SCHOOL OF GRADUATE STUDIES

.

EVALUATION OF FERMENTATION PERIOD AND STORAGE METHOD ON NUTRITIONAL QUALITY OF KOCHO, A TRADITIONAL FERMENTED FOOD OF ENSET (*Ensete ventricosum*)

MS.c THESIS

FEIRUZA AHMED

MARCH, 2025

WOLKITE, ETHIOPIA

WOLKITE UNIVERSITY

SCHOOL OF GRADUATE STUDIES

EVALUATION OF FERMENTATION PERIOD AND STORAGE METHOD ON NUTRITIONAL QUALITY OF KOCHO, A TRADITIONAL FERMENTED FOOD OF ENSET (*Ensete ventricosum*)

A Thesis Submitted to the School of Graduate Studies

In the Partial Fulfillment of the Requirements for the Degree of Master in Horticulture

FEIRUZA AHMED MUNAYE

Major Advisor: Helen Weldemichael (PhD)

Co-Advisor : Abraham Mulatu(Assistance Professor)

March ,2025

Wolkite Ethiopa

APPROVAL SHEET

WOLKITE UNIVERSITY

SCHOOL OF GRADUATE STUDIES

We hereby certify that we have read and evaluated this thesis entitled **"Evaluation of fermentation period and storage method on nutritional quality kocho, a traditional fermented food of Enset (*Ensete ventricosum*)"** prepared under our guidance by Feiruza Ahmed. We recommend that the thesis shall be submitted as fulfilling the requirement for the award of a MSc. Degree in Horticulture.

Helen W/Michael (PhD) ____________ 03/06/20/2025
Major Advisor Signature Date

Abreham Mulatu (Asst Prof) ____________ 03/06/2025
Co-advisor Signature Date

As the members of the Board of Examiners of the Master Thesis open defense examination, we have read and evaluated this thesis prepared by Feiruza Ahmed and examined the candidate. We hereby certify that, the thesis is accepted for fulfilling the requirements for the award of the Degree of Master of science (MSc.) in Horticulture.

1. Abreham Shumbulo (PhD) ____________ 03/06/2025
Name of External examiner Signature Date

2. Emikur Masresha ____________ 03/06/2025
Name of Internal examiner Signature Date

3. Assefa Menbere (PhD) ____________ 03/06/2025
Name of Chairman Signature Date

Final approval and acceptance of the thesis is contingent up on the submission of its final cop to the council of postgraduate program (CPGP) through the candidates department school graduate committee(SGC).

i

DEDICATION

This thesis is dedicated to my lovely Husband and family, who have provided encouragement, and showered me with love throughout my journey towards success in life.

STATEMENT OF THE AUTHOR

First, I declared that this thesis is a result of my genuine work and that I have duly acknowledged all sources of materials used for writing. I submitted this thesis to Wolkite University in partial fulfillment for the Degree of Master of Science in Horticulture and it is deposited in the library of the University to be made available to borrowers for reference. I solemnly declared that I have not so far submitted this thesis to any other institution anywhere for the award of any academic degree, diploma, or certificate.

Brief quotations from this thesis are allowed without requiring special permission provided that an accurate acknowledgement of the source is made. Requests for permission for extended quotations from or duplicate of this thesis in whole or in part may be granted by the Head of the department of Horticulture or the Director of Postgraduate Programs of Wolkite University when her judgment the proposed use of the material is in the interest of scholarship. In all other instances, however, permission must be obtained from the author of the thesis.

Name: Feiruza Ahmed Signature ____________

Place: Wolkite University,

Date of Submission: March,2025

BIOGRAPHICAL SKETCH

The author was born on June 2, 1983 from his father, Mr. Ahmed Munaye and her mother, Mrs. Zahra Negash. She attended her elementary education at Tiya Elementary School and completed her secondary education at Sodo Buee Senior Secondary School. After completing her secondary education, she enrolled at Welayta Sodo Agricultural Training Center in November 2001. She later joined Dilla Agricultural, Technical, Vocational, and Education Training (ATVET) College in October 2005, where she obtained a certificate in Development Agent and a diploma in Plant Science.

Following her graduation, she began working as a Horticulture Expert at the District Agricultural Office of Sodo Woreda in September 2008. After serving in this position for three years, she joined Dilla University in September, 2010 and earned a Bachelor of Science (BSc) degree in Plant Science in 2015. From 2015 until November 2021, she served as the Coordinator of the Extension Program at the Sodo Wereda Agricultural Office in the East Guraghe Zone. In 2021, she joined the Post Graduate Program at Wolkite University to pursue her master's degree in Horticulture.

ACKNOWLEDMENT

First and foremost, I would like to thank and praise the Almighty Allah for granting me the opportunity and strength to finalize this thesis. I would like to express my sincerer gratitude to my advisers, Dr. Helen Weldemichael and Asst. Prof. Abraham Mulatu for their invaluable guidance and support during my MSc thesis work. Furthermore, I extend my heartfelt thanks to my family and friends, especially Zehera Negash , Hailemariam Teklu, , and Redwan Keder for their unwavering support and encouragement during my study. Without their help, it would have been difficult to reach my goal.

Finally, I would like to extend my gratitude to the Sodo Wereda Administration Office for providing me with the opportunity to pursue my MSc studies. I am also thankful to the members of the Agriculture Office, Wolkite University, Department of Horticulture, Food Engineering laboratory and Biotechnology laboratory for their professional and personal support.

ABBREVIATION AND ACRONYMS

ANOVA	Analysis of Variance
AOAC	Association of official analytical chemist
CRD	Complete Randomized Design
CSAC	Central Statistical Agency of the Federal Democratic
FAO	Agriculture Food Organization
GHI	Global Hunger Index
ISO	International Organization for Standardization
LAB	Lactic Acid Bacteria
LSD	Least Significant Difference
SWFEO	Sodo Wereda Finance and Economy Office

TABLE CONTENT

Table of Contents

LIST OF TABLE

LIST OF APPENDIX

ABSTRACT

Enset (Ensete ventricosum) is an indigenous African crop that currently feed approximately 25 million peoples in Ethiopia. It has long been domesticated in the southern highlands of the country, where smallholder farmers cultivate hundreds of landraces across varied climatic and agro-ecological Zones. The objective of this study was to evalute the effect of fermentation period and storage method (pot and plastic jar). Both fermentation time and storage method had significant effects (p<0.05) on the pH, Ash, fiber, fat and carbohydrate content of kocho .The carbohydrate content ranged from 67.96 % to 91.61%. The highest carbohydrate content (91.606 %) was recorded in the sample fermented for 90 day and stored in pot, while the lowest (67.96%) was recorded in the sample fermented for 30 days stored in a pit. Another low value (66.95%) was recorded in the sample fermented for 120 days in pit .The microbial counts, including lactic acid bacteria (LAB) and yeast, increased during the first 30 days of fermentation but declined as the fermentation period extended beyond that. In conclusion, the combination of a 90 day fermentation period and pot storage method resulted in the highest carbohydrate content (91.61%), including that this combination is optimal for enhancing the nutritional quality of kocho.

