Ethiopian Southern Nations, Nationalities, and Peoples' Region (SNNPR). Guraghe Zone is one of the fantastic potential sites for cultivating Enset (Ensete ventricosum (Welw.). it is positioned between 7.80 - 8.50 North latitude and 37.50C - 38.70 East longitude of the equator. The zone includes altitudes ranging from 1,001 to 3,500 meters above sea level. The mean annual temperature of the zone ranges between 13-30 0c and the mean annual rain fall ranges 600-1600 mm.

Materials and Apparatus: The following materials were used in the study, beakers (different size), graduated cylinder, volumetric flask (different size), electrical balance, Erlenmeyer flask, mortar and pistil, auger, oven, polyethylene bags, desiccators, drying dishs, Kjeldahl flasks, muffle furnace , mesh with different size sieve, blending device (Moulinex, France),Ice box (portable), Erlenmeyer flask, Whatmann filter paper, flame photometer and flame atomic absorption Spectrophotometry (model AA-6800 Shimazdu).

Chemicals and Reagents: Reagents that were used in the analysis are all analytical grade. Chemicals which were used in the study are (69-72 %) HNO_3,Hydrochloric acid (HCl),sulfuric acid (H_2SO_4), $LnNO_3$. $ZnSO_4$, $CuSO_4$, $FeSO_4$, KCl, Na_2SO_4, $MgSO_4$, $CaSO_4$and $MnSO_4$ (or nitrate salts of the metals were used when available) are standard reagents that were used for the preparation of stoke standard solution of their metal.

Cleaning of glassware and sample containers: All glassware were cleaned with detergent and warm water, rinsed a number of times with tap water, and then soaked for 12 hours in 10% analytical grade nitric acid solution. Finally, they were rinsed with deionized water and dried in the oven at 105 ºC. The plastic containers were cleaned with detergent and tap water, soaked in 1:1 nitric acid. They were then dried in an open rack and stored safely in a locked dirt free storage area.

Sampling and fermentation: Samples of two enset variety (Lemat and Yedebreye) were randomly collected from enset farm (matured, that is, normal harvest for maximum kocho yield). The two enset varieties (Lemat and Yedebreye) were chosen based totally on the consumption frequencies and nearby preference for kocho preparation. The two types (Lemat and Yedebreye) of Enset ventricosum (Musaceae family) were processed into kocho, in accordance to the Gurage processing approach of kocho preparation in the enset farm at the outdoor of the farmer by means of following fermentation procedure of Gurage. This includes harvesting and removing leaves and older leaf sheaths from the designated plants for kocho preparation. The inner leaf sheaths were separated from the pseudo stem down to the real stem, which was part between corm and pseudo stem.
Then, the underground corm was separated from the real stem. The leaf sheath was once peeled and reduced into portions of about half meter size (approximate length), and cut up lengthwise, in order to shorten the leaf sheath to a manageable size. Then, the pseudo stem was decorticated the use of a domestically made bamboo scraper, whilst the leaf sheath is held on an incline in opposition to a wood plank. The working place used for decortication was covered with Enset ventricosum (Musaceae family) leaves. Then, the corm was grated one by one by serrated animal bone after uprooting and elimination of any soil from its surface with a domestically made knife. After the completion of decorticating and grating, the leaf sheath pulp was unfold on clean Enset ventricosum (Musaceae family) leaves overlaying the ground, after which the grated corm was mixed on the decorticated pulp. Until the blend is partly fermented, mixing, rinsing, and slicing of the decorticated pulp and starter was continued over a length of time, the last outcome is then referred to as Kocho. The first sample, referred to as zero month (0m) sample, was collected immediately after the mash was placed in the pit, thereafter samples were acquired at intervals of one month. 2kg kocho samples prepared from Lemat and 2kg kocho samples prepared from Yedebreye (total of 16kg) were collected. Samples were stored in plastic bags. Finally, the collected samples were air dried, crushed and passed via 2mm sieve and transferred to glass bottle till analysis.

