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# Effect of Enset variety (*Ensete ventricosum* (wele) cheesman) and fermentation time on the proximate composition of Kocho, an Ethiopian Traditional Fermented Food

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## Abstract

The experiment was carried out using two *Enset ventricosum* varieties (Lemat and Yedeberye) and three fermentation time, to investigate the effects of fermentation time and enset variety (Lemat and Yedeberye) on the proximate composition of kocho. In this study, kocho samples were prepared from yedbereye and Lemat enset variety, and processed by indigenous Gurage kocho processing methods. All processed kocho samples were then fermented for 30, 60 and 90 days. This studies output revealed that, the proximate composition of kocho were affected by fermentation time and *Enset ventricosum* variety. The contents of crude fat, crude fiber, moisture, crude protein and total ash in the Kocho that is prepared from yedebereye (1.74%, 4.90%, 54.84%, 4.57% and 3.14% respectively) was found higher Than the contents in Kocho prepared from Lemat (1.6%, 3.8%, 50.84%, 3.19% and 2.68% respectively). On the other hand, Kocho samples prepared from Lemat resulted in higher carbohydrate content (37.85%). As fermentation time increased, crude fiber, crude fat, carbohydrate and moisture contents were decreased; whereas, the total ash and crude protein contents were increased. The Fermentation time significantly ($p<0.05$) reduces the moisture and crude fiber content, whereas the reduction was not significant for the crude fat and carbohydrate content in Kocho prepared from both variety. Moreover, fermentation significantly ($p<0.05$) increases the crude protein and total ash content.

**Keywords:** Enset, Enset variety, fermentation, Kocho, proximate composition

## Introduction

*Enset ventricosum* (*Musaceae* family) [*Ensete ventricosum*] is a monocarpic short-lived perennial plant which is grown in southern and south-western parts of Ethiopia for human consumption. It tolerates prolonged drought periods, flooding and many diseases [1,2]. 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. Regions, where *Enset ventricosum* (*Musaceae* family) is used as staple food, are usually less affected by the periodic droughts that occur in Ethiopia [3].

*Enset ventricosum* (*Musaceae* family) is one of the potential indigenous crops for food production [4] and can be grown everywhere in Ethiopia. According to several authors [5,6], the *Enset ventricosum* (*Musaceae* family) cultivation system is economically viable and is one of the few successful indigenous and sustainable agricultural systems. It is sustainable because it has been providing food for humans for generation from the same plot and maintains the quality of life of the people.

It grows in a wide range of environmental conditions. Even though it is grown in many Administrative regions, the dwellers of the central and southwestern parts of Ethiopia are the only people that use *Enset ventricosum* (*Musaceae* family) as a staple and co-staple crop [5]. At present, *Enset ventricosum* (*Musaceae* family) is important for about one-fifth of the total population of Ethiopia and cultivation is estimated to cover more than 224, 400 Hectares of land. The majority of *Enset ventricosum* (*Musaceae* family) production is confined to Sidamo, Ghurage, Shoa, Keffa, Gamo Goffa and Illubabor administrative regions [5].

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 specimens 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 [7]. The edible parts are formed by the pseudostem and the underground corm rather than by the fruit [5]. Nutritive value of starchy foods depends mainly on their nutrient content, physico-chemical properties of their starches and the existence of anti-nutritional activities and toxic substances [8].

The most important foodstuffs obtained from *Enset ventricosum* (*Musaceae* family) are locally known as kocho and bulla. These are fermented foods as those foods which have been subjected to the action of micro-organisms or enzymes so that desirable biochemical changes cause significant modification to the food and its nutritional constituent. For human consumption, edible parts of *Enset ventricosum* (*Musaceae* family) are the pseudostem (squeezing and Fermentation time gives the main food source from *Enset ventricosum* (*Musaceae* family), a product called "kocho") and the corm (the underground stem) [9,10]. The length of Fermentation time period varies from a few weeks to several months, depending on temperatures of incubation [3,11]. In the cooler regions, it is kept in a hollow for years, and the quality is said to increase with increasing fermentation time. In warmer regions, Fermentation time is quick and is, therefore, completed about three to six months. Physical and chemical properties of the soil, application of natural (manure) and artificial fertilizers, storage methods and processing of Kocho, age of harvested (processed) *Enset ventricosum* (*Musaceae* family) plant, climatic condition of the region and other factors are the main contributors for the chemical and mineral contents of Kocho [11,12].

