Addis Ababa University
College of Natural Sciences
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Comparison of the Nutritional and Microbial composition of Kocho from Wild and Cultivated Enset from Bonga, Ethiopia.

By
Meseret Haile

A thesis Submitted to the School of Graduate studies of Addis Ababa University in partial fulfillment of the requirement for the Degree of Master of Science in Food Science and Nutrition.

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Declaration

I, the undersigned, hereby declare that this thesis is my original work and all sources of materials used for the thesis have been correctly acknowledged.

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Dedication

I dedicate this thesis to my Mother and Father, dear my family I would like to THANK YOU for all the things you have done for me since day one and FOR LOVING ME UNCONDITIONALLY. You were always there when there was a problem. You nursed me,raised me, educated me and loved me as much as you were able to with everything that you havebeen throughout the years, you have made it through. I am so happy that I got A Mother that I am always proud of. I can say for myself and everyone, that I am extremely proud to have you as my FATHER and MOTHER. I LOVE YOU BOTH FROM THE DEEPST OF MY HEART.

Dear my friend, brother and husband Yaredy, I don't know how to express my feeling for you.Although we did and will continue to encounter trials and hardship of various kinds, I am soblessed to endure it all with you. Starting out so young was scary for me. I had a tone of growingup to do…in many ways I still do. I am so much confident in who I am, what I believe and whatI am capable of because you have spoken that truth in my life. Your complement andencouragements have enabled me, and blessed me more than I deserve. Blessing started in our life even before graduation. I LOVE YOU! MAY GOD GIVE YOU LONG YEARS OF LIFE!!

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List of acronyms

AAU	Addis Ababa University
AOAC	Association of Official Analytical Chemists
WEPs	Wild edible plants
WHO	World Health Organization
PGRFA	Plant Genetic Resources for Food and Agriculture
LR	Landraces
CWR	Crop wild relatives
Ppm	Parts per million
AAS	Atomic absorption spectrometry
PFU	Colony Forming Units
LAB	Lactic Acid bacteria
FAO	Food and Agricultural Organization
ISO	International Organization for Standardization

Abstract

There are two types of Enset (Ensete ventricosum) plant in Ethiopia. The first one is cultivated enset and the second is wild enset which is seed propagated. Kocho prepared from wild enset was not used as food like kocho prepared from cultivated enset except during severe drought time without any investigation about the nutritional composition. The aim of this study is to evaluate and determine the nutritional, microbial composition and toxicity of kocho prepared from wild and cultivated enset. Three variety of kocho one from wild enset and two from cultivated enset were analyzed for their proximate, mineral, microbial composition and sub acute toxicity parameter using animal model. Microbial analysis for LAB, yeast and mold, Aerobic mesophilc bacteria, coliform and staphylococcus was made in 0, 10, 20 and 30th days of fermentation. Kocho sample from wild enset showed higher concentration of crude fat, crude fiber and Ash also the mineral concentration (K, Ca, Mg, Cu and Na) are higher as compared to the cultivated enset kocho.LAB were the dominant flora in all variety of kocho with total count of 12.4 lof cfu/ml, 12.1 log cfu/ml and 13.2log cfu/ml for White Bocho, Red Bocho and Epo, respectively at 30thdays of fermentation. Yeast also showed an increase in number from initial count of 2.6log cfu/ml to 8.9 log cfu/ml, 2.1log cfu/ml to 11.2 log cfu/ml and 3.4log cfu/ml to 10.3log cfu/ml for White Bocho, Red Bocho and Epo, respectively. Aerobic mesophilic bacteria and coliform decrease to the end of fermentation and staphylococcus are not detected in the course of fermentation. Sub-acute toxicity test showed that there is no sign of toxicity in skin and fur, eyes and mucous membranes, tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma of mice after administration of kocho prepared from wild Enset but there is an increase in mean weight of mice after the administration of test sample. Kocho prepared from wild enset can be used as a food and many other purposes. More ethno botanical investigations on wild Enset were recommended.

Key words: *Cultivated Enset, Wild Enset, Kocho, Nutritional Composition, Microbial composition, Toxicity*

CHAPTER ONE

1. INTRODUCTION

Now days the world is depending on few plant source foods which are not enough in balancing the food security**.** From different plants that can be used as a source of food some are grown in the forest and others are cultivated (Bharucha and Pretty, 2010). Those which are cultivated by the society are now getting more attention in investigating their content with respect to their nutritional value and improving food security.

Numerous wild edible plant species have been used by different communities in Ethiopia, mainly as supplement to conventional foods (Addis *et al.,* 2005; Awas, 2007; Giday *et al*., 2007; Assefa and Abebe, 2011; Feyssa *et al*., 2011; Molla *et al*., 2011; Addis *et al*., 2013). However, the biodiversity is threatened through replacement of forests with agricultural expansion and deforestation without cultivation and domestication of potential species. This situation could affect the biodiversity, climate condition and food insecurity throughout the country without knowing the important components of the plant. Diversification of production and consumption habits to include a broader range of plant species, particularly those currently identified as under-utilized, could significantly contribute to improve health and nutrition, livelihoods and ecological sustainability. Wild foods are relevant in household food security and nutrition in some rural areas, particularly in dry lands, where they represent relevant food sources during seasonal food shortage periods, and provide good nutritional supplies, notably micronutrients (Garí, 2003). Nutritional studies on neglected wild plants provide clues to aid the promotion of those species that have the best nutritional values which helps to ensure dietetic diversity and combat food insecurity (Tardio *et al*., 2006). According to Jaenicke and Hoschle-Zeledon (2006), over 50 percent of the world's daily requirement of proteins and calories is obtained from only three crops: wheat, maize and rice and this dependence on a few domesticated plant species will lead to limited resource use and also malnutrition result from micronutrient deficiency because many studies shows that wild vegetable have higher nutritional potential than some conventional cultivated green vegetables, particularly with respect to their mineral content (Yildirim *et al*., 2001).

Enset (*Ensete ventricosum* (Welw.) Cheesman) has been cultivated as a food and fiber crop in Ethiopia for several years and over 80% of the production is concentrated in the south and southwestern part of the country (Taye *et al*., 1967).There are different varieties of cultivated Enset found in Ethiopia both as the cultivated and wild. The wild species of Enset is distributed throughout much of central, eastern and southern Africa as well as Asia (Brandt 1996). This wild type of Enset was not eaten by the society in which the cultivated Enset is grown for a long time just because it is grown in the forest by natural way without human intervention. It is, therefore, worthwhile to note that focus should be provided to study kocho originated from wild Enset, to have scientifically explanation about this Enset. In this thesis, proximate compositions, mineral contents, Microbial population at different fermentation time and sub acute toxicology parameter were investigated.

1.1. Statement of the problem

From different types of crop growing in the southern Ethiopia Enset is the major staple food. The cultivated Enset is utilized by producing different byproducts but not the Forest one because of lack of any scientific investigation on the nutritional content of kocho prepared from the Forest Enset. In parts of Southern Ethiopia the consumption of wild-food plants seems to be one of the important local survival strategies and appears to have intensified due to the repeated climatic shocks hampering agricultural production and leading to food shortages.“Increased consumption of wild-foods enables people to cope better with erratic, untimely rains and drought for several consecutive years without facing severe food shortages, famine and general asset depletion as in other areas of Ethiopia (Mathys, 2000)”. The key to this strategy for survival is the collection and consumption of wild plants in uncultivated lowland areas such as bush, forest and pastoral land as well as the domestication of a great variety of these indigenous plants and trees for home consumption and medicinal use in the more densely populated and intensively used mid- and highlands.

The wild type of Enset which is at the study site is not used as a source of food by the society and it is just grown without giving any value for the society because they have spiritual believe that the Enset cannot be eaten and they even give it a name “The Devil Enset” and some people believe that when the kocho is baked the color is very dark by comparing it with the cultivated

variety of the Enset. Now a days in some parts of the community in which the research was conducted the people starts to cut the forest Enset and replace it with coffee plant because they believe it does have use. This activity will lead to the extinction of the plant.

With this understanding of the people about the wild Enset there is no scientific explanation about whether the plant have any nutritional value or not and does it have any relation with the nutritional content of the edible Enset beside the society believe. Little is known about the nutritional value or possible undesirable side-effects such as toxicity of kocho originating from wild, non-domesticated Enset. Therefore, it seems imperative to carry out applied research on the nutritional values of these plants. Information obtained from the study result may help to enhance knowledge needed to eradicate the negative attitude towards use and cultivation of the neglected variety of Enset and is critical in convincing the community and policy makers to assist their promotion for wider consumption and cultivation.

1. 2. Objectives

1. 2.1. General Objective

The main objective of this study is to evaluate and determine the nutritional, microbial composition and toxicity of kocho prepared from “Wild Enset “and “Cultivated Enset”.

1.2.2. Specific Objectives

- To determine the proximate composition of kocho from cultivated Enset and wild Enset.
- To determine the microbial population of the fermented kocho at 0, 10, 20 and 30 days of fermentation and isolation of Lactic Acid bacteria.
- To determine the mineral composition of kocho from cultivated Enset and wild Enset.
- To find out any type of health problem on consumption of kocho from wild Enset using sub acute toxicity parameters in animal model.

CHAPTER TWO

2. Literature review

2.1 The concept of wild and cultivated plant

Wild plants refer to plant that are not cultivated but are freely accessible within their natural habitats (Somnasang and Moreno-Black, 2000). There are also plants that gets a name wild edible plants (WEPs) which are neither cultivated nor domesticated but available from their natural source and used as a food (Beluhan and Ranogajec, 2010). Whereas, Gari (2003) defined Edible wild plants as 'Plants that grow in natural conditions and in some cases include semi-domesticated plants harvested for their human food and nutrition values'. Wild edible plant plays important role in closing gap during period of drought or food scarcity and in developing countries WEPs maintain livelihood security for many people (Afolayan and Jimoh, 2009). Cultivated plants are plants that the local species and their varieties are customarily used in agricultural and food systems. These plants are traditional in the sense that they are integrated and coevolving with the indigenous knowledge, agricultural practices, food habits, and cultural dynamics of the rural communities and peoples that hold them Gari (2003).

2.2. Contribution of edible wild plants in food security

The most widely used definition of food security states that food security exists when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food which meets their dietary needs and food preferences for an active and healthy life. Household food security is the application of this concept to the family level, with individuals as the focus of concern" (FAO, 2003). When the definition said "all people at all times" it shows that different people are food secure to varying degrees and will be affected by adverse events differently. We must assess variations in food security status between different groups of people. Most commonly, humanitarian and development agencies differentiate between groups according to their main livelihood (source of food or income), in addition to other factors such as geographical location and wealth.

This recognizes that people's food security situation may change. Even if your food intake is adequate today, you are still considered to be food insecure if you have inadequate access to food on a periodic basis, risking a deterioration of your nutritional status. Adverse weather conditions (drought, floods), political instability (social unrest), or economic factors (unemployment, rising food prices) may impact on the food security status (FAO, 2008).

"Three type of food security status include global, national and individual. Global food security requires that a sufficient quantity of food be present to feed the world's people, National food security is defined as an acceptable likelihood that food available for consumption within country is at least equal to biological needs throught the year, Individual food security is defined as an acceptable likelihood that each person's income, is broadly interpreted, is sufficient to satisfy all needs" (Ballenger *et al.*, 1990).

Using of wild edible plant is now becoming a key strategy of maintaining livelihood security by many developing countries (Afolayan and Jimoh, 2009). At its Twelfth Regular Session, the Commission on Genetic Resources for Food and Agriculture (Commission) urged that greater attention be given to crops that are essential for food security, and reiterated the importance of on-farm management of *Plant Genetic Resources for Food and Agriculture* (PGRFA) *in situ* conservation of crop wild relatives and wild plants for food, particularly in developing countries (Maxted *et al.,* 2011). *In situ* conservation means "the conservation of ecosystems and natural habitats and the maintenance and recovery of viable populations of species in their natural surroundings and, in the case of domesticated or cultivated plant species, in the surroundings where they have developed their distinctive properties" Convention on Biological Diversity (CBD, 1992) and FAO (2001). In this context *in situ* conservation encompasses two complementary approaches to the conservation of PGRFA: 1) on-farm management of traditional crops, or landraces and 2) management of wild populations in natural or semi-natural habitats.