Keywords:-*Fermentation period, Storage method, Kocho quality, Nutritional content*

1. INTRODUCTION

Enset (*Ensete ventricosum,*) is an African crop currently feed approximately 25 million peoples in Ethiopia and constantly been domesticated in the southern highlands of Ethiopian where smallholder farmers encourage hundreds of landraces across varied climatic and agro-ecological Zones (Yemataw *et al.,* 20014).Enset is a perennial monocarpic, herbaceous plant belonging to the Musaceae family *(*Nurfeta.*et al.,* 2008*;* Weldemichael, *et al., 2008).*The crop can be grown well at altitude ranges between 1400 to 3100 m.a.s.l and mainly propagated by vegetative means*(*Almaz and Admasu *et al., 2002*) and while the average temperature for enset growing areas is between 10 and 21°C and with relative humidity of 63 to 80 percent(Endale *et al*.,1996) . Enset is a crop that tolerates harsh environmental conditions including protracted drought periods, flooding, and various types of diseases and become a priority crop in Ethiopia, where it makes a major contribution to the food security. It is domesticated and under production majorly in Sidama, Gedyo, Gurage, Wolayita, Gammo, and Kembata that contributing for sustainable food security *(*Atnafu *et al., 2008).* The southern highlands' diverse agro-ecological and climatic conditions are ideal for the development of ensets and other root and tuber crops, which are crucial for both food security and revenue creation *(*Giorgis *et al.,2001).* Enset historically has been described as a 'tree against hunger' due to important characteristics played a back bone in supporting the food security of societies cultivating it and enset can be harvested and processed at any time of the year and stage to be stored for longer time as fermented product (STAT Ethiopia,2016)

Enset cultivation represents about 65 percent of the total crop production, and its yield is very high as related to other crops in the southern nation, nationalities, and people's regional state of Ethiopia (Birmeta *et al., 2004;* Mulatu, *2021).*According to CSA (2012-2020) report, number of tree to be harvested are increased. In the 2020/21 agricultural year, from all over the country is estimated to be 206,659,076 enset trees are harvested, yielding more than 122 million quintals of produce in the form of Amicho, Kocho, and Bula. Enset also provides important environmental and eco-systems services, by adding organic matter to the soil through a continuous accumulation of litter protection of the soil from erosion, and shade provision sustainability of agriculture systems in enset-producing localities *(*Haile *et al.,* 1996; Lee and Zawdie, 1997*;* Woldetensaye, 1997*).*

Enset has potential in industrial applications as a raw material for adhesives in the textile and paper industries, while its fiber can be used as a raw material in the production of sacking and string (Bezuneh, 2012; Brandt et al., 1997).Kocho is traditionally fermented enset product used as food in area of production and other part of the country (Tiruha *et al., 2014).* The plant has different parts such as pseudo stem, corm and stalk of inflorescence. The edible part of the plant found by fermenting the mixture of the scraped flesh of pseudo stem, pulverized corm and stalk of inflorescence under soil pit and above the ground using the leaves (Admasu and Struik.,*2001).* It is processed by different traditional tools done by indigenous knowledge of local people *(*Negash and Niehof, *2004*). Kocho fermentation is thought to comprise microbial disintegration of the grated corm and pseudo-stem and imparts flavor and textural qualities to the fermented food harvests (Urga et al., 1997). Microorganisms are active in kocho fermentation, which includes processes such as starch hydrolysis, proteolysis and lipolysis, and determine kocho product, odor, color, flavor and spoilage rate (Gizaw et al., 2016).

Fermentation is way of processing by which microorganisms propagate them utilizing their external medium as a source of nutrients *(*Gibson *1995).*It is the chemical transformation of an organic substance into simple compounds by the action of enzymes, complex organic catalysts, which are formed by a microorganism such as yeast, mold or bacteria. Microorganisms happening in raw material or in the environment of the production site are used for initiating the process *(*Oyewole, *1997).*

1.1. Statement of the Problem

Enset is a native root crop that is used to make both fermented (Kocho) and starch-based (Bulla) goods. Traditional food is one of the new food safety problems. They use outdated, unhygienic equipment and poor fermentation techniques to process enset .Processing enset are extremely unsanitary, labor-intensive, time-consuming, and harmful to the health of the women who do it. The fermentation process and the time required to finish it differ from one location to another, even within a single home. *(*Helen *et al.,* 2019)

Surface fermentation and pit fermentation are the two stages of the fermentation process that are frequently employed around the nation *(*Hunduma and Ashenafi, 2011). The temperature of the surrounding environment and the processing technique affect how long fermentation takes. The conventional fermentation process has a number of issues with food quantity, quality, and microbial instability (Hozlafel, 2002). Additionally, long-term pit fermentation permits the leaching of water-soluble proteins and amino acids (Tsegay, 2002). The average glucose content is significantly influenced by the fermentation duration. As fermentation time increases, the moisture content falls, and it is well known that the moisture concentration directly affects the end product's shelf life since it fosters microbial development (Ashenafi, 2008; Urga *et al.*1997; Zewdie *et al.*, 2011) . Fermentation duration and storage conditions enhance the nutritional stability, bioavailability, and quality of a fermented product (Chelule, Mokena, and Gqaleni, 2010). Thus, in this study was used pot and plastic jar storage method as an alternative at the treatment area to evaluate the nutritional quality of kocho. Therefore, the purpose of this study was to assess and suggest a suitable fermentation time and storage technique for improved kocho quality.

1.2. Objective

To examine the effect of fermentation period and storage method on the nutritional quality of Kocho at Sodo District in East Gurage Zone.

1.2.1. Specific Objective

Specific objective of this study are:

- To evaluate the optimum fermentation period for Kocho quality
- To evaluate storage method for better kocho quality
- To observe interaction effect of fermentation period and storage method on Kocho quality

2. LITERATURE REVIEW

2.1. Fermented Foods in Africa

Fermentation has been utilized in Africa for over 6,000 years as a traditional method of food preservation (Oyewole, 1997; Holzapfel, 2002). Fermented foods are highly valued in many developing nations due to their extended shelf life, affordability, safety, and cultural acceptance (Holzapfel, 2002). As a result, fermented foods and beverages make up a significant portion of the daily diet in African communities (Oyewole, 1997). Beyond preservation, fermentation enhances the sensory qualities and palatability of raw ingredients. It also plays a key role in reducing harmful substances such as linamarin in cassava (Edward, 2010) and antinutritional factors in legumes, while improving the digestibility of food. Thus, fermented foods are of vital nutritional and health importance across Africa. A wide variety of raw materials including cereals, root crops, legumes, and milk—are used to prepare essential staple foods and drinks. However, unlike other regions of the world, lactic acid fermentation of vegetables, fish, and meat is relatively uncommon in African food traditions (Steinkraus, 1996).

2.2. Challenges Associated with Traditionally Fermented Foods in Africa

Traditional fermented foods in Africa face several quality and safety challenges, starting with the inconsistent selection and inadequate cleaning of raw materials. This often results in contamination with foreign substances such as insects, stones, or plant debris, affecting both the safety and quality of the final product. Furthermore, many traditional fermentation processes lack standardized procedures, leading to poor hygiene during handling and processing. As a result, the end products frequently exhibit limited shelf life, inconsistent texture, flavor, and appearance, and minimal consumer appeal (Edward, 2010).

In addition, the absence of controlled fermentation environments increases the risk of contamination by undesirable microorganisms, which can compromise food safety and nutritional value. Most traditional producers rely on spontaneous fermentation using natural microflora, which, while culturally significant, leads to variability in product quality. The lack of packaging innovation and labeling further hinders marketability and scalability of these products. Moreover, there is limited application of modern food science and technology to improve these processes, and little effort has been made to upgrade traditional methods through training, equipment development, or the use of starter cultures.

Addressing these issues requires a more systematic approach to traditional fermentation, including improved raw material selection, sanitation, standardized procedures, and the introduction of appropriate technologies. This would not only enhance product consistency, safety, and shelf life but also open up opportunities for commercial production, value addition, and greater consumer trust both locally and internationally.