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Since *Enset ventricosum* (*Musaceae* family) products such as Bulla and Kocho are one of the main energy sources and serve as the day-today-life for many people in Guraghe, awareness of the fermentation process is of particular interest for proper utilization of the plant. Apart from some studies carried out to determine the chemical composition of some *Enset ventricosum* (*Musaceae* family) varieties [1,8]; there are no comprehensive studies to evaluate the impact of food processing on the nutritional quality of the food product (kocho) obtained by indigenous fermentation.

## Materials and Methods

### Description of the study area

The samples for the experiment were collected from Guraghe Zone in the Ethiopian Southern Nations, Nationalities, and Peoples' Region (SNNPR). Guraghe is a Zone in the Ethiopian Southern Nations, Nationalities, and Peoples' Region (SNNPR). This zone is named for the Gurage people, whose homeland lies in this zone. Geographically, Guraghe Zone is located between 7.80-8.50 North latitude and 37.50 C-38.70 East longitude of the equator. Wolkite, the capital of the zone, is 155 km away from Addis Ababa to south west direction. Gurage zone has a total area 5932 km [2]. It has 13 Woreda as with a total population estimated about 1,343,246. The zone comprises altitudes ranging from 1,001 to 3,500 meters above sea level. The mean annual temperature of the zone ranges between 13°C-30°c and the mean annual rain fall ranges 600-1600 mm. The climate in the zone is of three divisions.

### Sampling and fermentation

The study's experiment was conducted with two *Enset ventricosum* (*Musaceae* family) varieties (Lemat and Yedebreye) which are processed by indigenous method at three fermentation times (30 day, 60 day, and 90 day). The two *Enset ventricosum* (*Musaceae* family) varieties were collected from *Enset ventricosum* (*Musaceae* family) farm (matured, that is, normal harvest for maximum kocho yield). The samples were obtained from Gurage Zone, Gunchire woreda, in SNNP, Ethiopia.

The two *Enset ventricosum* (*Musaceae* family) varieties (Lemat and Yedebreye) were chosen based totally on the consumption frequencies and local desire of *Enset ventricosum* (*Musaceae* family) varieties for kocho preparation. The two varieties (Lemat and Yedebreye) of *Enset ventricosum* (*Musaceae* family) were processed into kocho, in accordance to Gurage processing approach of kocho preparation as described by [4]. During harvest, leaves and older leaf sheaths were first eliminated from the designated plants for kocho preparation. The inner leaf sheaths were separated from the pseudo stem down to the true stem, which was area between corm and pseudo stem.

After completion of harvesting and fermentation area preparation pseudo stem and the corm was separated and also the corm was splitted into 4-8 pieces based on their sizes, surface cover of leaf sheath (shafa) was peeled, and scraped by a locally made bamboo scraper. The upper half of the leaf sheath was then turned upside down, for scraping. Fibers were extracted as a bi-product through repeatedly scraping of the soft leaf sheath. It is chopped by locally made knife, and the corm was pulverized by traditional equipment called Cheko. Then, the chopped scraped leaf sheath and pulverized corm was mixed together and tightly packed on the surface by wrapping with fresh and dry enset leave. Then loaded with heavy material such as stones to create airtight conditions and facilitate the fermentation process. The samples were subjected for analysis after 30, 60 and 90 days of fermentation.

The samples were subjected for analysis after 30 days, 60 days, and 90 days of fermentation including the raw. 2 kg kocho samples prepared from Lemat and 2 kg kocho samples prepared from Yedebreye (total of 8 kg) were collected. Samples were kept in plastic bags. Finally, the collected samples were air dried, crushed and passed through 2 mm sieve and transferred to glass bottle until analysis.

### Chemicals and reagents

Reagents that were used in the analysis were all analytical grade. Deionized water, catalyst, perchloric acid, nitric acid, sulfuric acid, sodium hydroxide, and hydrogen peroxide, methyl blue, diethyl ether, ethanol, boric acid of analytical reagent grade which were purchased from JJ Laboglass St. Company were used acting as reagents and solvents throughout all procedures starting from sample collection to analysis.

### Characterization method

The methods of Association of Official Analytical Chemists (AOAC, 2000 and 1995) were used for determination of primary metabolites content [13,14]. Accordingly, AOAC 925.09 for moisture, AOAC 979.09 for protein, AOAC 920.39 for fat, AOAC 962.09 for fiber and AOAC 923.03 for ash contents were used with minor modification.

### Proximate composition analysis

The collected samples were air dried, crushed in to powdered size and surpassed via 0.425 mm sieve. The methods of Association of Official Analytical Chemists (AOAC, 2000) were used for determination of moisture, crude fiber, protein, fat, crude ash and carbohydrate content of the samples [13].