From these different types of PGRFA, LR and CWR are the most threatened and least well conserved-both *in situ* and *ex situ*. In recent years there has been increased recognition of their importance for food and livelihood security, particularly in the context of climate change. The

particular food security value of CWR has been recognized at least since Darwin discussed their conservation (1868), but it was Vavilov (1926) who was the first to address their conservation in practical terms. However, CWR have been widely neglected because the responsibility for their conservation has neither been adopted by agricultural agencies nor environment agencies and it is only relatively recently that systematic CWR diversity conservation been promoted (e.g., Maxted *et al*., 19971; Meilleur and Hodgkin, 2004; Heywood and Dulloo, 2005;Stolton *et al*., 2006; Maxted *et al*., 2008).

The increasing human population, periodic food shortages and current and expected effects of climate change have all led to raised awareness of the need for more attention to be paid to global and national food security. However, it became obvious that an adequate supply of food at the national or international level does not in itself guarantee household level food security. For example, the Green Revolution in Asia of the 1960s and 1970s, with its package of improved seeds, farm technology, better irrigation and chemical fertilizers, was highly successful at increasing food supplies, but this was not automatically translated into improvements in food security of all people.

This insight showed the problem of a lack of effective demand. From the early 1980's, the importance of food access was increasingly recognized as a key determinant of food security. Hence, food production is just one of several means that people have to acquire the food that they need (FAO, 2008).

Globally, agriculture is being practiced in more adverse or marginal environments, whether due to human degradation of habitats, the demand for food forcing the expansion of agricultural lands or the effects of climate change. As a consequence, there is growing demand for the development of new varieties that can be adapted to these marginal environments and to the changing environmental conditions that have been rapidly evolving in recent years (Heywood *et al.*, 2007), as well as those expected in the coming decades due to the effects of climate change.

Forests have a large and indispensable role to play in improving food security of tribes (Yesodharan *et al.*, 2007). Crop wild relatives are species closely related to crops, including crop progenitors, and are defined by their potential ability to contribute beneficial traits to crops such

as pest or disease resistance, yield improvement or stability (Maxted *et al.*, 2006). Almost all modern varieties of crops contain some genes derived from a crop wild relative and possibly all contain genes from landraces, so they are both a critical resource with a vital role in food security for the 21st century. Also since crop wild relatives are components of natural and semi-natural ecosystems, they also play a role in ecosystem functioning and thus in broader environmental sustainability and maintenance of ecosystem services (Maxted *et al*., 2011).

Farmers are now producing new varieties with the help of plant breeders to increase the production of food crops and insuring food security. Also the cultivation of Landraces by farmers is likely to continue to be of direct importance for food and livelihood security for individual families and communities; particularly the poorest people living in rural and marginal areas. Landraces are adapted to local environmental conditions and may be more productive, more nutritious, have a wider range of culinary uses, are less likely to suffer from pests, diseases and biotic stresses, and have a wider cropping window (Maxted *et al.,* 2011).

With all different activities made by different people to increase the production of food and balance the growing of gap between the population and food production there is big problem with the majority of different society not even to bring wild plant as a food but also using WEPs as a food is considered by some communities as indicator of poverty and backwardness. This perception is the reflection of lack of knowledge on the nutritional importance of wild and traditional vegetables (Addis *et al*., 2013).

2.3. Ethnobotanical studies on edible wild plants in Ethiopia

Wild edible plants (WEP) provide staple and supplement foods, as well as cash income to local communities, thus favoring food security. However, WEP are largely ignored in land use planning and implementation, economic development, and biodiversity conservation. Moreover, WEP-related traditional knowledge is rapidly increasing within the rural community who use these plants as a food source (Uprety *et al*., 2012).

In recent decades WEPs have been a focus of research for many ethnobotanists.Now a days, there is renewed global interest in documenting ethnobotanical information on neglected wild edible food sources (Bharucha and Pretty, 2010).

A major objective of ethnobotanical investigation into wild food plants is the documentation of indigenous knowledge associated with these plants. Comparative studies on WEPs in different cultures or among different countries contribute to the identification of the most widely used species for further nutritional analysis (Termote *et al.*, 2009; De Caluwé, 2010). Nutritional analysis results provide clues to aid the promotion of those species that have the best nutritional values which helps to ensure dietetic diversity and combat food insecurity (Tardio *et al.*, 2006).

"A considerable amount of research has been conducted worldwide on WEP ethnobotany with an emphasis on field surveys and documentation, to cite but a few: Asfaw and Tadesse (2001); Pieroni *et al.* (2002); Ertug (2004); Reyes-Garcia *et al.*(2005); Balemie and Kibebew (2006); Tardio *et al.* (2006); Arenas and Scarpa (2007); Rashid *et al.* (2008); Asfaw (2009); Giday *et al.* (2009); and Teklehaymanot and Giday (2010)" (*Lulekal et al.*,*2011).*

2.4. Positive and Negative Perceptions of IWFPs

Table 1: the negative and positive perception toward edible wild food plant (Grosskinsky *et al*., 2000)

Positive Perceptions	**Negative Perceptions**
Food diversity	Uncivilized
Income generating	Famine food
Long shelf life/storage	Poor people's food
Seasonal	Toxic
Famine food	Labor intensive
Medicinal value	Difficult (long distances) and dangerous (wild animals) to access
Are good for soil and water	Low production
Diversity of usage	Poor quality
"Freely" available	Cultural stigma
No pesticides or other chemicals necessary	Low nutritional value

Easily digestible	Equated to children's diseases
Nutritious	Not palatable
Low cost	Seasonality (not available during certain times)

2.5. Wild plant and nutrition

Mainly wild edible trees and shrubs are consumed during famine. Famine foods are used only when preferred alternatives are not available, and in situations where chronic food shortage prevails (Amare Getahun, 1974; Guinand & Dechassa Lemessa, 2000; Kebu Balemie & Fassil Kebebew, 2006).

Traditional knowledge of wild plants in Africa is in danger of being lost, as habits, value systems and the natural environment change there is a widespread decline in knowledge about wild food plants, especially among young people and urban dwellers. Study made by Addis *et al.*, 2013 showed that the green leafy vegetable of wild origin have a good sources of nutrients including minerals such as Ca, Mg, Fe, Zn and Mn, which are comparable with or sometimes higher than conventional green leafy vegetables. They also have good fiber content and quality protein which is rich in lysine, an amino acid limiting in cereals.

Studies made by Getachew Addis *et al.*, 2005; Kebu Balemie & Fassil Kebebew, 2006 wild plants can supplement nutritional requirements, especially vitamins and micronutrients. Analysis of some wild food plants demonstrates that, in many cases, their nutritional quality is comparable to domesticated varieties.

2.6. Overview of the study plant

2.6.1. What is Enset?

Enset is a genus of monocarpic flowering plant native to tropical regions of Africa and Asia. It is one of the two genera in the banana family, Musaceae, and includes the false banana or Enset (*E.ventricosum*), an economically important food crop in Ethiopia. Enset is a very tall plant. It

consists entirely of soft plant material but reaches up to 12m in height and the trunk (or "pseudo-stem") can be nearly a meter thick at the base.

It looks a bit like a giant extruded onion. Its main product is the starchy pith of its massive "pseudo-stem", which is pulped and then fermented in a big bundle, buried underground for 20days up to 6 months, to produce "kocho" a solid staple a bit like heavy bread which is eaten with milk, cheese, cabbage, meat and/or coffee**.**

2.6.2 Origin and type

One of the first European travelers to notice its economic salience and cultural distinctiveness was James Bruce (1790). Murdock (1959) pointed out that the Enset was cultivated as a food source only in the south-western Ethiopia, being almost unknown in the rest of Africa. In the past some historians and botanists argued that the origin of the Enset should be traced back to the ancient Egypt (to the Nile's source). On the contrary, others (Smeds, 1955; Simoons, 1960; Stanley, 1966) have argued is for sure a native plant of Ethiopia. The hypothesis is that the original core of the cultivation is the western edge of the Rift Valley, particularly the plateau area. This hypothesis has then been improved (Brandt, 1997): over the millennia the southwestern highlands have eventually become an environmental refuge with the emergence of complex systems of hunting and gathering (highly dependent on the use of certain plants and animals) (Valentina, 2012).

In 1947 about 20 species were recognized in the genus *Enset* but in 1962 *E. ventricosum* was placed in the genus *Enset* together with only 5 other species, namely: *E. gilletti*, *E. homblei*, *E. perrieri*, *E. glaucum* and *E. superbum*, and noted that *E. glaucum* and *E. ventricosum* perhaps should be treated as a single species (Genet *et al*., 2004).

Of the six commonly recognized species of *Enset*, *E. superbum* and *E. glaucum* grow wild in Asia, *E. perrieri* in Madagascar and *E. gilletii*, *E. homblei* and *E. ventricosum* in eastern Africa. Some species of *Ensete* are also reported to grow in North America. *Ensete ventricosum* is considered to be the only wild species growing in Ethiopia (Genet, 2004 and Brandt *et al.*, 1997).

Ensete ventricosum was previously cultivated only in the south and south-western parts of Ethiopia, but the recurrent droughts have led to the expansion of Enset cultivation to other parts of the country. A wide adaptation within the species to altitude, soil and climate has allowed widespread cultivation in western Bale, south-western Oromia including south and east Shewa, Jimma, Illubabor and Welega. Wild Enset grows at altitudes of 1200–1600 m above sea level while domesticated Enset is cultivated at altitudes of 1100–3100 m above sea level. The optimal conditions for Enset cultivation occur at 2000–2750m with 1100–1500mm rainfall, a temperature range of 10–21 ºC and a relative humidity of 63–80%. Lack of sufficiently high humidity is more limiting for good growth than high temperatures (Genet, 2004 and Brandt *et al.,* 1997).

2.7. Distinction between wild and cultivated Enset

The wild relatives of *Ensete ventricosum* occur across tropical Africa in high-altitude regions. Wild *Enset* still grows in Ethiopia and is found in close proximity to gardens of cultivated forms. Wild Enset grows at an altitude 1200-1600m above sea level while domesticated Enset grows at altitude of 1200-1600. The first study of wild *Enset* appears to be Wittmack (1867) who also reports on the growth habit of a living plant transported to Kew Gardens in the 1860s.

Smeds (1955:15) reviews a wide range of sources for the distribution of wild *Enset*. Wild *Enset* reproduces sexually, in contrast to the cultivated types (Blench *et al*., 2003).Hildebrand (2001) describes the different morphologies of wild and domestic enset in Sheko, where processing technologies of wild Enset are relatively underdeveloped.

Wild Enset Cultivated Enset Wild Enset

Fig 1: distinction between wild and cultivated Enset (March 15/2015, Kayekela kebele, Bonga, Ethiopia)

Only a very few vernacular names for the wild plant have been recorded, but they are usually quite distinct from names for the cultivated form, for example in Keffa zone it has got a local name called “Epo” or “Kocho Seytan”.

Study made by Hilderbrand (2001) in northeastern Sheko showed that “Forest Enset with local name Erfu” consumption did not cause any unusual digestive disturbance and suggested that “Erfu” probably not poisonous but certainly tough. “Also the south western sheko elders’ reluctantly recalled a period when tribal hostilities had forced them to flee their farms. They lived in the forest for a year and ate “erfu” after other resources ran out. Elders advised me that smaller individual, with pseudostems about 2 m tall, were better than large ones. A second set of

cooking experiment at Optika bore out these statements: steamed corm from a 2 m tall "erfu" attained a starchy consistency no worse than domestic "udu-babu"."

Table 2: Morphological characterization of domestic vs. forest-growing Enset**.** Source: Hilderbrand, E. (2001).

Traits	Domestic Enset (udu)	Forest growing Enset(erfu)
White, dusty coating(wax bloom) on ventral leaf blade and midrib and upper pseudostem	Present	Absent
Darkening of liquid and tissue in pseudostem shortly after cutting	Absent	Present
Adventitious roots emanating from corm	Thin	Thick
Pseudostem basal width (compared to width of upper pseudostem)	Base is same width as upper parts	Base is fat or swollen compared to upper parts
Number of seedling under fruiting parent	None or few	Many
Color and palatability of corm after cooking	White,soft,tasty	Black,hard,bad

Hilderbrand, E.2001 also stated that farmers from Bench elders said that both cultivated and uncultivated Enset were originally brought from the forest and planted in gardens. Udu-bai (cultivated) Enset was domesticated in distant past, whereas Udu-babu was brought from forest to garden much more recently to protect Udu-bai from leaf cutting.