2.3. Origin and Distribution of Enset

Ethiopia is a center of origin for Enset and it is geographically dispersed in many parts of Sub-Shara Africa and Asia as wild species (Olango *et al., 2014).* The first scientific indication of enset in Ethiopia dates back to 1700 by travelers .Chessman made the genetic taxonomy and related investigations of the 25 African and Asian Musa species to the genus Enset(Yemataw, *2018*). E. *ventricosum* is commonly cultivated in Ethiopia for food and fiber, among other species in mountains and lowland. Ethiopia, Uganda, Tanzania and Sudan are the centers for origin of enset *(*TesfayeandLudders, *2003).*

2.4. Traditional use of Enset

Enset is an indigenous, multipurpose plant in Ethiopia particularly in enset cultivated area *(*Olango *et al.*, 2014*).* It is also considered as tree against hunger because; it can be harvested at any time in the case of food storage and also known as drought resistance plant. During drought and famine prone decades 1970’s and 1980’s in Ethiopia population depend up on enset cultivation survived. It also said to be enemy of hunger by local farmers because, human and livestock life is impossible without it *(*Mohammed *et al.*, 2013). Enset serve as a food, fibers traditional medicine (Mohammed *et al*, 2013*)* and it may be used as a partial substitution of barley malt in beer production *(*Temesgen *and* Getasew, 2015*)*. Some societies, particularly the Wolita zone, use food derived from enset as a source of nutrition and cultural value. The fiber that is extracted from enset is used to make fencing, house decoders, filters, rope, strings, basket sacks, and carpet for floors. *(*Olango *et al.,* 2014). Enset used as medicinal plants for example, porridge made from bulla used for strengthen women after delivery and healing of bone fractures in human, highly fermented kocho used for curing stomach cramps, amicho (boiled corm) used for birth control and abortion in human. Fiber and carbohydrate of enset foods may help to reduce blood glucose and blood fat level *(*Olango *et al*., 2014). Kocho is the bulk of the fermented food obtained from the mixture of the decorticated (scraped) leaf sheaths and grated corm (Tiruha *et al.,* 2014 *)*

2.5. Socio-Economic and Cultural Values of Enset

Enset could be a perennial evergreen crop with many aesthetic values and multipurpose within the south and southwestern Ethiopia. Its many material uses and socio-cultural values. An oversized number of enset plants around houses provide comfort, shading for people and a few crops like coffee, which require only moderate sunshine *(M. Shigeta etal, 1991*) The employment of enset and its products for various purposes like food (amicho (corm), bulla, and kocho, medicine, rituals, and construction purposes could attribute to the existence of assorted enset varieties (*Y. Tsehaye and F. Kebebew, 2006).* Enset garden is usually an indicator of the economic status of farmers; many types more mature and enormous number of enset plants are found within the gardens of wealthier households (*A. Negash and A. Niehof, 2004*) Gurage people depend much on enset socially and economically to get their essential needs (*E. Westphal, etal 1975).* Once it reaches certain stage of growth, cultivated enset will utilized for several purposes throughout a year (*E.Westphal,etal 1975)* Furthermost botanical parts of enset are good fodder for livestock. It is drought resistant because it contains plenty of water in pseudo-stem and used for cattle stall-feeding especially within the season when grass is scarce. It provides good fiber, a by-product obtained from decorticating the leaf sheaths and therefore the dried fibers are strong enough to create prime quality ropes. The pseudo-stem yields very strong fibers, even the unprocessed leaf sheaths is employed for tying livestock, bundling harvests from fields, and fencing (*Brandt ,etal 1975*)The fiber has excellent structure and its strength adores the fiber of abaca, excellent fiber crop. In rural areas, the fiber has employed to create sacks, bags, ropes, cordage, mats, construction materials, and sieves. About 600 heaps of enset fiber each year is shipped to factories for processing (*E. Taboje,eta 1997*)Fresh enset leaves used for wrapping foods, portion plates, and pit linings to store kocho for fermentation. Men sometimes make cap from enset and therefore the fresh leaves are used as clothes for women skirt, infrequently worn within the markets and ceremonies (*M. Shigeta etal,1991*)The dried petioles and midribs has used as firewood, to form mats, and tying materials for house construction. For cleaning rags, brushes, baby cushions, pot stands, as wrappers for butter, kocho, and other items to move to local market (*Brandt ,etal 1975).* Specific varieties and enset parts are used medicinally for both human and livestock for problems like diarrhea, contraception (as an abortifacient), and supporting to discharge placenta (*E. Taboje etal 1997*). Enset has cultural values through wedding and funeral ceremonies. During wedding, enset leaves will used on tables for serving food and as skirts for girls in some cases additionally to being the main food for the ceremony. Elucidated that at funeral ceremony, people beat the psuedostems of enset laid on the bottom in circle like drums and that they associate with enset leaves on their hands. The members of the lineage cut all the enset plants when the top of the household dies, to specific their sorrow and desperation (*M. Shigeta etal, 1991*) the enset plant may also sold in some cases and the processed products like kocho and bulla have sold anytime within the rural markets and towns. Therefore, it has an on the spot cash income source for a family to shop for their daily needs. Enset is widely used as food source and traditional medicine, scarce information is available on the antioxidant activities of food products of this plant (Forsido et al., 2013).

2.6. Production Capacity of Enset

Enset is a starchy crop and a staple food in Ethiopia, where it provides food for 20 million people. According to the FAO report, it provides more amount of foodstuff per unit area than most cereals (FAO). Unlike a banana, however, its fruit is inedible, but its stems and roots can be fermented and are commonly used to make porridge and bread. It’s an important multipurpose crop. The plant is used for animal feed, mostly during the dry season of the year since the pseudo-stem contains a lot of water (Nurfet a*et al., 2008*). The plant is an income source for the household and an indication of wealth and status in society. All parts are used for multiple purposes social value. The Global Hunger Index (GHI) in 2021 shows Ethiopia holds the 90th position among 116 countries. However, the Enset plant can feed more than 100 million people and increase food security in Ethiopia and other African countries. Advancing and standardizing of processing conditions could increase addressability of enset for food security issues. Enset grows in a wide range of agro ecological zones and it has flexibility and adaptability in nature. According to a World Economic Forum report, to decrease hunger on a global basis, integrating Enset might become particularly practical and distribute climate resilience. Additionally, enset-based practices eliminate chemical fertilizers and pesticides, increase the use of farmyard manure, and produce a variety of food and other products for millions of households. The productivity and area coverage of the crop are continuously declining due to various environmental and management factors (Birmeta *et al.*, 2004). Enset bacterial wilt disease is a prior threat or problem to the enset production system in Ethiopia (Zerfu *et al*., 2018).

2.7. Enset Processing

Enset processing, particularly the extraction of its edible and non-edible parts, is one of the most labor-intensive household activities traditionally performed by women. Despite some regional variations, the core steps such as decortication and pulverization of the pseudostem and corm, and wrapping the fermentable mass with enset leaves are generally consistent across communities (Hunduma & Ashenafi, 2011). However, the fermentation methods differ among regions and even between individual processors, though the final product remains similar.

In some areas, the process begins with digging a fermentation pit. The fermentable mass is placed inside, lined with fresh enset leaves, and covered with heavy stones to create anaerobic conditions (Mehtzun & Yewelsew, 1994). After several days or weeks, traditional fermentation enhancers may be added to accelerate the process (Gashe, 1987). In lower-altitude regions, the mass is first tightly wrapped in fresh enset leaves and left at ambient temperature for a few days to weeks to initiate fermentation before being transferred to the pit and sealed in the same manner (Hunduma & Ashenafi, 2011). In southern Gedeo, the fermentable mass is surface-fermented by tightly wrapping it with a combination of fresh and dry enset leaves, then weighted down with heavy materials like stones to create airtight conditions (Zerihun & Brihanu, 2015). A traditional starter culture may be added either at the beginning or after about 15 days of surface fermentation.