Sample preparation: The mineral analysis ofkocho sample was conducted after sample preparation by wet digestion procedure. (Minaleshewa and Bhagwan, 2008) has optimized different conditions by varying reagent volume, digestion time, volume ratio ofreagents and digestion temperature for processed edible part of *Ensete ventricosum* (kocho and bulla). Finally he selected the procedure involving 2 mL of HNO_3 (69-70%) and 2 mL of $HCLO_4$ (70%) for digestion time of 2 hoursfor a temperature adjusted at an approximately 240 ^{o}Cfor the whole duration. Having this as a base, his procedure was checked whether it works for the processed enset sample or not. All his procedures were evaluated and it was effective for Kocho.Therefore, based on the listed criteria on his paper and presently confirmed one, the optimum digestion procedure chosen was the one that fulfilled thestated criteria for complete digestion of 0.5 g of the dry sample, with 2 mLHNO_3(69-72%) and 2 mL $HCLO_4$(70%) for a total of 2 hrs. for a temperature adjusted at an approximately 240 ^{o}C.Blank solutions were prepared following the same digestion procedure as the sample. Finally, cool and filter into the volumetric flask. Then, the solution was used for the determination ofall the minerals.

InstrumentCalibration and Operating Conditions: Calibration ofthe instrument was carried out with a range of standard solutions prepared. The calibration curves for each minerals were set to ensure the accuracy of the atomic absorption spectrometer and to confirm that the results of determination are true and reliable.Secondary standard solutions containing 50 mg/L were prepared from 1000 mg/L standard stock solutions. The working solutions (50mg/L) of each minerals were then freshly prepared by diluting with deionized water to obtain five working standards for each mineral of interest. Ca, Mg, Mn, Zn, Fe, and Cu were analyzed with atomic absorption spectroscopy (model AA-6800 Shimazdu) Na and K were determined by using the Flame photometer methods (AOAC, 2000).Acceptability of linearity data is often judged by examining correlation coefficients (Table 2.1).The instrumentaloperating conditions (Wavelength, lamp and burner alignment and slit width) for minerals (Ca, Mg, Fe, Zn, Cu, and Mn) are presented in Table 2.2.The same analytical procedure was employed for the determination ofelements in digested blank solutions.

Method validation: The capability to furnish timely, accurate and reliable information is central to the role of analytical chemistry. Method validation is the process of providing that analytical method is acceptable for its intended purpose. Therefore, Validating analytical procedures and estimating uncertainties related to the result are an important concerns for analysts. As there is no certified reference material (Kocho) in our laboratory, the validity of the optimized digestion process for Kocho were checked with the help of carrying out with a lower level of traceability, such as spiked samples. The percentage recovery test for the kocho sample shewed that the value of % recovery lies between 90 to 110% (100± 10), which are within the acceptable range for all metals.

Statistical analysis: Results were expressed as mean±standard deviation of three replicates. Data was analysed by the analysis of variance (ANOVA) using SPSS/20.0 softwareto check whether there is significant difference or not between means at 95% confidence interval. Significance was accepted at ($p<0.05$) level ofprobability.

RESULTS AND DISCUSSION

Effect of enset variety and fermentation time on the Mineral composition of Kocho: The effect of indigenous fermentation time (30, 60 and 90 days of fermentation time) and Enset ventricosum (Musaceae family) varieties *(Lemat and Yedebreye)* on the mineral composition of kocho (Calcium, sodium, potassium, magnesium, copper, manganese, iron and zinc) are presented in Table 4.1. As can be seen from Table 5.1, the results of this study shows that the content of Ca, Mg, K, and Zn content (190.11mg/100g, 117.47mg/100g,860.99mg/100g and 3.87mg/100g respectively) was found higher in the unfermented kocho prepared from *yedebreye* than inthe unfermented Kocho prepared from *Lemat (*183.89mg/100g, 112.68mg/100g, 857.06mg/100g and 2.17mg/100g respectively),but the Na, Fe, Cu, and Mn content of Kocho prepared from *Lemat* (27.94mg/100g, 3.58mg/100g, 3.3mg/100g and 2.95mg/100g respectively) was found higher than in kocho prepared from *yedebreye*(22.54mg/100g,2.66mg/100g, 2.41mg/100g and 1.56 mg/100g respectively). The Ca, Mg, Na and Fe content differ significantly ($P<0.05$), whereas, the K, Cu, Zn and Mn content does not differ significantly ($P>0.05$) among the two verities. The pattern of concentration of minerals in the unfermented kocho prepared from *Lemat* was decreased as; K > Ca> Mg> Na > Fe > Cu>Zn >Mn. Likewise the pattern of concentration of minerals in the unfermented kocho prepared from *yedebreye* was decreased as; K > Ca> Mg > Na > Fe> Zn > Cu >Mn.