The lack of a wax bloom on forest and most naturalized Enset does not signify a lack of epicuticular wax, it indicates that light scattering is minimized because dimension of wax bodies on uncultivated Enset differ from the wavelength of light. This could be due to a slight dimensional disparity, or to gross difference in wax body configuration or composition, as the ulterstracture of wax bodies is often linked to their chemical constituents (jefferee 1986; Jefferee *et al*., 1976).

Table 3: Difference between wild and domestic Enset in areas inhabited by the Ari, southwest Ethiopia.

Shigeta category/Ari name	Domestic Enset/Agemi	Wild Enset/Gela
Relation to humans	Cultivated	Uncultivated
Use by Ari	Food, other multipurpose use	Not utilized
Context	Gardens near house,1200-2800m	Swamp,riverbank,ritually taboo areas,1200-1600m
Base of pseudostem	Enlarged	Not Enlarged
Corm size, taste	Big, not bitter	Smaller, bitter
How shigeta's categories of "domestic "and "wild" Enset may both contain naturalized Enset (from Shigeta 1991 text, passim)	Category includes Enset grown from seeds and tolerated or encouraged in Ari gardens; farmers may later reclone a seedling with traits they desire.	Category includes spontaneous Enset appearing near agricultural settlement; this Enset may be naturalized rather than wild.

2.8. Enset Utilization in Ethiopia

Enset is a multipurpose crop with all plant parts being utilized for human food, animal forage, medicinal and ornamental uses. Enset has high significant value in day to day activity of peasants who cultivate this plant and it's a major source of food. Farmers that cultivate Enset showed that Enset is their food, their cloth, their house, their bed, their cattle feed and their plate.

The product from Enset Kocho (Wol. Uncha), Bulla (Wel. Itima), Workay (Wel.Godeta) and Amicho (Wel. Doysetida utta) are eaten by humans. During the dry season the domestic livestock are fed on remnants of Enset parts, which are not normally eaten by humans also the fibre (Wel.golla) from Enset is used in the weaving of products such as shopping bags, handbags, suitcases, sieves, pouches and mats. The variety, the age of the plant, and the way in which the fiber is extracted and stored all determine its length and quality (Kefale and Sandford, 1991).

Farmers strongly believe that fiber extracted from the male is of a very high quality and strength. Some enset cultivars are believed to have medicinal value. Farmers use enset plants as medicines not only for human beings but also for their animals. Moderately dried enset leaf sheaths and

midribs are used for local house in enset growing areas. Farmers say Fiber is the most important raw material obtained from an enset and indispensable for fencing, wrapping and packing of every material and product and for tying and keeping animals in and around the house.

Enset is a decorative plant that gives grace to the homesteads and is used as a shade for humans and domestic animals. It is also a good windbreak to protect the small grass roofed farmers' houses from strong wind, conserves soil and moisture. The leafs can be used as an umbrella during rains, for serving oily food, for rapping butter, and spices, for sleeping and sitting. Also stores water for Small domestic animals like chicken at the bottom part of the loose leaf sheathes. With all these important Kena (1993) also indicated that enset fields when compared to other cop fields are less subject to erosion. In addition it protects the soil from direct sun's heat and decreases evapotranspiration both from the plants and from the land surface. It also has profound importance as a windbreak by decreasing the velocity of the wind (evapotranspiration will be considerably decreased). As a homestead plant it protects the farmer's local houses from strong wind destruction. It is also the guardian of small coffee seedlings (Shack 1963).

2.9. Classification of Enset as male and female

As a botanist divides plants and each subdivision is further classify Enset based on their physical appearance which is nothing to do with the type of reproduction. According to their perceived characteristics of strength they are classified strength as male and tenderness female.

Table 4: Classification of Enset as Male and Female

Criteria	Male	Female
Maturity	Late maturing	Early maturing
Fibrousity	Strong, high in quality and quantity	Low strength, low in quality and quantity
Size	Big	Smaller
Susceptibility to diseases and pests	Resistance	Susceptible
Corm	Fibrous (unpalatable)	Delicious, low fiber
Kocho	Ferment slowly	Ferments Quickly

Leaves	Hard and stiff	Soft
Pseudostem and leaf sheaths	Hard and stiff	Soft
Average yield	High	Lower

2.10. Nutritional Value of Kocho

Kocho, which can be baked to thin bread, provides a good source for Ca and Fe (Abebe *et al*., 2007; Atlabachew and Chandravanchi, 2008) but is low in protein; this can be substantially improved by making complementary food to improve its protein content(Abebe *et al*., 2006). Kocho is a calorie-dense food forming the basis of many people's diets.

Highly adapted to Ethiopia's highlands, Enset is capable of weathering drought and has helped many Ethiopians survive famine in years of bad weather. In fact a group of researchers from the American Academy for the Advancement of Science went so far as to call it the "Tree Against Hunger" in a 1997 report (Daniel 2010).

Study made by Atlabachew *et al*., 2008 the concentration of K was highest followed by Na, Ca, and Mg in Kocho and Bulla. From trace elements analyzed, Zn was found to be highest next to Fe. Generally, Kocho contained higher concentration compared to Bulla for the majority of the mineral nutrients. In general, Kocho and Bulla are rich in Ca and Zn compared to other similar foodstuffs and contains comparable concentration of Cu, Fe, and Mn. The study also showed Kocho and Bulla are free of heavy metal (Cd and Pb) contaminations compared to others.

CHAPTER THREE

3. Materials and Methods

3.1. Selection of vegetable species

The study was conducted in Kayakella Kebele, Gimbo woreda which was located at Kaffa zone, Southern Nations Nationalities and People's Region (SNNPR). It is found within the southwestern plateau of Ethiopia and 450km and 725km far from Addis Ababa and Hawassa respectively. The area lies within 7°00'- 7°25'N Latitude and 35°55'-36°37'E Longitude. The altitude of the study area is 1750 m.a.s.l. The topography is characterized by slopping and rugged areas with very little plain land. The area experiences one long rainy Season, lasting from March, April to October. The mean annual rainfall ranges from 1710 mm to 1892mm. Over 85% of the total annual rainfall occurs in 8 months rain season**.**

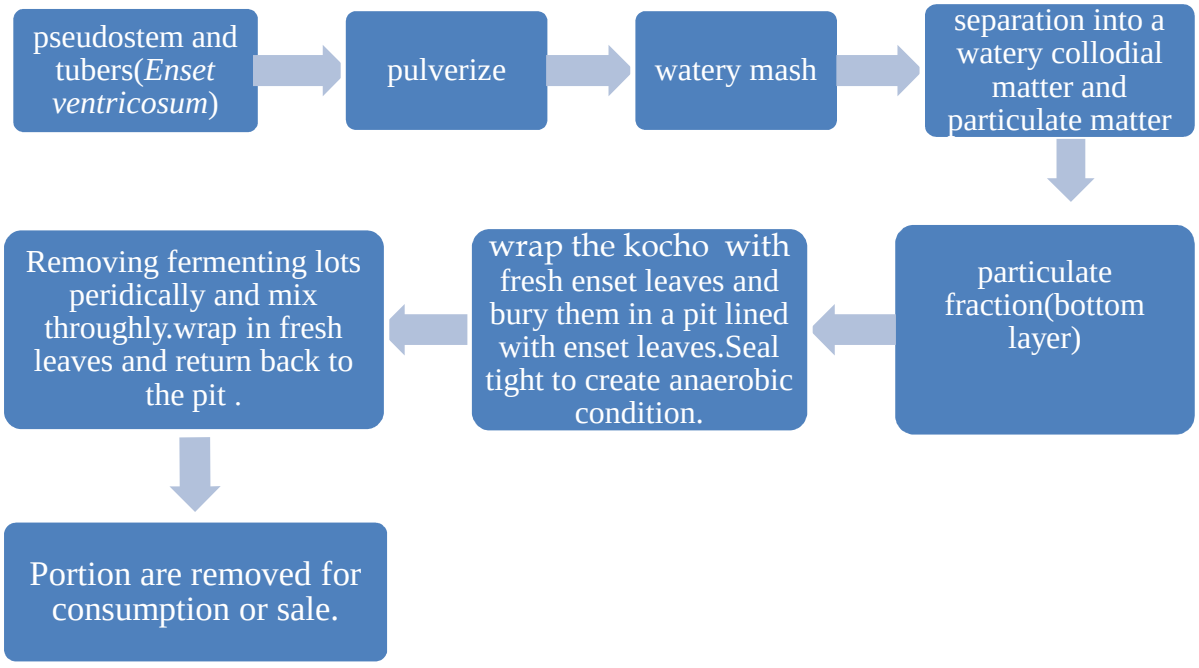
Selection of species was based on ethnobotanical study findings in the study zone (Tsehaye *et al*., 2006). "**White Bocho"** and **"Red Bocho"** *variety of Enset* were selected based on their wider utilization by the community in the study area and "**Epo**" were selected based on its relatedness to cultivated Enset in morphology and age. The first two varieties are cultivated and the last one is under cultivation and wild stand. The white Bocho variety of Enset is used by the community widely and the Red Bocho variety is used as an option when there is no White Bocho Enset and it's the least used by the community.

3.2. Sample collection

Three varieties (White Bocho, Red Bocho and Wild) of Enset were processed into *kocho*, according to Keffa processing method of *kocho preparation*. During harvest, leaves and older leaf sheaths were first removed from the designated plants for *kocho preparation*. The internal leaf sheaths were separated from the pseudostem down to the true stem, which was section between corm and pseudostem. Then, the true stem was separated or stumped from the underground corm. The concave side of the leaf sheath was peeled and cut into pieces of about half meter length (approximate length), and split lengthwise, in order to shorten the leaf sheath to

a workable size. Then, the pseudostem was decorticated using a locally made bamboo scraper, while the leaf sheath is held on an incline against a wooden plank and it's mixed with fermenter with the local name "kisso" which used as catalyst for facilitating fermentation. After the completion of decorticating and grating, the leaf sheath pulp was spread on fresh Enset leaves covering the ground, after which the grated corm was mixed on the decorticated pulp. The kocho were placed inside polyten bag and put in ice box and transported to Addis Ababa University 4kilo campus.

3.3. General flow chart of Kocho preparation



3.4. Chemical analysis of selected vegetables

Proximate compositions (moisture, crude fiber, crude protein, crude fat, and ash), minerals (Ca, Cu, Fe, Zn, K, Na) were determined from fermented Kocho sample.

3.4.1. Determination of the moisture content

Empty drying dishes (made of porcelain) were dried using a drying oven (Germany, Memmert) for 1 hour at 105 ºC. The dishes were cooled for 30 minute in desiccators with granular silica gel and weighed using a digital analytical balance to the nearest milligram (W_1). About 5g of fresh samples were weighed (W_2) in dried and pre-weighed drying dishes. The dishes and their contents were then placed in drying oven and dried for 5 hours at 105 ºC. The dishes and their contents were cooled in desiccators to room temperature and weighed (W_3). The procedure was repeated until a constant weight was attained (AOAC, 2005).

$$\% \text{ Moisture content} = \frac{W_2 - W_3 \times 100}{W_2 - W_1}$$

3.4.2. Determination of crude protein

The protein content of the samples was determined on the basis of total nitrogen content by micro Kjeldahl method of crude nitrogen determination (AOAC, 2005). Two grams of dried sample was digested in a 100-mL Kjeldahl digestion flask by boiling with 6mL of concentrated H_2SO_4 acid and 3g of Kjeldahl digestion tablet (selenium: potassium sulphate mixture) as a catalyst and boiling point raising agent. 3.5ml of 30% hydrogen peroxide was added to the digestion mixture after which the teccator tube containing the mixture was set with teccator digester. The digestion was continued for 4 hours at 37 ºC until the mixture became a clear solution. The digested sample solution was made up to 50ml with distilled water and 30ml of 40% sodium hydroxide.

The solution was slowly and automatically added to mixture by Kjeldhal titration apparatus. The ammonia liberated was collected in 30ml of 1% boric acid solution containing a mixed indicator. Steam was applied to the solution to distill out ammonia evolved with the distillate collected into the boric acid solution. Ammonia was estimated by titrating with standard 0.1N Hcl solution. Blank nitrogen determination was carried out in a similar manner and substandard 0.1N Hcl solution. Blank nitrogen determination was carried out in a similar manner and subtracted from the sample nitrogen. Crude protein was determined by multiplying the value obtained for percentage nitrogen by a factor of 6.25.