The duration of fermentation varies widely, ranging from a few weeks to several months or even years, depending on local practices and ambient temperatures. In cooler regions, the mass may be left to ferment for years, with quality believed to improve over time. In warmer areas, fermentation progresses faster and is usually completed within three to six months (Gashe, 1987). For example, in the Gedeo region, fermentation typically lasts from 15 days to one month (Zerihun & Brihanu, 2015). Once fermentation is complete, a portion of the mass is removed from the pit, and the liquid is squeezed out to yield moist, fibrous kocho. This is then kneaded, shredded, and sifted to remove residual fibers, resulting in a fine kocho powder (Gashe, 1987).

2.8. Utilization of Processed Enset Based Products

The fermented products of the enset plant are used to make different dishes such as thin, unleavened kocho bread, bulla porridge, thick cooked bulla gruel, and a shredded flake made of a mixture of kocho and bulla (Ashenafi, 2006). The fermented products of the enset plant are basically energy foods and are often blamed for causing protein deficiency disease when eaten alone as a staple. However, it should be noted that dishes made of these products are traditionally served with other protein and vitamin sources. The various kocho and bulla dishes are supplemented with milk, traditional butter, fenugreek, traditional cottage cheese, meat, kale or beans separately or in combination. Unfermented, fermenting or completely fermented kocho is baked or cooked and eaten alone or in combination with various indigenous foods. Unfermented kocho is consumed only when there is a shortage of fermented or fermenting material (Ashenafi, 2006; Zerihun and Brihanu, 2015).

2.9. Impact of Fermentation Period and Storage Methods on Kocho Quality

Fermentation method and length of time to complete the process vary from place to place. The two commonly used methods in enset producing area are surface and pit fermentation which is generally used in different parts of the country (Hunduma and Ashenafi, 2011a). Traditional fermentation method has several problems in terms of food quality, quantity and microbiological instability (Hozlafel, 2002).The growth of mold in enset production is totally undesirable because; they have capacity to produce mycotoxin, which is cause, for cancer vomiting and liver disease (Brase*et al., 2013).*

2.9.1. Impact of Fermentation Period on Kocho Quality

The quality of fermentation culture used is one of the factors responsible for the final quality and protection of the fermented food *(*Motarjemi, *2002).*Microbial are the dynamics of growth, survival, and biochemical activity of microorganisms in fermented kocho and food fermentation, which provide the favorite quality of fermented food. Also, microbial dynamics are the driving force of the traditional food fermentation

That play a major role in the fermentation process of traditional food, which can contribute for tastiness, wholesomeness, enhancing nutritional quality, and prolonging shelf-life *(*Braide *et al., 2018).* Fermentation time has an influence on the average carbohydrate content. The moisture content decreases as the fermentation time increases and it is known that the concentration of moisture has a direct relation to the shelf life of the end product as a conducive environment is created for microbial growth (Ashenafi, *2008;* Urga *et al.1997;* Zewdie *et al., 2011).*

2.9.2. Impact of Storage Methods on Kocho Quality

Pit walls and storage duration of in the pit, allows leaching of water-soluble proteins and amino acids *(*Tsegay, *2002).* The average amount of carbohydrates is significantly influenced by the length of the fermentation process. As fermentation time grows, the moisture content drops, and it is well known that the concentration of moisture directly affects the final product's shelf life since it creates an environment that is favorable for microbial development.*(*Ashenafi, 2008; Urga *et al.*1997; Zewdie *et al.,* 2011).

2.9.3. Interaction Effect of Fermentation Period and Storage Methods on Kocho Quality

The microorganisms are responsible for fermentation, change of chemical composition of raw material, which in some cases improves the nutritional value of fermented products (Tamang Watanabe, and Holzapfel*, 2016)* by removing anti-nutritionals and breaking down complex components. Furthermore, microbial communities introduced during processing are frequently critical to food safety and preservation by preventing growth of spoilage and toxic organisms. These microorganisms, often occurring as communities in food products are poorly known in orphan and minor tropical crops cultivated by subsistence farmers (Tamang *et al., 2016).* However, improvement of these cultures represents a relatively accessible opportunity to improve the nutritional stability, bioavailability and quality of neglected food products (Chelule, Mokoena,&Gqaleni, *2010).*

2.10. Nutritional Composition of Kocho

The kind, variety, age of the plant, agro climatic and geographic conditions, harvesting season, processing conditions, and fermentation duration all affect the nutritional makeup of the components. The enset plant regularly exhibits a wide range of varieties (Tesfaye and Lüdders, 2003; Tsehaye and Kebebew, 2006; Yemataw et al., 2016).

2.10.1 Carbohydrate Composition of Kocho

The nutritional composition kocho depends on many factors, type, variety of the enset, age of the plant, geographical and agro-climatic conditions, period of harvesting, processing conditions and fermentation time. Regularly, enset plant is greatly diverse in variety (Tesfaye and Lüdders, 2003; Tsehaye and Kebebew, 2006; Yemataw et al., 2016).

2.10.2. Kocho pH

The pH decrease, for the period of fermentation due to the active participation of fermenting microorganism, this is also happen in the case of enset fermentation to obtain kocho since, the process is lactic acid fermentation. The pH of end product (kocho) reaches around 4.0 - 4.3 *(*Hunduma*and* Ashenafi*, 2011a;* Karssa*et al., 2014)* while*,* Yirmaga*, (2013)* reported 3.79 at 30 day fermentation*. (*HundumaandAshenafi*, 2011 a)* concluded that both pre -fermentation and starter culture reduce the pH rapidly.

2.10.3 .Moisture Contents of Kocho

During fermentation time, the moisture content of fermenting enset reduced. In both high altitude and mid altitude region the initial moisture content of fermenting mass was around 80-86% and dropped to about 66-60% at the end of fermentation *(*Hunduma and Ashenafi, 2011 a*).* During pre-fermentation phase the moisture content lies between 84-68.6%, while at the end of pit fermentation it decrease to around 60% *(*Karssa *et al., 2014;* Bosha*et al., 2014).*

2.10.4 Ash Content of Kocho

The ash content dependency on inorganic residue and mineral composition of food Fermentation time considerably affect ash content and its content was reduced as fermentation time increased *(*Yirmaga*, 2013;* Desta *et al., 2021).*

2.10.5. Protein Content of Kocho

Kocho, the fermented starch product derived from enset (*Ensete ventricosum),* is generally low in protein, with reported crude protein contents ranging from approximately 2.5% to 5.09%, depending on various factors (Yirmaga, 2013; Desta et al., 2021). Despite its importance as a staple food in many parts of Ethiopia, kocho's nutritional profile is limited in terms of protein, making it necessary to complement it with protein-rich foods in the diet.

Several studies have indicated that the protein content of kocho tends to increase with the duration of fermentation. Extended fermentation periods likely enhance microbial activity, leading to the accumulation of microbial biomass and protein-rich metabolites, which contribute to the overall protein content (Yirmaga, 2013).

In addition to fermentation time, the specific variety of enset used has a significant influence on protein levels. Research has shown that different enset cultivars exhibit considerable variation in nutrient composition, including protein content (Krassa et al., 2018; Nuraga et al., 2019; Yirmaga, 2013). This highlights the importance of cultivar selection in efforts to improve the nutritional quality of kocho.

Although kocho is not a rich source of protein compared to legumes or animal products, understanding and optimizing factors such as fermentation practices and enset variety selection can contribute to improving its nutritional value, especially for communities that rely heavily on it as a dietary staple.