The variation in the mineral content in the two varieties could be because of genetic differences, immaturity, variation in the soil fertility, root distribution of the plant, physical and chemical nature of the soil, and method of cultivation (Igbabul, 2014). A high accumulation of potassium, calcium and magnesium relative to others could be due to their higher natural abundance in the soil (Fekadu and Ledin, 1997). Additionally, since the soils, which have been used for cultivating the plant are highly fertilized with manure and organic residues, they were high in potassium, calcium and magnesium. Hence, the plant has high amount of these metals. Whereas, in all the samples copper concentration was the least, this may be due to low natural abundance in the soil, low absorption constant and liable complexion with organic matter in the soil (Nurfetaet. al, 2008 and, Minaleshewa and Bhagwan, 2008)). The effect of the fermentation time in the mineral composition was studied. The current study indicated a decreasing pattern in the Iron, Zinc, and sodium content as the fermentation time increases in both varieties. However, the reduction was not significant ($p>0.05$) in both kocho samples (Table 4.1). The possible reason for the reduction ofthe minerals could be due to loses during and after fermentation i.e. when the edible part of pseudostem and corm are grated, decorticated and peeled, losses that result from squeezing out of the watery part from Kocho, losses due to inefficient processing or storage systems, and uptake of the minerals by the fermenting microorganisms (Minaleshewa and Bhagwan, 2008). Even if there is no significant different ($p>0.05$), fermentation time resulted slight increment in the calcium, magnesium, potassium, copper, and manganese content of the kocho prepared from both varieties. The increase in the concentration ofcalcium, magnesium, potassium, copper and manganese could be due to the minerals of the Kocho that are not readily available for microorganisms as they are

Table 2.1. Instrumental operating conditions for the determination of minerals by FAAS

Instrumental parameters	Metals					
	Ca	Mg	Fe	Zn	Cu	Mn
Wavelength (nm)	422.7	285.2	248.3	213.9	324.7	279.5
Slit width (nm)	0.7	0.5	0.4	0.4	0.4	0.2
Lamp current (mA)	2.0	4.0	0.2	3.0	3.0	2.0
Instrumental Detection limit (pg/ml)	0.01	0.001	0.03	0.005	0.005	0.001

Table 2.2. Standard solution and correlation coefficient

Metals	Concentration of Standard solution (mg/kg)	Correlation coefficient (R^2)
Cu	0, 0.25, 0.5, 1.5, 2	0.99984
Zn	0, 0.25, 0.50, 1.00, 1.50, 2.00	0.9995
Mn	0, 0.01, 0.05, 0.1, 0.5	0.9987
Fe	0, 0.5, 1, 2, 3, 4,	0.9993
Ca	0, 0.5, 2, 4, 6, 8	0.9989
Mg	0, 0.05, 0.1, 0.2, 0.3, 0.4	0.9987
Na	0, 2, 4, 6, 8, 10.	0.9997
K	0, 2, 4, 6, 8, 10	0.9998

Table 2.3 Recovery test for Kocho samples

Metals	[a]Conc. In sample	Amount spiked (in mg/kg	[b] Concentration in spiked sample (in mg/kg)	[c] %R±SD
Ca	183.89	50	234.89±0.3342	102% ± 1.3342
Mg	112.68	50	161.68±0.2352	98% ± 1.2352
K	857.06	100	958.497±0.3561	101.44%±0.3561
Na	27.94	20	48.56±0.4323	103.1% ± 0.4323
Cu	3.3	5	8.10±0.78	96%±3.45
Zn	2.17	10	12.12±0.09	99.5%±2.45
Fe	3.58	5	8.32±0.49	94.8%±3.50
Mn	2.95	10	12.42±0.32	94.9%±1.55

[a] Average value of triplicate measurements
[b] Values are mean ± SD of triplicate readings of triplicate analyses.
[c] Values are mean ± SD of triplicate readings of triplicate analyses.