Calculation:

$$\%N = \frac{14 \times M \times Vt \times V100}{\text{Weight of sample (mg)} \times Va}$$

%Crude Protein = % N (Nitrogen) × 6.25

Where, M = Actual molarity of Acid

V = Titer value (Volume) of Hcl used

Vt= Total volume of diluted digest

Va = Aliquot volume distilled

3.4.3. Determination of crude fat

Ether extractives as an estimate of crude lipid was determined using Soxhlet extraction apparatus by exhaustively extracting a known weight of sample by diethyl ether. Two gram of dried sample was weighed in extractor thimble (W_1). A clean, dried round bottom extraction flask containing a few granules of boiling chips were weighed (W_2). The extraction thimble and flask was fitted on the extractor unit and 60 ml of diethyl ether was poured into the flask using a tube connected on the top of the extraction unit.

Condenser was connected to the Soxhlet extractor and cold water circulation was put on. The heating mantle was switched on and the heating rate adjusted until the solvent was refluxing at a steady rate. Extraction was carried out for 4 hour. The solvent was recovered and the oil dried in an oven set at 70 ºC for 1 h.

The round bottom flask and oil was cooled in a disccator and the weighted (W_3) (AOAC, 2005).The ether extract was calculated as:

$$\% \text{ Crude fat} = \frac{W_3 - W_2 \times 100}{W1}$$

3.4.4. Determination of crude fiber

Acid-Base digestion method was used to determine the crude fiber content. Two gram sample was weighed in 600ml beaker,200mL 1.25% H_2SO_4 was added and the mixture boiled for 30 minutes on hot plate in the fume hood. After 30 minutes, 20 ml 28% KOH was added and the mixture again boiled gently for further 30 minutes, while stirred occasionally. The hot solution was quickly filtered under suction. The residue was washed several times with hot distilled water and then by 1% H_2SO_4 and filtered after rinsing the residue with distilled water, it was washed with 1%NaOH. The crucible containing the residue was once again washed with distilled water and finally by 1% acetone. The washed residue was dried in an oven at 100 °C to constant weight and cooled in a desiccators and weighed (C_1). The weighed residue was ashed in a muffle furnace at 550°C for 2 h, cooled in desiccators and reweighed (C_2). Crude fiber content was expressed as percentage loss in weight on ignition (AOAC, 2005).

Calculation: the loss in weight on incineration = C_1-C_2

$$\textbf{\% Crude fiber} = \frac{C_1 - C_2}{\text{Weight of original sample}} \times 100$$

3.4.5. Determination of total ash

Ash was determined using AOAC (2005). The porcelain crucible was dried in an oven at 100 ºC for 10 min, cooled in desiccators and Weighed (W_1). Two grams of the finely ground sample was placed into a previously weighed porcelain crucible and reweighed (W_2); it was first charred on the hot plate in the fume hood until it was completely decarbonized and then transferred into a furnace which was set at 550°C. The sample was left in the furnace for six hours to ensure proper ashing. The crucible containing the ash was then removed; cooled in desiccators and Weighed (W_3). The percentage ash content was calculated as follows:

$$\textbf{\% Ash Content} = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

3.4.6. Determination of carbohydrate

Carbohydrate was estimated by difference as described by FAO (1998) as follows:

% Total carbohydrate = 100 - (% moisture + %Ash + % fat + % Protein + % Fiber)

3.4.7 .Determination of minerals

Wet digestion methods were used for elemental analysis. Wet digestion methods for elemental analysis involve the chemical degradation of sample matrices in solution, usually with a combination of acids to increase solubility. The various acid and flux treatments were carried out at high temperatures in specially designed vessels that help to minimize contamination of the sample with substances in the air, the local environment, and from the vessel walls losses from the sample may occur due to adsorption onto the vessel walls, volatilization, and coextraction.

Iron (Fe), Calcium (Ca), and Magnesium (Mg) by Atomic Absorption Spectrometry. In this technique the atoms of an element was vaporized and atomized in the flame. The atoms then absorb the light at a characteristic wavelength. The source of the light is a hollow cathode lamp, which is made up of the same element, which has to be determined. The lamp produces radiation of an appropriate wavelength, which while passing through the flame is absorbed by the free atoms of the sample. The absorbed energy is measured by a photo-detector read-out system. The amount of energy absorbed is proportional to the concentration of the element in the sample (Gul *et al.,* 2009).

The dilution factor for all minerals except P and Mg is 100. For determination of Mg, further dilution of the original solution was done by using 0.5 ml original solution and enough distilled water was added to it to make the volume up to 100 ml. Also for the determination of Ca, 1.0 ml lithium oxide solution added to the original solution to unmask Ca from Mg.The concentrations of minerals recorded in terms of “ppm” converted to milligrams (mg) of the minerals by multiplying the ppm with dilution factor and dividing by 1000, as follows:

$$MW = \frac{\text{Absorbency (ppm)-blank x v *cf}}{\text{Wt.of sample}}$$

Determination of Sodium (Na) and Potassium (K) made by flame photometer. The flame photometer measures the emission of radiant energy when atoms of an element return to their ground state after their excitation by the high temperature of the flame. The degree of emission is related to the concentration of the element in the solution.

The same wet digested food sample solutions as used in AAS used for the determination of Na and K.The calculations for the total mineral intake involve the same procedure as given in AAS (Gul *et al*., 2009).

3.5. Determination of microbial Population

3.5.1. Total microbial count using plate count agar (PCA)

The aerobic plate count (APC) is intended to indicate the level of microorganism in a product. Microbiological assessment for quality and safety of foods traditionally relies upon the enumeration and specific detection of pathogenic and spoilage microorganisms. A common requirement for all samples is to bring them into suspension aseptically. After aseptically mixing the samples thoroughly, a 10g sample was aseptically blended in 90ml of sterile water using a laboratory blender (Wagtech international Ltd, UK). Serial dilution was made aseptically for all three samples (ICMSF 2001).

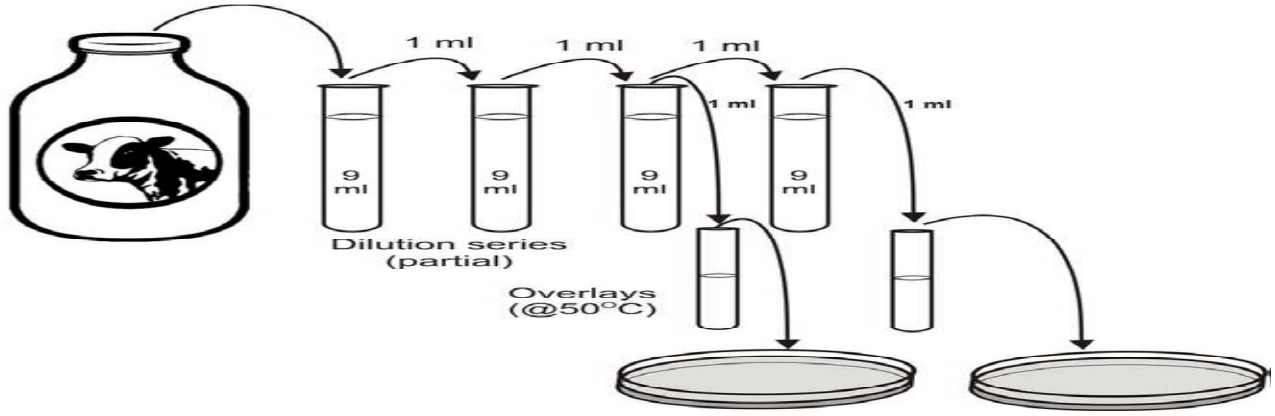


Fig 2: method of serial dilution

The objective is to estimate the total number of bacteria, a complex medium called Plate Count Agar was used since it can support growth of many different types of bacteria. The result was

expressed as number of Colony Forming Units (CFU), not total bacteria. This is because no single culture medium will support all different types of bacteria.
The result was expressed as follows:

Colony forming unit:

$$\frac{\text{Number of CFU}}{\text{Volume plated (mL) x total dilution used}} \longrightarrow \frac{\text{Number of CFU}}{\text{mL}}$$

Dilution of samples

The samples were processed using food dilution plate method. One gram of kocho sample was serially diluted with sterilized distilled water. The final dilution factor 10^{-3}, 10^{-4} was used for yeast and mold and final dilution of 10^{-6}, 10^{-7} was used for Bacteria count and 1 ml of each dilution was added to 20ml of pre dried agar medium in 90mm diameter sterile Petri dishes (Nandhinit *et al*.,2013).

3.5.2. Coliforms

Violet Red Bile Agar(VRBA) is a selective medium used to detect and enumerate lactose-fermenting coliform microorganisms .The composition of the media are Yeast Extract(3g),Enzymatic Digest of Gelatin(7 g) ,Bile Salts Mixture(1.5 g),Lactose(10 g),Sodium Chloride (5 g),Neutral Red(0.03g),Crystal Violet(0.002 g) and Agar(15 g).For preparation of the medium the direction showed 41.5 g of the medium in one liter of purified water. For my work in duplication of result 6 plates was used for three sample of Kocho. 120ml of water which is 20ml in each plate was used (Downes 2001).

4.98g of VRBA was mixed with 120ml of water and autoclaved at 121^{o}c for 15min. VRB agar plates which are prepared in a duplicate and incubated at 30 to 32°C for 24 hrs. Purplish red colonies surrounded by reddish zone of precipitated bile were counted as coliforms (Downes 2001).

3.5.3. Yeasts and molds

Yeast and molds were counted by spread-plating 1ml of appropriate dilutions in duplicate on Pre-dried surfaces of Potato dextrose agar. The ingredients include Potatoes, infusion from 200gm/Lt, Dextrose 20gm/Lt and Agar 15 gm/Lt and the final pH adjusted at at 25°C, 5.6±0.2. Suspend 39 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Plates were incubated at 25 to 28^{o}C for three to five days. Smooth (non-hairy) colonies without extension at periphery (margin) were considered as yeasts whereas hairy colonies with extension at periphery were considered as molds (ISO/DIS 2002).

3.5.4. Aerobic bacterial spores

To count aerobic bacterial spores, homogenized samples were heated at 80^{0}C for 10 minutes in a water bath to kill vegetative cells. A volume of 1ml of appropriate dilutions was spread plated in duplicate on pre-dried surfaces of Nutrient Agar (NA) plates. The plates were incubated at 30 to 32°C for 24 to 48 hrs after which colonies were counted as aerobic bacterial spores (ISO procedure 4833:1991).

3.5.5. Lactic acid bacteria (LAB)

Lactic acid bacteria (LAB) were counted by spread-plating 1 ml of appropriate dilutions in Duplicate on pre-dried surfaces of MRS (de-Mann Rogosa and Sharp) agar plates.Ingeredient composition of MRS include ,0.8 % egg extract,0.4 % yeast extract,2.0 % glucose,0.5 % sodium acetate trihydrate,0.1 % polysorbate 80 (also known as Tween 80),0.2 % dipotassium hydrogen phosphate,0.2 % triammoniumcitrate,0.02 % magnesiumsulfate heptahydrate,0.005 % mangane sulfate tetrahydrate,1.0 % agar and the final pH adjusted to 6.2 at 25°C.The direction showed that 66.2 g in 1 liter of purified water and heat to boiling to dissolve completely and autoclaved for 15 minutes at 121°C.The plates were incubated under anaerobic condition, using anaerobic candle jar at 30 to 32°C for 48 hrs. All colonies were counted as lactic acid bacteria. Ten colonies from each three samples were isolated and transferred into MRS broth for further

biochemical test. After incubation for 30 to 32°C for 48 hrs using a sterile loop the colony transferred into pre dried MRS agar and incubated at 32^{o}C for 24 hrs. Purity of the isolates was checked by streaking again on fresh agar plates of the isolation media (Omemu 2011).

3.5.5.1. Biochemical Test of Isolated colony

3.5.5.1.1 Carbohydrate Fermentation

Most of bacteria will ferment a variety of sugars to form one or more acid end products. The test cultures inoculated into sugar medium in this case MRS broth and incubate at 37^{o}C for 24 hr. Acid production is shown by change in the color of Andrade's indicator to pink. Gas, if produced, accumulates in the Durham tube (Samelis *et al*., 1994).

3.5.5.1.2. Catalase Test

Catalase test was performed by adding few drops of 3% hydrogen peroxide to a slide which contain one isolate from 24hour old culture. The result was expressed as catalase positive for producing bubble and negative for not producing bubble (Gregersen 1978).