2.11. Microbial Content of Kocho

The first microbial load of processed enset is depends on the property of soil, tools, starter culture and hand contact *(*Gorrens*, 2018).*The fermentation procedure is fast the microbial load is deterioration except lactic acid bacteria and some spore forming bacteria the other microbes cannot survive the acidic environment .The reason for deterioration in microbial load at the end of fermentation day. There was not much difference in aerobic and an anaerobic microbes on the first day of fermentation and as fermentation proceed *(*Gorrens, *2018)* Lactic acid bacteria are the major microbes in kocho fermentation process *(*Gorrens, *2018).*

3. MATERIALS and METHODS

3.1. Description of Study Area

The experiment was conducted at Central Ethiopia Regional Government East Gurage Zone Sodo district Suten Zuriya Kebele located at (8˚ 19′ N and 38˚ 39′ E,) and1800 m.a.s.l, the area has annual rainfall of 800 - 1200 mm and annual average temperature of 16 to 26 ^{0}C.The area is located 8 km North of Buee town the capital of Sodo woreda located at 40 km Northern of Butajera town and 225 km Northern Hosena and 103 km far from Addis Ababa at the Southern part.

3.2. Experimental Design and Treatment

The experiment consists of four fermentation period and three storage methods that was be arranged in Factorial arrangement random complete block design(RCBD) and triplicated. The local storage method pit with leave was used as check for the experiment. Each pit, plastic jar (baldi) and pot was 0.003m^{3}containing 5kg of fresh decorticated enset product; next the pit and plastic jar (baldi), pot was covered with fresh enset leaf and compacted with stone to protect aerobic process.

Table 1 Treatment layout of the experiment

Storage method	Fermentation days			
	30 (D30)	60 (D60)	90 (D90)	120 (D120)
Pit (Pi)	PiD30	PiD60	PiD90	PiD120
Plastic-Jar (P)	PD30	PD60	PD90	PD120
Pot (Po)	PoD30	PoD60	PoD90	PoD120

3.3. Sample Preparation

The chosen plant's younger and older leaf sheaths were removed at harvest. The pseudo-stem and the genuine stem (corm) were separated by the leaf sheath. To make the leaf sheath manageable, the concave side was cut to a length of 1 m and then divided lengthwise .Using a traditional bamboo (Sibisa) scraper, pulp decortications were completed by slanting the leaf sheaths on a wooden plank (Watan) at an angle of 45 to 80 degree (Hunduma and Ashenafi, 2011).The fresh and green leaves that had been separated served as the bottom sheets for the scraping procedure. At the same time, the corm was detached and combined with the scraped leaf sheaths using a traditional wooden tool called a Jagba (Karssa *et al.*, 2014). Following that, the surface fermented kocho sample was moved to the appropriate fermentation treatments.

3.4. Proximate Composition Analysis of Kocho

The moisture contents, ash, crude protein, and total fat were evaluated according to the standard methods of the Association of Official Analytical Chemists (AOAC, 2000) and the methods used by (Bosha et al., 2016;Yirmaga, 2013).

3.4.1. Moisture Content

Moisture content of the kocho samples was determined according to AOAC (2000). An empty dish was dried using drying oven for 1h at 100 ^{o}C, transferred to the desiccator (with granular silica gel), cooled for 30 min, and weighed. The prepared samples were mixed thoroughly and about 5 g of sample was transferred to the dried and weighed dishes. Then after placed in the vacuum oven and dried for 5 h at 105 ^{o}C. Then the dishes and their contents were cooled in desiccators at room temperature and reweighed. Duplicates of each sample were determined. The amount of water present in a sample is considered to be equal to the loss of weight after drying the sample to constant weight at a temperature near the boiling point of water.

3.4.2. Ash Content

Ash content determination was performed following the standard method of the AOAC (2000). The crucibles were dried in an oven at 105 ^{o}C for 30 min and cooled in a desiccators for 30 min (M_1). Then, about 2 g of kocho sample was transferred to each of the dried crucible and weighed (M_2). The entire contents were then ignited in a muffle furnace at 550 ^{o}C for 2 h to obtain ash. Thereafter, the crucibles were cooled in a desiccator and weighed (M_3). The ash contents were calculated using the following equation:

$$\%\ \text{Ash} = \frac{M_2 - M_1}{M_2 - M_3} \text{X}100 \qquad \text{Eq.1}$$

Where: M_1 - weight of dry crucible, M_2 -weight of fresh sample and crucible and M3 -weight of dry sample and crucible.

3.4.3. Total Fat

Total fat was determined using a standard method of AOAC (2000). Accordingly, about 2 g of the kocho samples was transferred to each of the 100 ml extraction flask. The fat content of kocho samples were determined by Soxhlet extraction method using 70 ml diethyl ether as extraction solvent. The ethyl ether was evaporated from the extraction flask. The amount of fat in each kocho sample was calculated using the following equation:

$$\% \text{ Fat} = \frac{W_2 - W_1}{W} \text{X } 100 \qquad \text{Eq. 2}$$

Where: W_1= weight of extract cylinder , W_2 = weight of fat after extraction, W= weight of sample.

3.4.4. Total Nitrogen

Crude protein was analyzed by micro Kjeldahl method. The general procedure included the following steps of digestion: about 2 g sample was digested by adding 5 ml of concentrated sulfuric acid, in the presence of potassium sulfate catalyst in a Kjeldahl flask, then diluted with 30 ml distilled water. 25 ml of NaOH (40%) was added to neutralize the sulfuric acid. Upon addition of NaOH, the ammonium was distilled off and trapped into a boric acid and solution containing methyl blue and methyl red indicators. Finally, titration of the ammonium attached to borate anion was titrated with standardized HCl, and total crude protein of kocho was calculated using the following equation :

$$N(\%) = \frac{a - b * N * 0.014 * 100}{S} \text{mcf} \qquad \text{Eq. 3}$$

Where: a= ml of HCl required for titration, b= ml of HCl required for titiration of blank, N= normality of HCl (0.1), S= dry sample weight in gram (0.5g), 100= ml of solution 4 0.014= molecular wight of nitrogen , mcf= moisture correction factor where, the moisture analysis is done in wet moisture and % protein = % N x 6.25.

3.4.5. Fiber Content

Kocho, a fermented starch-rich product obtained from enset, contains varying levels of crude fiber, which contributes to its role in promoting digestive health, though the content is generally moderate. The crude fiber content is determined by sequential digestion of the sample using dilute acid and alkali solutions, followed by filtration, drying, and incineration to isolate the indigestible fiber fraction (Hiwot Bekele, 2015).

In the standard procedure, a 2-gram sample of dried kocho is subjected to acidic digestion using 1.25% sulfuric acid, followed by washing and subsequent alkaline digestion with 1.25% sodium hydroxide. The residue is then filtered using a coarse porosity crucible under vacuum (approximately 25 mm Hg). To ensure thorough removal of soluble components, the residue is washed again with hot dilute sulfuric acid, then dried at 95°C overnight and cooled in a desiccator for weighing (M1). The dried residue is incinerated in a muffle furnace at 500°C for about two hours, cooled, and weighed again (M2). The difference in mass before and after incineration represents the crude fiber content, calculated as:

$$\text{Total Crude Fiber (\%)} = \left(\frac{M_1 - M_2}{M_3}\right) \text{X100} \qquad \text{Eq. 4}$$

where: M_1 = mass after drying, M_2 = mass after incineration, and M_3 = original mass of the sample.

3.4.6. Carbohydrate Content

The carbohydrate content of kocho, the main energy-yielding component of the food, is typically calculated by difference a standard method in food composition analysis. This approach assumes that all components not accounted for by direct measurement (moisture, ash, crude protein, crude fiber, and fat) are carbohydrates. The formula used is:

Total Carbohydrate (%) = 100 - (% Moisture + % Ash + % Protein + % Fiber + % Fat)

This method provides an estimate of the total carbohydrate content, including both digestible carbohydrates (such as starches and simple sugars) and some indigestible carbohydrates like resistant starches, which may play a functional role similar to dietary fiber.