Moisture content: A drying dish was dried in an oven at 105°C for 1 hr. and placed in desiccator to cool. The weight of the drying dish (W1) was determined. 5 g of kocho samples were weighed in the dry dish (W2), oven dried at 105°C for 3 hr. and after cooling in a desiccators to room temperature, it was again weighed (W3). And brought to a constant weight by put it again in an oven for extra one hour.

%moisture content=original wt.-final wt. x100/Original wt.

Crude fiber: Crude fiber content was determining by using Fibertec. The sample were analysed by the steps of digestion, filtration, washing, drying and combustion. 2 g kocho sample were transferred to 400 ml beaker. After digestion with 1.25% $H_2SO_4$ and washed with distilled water, it was digested by means of 1.25% NaOH, was filtered in coarse porosity crucible apparatus, at a vacuum of about 25 mm. The residue left after refluxing was washed again with 1.25% sulfuric acid near the boiling point. The residue was then dried at 95°C overnight, cooled in desiccators, and weighed (M1). After incineration for 2 hrs. At 500°C, it was cooled in desiccators, and weighed again (M2) [9]. The total crude fiber will be expressed in percentage as

Total crude fiber (%)=((M1-M2)/ M3) x 100

Where, M3 is the weight of sample

Crude fat: Crude fat test were carried out on soxhlet extractor method utilizing diethyl ether. 3 g dried samples of kocho sample were extracted with 100 ml diethyl ether, for a minimum period of 4 hrs. in the soxhlet extractor. The solvent was once then evaporated through heating on a steam bath. The flask containing the extracted fats was dried on steam bath to a constant mass. The total crude fat was then calculated as percentage by weight:

Crude fat, percent by weight=((W2-W1)/w) x 100

Where, W1=weight of the extraction flask

W2=weight of the extraction flask plus the dried crude fat (g)

W=weight of the sample

**Crude protein:** Protein content was determined by means of the Kjeldahl method. The universal procedure included the following steps of digestion at 370oc for 4 hrs. 2 g sample was digested by adding 6 ml of concentrated sulfuric acid and then, 3.5 ml of (30%) H2O2 was introduced step by step. When violent reaction stopped 3 gm of catalyst (mixture of potassium sulphate and copper sulphate) was added and left for 30 minutes. The mixture was digested in digestion stove for 4 hrs at 370o c. After digestion was once completed, the content in the flask was once diluted by 50 ml of water and neutralized using 35% of NaOH. Upon addition of NaOH, the ammonium was distilled off and trapped into a boric acid and solution containing methyl blue and methyl red indicators. Finally, titration of the ammonium attached to borate anion was titrated with standardized 0.1 HCl, and the quantity of HCL consumed was recorded and total crude protein of kocho was calculated as total nitrogen.

%N=(S-B x 14 x NHCl)/10 x V

Crude protein=%N x 6.25

Where, S-is sample titration reading

B-is blank titration reading and 14 is the molecular weight of N

V-Is volume taken for titration?

N-is normality of HCl

Total ash content: the ash content was measured in accordance to dry ashing procedure. Clean dcg dish was dried at 105°C in hot air oven, cooled in a desiccator and weighed using analytical balance (M1). Then, 2.5 gram of kocho powder sample was put and weighed (M2). The sample was once charred on a hot plate till the contents flip black. The dish with its contents was transferred to a muffle furnace (S30 2RR England) and ignited at 550°C for 5 hrs. Lastly, the residue was weighed (M3) and the total ash was expressed as percentage on dry basis as follow:

Total Ash (%)=[(M3-M1)/(M2-M1)]*100

Carbohydrate content:Total carbohydrate content of the samples were determined by difference:

Total Carbohydrate (%)=100-(%M+%P+%F+%Fb+%A)

Where, %M=Moisture content in percent

%P=Crude protein content in percent

%F=Crude Fat content in percent

%Fb=Fiber content in percent

%A=Ash content in percent

**Methods of data analysis**

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

## Result and Discussion

The effect of fermentation time (0, 30, 60, and 90 days) and Enset varieties (Lemat and Yedbriye) on the proximate composition of Kocho are presented in Table 1