3.5.5.1.3. Gram Stain

Potassium hydroxide (KOH) test used detect gram-negative bacteria. A loop of growth from a colony is emulsified on the surface of a glass slide in a suspension of 3% KOH and stirred continuously for 60 seconds. Gram-negative cell walls break down while the cell walls of gram positive bacteria are not disrupted and when the loop is gently pulled from the suspension it will be thick and stringy (Gregersen 1978).

3.5.5.1.4. Cell morphology and Motility

Microscopic examination of cell morphology and classification as rod and cocci was made based on shape of bacteria and Motility (unidirectional movement) were characterized by Microscope (Gerhardt *et al*., 1981)

3.6. Analysis of Sub Acute toxicity

In traditional methods for assessing acute toxicity use death of animals as an endpoint. In 1984, a new approach to acute toxicity testing was suggested by the British Toxicology Society based on the administration at a series of fixed dose levels. The approach avoided using death of animals as an endpoint, and relied instead on the observation of clear signs of toxicity at one of a series of fixed dose levels. Following UK and international in vivo validation studies the procedure was adopted by the Council as a Test Guideline in 1992(Van den Heuvel et al., 1987). .Together, the in vivo and modeling studies have demonstrated that the procedure is reproducible, uses fewer animals and causes less suffering than the traditional methods and is able to rank substances in a similar manner to the other acute toxicity testing methods. We use five mice for each cultivated and wild kocho. The test animals are females for their being sensitive. Groups of animals of a single sex are dosed in a stepwise procedure using the fixed doses of 5, 50, 2000 and 5000mg/kg. 5mg/Kg and 50mg/kg dose level was used for wild kocho type because we don't have any information on its toxicity dose level. This procedure continues until the dose causing evident toxicity or no more than one death is identified, or when no effects are seen at the highest dose or when deaths occur at the lowest dose.

The animals are randomly selected, marked to permit individual identification, and kept in their cages for 5 days prior to the start of dosing to allow for acclimatization to the laboratory conditions. Animals fasted prior to dosing for 3-4 hours. Following the period of fasting, the animals weighed and the test substance administered. After the substance has been administered, food withheld for 1-2 hours (OECD 2001).

3. 7. Statistical analysis

Descriptive statistics such as means and standards deviation were calculated using SPSS version 16 software. Colony forming unit result was directly used to compare different microbial population of kocho variety.

CHAPTER FOUR

4. Results and Discussion

4.1. Morphological difference of Enset varieties

It is evident from Table 6 that wild Enset differs from cultivated Enset in different aspects. The white dusty coating which is found in the leave parts of cultivated Enset is not present on the leave of wild Enset. Epicuticular wax forms crystalline projections from the plant surface, which enhance their water repellency, create a self-cleaning property known as the lotus effect and reflect UV radiation Hilderbrand (2001). The shapes of the crystals are dependent on the wax compounds present in them. Waxes prevent moisture loss during fruit storage. Although natural waxes on fruits are effective in preventing water loss, the application of commercial wax can further decrease water loss during prolonged storage.

Holloway (1969) and Barthlott & Neinhuis (1997) have observed that application of the wax formulations to Red Delicious apples by the usual packing house operations used in Washington increased the resistance of the fruit surface to water loss and measurably reduced water loss during six month storage of the fruits.

Tissue discoloration data do not bear out dichotomous characterization of domestic Enset as white and seed propagated Enset as dark. Although forest Enset have universal discoloration after cutting, several domestic landraces although show significant tissue darkening. But heavy discoloration is uncommon in this landraces and also in cultivated Enset. Among both cultivated Enset and wild Enset the final color and speed of discoloration is different. In wild Enset discoloration start to be observed immediately after cutting of the corm and during scrapping of the leave but in cultivated Enset tissue discoloration was observed after several minute of scrubbing when it was exposed to air and the result is agreement with Hilderbrand (2001).

In some family they grow wild Enset in their yard to use the leave part which is important in baking bread. Farmer in the study area said the wider leaves of wild Enset are very comfortable

to cover the whole part of flour so that that air can't enter during baking and gives the bread good color and sensory acceptance.

In cultivated Enset a new plant shoot that develop at the base of the leaf and at the juncture of the psuedostem and the corm, more properly called a sucker since it is not developed from a seed. So seedlings are not observed in cultivated Enset but in case of wild Enset during the end of maturation seedling was observed with 90-100 in number of seed. Similarity, Karlsson *et al*. (2013) have indicated that one seed batch of wild origin have higher seedlings than the cultivated origin.

Table 6: The difference between wild and cultivated Enset as characterized by Keffa farmers

Traits	Cultivated Enset	Wild Enset
White, dusty coating(wax bloom) on ventral leaf blade and midrib and upper pseudostem	Present	Absent
Darkening of liquid and tissue in pseudostem shortly after cutting	Absent	Present
Adventitious roots emanating from corm	Thin	Thick
Pseudostem basal width (compared to width of upper pseudostem)	Base is same width as upper parts	Base is fat compared to upper parts
Number of seedling under fruiting parent	None or few	Many
Leaf size and width	wide	Narrow

4.2. Proximate Composition of Kocho from Cultivated Enset

4.2.1. Ash content

Kocho from wild Enset has exceptionally highest level of total ash (11.9g/100g) than cultivated Enset with the lowest (5.03g/100g) ash content observed in kocho processed from Red Bocho Enset variety. As table 7 indicated the total ash content, which is an index of mineral contents of kocho, gives insight that the kocho from wild Enset is reach source of minerals. The result is different from the other author finding which is mainly because of different variety and duration

of fermentation (Meles 2013). The high ash content of kocho from wild Enset as compared to kocho from cultivated Enset argue with the result of Yemane *et al*., (2006) which is 12.02g/100g. The difference in ash content may be due to genetic or cultivar variation of Enset varieties (Nurfeta *et al*., 2008). It was also indicated that fermentation decreased the ash content of Enset, and thus, *kocho* has lower ash content than Enset plant (Urga *et al*., 1997) this could due to fermentation, which imparts the solubility of minerals and other organic constituents, which contribute the total ash content in *kocho*.

Table 7.Ash content of kocho sample prepared for the study.

Variety	Ash (Db)
White Bocho	7.71%
Red Bocho	5.03%
Epo	11.9%

Db =dry bias

4.2.2. Crude fiber content

As indicated in Table 8 Kocho from cultivated Enset showed fiber content of 8.6% and 8.3% for white and red bocho, respectively. Kocho from wild Enset shows high (23.2%) content of fiber. The result of high fiber content in Kocho from wild Enset is close to the finding (21.48%) made by Yemane *et al*., 2006. The difference relays on wide range of different variety within the wild Enset. Fermentation has a significant effect on decreasing the total crude fiber content of Kocho (Melese 2013**)**. 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 (Wizna *et al*., 2009) and in this also, fermentation increases the breakdown of dietary fiber to soluble and digestible form, and which decrease the total indigestible crude fiber.

According to current recommendations (Food and Nutrition Board, Institute of Medicine, 2001), the average daily requirement of dietary fiber is 25 g per day for women younger than 50, 21 g per day for women older than 50; 38 g per day for men younger than 50, and 30 g per day for men older than 50 or the fiber recommended dietary allowance values for children, adults,

pregnant and breast-feeding mothers are 19–25%, 21–38%, 28% and 29%, respectively. Thus, the kocho from wild Enset could be a valuable source of dietary fiber human nutrition.

Table 8.Crude fiber content of kocho prepared for the study.

Variety	Fiber (Db)
White Bocho	8.6 %
Red Bocho	8. 3 %
Epo	24.4 %

4.2.3. Crude fat content

Table 9 showed that highest fat content was observed in Kocho from wild Enset (8.22%) followed by kocho from Red Bocho and White Bocho, 6.65% and 0.74%, respectively. There is high variation between fat content of different variety of kocho. The result is different from the result of the other author which is mainly because of variety and different time of fermentation (Yemane *et al*., 2006; Melese 2013).

The study made by Melese (2013) showed there is a positive correlation between variety and fermentation period on fat content of *kocho* sample. 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 which is 1.60% and 1.52% for 20 and 30 days of fermentation time.

This could be due to fermentation, which increase the degradation of fats by lepolytic enzymatic activities and microbial proliferation causing the consumption of nutrients. As compared to cultivated Enset kocho, wild Enset kocho can give higher amount of daily fat requirement of human.

Table 9. Fat content of kocho prepared for the study

Variety	Fat(Db)
White Bocho	0.74%
Red Bocho	6.65%
Epo	8.22%

4.2.4 .Crude protein content

Table 10 showed that crude protein content of *kocho* from White bocho shows high value (7.6%) followed by Epo and Red Bocho kocho, 6.8% and 5.6%, respectively. Study made by Meles 2013 showed that "There was remarkable increase in crude protein content of *kocho* samples as fermentation time increased from 10, 20 and 30 days. The highest crude protein content (5.09%) was obtained at 30 days of fermentation, while the lowest value (4.42%) was recorded at 10 days of fermentation." Kocho from wild Enset has higher value of protein as compared to the cultivated Enset kocho of the other findings Yemane *et al*., (2006) also showed that kocho from this variety has highest protein 7.89% than other five kocho from cultivated origin (Table 12).

The crude protein content of *kocho* increased due to the action of fermenting microorganisms in the synthesis of some amino acids, and improved the quality of the protein, as determined by amino acid profiles. It is also known that fermentation had the general effect of increasing the essential amino acid content of *kocho*. The same trends in increasing of crude protein were observed in barley fermented product, fermented barley contained about 22% of crude protein and about 16% of true protein on a dry matter basis Mathot *et al*., (1992).

Table 10.Crude protein content of Kocho prepared for the study.

Variety	Protein (Db)
White Bocho	7.6%
Red Bocho	5.6%
Epo	6.8%

4.2.5. Carbohydrate

In this study there was significant difference in carbohydrate content among the varieties of Kocho. The maximum carbohydrate content (72.3%) for *kocho* samples was obtained from Red Bocho, while the minimum carbohydrate content (46.5%) was obtained kocho from wild Enset (Table 11).

Difference in the result of kocho carbohydrate value was recognized due to different variety and fermentation time. Melese *et al*., 2013 showed a reduction in the carbohydrate content with

increasing of fermentation time which was possibly due to the breakdown of more complex components enzymes produced by fermenting microorganisms.

Table 11.Carbohyderate content of kocho prepared for the study.

Variety	Total carbohydrate
White Bocho	71.7%
Red Bocho	72.3%
Epo	46.5%

Similarly, Tshaye *et al*., (2006) have showed proximate composition of wild Enset variety was characterized by having high fiber, crude protein, ash, and low carbohydrate content.

Cluster	Fat	Fiber	Nitrogen	Protein	Ash	Total Carbohydrate
A	0.27	8.25	1.05	6.53	10.42	70.75
B	0.30	8.89	0.95	5.91	9.30	73.03
C	0.29	21.48	1.26	7.89	12.02	69.30
D	0.23	6.89	0.71	4.41	6.81	76.99
E	0.21	5.46	0.55	3.43	5.99	78.92
F	0.21	5.62	0.48	2.96	5.83	78.73
Total Mean	0.24	7.14	0.71	4.42	7.28	76.34

Cluster A-this group encompass a two-colored pseudostem with a deep red at the base and brownish yellow above the base, B-contain dark red pseudostem, midrib, and petiole color,C-wild type of Enset called Koch Seytana (meaning the devil's enset),D-Members of this group have a greenish dark pseudostem and light red midrib and petiole color,E-Members of this cluster comprise varieties having a deep green colored pseudostem, midrib, petiole and leaves and dark brown patches in the pseudostem,F- two colored pseudostems with a deep red at the base and brownish yellow above the base and deep red midrib and petiole color. Source Tshaye *et al*., (2006).

4.3. Mineral Analysis

Table 13: Concentration of elements in different variety of Kocho

Kocho variety	Elements (mg/100g)					
	Ca	**Mg**	**Fe**	**Cu**	**Na**	**K**
White Bocho	1600	409.7	706.3	1306.7	470	2233.3
Red Bocho	973.3	449.3	449.3	1850	375.9	1800
Epo	1500	1197.3	1197.3	1486.7	239	2500

The pattern of concentration of elements in Kocho prepared from White Bocho was decreased as K> Ca> Cu> Fe> Na> Mg, kocho prepared from Red Bocho Cu>K>ca>Fe=Mg>Na and for kocho prepared from wild Enset K>Ca>Fe=Mg>Cu>Na

In all variety of Enset kocho contain higher amount of K followed by Ca and Fe. According to Marschner (1995) was due to the fact that nutrient elements such as N, P, K, S, and Mg are highly mobile in the plant tissue and trans-located from old plant tissue to new plant tissue.