Table 4.1: The effect of Fermentation time and variety on the mineral composition of *Kocho in mg/100g*

Minerals	Varity	Fermentation time period			
		Raw	30 day	60 day	90 day
Ca	*Lemat*	183.89±2.04	178.99±0.65	180.00±0.98	181.80±0.67
	Yedebreye	190.11±1.83	188.83±1.24	189.67±1.87	190.11±2.70
Mg	*Lemat*	112.68±2.97	108.64±3.75	109.11±4.5	110.60±3.5
	Yedebreye	117.47±5.2	110.88±7.8	113.05±4.4	115.11±4.44
K	*Lemat*	857.06±7.60	844.26±3.668	848.77±7.00	50.8±8.90
	Yedebreye	860.99±6.55	850.26±5.33	855.60±7.84	858.21±8.06
Na	*Lemat*	27.94±0.28	23.76±0.34	20.11±0.40	18.64±0.50
	Yedebreye	22.54±0.19	20.31±0.16	18.55±0.065	17.36±0.475
Cu	*Lemat*	3.3±0.03	2.31±0.02	2.82±0.09	3.36±0.05
	Yedebreye	2.41±0.04	2.10±0.04	2.25±0.06	2.45±0.023
Zn	*Lemat*	2.17±0.07	3.50±0.0	2.86±0.07	12.77±0.07
	Yedebreye	3.87±0.04	4.85±0.02	4.58±0.02	3.90±0.01
Fe	*Lemat*	3.58±0.09	4.24±0.27	3.68±0.31	3.67±0.22
	Yedebreye	2.66±0.09	3.00±0.07	2.67±0.08	2.66±0.12
Mn	*Lemat*	2.95±0.11	2.65±0.02	2.70±0.06	2.87±0.09
	Yedebreye	1.56±0.06	1.65±0.05	1.71±0.025	1.92±0.18

complexed with phytate, and the hydrolyses of phytate and the minerals are released from the complex (Igbabul, 2014).

The effect of fermentation time in the mineral composition was compared with different studies in literatures. Therefore, the effect of fermentation time on thecalcium and magnesium contents reported in this study were in agreement with (Hiwot, 2015, Nwachukwu et. al., 2018, Hassan *et al*, 2015 and Makinde *et al*, 2013).The calcium contents ofthis study was also in agreement with (Murwan and Ali, 2011). The effect of fermentation time for the potassium and magnesium contents reported in this study were in agreement with (Makinde *et al*, 2013, Hassan *et al*, 2015 and Murwan and Ali, 2011) and (Hiwot, 2015, Nwachukwu et. al, 2018 and Hassan *et al*, 2015) respectively.

The effect of fermentation time for the manganese contents of this study was in agreement with (Makinde *et al*, 2013). The effect of fermentation time on the iron and zinc contents reported in this study were in agreement with (Hassan *et al*, 2015, Murwan and Ali, 2011 andEmire andButa, 2015) and the iron content was also in line with (Kelbessa, 1997). The Sodium content in this study was in agreement with the study reported by (Hassan *et al*, 2015).

Conclusion

In this study, kocho prepared from two varieties of enset were analyzed for eight mineral contents. Moreover, the effect of fermentation time (0, 30, 60, and 90 days) on the parameters of interest for all kocho was observed.

The mineralsanalysed were calcium, magnesium, potassium, sodium, iron, zinc, copper and manganese. In conclusion, extending the fermentation time and different Enset variety had resulted in differences in mineral composition of kocho. Kocho fermented for 90 days had showed better nutritional quality. Kocho prepared from *yedbreye* variety was better in minerals such as Calcium, potassium, magnesium, and zinc content whereas Kocho prepared from *Lemat* variety was better in minerals like sodium, copper, manganese and iron content. Generally, the selection of appropriate varieties and use of recommended fermentation time were found to be good practice for the processing of traditional enset products such as kocho which is a traditional fermented food in Ethiopia.

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