3.5. Kocho pH

A digital pH analyzer (Meltler Toledo MPC-227) was used to measure the pH of the samples after they had been homogenized with 90 milliliters of distilled water for each treatment. Standard buffer solutions with pH values of 4 and 7 were used to calibrate the pH meter initially. Moreover, 10 g of sample.

3.6 Microbial Content (yeast and lAB)

Classical plate counts (culture dependent analysis) was carry out according to the ISO, 1446 (2001) standards for microbial analysis of food (Gorrens, 2018). Initially, the required media was prepared. de Man Rogosa Sharpe agar (MRS) and potato dextrox agar (PDA) were used to measure lactic acid bacteria and yeast, respectively. The medium was then autoclaved at 121°C for 15 minutes. The growing media was placed in a water bath at 40°C until the agar solidified after the autoclaving process was finished. For microbiological studies, ten grams were aseptically transferred into a sterile test tube.

A sterile test tube holding 10 g of sample was then filled with 90 ml of distilled water, shaken with a test tube shaker until the sample was thoroughly diluted, and then serially diluted from $10^{-1}$to 10^{-7} g . Following dilution, 20 ml of the growth medium was added to a sterile plate along with 1 milliliter of the diluted sample. A duplicate plate was used for each suitable dilution. Each medium containing the material was cultured for three days at 37°C after the agar solidified. Yeast are incubated for three days at 25°C. Following the specified incubation period, each plate's colony count was determined using a colony counter and reported as log cfu/g.

3.7. Statistical Analysis

When the treatment effects were significant at the 5% probability level, the means were separated by LSD, and correlation analysis was performed using Pearson's simple correlation coefficients for the dependent variables, the fermentation period and storage methods. The data was analyzed using the general linear model (GLM) and analysis of variance (ANOVA) using SAS software (SAS Version 9.4).

4. RESULT AND DISCUSSION

4.1. PH of Kocho

Table 2 showed how the pH of the kocho samples was affected by the combination of fermentation time and storage techniques. According to the analysis of variance, the interaction effect had a significant ($p<0.01$) impact on the pH of the kocho. The pot had the lowest pH of the kocho, which was lower than the control, at (3.9), while the plastic jar, which was used for storage, had a record of (4.11). When the fermentation duration was used to compare the pH of kocho, the greatest pH was observed at 90 days, which is 4.14; the lowest pH was obtained at 60 days. The greatest pH of kocho was observed at 90 days using in-pit storage techniques among the interaction effects (4.24). In contrast, the 60-day pot storage method yielded the lowest pH value. As a result, the pH of the kocho lowered as the fermentation period increased. This was because the active engagement of the fermenting microorganisms increased and the lactic acidity reduced. This conclusion is similar to a study by (Gashe, 1987; Hunduma & Ashenafi, 2011a; Karssa *et al.*, 2014; Andeta *et al.*, 2018; Gorrens, 2018).pH was observed due to the activities of fermenting microorganism especially LAB. To facilitate the fermentation process the fermenting microorganism important favorable temperature and one of the predominant lactic acid bacteria *(L. Planetarium).*

Table 2. Interaction effects of fermentation time and storage methods on pH

	Fermentation days			
Parameter	Ph			
Storage method	30	60	90	120
Pit	4^{fg}	4.017^{f}	4.24^{a}	4.125^{c}
Plastic jar	4.18^{b}	4.02^{f}	4.086^{de}	4.07^{e}
Pot	3.34^{cd}	4.08^{ab}	4.11^{cd}	3.96^{hi}
CV(%)	0.304			
LSD (5%)	0.012			
Mean	4.05			

4.2. Moisture Content of Kocho

As shown in Table 3 below, there was a significant difference ($p<0.01$) in moisture content between the treatment's interaction effects. The lowest moisture level of kocho was obtained using the in-pot storage method (65.64%), while the maximum moisture content was recorded using the plastic jar (73.83%). The 90-day fermentation phase had the highest moisture content of kocho (69.28), while the 120-day fermentation period had the lowest moisture content (66.68).Similar to other researchers' findings, the 120-day fermentation period and plastic jar storage method produced the highest moisture content of kocho among the interaction between storage method and fermentation period (73.88), while the pit storing method produced the lowest moisture content (62.07).(Gashe, 1987; Krassa *et al*., 2014; Andeta *et al.*, 2018; Weldemichael *et al.*, 2018) described that moisture content of kocho decreased as fermentation time increased. These ends equal were relatively high compared to earlier studies, where the moisture contents dropped to approximately 60% (Gashe, 1987; Krassa *et al.*, 2014).

Table 3. Interaction effects of fermentation time and storage methods on moisture content of kocho

	Fermentations days			
Parameter	Moisture content			
storage methods	**30**	**60**	**90**	**120**
Pit	67.48ef	69.1325d	70.33c	62.32jk
Plastic jar	65.88fg	71.88bc	73.443ab	73.83a
Pot	64.92ghi	68.39de	64.07hi	65.53fgh
CV (%)	1.1			
LSD (5%)	0.65			
Mean	67.7			

4.3. Ash Content of Kocho

Significant changes in ash content were observed between the treatment's interaction effect at ($P < 0.01$). Of all the storage methods, the pit method had the highest recorded ash content of kocho (1.27), while the pot method had the lowest. The smallest amount of kocho was observed in 60 days (0.28), while the largest amount was recorded in 30 days (1.15) when comparing the ash of kocho utilizing fermentation duration.

The 60-day fermentation period and pit storage method had the greatest ash content of kocho among the interaction between storage method and fermentation period (1.14), while the 90-day pit storage method had the lowest ash content (0.79). In the other investigation, the ash concentration dropped as the fermentation period increased. (Yirmaga, 2013; Desta *et al*., 2021).

Table 4. Interaction effects of fermentation time and storage methods on Ash content of kocho

	Fermentation days			
Parameter	Ash content			
storage method	30	60	90	120
Pit	1.28ab^{c}	1.41^{a}	0.796^{e}	1.17^{cd}
Plastic jar	1.06^{d}	1.26a^{bc}	1.17^{cd}	1.165^{cd}
Pot	1.1266^{cd}	1.18^{cd}	1.215^{bc}	1.215^{bc}
CV (%)	6.46			
LSD (5%)	0.0747			
Mean	1.163			

4.4. Fat Content

The kocho products showed a significant difference ($p<0.05$), and the interaction effect had an impact on the fat content. The fat content ranged from 0.42 to 0.64, with the kocho sample fermented on day 120 and stored in a plastic jar having the highest value (0.64) and the sample fermented on day 60 and stored in a pit having the lowest value (0.42). The outcome was similar to the research of (Weldemichae*l et al.*, 2018) which ranges (0.5-1.5) and (Bosha *et al*., 2016). The possibility that the fermenting microorganism may secrete microbial oil as a result of the extensive breakdown of large fat molecules into simpler fatty acid units due to the high activity of the lipolytic enzymes could be the reason for the increase in the fat content of kocho as the fermentation time increases.

Since food products with high fat content are prone to both hydrolytic and oxidative or enzymatic rancidity and are in charge of the product's overall acceptability and storage stability, kocho's lower fat level might contribute to its longer shelf life. A class of substances known as lipids is soluble in water, organic solvents, and chloroform. Foods vary greatly in their lipid content; however because of functional qualities, nutritional worth, and regulatory requirements, quantification is crucial. *(Hiwot, 2015)*

4.5. Fiber Content

The fiber of Kocho was considerably ($p<0.01$) impacted by the interaction effect. The Kocho sample that was fermented for 120 days and stored in a plastic jar had the lowest fiber content (1.38%), while the sample with the highest fiber content (4.04%) was obtained using the pit storage method and a Kocho sample that underwent a 30-day fermentation period. The content of the other study dropped from 2.67 to 1.98 percent. (Yimerga, 2013). The partial breakdown of cellulose and hemicellulosic materials in fermentation water by microbial enzyme activity may be the cause of the potential decrease in fiber content throughout the fermentation process. Therefore, fermentation reduces the overall amount of indigestible fiber by increasing the breakdown of dietary fiber into soluble and digestible form (Yimerga, 2013).