The concentration of Fe and Mg is found to be equal in kocho prepared from Red Bocho and white Bocho. Beyene, (1988) have showed that the soil types of Enset growing areas of Ethiopia are moderately acidic to slightly basic with the pH ranges from 5.6 to 7.3, the plant is expected to have a better accumulation of micronutrients like iron and zinc.

It has been reported that Fe, Cu, and Zn are the main elements that plant could accumulate and be passed up the food chain. Therefore, the detection of Cu may be because of the fact that these ions are readily transferred from the soil to plants, and accumulate in the root and tuber of the false stem of Enset plant and hence in Kocho (Minaleshewa *et al*., 2008)

The mineral content of Ca, Zn, and Fe of kocho are reported by Abebe *et al*., (2007), Agren and Gibbson (1968), Amede *et al*. (2004) and Wu Leung *et al.* (1968) which is different from the present study that I conducted. The variation may be attributed from different reason. The

mineral concentration of Kocho is affected by different reasons, of these, variety of Enset plant, precaution taken during processing, physical and chemical nature of the soil and its mineral nutrients, etc. Second, there is also a variation in the sample preparation for the analysis using dry ashing as sample decomposition method, which is known to have a problem in analyte loss.

Except for few metals, the trends for metal accumulation in Kocho prepared from different Enset variety were almost the same and for some metals their concentrations were almost comparable. This is because these areas are located within the same geographical location. As a result of this, they will have comparable climatic conditions such as temperature, soil, PH and relative humidity.

Table 14.Comparison of levels of some elements with the available data in literature.

Food type	Elements (mg/100g)			
	Zn	Fe	Ca	Reference
Bulla	0.7–0.9	36–65	400–470	Abebe *et al*. (2007)
	–	58	910	Agren and Gibbson (1968)
	–	11–77	440–650	Wu Leung *et al*. (1968)
Kocho	3.2–7.2	36–101	1400–2260	Abebe *et al*. (2007)
	–	70	600	Agren and Gibbson (1968)
	6	37	320	Amede *et al*. (2004)
	–	53	1200	Wu Leung *et al*. (1968)

Kocho prepared from wild Enset contain higher concentration of K, Mg, Fe, Cu and almost comparable concentrations Na with kocho prepared from white bocho so kocho prepared from Epo can be good source of major, minor and trace metals that are essential to human.

4.4. Result of microbial Analysis

4.4.1. Lactic Acid Bacteria

As indicated in table 15 the initial stage of lactic acid bacteria count was 3.1 log cfu/ml in White bocho, 3.5 log cfu/ml in Red bocho and 5.2 log cfu/ml in Epo but after 10days of fermentation the number increase into 9.6 log cfu/ml, 7.6 log cfu/ml and 6.4 log cfu/ml, respectively. Maximum count of LAB and yeasts were recorded at day 20th and day 30th day of fermentation in all variety of kocho. Gashe (1987) also indicated the dominance of LAB during the active stages of Enset fermentation.

The LAB achieves prominence and dominated the rest flora during the entire kocho fermentation. This may explain their tolerance to acid. In addition, during fermentation the anaerobic condition created in the pit may favor their growth. Similarly, Lu *et al*., (2008), have reported there is a general agreement on the dominance and beneficial effects of LAB on the fermentation process of starchy food products. The simultaneous increment of lactic acid bacteria and yeasts likely to be due to their co-metabolic activities. Paramithiotis *et al*., 2006; Nyanga *et al*., 2007; Vogelmann *et al*., 2009 sebsequently,reported the co- occurrence of LAB and yeasts in many foods.

High numbers of lactic bacteria contribute to the microbiological stability and safety of kocho by inhibiting the growth of pathogenic bacteria and deteriorative bacterial flora. The suppression of competitors by lactic bacteria occurs mainly due to changes caused by the production of organic acids.

Table 15.Lactic acid bacteria count (log cfu/ml) for different variety of kocho at different time of fermentation

Variety	Days of fermentation			
	0	10	20	30
White Bocho	3.1	9.6	10.2	12.4
Red Bocho	3.5	7.6	8.5	12.1
Epo	5.2	6.4	7.4	13.2

4.4.2. Total Aerobic Mesophilic bacteria

The initial stage of kocho fermentation was dominated by aerobic mesophilic micro organisms (Table 15). The fermentation of kocho caused a significant change in aerobic mesophilic bacteria in all variety of Enset. Aerobic Mesophilic bacteria was counted as 3.5log cfu/ml in white bocho, 2.5 log cfu/ml in Red bocho and 3.7log cfu/ml in Epo at the initial stage of fermentation and at the 10^{th} and 20^{th} day of fermentation the number increase but it shows a slight decrease around the end of fermentation at 30^{th} days of fermentation, reaching counts around 3.1 log cfu/ml, 4.8 log cfu/ml and 2.4 log cfu/ml, respectively.

The difference in total bacterial count between varieties was due to slow time in fermentation process, difference in production of organic acids, dominantly lactic acid (Melese 2013). Therefore, fermentation starts earlier in cultivated Enset than in wild, or wild Enset had more delay time of fermentation but the highest count of aerobic bacterial count in wild variety of Enset may have reason that the Enset is susptible to aerobic bacteria since the kocho start a significant change in its color after cutting.

Table 16. Total Aerobic Mesophilic bacterial count (log cfu /ml).

Variety	Days of fermentation			
	0	10	20	30
White Bocho	3.5	6.2	4.3	3.1
Red Bocho	2.5	4.2	3.1	2.4
Epo	3.7	5.2	5.1	4.8

4.4.3. Total Coliform

Table 16 indicated that the initial count of coliform were 2.8 log cfu/ml,3.1log cfu/ml and 4.1log cfu/ml for White Bocho, Red Bocho and Epo, respectively. However these groups of microorganisms were later not detected following the proliferation of LAB. Coliforms were called “pioneer species” of the sauerkraut fermentation as they produce acids and lower pH of the fermenting sauerkraut, setting appropriate condition for the lactic acid bacteria to colonize the system (Scott and Sullivan, 2008).

LAB has been known to produce antimicrobial substances during fermentation of different foods (Steinkraus, 1992; Jay *et al*, 2005; Tadesse *et al*., 2005) and play important role in the preservation and production of wholesome foods.

Melese 2013 showed as the fermentation time increased from 10 to 30 days, counts of coliform in *kocho* was significantly decreased, as counts of coliform in *kocho* sample from log 7.26 cfu/g at 10 days of fermentation time to log 3.70 cfu/g at 30 days of fermentation time. This clearly showed that counts of coliform in *kocho* decreased as the fermentation time increased. Similarly, coliform counts in *kocho* from different Enset varieties had showed variation. The highest count of coliforms (log 2.77 cfu/g) was recorded from *kocho* of Astare variety and the lowest count of coliforms (log 2.71 cfu/g) was recorded from Kinnare *kocho*.

The increase in LAB counts resulted in low levels of these groups of bacteria and they could not be encountered with the limit of detection used in this study. Despite the unhygienic handling of the fermenting mass and the possible introduction of food borne pathogens, the fermentation appeared to reduce possible food borne pathogens.

Steinkraus 1996 has also showed that Enterobacteriaceae were undetectable at day 10 and day 20 of possibly due to unfavorable conditions created due to reduction in pH over fermentation time. A pH of 3.5-4.0 has been reported to inhibit Enterobacteriaceae and other Gram negative bacteria. The elimination of Enterobacteriaceae may indicate safety of kocho from enteric pathogens.

Table 17.Total coliform count (log cfu/ml).ND-non detectable

Variety	Days of fermentation			
	0	10	20	30
White Bocho	2.8	2.3	ND	ND
Red Bocho	3.1	1.7	ND	ND
Epo	4.1	3.2	2	ND

4.4.5. Yeast and Mold

Yeasts had initial counts of of 2.6 log cfu/ml in white bocho, 2.1 log cfu/ml in Red Bocho and 3.4 log cfu/ml in Epo. Later, they reached counts around 8.9 log cfu/ml, 11.2 log cfu/ml and 10.3 log cfu/ml, respectively at around day 30 Table (18).

There was a predominance of yeast growth compared to that of the molds in all samples. Table (18). The highest count of mold is shown in Epo variety that is 1.7 log cfu/ml and the lowest count was also observed in White bocho 1.2 log cfu/ml. Tiruha *et al*. (2005) have observed the maximal count around 8.3 log cfu/ml, and in some cases, the presence of molds is below detectable level and lowest count of 2.5 log cfu/ml.

The proliferation of yeasts requires an abundant continuous supply of oxygen. The low number of yeasts in fermenting Enset could be due to unavailability of sufficient oxygen in the tightly packed and sealed fermenting mass.

Bozkurt and Erkmen (2002) have evaluated the effects of the addition of starter cultures and additives, observed that there was an increase in the number of molds and yeasts during the first days of starter culture addition 6 $\log_{10}$ cuf/g and around 4 $\log_{10}$ ufc/g in treatments without the addition of additives. During kocho preparation"Kisso" as starter culture was added to the mass as the community believe that the addition help to speed up the fermentation process. So the increase in yeast count may be the result of the addition of "kisso". Steinkraus (1996) reviewed that, LAB create an acidic environment conducive to yeast proliferation, while the yeasts provide vitamins and other growth factors for the LAB. Therefore, the simultaneous increment of lactic acid bacteria and yeasts likely to be due to their co-metabolic activities.

Table 18.Yeast count log cfu/ml

Variety	Days of fermentation			
	0	10	20	30
White Bocho	2.6	7.4	7.5	8.9
Red Bocho	2.1	7.2	8.1	11.2
Epo	3.4	5.8	6.7	10.3

Table 19.Mold count log cfu/ml

Variety	Days of fermentation			
	0	10	20	30
White Bocho	1.2	<1	ND	ND
Red Bocho	1.4	<1	ND	ND
Epo	1.7	1.3	<1	ND

4.4.6. Staphylococcus

Staphylococcus growth was not detected in all varieties of kocho. Staphylococcus *aureus* does not compete well with indigenous microbiota in raw foods, contamination is mainly associated with improper handling of cooked or processed foods, followed by storage under conditions which allow growth of *S. aureus* and production of the enterotoxin(s) (Schelin *et al*., 2011). During the processing of Kocho it has been taken a very hygienic condition including cleaning of hands of women who scrubbed the kocho so the hygienic processes help to minimize the introduction of Staphylococcus species to undetectable point. But if there is any contamination of Staphylococcus in the food later stage of processing which is baking of kocho will contribute a significant decrease for this type of microorganism.

4.4.7. Result of Biochemical test

Table 20: Result of Biochemical test for Isolated Lactic Acid Bacteria from "White Bocho "at 0, 10, 20 and 30th days of fermentation

No	Catalase test				Lipopolsaccaride test using KOH				Carbohydrate fermentation				Cell Shape				Motility			
	0	10	20	30	0	10	20	30	0	10	20	30	0	10	20	30	0	10	20	30
1	-	-	-	-	+	+	+	+	Ht	Hm	Hm	Hm	c	C	c	r	nm	nm	nm	nm
2	_	_	_	_	+	+	+	+	Ht	Hm	Hm	Hm	c	R	c	c	nm	nm	nm	nm
3	_	_	_	_	+	+	+	+	Ht	Hm	Hm	Hm	c	C	c	c	m	nm	nm	nm
4	_	_	_	_	+	+	+	+	Ht	Hm	Hm	Hm	c	C	c	c	m	nm	nm	nm
5	_	_	_	_	+	+	+	_	Ht	Hm	Hm	Hm	c	C	c	c	nm	nm	nm	m
6	_	_	_	_	+	+	+	_	Ht	Hm	Hm	Hm	r	C	c	r	nm	nm	nm	m
7	_	_	_	_	+	+	+	_	Ht	Hm	Hm	Hm	c	C	c	r	nm	nm	nm	nm
8	_	_	_	+	+	+	+	_	Ht	Hm	Hm	Hm	c	C	r	c	nm	nm	nm	nm
9	_	_	_	_	+	+	+	_	Ht	Hm	Hm	Hm	r	C	r	r	nm	nm	nm	nm
10	_	_	_	_	+	+	+	_	Ht	Hm	Hm	Hm	c	R	c	r	nm	nm	nm	nm

Hm-homofermentative, Ht-hetrofermentative, nm-Non motile, m-motile, c-cocci, r-rod

As the biochemical test result showed all isolates are catalase negative except for one isolate at 30th day of fermentation, Since all lactic acid bacteria are catalase negative (Fooks *et al*., 1999; Holzapfel *et al*., 2001).