Moisture content

The moisture content of Kocho sample significantly ($p<0.05$) varied between the two varieties of *Enset ventricosum* (*Musaceae* family). Kocho prepared from Yedebreye (54.84%) had higher moisture content than Kocho prepared from Lemat (50.84%). The significant variation in moisture content among the Kocho samples prepared from Lemat and Yedebreye variety greatly reflects the genetic difference and maturity (age) difference among the varieties [6]. The moisture content of Kocho prepared from both variety (Lemat and Yedebreye) were significantly ($p<0.05$) affected by fermentation time (Table 1). The moisture content of Kocho prepared from both variety (Lemat and Yedebreye) significantly reduced from raw, fermented for 30 days, 60 days and for 90 days. This could be due to leakage or/and evaporation at a time of fermentation. Moreover, the reduction of the moisture contents has

| | Metabolites | Varity | Fermentation time period | | | |
|---|---|---|---|---|---|---|
| | | | Raw | 30 day | 60 day | 90 day |
| % | Crude fat | Lemat | 1.60 ± 0.01 | 1.75 ± 0.12 | 1.70 ± 0.18 | 1.69 ± 0.99 |
| | | Yedebreye | 1.74 ± 0.19 | 1.84 ± 0.24 | 1.82 ± 0.73 | 1.80 ± 0.7 |
| | Crude fiber | Lemat | 3.84 ± 0.22 | 2.85 ± 0.14 | 2.77 ± 0.67 | 2.55 ± 0.12 |
| | | Yedebreye | 4.90 ± 0.02 | 3.11 ± 0.44 | 2.85 ± 0.40 | 2.68 ± 0.8 |
| | Moisture | Lemat | 50.84 ± 0.35 | 40.57 ± 0.19 | 40.08 ± 0.57 | 39.82 ± 0.23 |
| | | Yedebreye | 54.84 ± 0.30 | 45.57 ± 0.19 | 45.24 ± 0.57 | 44.02 ± 0.23 |
| | Crude protein | Lemat | 3.19 ± 0.02 | 5.80 ± 0.99 | 6.32 ± 0.17 | 7.60 ± 0.12 |
| | | Yedebreye | 4.57 ± 0.20 | 5.05 ± 0.16 | 5.55 ± 0.15 | 6.80 ± 0.17 |
| | Ash | Lemat | 2.68 ± 0.99 | 2.78 ± 0.12 | 2.99 ± 0.99 | 3.99 ± 0.27 |
| | | Yedebreye | 3.14 ± 0.45 | 3.36 ± 0.01 | 3.58 ± 0.02 | 4.85 ± 0.02 |
| | Carbohydrate | Lemat | 37.85 ± 0.01 | 46.25 ± 0.11 | 46.14 ± 0.12 | 44.67 ± 0.17 |
| | | Yedebreye | 30.81 ± 0.19 | 41.07 ± 0.04 | 40.86 ± 0.06 | 39.53 ± 0.23 |
| Values are mean of duplicate ± SD | | | | | | |

**Table 1:** Effect of Fermentation time and enset variety on the proximate composition of kocho.

been related to the use of water by microbes for metabolic and growth activity, and due to a function of factors such as temperature, time and humidity [9,15,16]. The effect of fermentation time and variety on the moisture contents of Kocho reported in this study were in agreement with [4,9,11,15].

**Crude fiber content**

As it can be seen from Table 1, the crude fiber content of kocho samples showed significant difference ($p<0.05$) due to the enset variety and fermentation time. Kocho samples made from Yedebreye (4.90%) variety had more crude fiber content than kocho from Lemat (3.84%) variety. The difference in crude fiber content among kocho prepared from the two varieties was most probably attributed by genetic variation and by maturity (age) difference [9]. As the fermentation time elongates, the crude fiber content of kocho was decreased in variety, 0, 30, 60, and 90 days of fermentation. The decrease in fiber content during fermentation could be attributed to the partial solubilization or degradation of cellulosic and hemi-cellulosic structural materials in the plant (pulp) by microbial enzymes [15] and in this also, fermentation increases the breakdown of dietary fiber to soluble and digestible form, and which decrease the total indigestible crude fiber content. The effect of fermentation time and variety on the crude fiber contents of Kocho reported in this study were in agreement with [4,9,17,18].

**Crude fat content**

The crude fat content of kocho samples showed no significant difference ($p>0.05$) among the two enset variety and fermentation time. Kocho samples made from Yedebreye (1.94%) variety had more crude fat content than kocho from Lemat (1.80%) variety. This could be due to genetic variation. An effect was observed due to fermentation time difference on the kocho in crude fat content. When fermentation time elongates, the crude fat content in kocho had decreased in both variety. However, the reduction was not significant in both kocho samples (Table 1). This could be due to the increase in degradation of fats by lepolytic enzymatic activities and microbial multiplication of microbial causing the consumption of nutrients during fermentation [4].