Table 5. Interaction effects of fermentation time and storage methods on fat and fiber content of kocho

Storage methods	Percentage of Fat and fiber content	**Fermentation days**			
		30	**60**	**90**	**120**
Pit	Fat (%)	0.6^{b}	0.42^{g}	0.56^{c}	0.54^{de}
	Fiber (%)	4^{a}	1.6^{bcd}	1.8^{bcd}	2.22^{b}
Plastic jar	fat (%)	0.55^{d}	0.5^{f}	0.59^{b}	0.64^{a}
	Fiber (%)	2^{bcd}	0.9^{de}	1.2^{cde}	1.38^{bcde}
Pot	Fat (%)		0.53^{def}	0.54^{de}	0.59^{bc}
	Fiber (%)	2^{bcd}	0.5^{e}	1.2^{cde}	1.45^{bcde}

4. 6. Protein Content

The protein content of kocho was non-significant but it is important for carbohydrate calculation the result of protein content of kocho table 6 below

Table 6. Interaction effects of fermentation time and storage methods on protein content of kocho

	Fermentation days			
Parameter	Protein content			
storage method	30	60	90	120
Pit	0.01^{b}	0.02^{g}	0.034^{c}	0.005^{d}
Plastic jar	0.013^{d}	0.04^{f}	0.024^{a}	0.025^{a}

Pot	0.02^{e}	0.06^{e}	0.021^{ac}	0.034^{bc}
CV(%)	0.304			
LSD (5%)	0.012			
Mean	3.05			

4.7. Carbohydrate Content

The amount of carbohydrates in the various kocho samples varied significantly ($p<0.05$), and the amount of carbohydrates varied from 91.606% to 67.96% depending on the fermentation duration and storage technique. The Kocho sample fermented for 90 days and stored in a plastic jar and pot yielded the maximum value (91.42 and 91.606 percent, respectively), while the lowest value (67.96 percent) was achieved produced by fermenting the sample for 30 days using pot storage techniques, and (66.95%) was produced by fermenting the sample for 120 days using pit storage techniques. The previous study produced a relative result, *(*Weldemichael *et al.*, 2018; Bosha *et al.* 2016), the variation in carbohydrate content might be due to the use of different methods of analysis to determine the total carbohydrate content. The food products prepared from *enset* are rich in carbohydrate (Bosha *et al*., 2016). The carbohydrate content depends on the sum of all other proximate content.

Table 5 . Interaction effects of fermentation time and storage methods on carbohydrate content of kocho

	Fermentations days			
Parameter	carbohydrate content(%)			
storage methods	30	60	90	120
Pit	72.63ab	73.272ab	74.36ab	66.95b
Plastic jar	70.013ab	75.23ab	91.444a	77.615ab
Pot	67.96b	71.565ab	91.606a	69.234ab
CV(%)	16.8			
LSD(5%)	12.2			
Mean	72.41			

4.8. Microbial Content

4.8.1. Lactic Acid Bacteria

The interaction effect showed a significant difference at $p<0.05$. Among the storage methods, the pit storage method had the greatest LAB count (5.79 log cfu/ml), while the plastic jar method had the lowest LAB count (5.28 log cfu/ml). When comparing LAB (Table 5 fermentation period), the maximum LAB of kocho was recorded at 30 days, with the smallest LAB being 7.76 log cfu/ml. LAB count of 3.39 log cfu/ml after 120 days The 30 day fermentation duration and pot storage method had the greatest LAB (7.76 log cfu/ml) among the interactions between storage method and fermentation time, while the 120-day pot storage method had the lowest LAB (3.36 log cfu/ml). During surface fermentation, the total mesophilic bacterial load rose, while during pit fermentation, it decreased (Krassa *et al*., 2014).

4.8.2. Yeast

There was a noteworthy distinction ($p<0.05$) between the interaction effect Out of all the storage methods, the plastic jar storage method had the highest yeast count (5.56 log cfu/ml). 5.25 log cfu/ml was the lowest yeast count obtained using the pot method. When comparing fermentation times, the largest amount of Kocho yeast (7.62 log cfu/ml) was found after 30 days. 3.39 log cfu/ml, the lowest yeast count after 120 days The 30 day fermentation length and pot storage method had the highest yeast count (7.8 log cfu/ml) among the interactions between storage method and fermentation time. Whereas the lowest yeast was counted in pot storing method with 120 days (3.34log cfu/ml)

Table 8. Interaction effects of fermentation time and storage methods on microbial content of kocho

Storage methods	Microbial load(log)cfu/ml	Fermentation days			
		30	60	90	120
Pit	LAB	7.52^{b}	6.49^{d}	4.47^{e}	3.43^{f}
	Yeast	7.51^{c}	6.46^{e}	3.37^{h}	3.38^{h}
Plastic jar	LAB	7.59^{b}	6.49^{d}	4.453^{e}	3.41^{f}
	Yeast	7.6^{b}	6.51^{e}	4.36^{g}	3.38^{h}
Pot	LAB	7.76^{a}	6.58^{c}	4.93^{e}	3.36^{f}
	Yeast	7.83^{a}	6.63^{d}	$4.45f^{g}$	3.34^{h}

5. CONCLUSION AND RECOMMENDATION

The experiment was carried out at Suten Zuriya Kebele, Sodo district, East Gurage Zone, Central Ethiopia Regional Government. The experiment includes three storage techniques and four fermentation times, all of which were set up in triplicate and in a factorial lay out. The experiment was checked using the local storage method pit with leaves. Five kilograms of fresh decorticated enset product were placed in each 0.003 m^3 pit, plastic jar (baldi), and pot. To safeguard the aerobic process, fresh enset leaves were then placed over the pit, plastic jar (baldi), and pot and compacted with stone.The pH, ash, fiber, and carbohydrates are all strongly ($p<0.05$) impacted by the fermentation period and storage technique. The range of the carbohydrate content was 91.606% to 67.95%. The sample fermented for 90 days had the highest value (91.58%) in pot methods of storage, while the sample fermented for 30 days had the lowest value (67.95%) in pit storage methods, and the sample fermented for 120 days had the lowest value (66.95%).The number of microbes, including the number of lactic acid bacteria and yeast grew over the first 30 days of fermentation, whereas these numbers declined as fermentation time progressed. In conclusion, the maximum (91.606%) carbohydrate content was recorded at the 90-day fermentation date using the pot storage method. Then, for Kocho nutritional quality, the 90-day fermentation date and the pot storage method worked well. Then For minimizing leaching of water soluble Proteins and amino acid, to move from place to place for safety for easily accessibility the Pot storage method was conducted as an alternatives at the treatment area and further movable and a new material which will be used as a pot storage method will be innovated and provided for the community with concerned researchers and other Governmental bodies.

6. Reference

Admasu P. Struik (2001).Enset (Enseteventricosum (Welw) Cheesman) kocho yield under different crop establishment methods as compared to yields of other carbohydrate rich food crops. Netherlands J AgriSci 49: 8194.

Ajebu N., Lars O.E., Adugna T., and Frik S. 2008. Chemical composition and in sacco dry matter degradability of different morphological fractions of 10 enset (Enseteventricosum) varieties. Animal Feed Science and Technology 146: 55-73.

Almaz.Admasu, T.Tureen, R., and Visor, B. (2002). AFLP analysis of Enset clonal diversity in south and south western Ethiopia for Conservation Crop Sci. 42:11051111.