Some of the LAB are homofermentative, and produce lactic acid as the main product of glucose fermentation, while others are heterofermentative and produce carbon dioxide and ethanol in addition to lactic acid (Blandino *et al*., 2003). The volatile compound and alcohol generated in heterofermentation could provide particular tastes and aromas to final fermented foods. Hence, the characteristics of many fermented foods are dependent on LAB metabolic process Varadarajan and Miller (1999).

Heterofermentative cocci were dominant during the initial stage of fermentation and plays important role in initiating Enset fermentation. After 10days of fermentation all the hetrofermentative are replaced with homofermentative. Hence, more acid would be produced per mole of fermentable sugar and the rate of pH fall would be faster. The lowest pH achieved during the end of fermentation in all variety of Kocho.

Table 21: Result of Biochemical test for Isolated Lactic Acid Bacteria from "Red Bocho "at 0, 10, 20 and 30th days of fermentation

No	Catalase test				Lipopolsaccaride test using KOH				Carbohydrate fermentation				Cell Shape				Motility			
	0	10	20	30	0	10	20	30	0	10	20	30	0	10	20	30	0	10	20	30
1	_	+	_	+	+	_	+	_	Ht	Hm	Hm	Hm	c	r	c	r	nm	nm	nm	nm
2	_	+	_	_	+	_	+	+	Ht	Hm	Hm	Hm	c	r	c	c	nm	nm	nm	nm
3	_	+	_	+	+	+	+	+	Ht	Hm	Hm	Hm	c	c	c	c	nm	nm	nm	nm
4	_	+	_	_	+	+	+	+	Ht	Hm	Hm	Hm	c	c	c	c	nm	nm	nm	nm
5	_	_	_	_	+	+	+	+	Ht	Hm	Hm	Hm	c	c	c	r	nm	nm	nm	nm
6	_	_	_	_	+	+	+	+	Ht	Hm	Hm	Hm	r	c	r	c	nm	nm	nm	nm
7	_	_	_	_	+	+	+	+	Ht	Hm	Hm	Hm	c	c	c	c	nm	nm	nm	nm
8	_	_	_	_	+	+	+	+	Ht	Hm	Hm	Hm	c	c	c	c	nm	nm	nm	nm
9	_	_	_	_	+	+	+	+	Ht	Hm	Hm	Hm	c	c	r	c	nm	nm	nm	nm
10	_	_	_	_	+	+	+	+	Ht	Hm	Hm	Hm	c	c	c	c	nm	nm	nm	nm

Hm-homofermentative, Ht-hetrofermentative, nm-Non motile, m-motile, c-cocci, r-rod

As the result showed isolates from "Red Bocho", they showed catalase negative reaction except for four isolates at 10th day and 2 at 30th day of fermentation but almost all isolate shows catalase negative and gram positive reaction which makes it close to be Lactic acid bacteria. Since all lactic acid bacteria are catalase negative (Fooks *et al*., 1999; Holzapfel *et al*., 2001).

The carbohydrate fermentation result is similar with the isolate from White Bocho having the same type of explanation. Heterofermentative cocci were dominant during the initial stage of fermentation and plays important role in initiating Enset fermentation. After 10days of fermentation all the hetrofermentative are replaced with homofermentative.

Table 22: Result of Biochemical test for Isolated Lactic Acid Bacteria from "Epo "at 0, 10, 20 and 30th days of fermentation

No	Catalase test				Lipopolsaccaride test using KOH				Carbohydrate fermentation				Cell Shape				Motility			
	0	10	20	30	0	10	20	30	0	10	20	30	0	10	20	30	0	10	20	30
1	_	+	+	+	+	_	_	+	Ht	Hm	Hm	Hm	c	r	r	c	nm	nm	nm	nm
2	_	+	+	+	+	_	_	_	Ht	Hm	Hm	Hm	c	c	r	r	m	nm	nm	nm
3	_	+	+	+	+	_	_	_	Ht	Hm	Hm	Hm	c	r	r	r	nm	nm	nm	nm
4	_	+	+	+	+	_	_	_	Ht	Hm	Hm	Hm	c	r	c	c	nm	nm	nm	m
5	_	+	+	+	+	_	_	_	Ht	Hm	Hm	Hm	c	r	r	r	nm	nm	m	nm
6	_	+	+	+	+	+	_	_	Ht	Hm	Hm	Hm	c	c	r	c	nm	nm	nm	m
7	_	_	+	+	+	+	_	_	Ht	Hm	Hm	Hm	c	c	c	c	nm	nm	nm	nm
8	_	_	+	+	+	+	_	+	Ht	Hm	Hm	Hm	r	c	r	r	nm	nm	nm	nm
9	_	_	+	+	+	_	_	+	Ht	Hm	Hm	Hm	c	c	c	r	nm	nm	nm	nm
10	_	_	+	+	+	_	_	+	Ht	Hm	Hm	Hm	c	r	c	r	nm	nm	nm	nm

Hm-homofermentative, Ht-hetrofermentative, nm-Non motile, m-motile, c-cocci, r-rod

The table showed after 10days of fermentation all the catalase negative are replaced by catalase positive. Sharp, 1979 has studied the Gram-negative and catalase positive strains were regarded as non-LAB because Lactic Acid Bacteria (LAB) are Gram positive, fastidious, acid tolerant, generally non-sporulating and catalase negative (Fooks *et al*. 1999; Holzapfel *et al*. 2001).

It has been showed that some lactic acid bacteria as catalase positive. Some lactic acid bacteria including lactobacilli, enterococci and pedococci can produce Pseudocatalase. This can result in a false positive reaction for catalase, creating problems in isolate classification. The activity of

catalase is due to its haem group. Pseudocatalase does not contain a haem group but does contain manganese. Heterofermentative cocci were dominant during the initial stage of fermentation and plays important role in initiating Enset fermentation but after 10days of fermentation all the heterofermentatives are replaced by homofermentative which is similar to that of Kocho from cultivated Enset.

4.6. Sub -acute Toxicity in mice

Sub acute toxicity was done in Swiss albino mice using observation for any sign of toxicity and the result showed that there is no single type of toxicity sign after the administration in 5, 50, 300, 2000 and 5000mg/kg dose level. The mice were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total of 14 days. Individual weights of animals also determined shortly before the test substance is administered and weekly thereafter. Even if the animals didn't show any sign of toxicity changes in skin and fur, eyes and mucous membranes with special attention directed to observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma was observed.

As the result from table 22 showed there is no any sign of toxicity observed in mice after the administration of Kocho from different varieties of Enset within different amount of dose level as expressed in the methodology part. Observation of each test mice for skin and fur, eyes and mucous membrane, tremors, convulsions, salivation, diarrhea, lethargy and sleep in every 24 hours for 14 days shows no difference in comparison to the control group.

The result for kocho prepared from "Epo" didn't show any sign uncomfortable stomach condition is in accordance with Hildebrand (2001). "Forest Enset with local name Erfu" consumption did not cause any unusual digestive disturbance and suggested that "Erfu" probably not poisonous but certainly tough". Significance difference was observed in weight change before and after the administration of test sample.

From the result observed as compared to the control group kocho prepared from wild Enset didn't have any effect on the tested mice.

Change in weight at 5, 50 and 2000mg/kg and level of administration

Table 23: Body weight of mice before administration of kocho sample

Control group	5mg/kg from Epo	50mg/kg from Epo	2000mg/kg from WB	2000/kg from RB
35.1g	**33.9g**	33.4g	29.00g	33.5g
30.1	**36.9g**	32.2g	26.42g	26.3g
35.8	**30.2g**	30.1g	35.7g	22.2g
37.4	**35.2g**	36.5g	25.97g	30.6g
32.3	**32.56g**	30.6g	24.15g	26.6g
Total=107.7 **Mean=34.14**	Total=168.76 Mean=33.7	Total=162.8 Mean=32.56	Total=141.24 Mean=28.25	Total=139.2 Mean=27.8

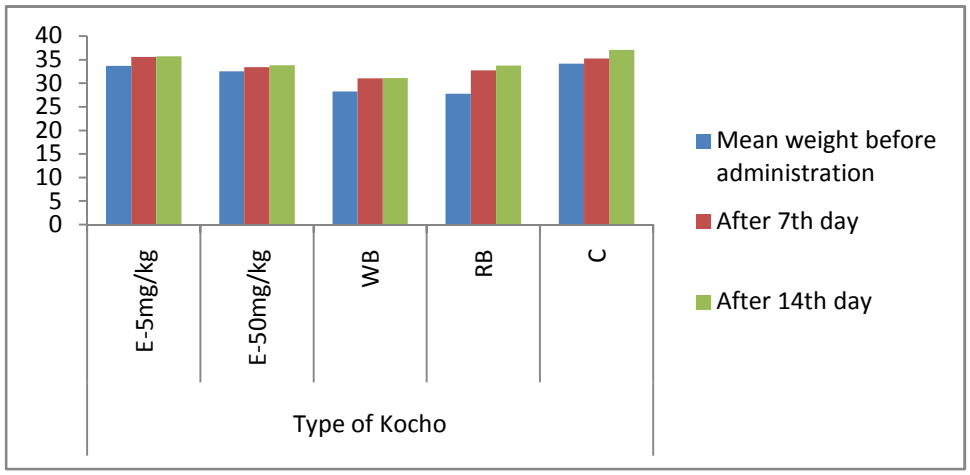
Epo=kocho prepared from wild Enset, WB=kocho prepared from White Bocho, RB=kocho prepared from Red Bocho.

Table 24: Change in Body Weight of mice after seven days of administration of kocho sample.

Control group	5mg/kg from Epo	50mg/kg from Epo	2000mg/kg from WB	2000/kg from RB
37.1g	**35.3g**	33.7g	30.2g	36.5g
31.4g	**37.4g**	34.4g	30.1g	27.6g
36.5g	**34.1g**	29.9g	37.2g	30.8g
37.8g	**37.2g**	37.2g	31.4g	40.5g
33.3g	**33.8g**	31.9g	26.15g	28.4g
Total=176.1 **Mean=35.22**	Total=177.8 Mean=35.56	Total=167.1 Mean=33.42	Total=155.05 Mean=31.01	Total=163.8 Mean=32.76

Table 25: Change in body weight of mice after 14 days

Control group	5mg/kg from Epo	50mg/kg from Epo	2000mg/kg from WB	2000/kg from RB
39.3g	**38.2g**	34.1g	30.4g	36.6g
34.2g	**37.2g**	35.2g	29.2g	28.3g
37.5g	**30.7g**	30.3g	37.6g	33.2
39.4g	**38.5g**	37.3g	31.7g	41.4
35.1g	**34.1g**	32.1g	26.7g	29.3
Total=185.5 **Mean=37.1**	Total=178.7 Mean=35.74	Total=169 Mean=33.8	Total=155.6 Mean=31.12	Total=168.8 Mean=33.76



E-Epo,WB-White Bocho,RB-Red Bocho and C- Control

Fig 3: Graphical view of change in mean weight at different dose level and variety of kocho.

The figure showed that there is a slight increase in weight mean before and after the administration of kocho variety. From the result given by change in weight I can say that the kocho prepared from wild Enset doesn't bring any decrease in weight of test animal which is one of acute toxicity testing parameter.

Change in weight at 5000mg/kg level of administration

Table 26: Change in body weight of mice before administration of kocho

Weight of mice before administration (control group)	Wight of mice before administration of Kocho from cultivated Enset	Wight of mice before administration of Kocho from wild Enset
32	33.1	26.1
37.1	36.6	29.4
28.2	29.9	29.6
33.9	36.6	32.2
43.1	28.6	29.1
Total=174.3	Total=164.8	Total=146.4
Mean=34.86	Mean=32.96	Mean=29.28

Table 27: Change in body weight of mice after seven days of administration of kocho sample

Weight of mice before administration (control group)	Wight of mice before administration of Kocho from cultivated Enset	Wight of mice before administration of Kocho from wild Enset
33.8	34.1	27.5
37.2	39.1	33.4
29.1	34.7	35.6
35.1	41.5	30.6
44.1	28.4	30.8
Total=179.3	Total=178.2	Total=159.9
Mean=35.86	Mean=35.64	Mean=31.58

Table 28: Change in body weight of mice after 14 days of administration of Kocho sample.