The decreased fat content of Kocho may help to extend its shelf life because food products containing high fat are susceptible to both hydrolytic and oxidative or enzymatic rancidity and responsible for both the general acceptability and storage stability of the product [9]. The effect of fermentation time and variety on the crude fat contents of Kocho reported in this study were in agreement with [4,19].

**Total ash content**

The total ash content of kocho showed no significant difference ($p>0.05$) among the enset variety and fermentation time. Kocho samples made from Yedebreye (3.14%) variety had more ash content than kocho from Lemat (2.68%) variety. The difference in ash content may be due to genetic variation of *Enset ventricosum* (*Musaceae* family) [19]. An effect was observed due to fermentation time difference on the kocho in ash content. As fermentation time increases, the ash content in kocho sample was increased in both varieties.

However, the increment was not significant ($p<0.05$) in both kocho samples (Table 1). The possible reason for the increment could be the solubility of minerals and other organic constituents. The higher the fermentation time, the higher the amount of Organic and mineral constituents which contribute the total ash content in kocho [4]. The effect of fermentation time and variety on the ash contents of Kocho reported in this study were in line with [4,9,15].

**Crude protein content**

The Crude protein of kocho showed significant difference ($p<0.05$) among the enset variety and fermentation time. Kocho samples made from Yedebreye (4.57%) variety had more protein content than kocho from Lemat (3.19%) variety. The difference in protein content could be due to genetic/maturity level variation of the *Enset ventricosum* (*Musaceae* family) varieties [20]. An effect was also observed due to fermentation time difference on the kocho in protein content. As fermentation time elongates, the protein content in kocho sample was increased in both variety. The observed increase in protein content of fermented kocho samples could be attributed to the increase in microbial mass during fermentation, causing extensive hydrolysis of protein molecules to amino acid and other simple peptides. Secondly, the enzymatic hydrolysis of some protein inhibitors during fermentation, for instance, the degradation of anti-nutritional factors especially phytate may contribute to the increase in protein content due to the breakage of phytate-protein complexes. The increase may also be due to the structural proteins that are an integral part of the microbial cell. It is also known that Fermentation time had the general effect of increasing the essential amino acid content of kocho [11,16]. The effect of fermentation time and variety on the crude protein contents of Kocho reported in this study were in agreement with [4,9,15,18,21].

**Carbohydrate content**

As the finding of the study, there was significant difference ($p<0.05$) in carbohydrate content among the enset varieties. The maximum carbohydrate content (37.85%) for kocho samples was obtained from Lemat, while the minimum carbohydrate content (30.81%) was obtained from Yedebreye. The difference in carbohydrate content may be due to genetic and/or maturity level variation [9]. The current study indicated a decreasing pattern in the carbohydrate content as the fermentation time increases in both varieties. However, the reduction was not significant ($p>0.05$) in both kocho samples (Table 1).

The reduction of carbohydrate content during the fermentation process was possibly due to the breakdown of more complex components by enzymes produced by the fermenting microorganisms. In connection with this the soluble starch and sugar are principal substance for fermenter microorganisms. Therefore, degradation and subsequent decrease in starch content are expected to occur. Fermentation will activate enzymes which act on polysaccharides. These enzymes degrade polysaccharides and latter leads to reduction of carbohydrate [4,9]. The effect of fermentation time and variety on the carbohydrate contents of Kocho reported in this study were in agreement with [4,9,18] respectively.

## Conclusion

In this study, kocho prepared from two varieties of enset by fermenting for 30, 60 and 90 days were analyzed to investigate the effect of fermentation time and enset variety on the composition of proximate compositions. Secondary metabolites such as moisture, crude fat, crude fiber, crude protein, total ash and carbohydrate content were studied.

In conclusion, having different fermentation time and different *Enset ventricosum* (*Musaceae* family) variety had resulted differences in proximate composition. Extending the fermentation time and different *Enset ventricosum* (*Musaceae* family) variety had resulted change in the composition of proximate compositions. Kocho prepared from Yedebreye variety was better in crude fat, crude fiber, moisture, ash and crude protein, whereas kocho prepared from Lemat variety was better in carbohydrate. Combining all the results of this study, the most commonly used fermentation had resulted in tremendous reduction of moisture and crude fiber and slight reduction in crude fat and carbohydrate contents of kocho. Moreover, it also enhanced the protein and ash the raw sample. Kocho fermented for 90 days had showed better nutritional quality in terms of crude protein and ash content.

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