Weight of mice before administration (control group)	Wight of mice before administration of Kocho from cultivated Enset	Wight of mice before administration of Kocho from wild Enset
34.2	36.2	28.2
38.1	39	34.3
30.2	36.2	36.5
37	43.7	32.4
44.6	30.8	31.3
Total=184.1	Total=185.9	Total=162.7
Mean=36.82	Mean=37.18	Mean=32.54

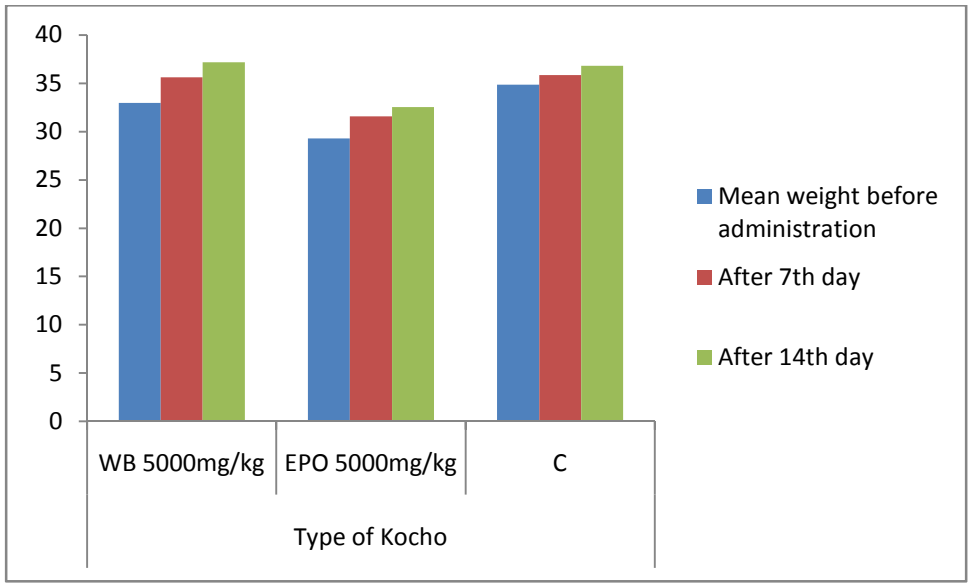


Fig 4: Change in mean body weight of mice after 5000mg/kg administration. WB-White bocho, c-Control

OECD (1998) has stated that only when justified by specific regulatory needs, the use of an additional upper fixed dose level of 5000 mg/kg may be considered. Recognizing the need to protect animal welfare, testing at 5000 mg/kg only considered result of the test have a direct relevance for protecting animal or human health. Although it is prohibited to dose the animal at the maximum amount, the test was performed as there is a strong likelihood that the results of such a test would have a direct relevance for protecting animal or human health.

It is indicated in Fig 4 after the dose of kocho was increased into 5000mg/kg the mean average weight of the mice showed a slight increase. Every 24hours the changes in skin and fur, eyes and mucous membranes with special attention directed to observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma was observed and we didn't find any sign of toxicity for 14 days and even the mice are still alive which is now 3month.

As Hildebrand (2001) showed that consumption of kocho prepared from wild Enset doesn't show any sign of toxicity or digestive disturbance since there was no animal showing moribund condition or animals showing severe pain or enduring signs of severe distress, the result of toxicity in this thesis is in agreement for not showing any toxicity sign in the mice. Since animals didn't show any sign of toxicity gross necroscopy change test was not done.

CHAPTER FIVE

5. Conclusion and Recommendation

5.1. Conclusion

The nutritional evaluation studies on cultivated variety of kocho consumed by the Keffa community indicated that they are good sources of crude fiber, carbohydrate essential minerals. Also Kocho from wild Enset have got a good source of protein, fiber content but low carbohydrate with higher concentration of minerals as compared to Kocho prepared from cultivated Enset. Kocho from wild Enset is rich in potassium and can contribute in maintaining normal blood pressure and its heart protective role.

The high crude fiber and minerals, it is suggested that kocho from Wild Enset may be used in enhancing the starchy staple foods of the south community that used kocho as daily diet source.

Depending on variation of variety of plant, geographical position, ways of processing, hygienic practice, starter culture used there is a difference on the nutritional composition and microbial dynamics of kocho studied in this experiment.

5.2. Recommendation

The wild variety of Enset “Kocho seytana” is believed to contain high variability within itself and from the other cultivated ones. Breeding programs on Enset particularly focusing on the bacterial wilt resistance should seek the possibility of utilizing this variety as a source of reliable resistant genes.

Wax from the leaves of cultivated Enset has not been studied yet so other experimental researches have to be done on the chemical composition and its potential application.

The study result showed that kocho from wild Enset contain high amount of protein as compared to the cultivated Enset Kocho except for Kocho prepared from White Bocho. In areas where

kocho used as daily part of diet are at high risk of being malnourished because of low protein content of kocho, so using kocho from wild Enset can be a better way to minimize this type of malnutrition.

Kocho from wild Enset become dark when it's cooked and is one of the factor that the society don't want to use this kocho. As food scientists there are lots of ways that the color of food can be changed by blending with other cereal.

More ethno botanical studies has to be made by biologist so that it will be easy to safe this Enset from extinction by creating awareness to the society to plant this variety of Enset.

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Annex 1: Preparation of test substance for toxicity test

Test substance was prepared based on the average weight of animals determined before the test Substance was administered. Weight of animals determined before the test substance is administered

Cage number	Test animal weight in gram					Average weight
1	29.00g	26.42g	35.7g	25.97g	24.15g	28.25g
2	33.5g	26.3g	22.2g	30.6g	26.6g	27.8g
3	33.9g	36.9g	30.2g	35.2g	32.6g	33.7g
4	33.4g	32.2g	30.1g	36.5g	30.6g	32.56g
control	35.1g	30.1g	35.8g	37.4g	32.3g	34.14g

Cage one and two Koho from Cultivated variety (white Bocho), Cage three and four kocho from wild variety and the last for control group.

The kocho sample was prepared based on the average weight of mice. For group one which are going to be tested by kocho from cultivated Enset 2000mg was chosen since the kocho is eaten by human being. The calculation was made below:

Cage one

2000mg is administered for 1000g average body weight of mice since the average weight of mice for cage one was 27.8g then 55.6mg kocho sample was prepared and changed into gram by dividing by 1000.

55.6/1000=0.0556g .Seven sample of test substance was prepared for five mice if there is any loss of sample in case.

7*0.0556=0.3892

0.3892 g with 0.2ml water

0.2ml *7=1.4ml water

Cage two

In cage two the mice were also tested by kocho prepared from cultivated Enset (Red Bocho) for 2000mg/kg. The Average weight of mice was 28.25g and 56.5mg kocho sample was prepared and the same procedure was used like preparation for cage two.

56.5mg/1000g =0.0579g

0.0579*7=0.399g

7*0.2ml=1.4ml water

Cage Three

Here the mice were tested for kocho from wild Enset, since we don't have any information weather the kocho is toxic or not I started with the smallest amount which is 5mg/kg.

5mg=1000g

? 33.7g (Average weight of mice).

0.169g

0.169*7=1.179g

0.2*7=1.4ml water

Cage four

In this group the mice was also tested for kocho from wild Enset but with increased amount 50mg/kg and the average weight 32.56g and 1.63mg kocho sample was prepared and changed in to gram by dividing with 1000.

1.63mg/1000g=0.002

0.002*7=0.0119g

0.2*7=1.4ml water

Cage Five

Cage five contains mice that are used as a control and we gave them clean water.

0.2*7=1.4ml water

Since the mice didn't show any evident toxicity we increase the dose to 5000mg using the same procedure used for the previous investigation.

At the next stage of comparison because it's was difficult to find mice so we made the comparison only for two Enset variety of Kocho which are White Bocho and Epo. Weights of test substance were determined before the administration of test substance.

Cage number	Test animal weight in gram					Average weight
1	33.1g	36.6g	29.9g	36.6g	28.6g	32.9g
2	26.1g	29.4g	29.6g	32.2g	29.1g	29.28g
control	32g	37.1g	25.6g	33.9g	43.1g	34.34g

Cage one-kocho from Cultivated Enset, cage two kocho from Wild Enset

The calculation was made below:

Cage one

5000mg=1000g

? = 32.9g (average weight of mice)

164.5mg/1000g

1.1515mg

0.2*7=1.4ml water

Cage two

5000mg=1000g

? = 29.28g (average weight of mice)

146.4mg kocho sample divided by 1000g.

0.1645mg*7ml

146.4mg/1000g

0.1464mg*7ml

1.0248mg

Controlled group

Water was used for the control group. 0.2ml of water was given for the controlled group of mice.

0.2*7=1.4ml water

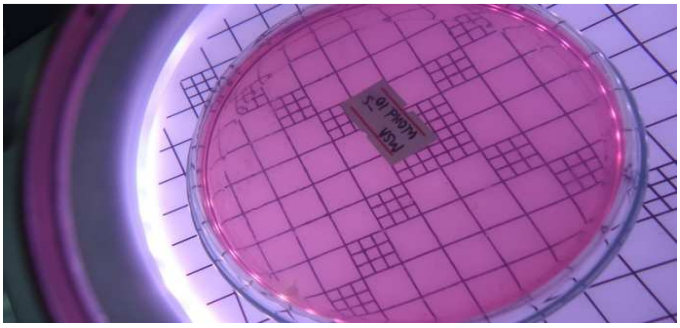
Annex 2: Processing of Kocho in the study site

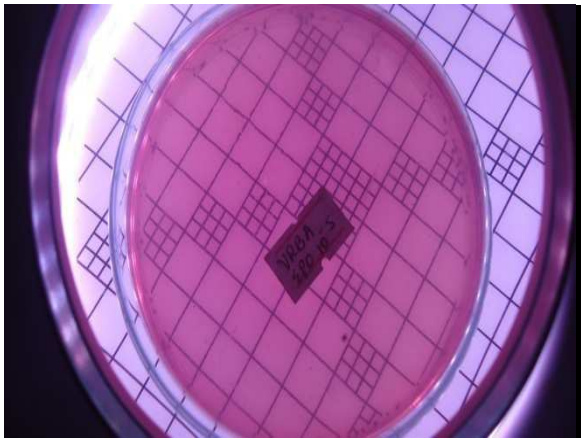
Annex 3: Interviewing people about wild enset

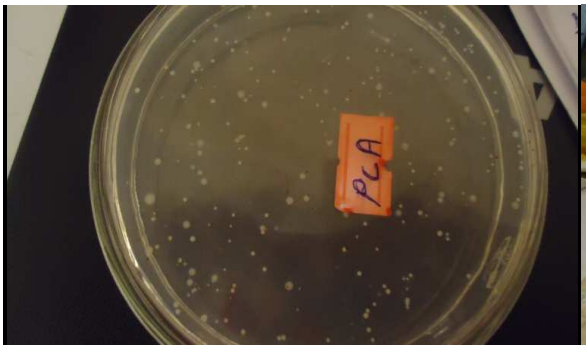
Annex 4: kocho from wild Enset and cultivated Enset

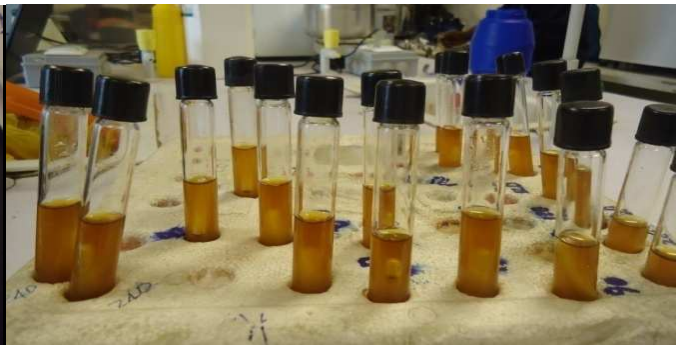
Wild Enset **Cultivated Enset**

Annex 5: microbial Analysis









Annex 6: Microscopic Examination of shape of Lactic Acid Bacteria