Atnafu, B, Ermias, T., Endale, T., Mikiyas, Y., Mulgeta, D and Yohannes, T. (2008).Enset varietal evelopment. In: Root and tuber crops: the untapped resources. (GebremedihinWoldegiyorgis, EndaleGebre and BergaLemaga.,eds). EIAR,Addis Ababa.

A. Negash and A. Niehof, "The significance of enset culture and biodiversity for rural household food and livelihood security in South Western Ethiopia," Agriculture and Human Values, vol. 21, pp. 61-71, 2004.

Brase, S., Glaser, F., Kramer, C. S., Lindner, S., Linsenmeier, A. M., Masters, K. S., Zhong, S. (2013). The chemistry of mycotoxin. London: springer

Brandt, S.A., A. Spring, C. Hiebsch, J.T. McCabe, E. Tabogie, M. Diro, G. Wolde Michael, G. Yntiso, M. Shigeta and S. Tesfaye. 1997. The 'Tree against Hunger': Enset-Based Agricultural Systems in Ethiopia.

Bosha,A.,Dalbato,A.L.,Tana,T.,Mohammed,W.,Tesfaye,B.andKarlsson,L.M.(2016), “Nutritional and chemical properties of fermented food of wild and cultivated genotypes of enset (Enseteventricosum)”,FoodResearchInternational,Vol.89,pp.806-811.

Birmeta, G., Nybom, H., &Bekele, E. (2004).Distinction between wild and cultivated enset (Enseteventricosum) gene pools in Ethiopia using RAPD markers.Hereditas, 140(2), 139– 148. https://doi. org/10.1111/j.1601-5223.2004.01792.x.

Bosha, A., Dalbato, A., Tana, T., Mohammed, W., Tesfaye, B., &Karlson, L. (2016). Nutritional and chemical properties of fermented of food wild and cultivated genotype of enset. Food research international,89,801-811.

Chelule, P. K., Mokoena, M. P., &Gqaleni, N. (2010).Advantages of traditional lactic acid bacteria fermentation of food in Africa, 1160–1167.

CSA (2009-2020): Agricultural sample survey; crop and livestock product utilization

Country STAT Ethiopia (2016).Production quantity of major agricultural national commodities. Southern N ations, Nationalities, and Peoples’ Region Way back Machine.

E. Taboje, "Morphological characterization of enset (Ensete Ventricosum (Welw.) (Cheesman) clones and the association of yield with different traits," MSc. Thesis, Alemaya University of Agriculture, Ethiopia, 1997.

Endale, Tabogie (1996). Morphological Characteristics of Enset (Enseteventricosum), Clones and the association of yield with different traits. MSc Thesis, Alemaya University of Agriculture. Alemaya, Ethiopia

E. Westphal, Agricultural systems in Ethiopia. Wageningen, The Netherlands: Pudoc, 1975.

Gorrens E., (2018). Fermentation of Enset (Ensetventricousm) in the Gamo highland Ethiopia. Introduction of sauerkraut Jars. M.Sc. Thesis, Ariba Minch University, Ethiopia.

Giorgis , G Gebre E, Bedane K, Kassa B (2001). Country Profile on Potato Production and Utilization: Ethiopia.Ethiopian I nstite of Agricultural Research, Holetta Agricultural Research Centre, National Potato Research.

Gibson, S. A. W. (editor) (1995). Human Health: The Contribution of microorganisms. London: Springer- Verlag.

Helen, W., Dominic, S., Christoph, W., Tesfemariam, B., Shimelis, A.,Melaku, A., Melanie Huch (2019). Characterization of the microbiota and volatile components of kocho, a traditional fermented food of Ethiopia.Heliyon 5 (2019) e01842.

Hiwot Bekele (2015) Effect of Enset(Ensetventricosum (Wele) cheesman) Variety and fermentation on nutritional composition,anti-nutritional factors. Physicochemical characteristics and functional property of Bulla.Food and nutritional sciences .Adis Ababa. Ethiopia

Hunduma, T., &Ashenafi, M. (2011a).Effect of Altitude on microbial succesion during traditional Enset (Enseteventricosum) fermentation. International Journal of Food, Nutrition and Public Health,4(1),35-55.

Holzapfel, W.H. (2002). Appropriate starter culture technologies for small-scale fermentation in developing countries. International Journal of Food Microbiology; 75, 197–212.

Karssa, T. H., Ali, K. A., &Gobena, E. N. (2014). The microbiology of Kocho: An Ethiopian Traditionally Fermented Food from Enset (Enseteventricosum). International Journal of Life Sciences,8(1),7-13.

Karssa, T &Papini, A. (2018). Effect of colonial variation on quality of kocho traditional fermented food from Enset (Enseteventricosum). International Journal of Food Sciences and Nutrition Engineering,8(3),79-85.

Mohammed, B., Martin, G., &Laila, M. (2013). Nutritive values of the drought tolerant food and fodder crop enset. African Journal of Agricultural Research,8(20),23262333.

M. Shigeta, "The ethnobotanical study of enset (Ensete Ventricosum) in the Sothern Ethiopia," Dissertation, Kyoto University, 1991.

Motarjemi, Y. (2002). Impact of small scale fermentation technology on food safety in developing countries.International Journal of Food Microbiology, 75(3), pp.213-229

Negash, A., &Niehof, A. (2004).The significance of enset culture and biodiversity for rural household food and livelihood security in southwestern Ethiopia. 21(1), 61-71.

Nuraga, G. W., Feyissa, S., Demissew, k., Tesfaye, &Woldegiorgis, A.Z. (2019). Comparison of proximate, mineral and phytochemical composition of enset (Enseteventricosum (Welw.) Cheesman) landraces used for a different purpose. African Journal of Agricultural Research,14(30),1326-1334.

Nurfeta, A.Tolera, A., Eik, L. O., &Sundstøl, F. (2008). Feeding value of enset (Enseteventricosum), Des modiumintortum hay and untreated or urea and calcium oxide treated wheat straw for sheep. Ethiopian Journal of Applied Technology; 3: 13–24.

Olango, T. M., Tesfaye, B., Catellani, M., &Pè, M. E. (2014).Indigenous knowledge, use and on-farm management of enset (Enseteventricosum (Welw.)Cheesman) diversity in Wolaita, Southern Ethiopia.Journal of Ethno biology and Ethno medicine, 1, 10-40.

Oyewole, O. (1997). Lactic fermented foods in Africa and their benefits. Food Control; 8, 289– 297.

Tamang, J. P., Watanabe, K., &Holzapfel, W. H. (2016). Review: Diversity of microorganisms in global fermented foods and beverages. Frontiers in Microbiology, 7.

Tesfaye, B. and Lüdders, P. (2003), “Diversity and distribution patterns of enset landraces in Sidama, SouthernEthiopia”,GeneticResourcesandCropEvolution,Vol.50No.4,pp.359-371.

Tiruha,H. K., Kebede, A. A. and Edessa, N. G (2014).The microbiology of Kocho: An Ethiopian Traditionally Fermented Food from Enset.

Tsehaye, Y. and Kebebew, F. (2006), “Diversity and cultural use of enset (Ensetventricosum (Welw.)cheesman)inbongainsituconservationsite,Ethiopia”,EthnobotanyResearch andApplications, Vol.4,p.147.

Tsegaye, A., &Struik, P. (2002).Analysis of enset (Enseteventricosum) indigenous production methods and farm-based biodiversity in major enset-growing regions of southern Ethiopia. Experimental Agriculture, 38(3), 291-315.

Welde-Michael. G, Bobosha. K., Blomme. G., Addis. T., Mengesha.T., and Mekonnen. S. 2008. Evaluation of Enset Clones against Enset Bacterial Wilt. African Crop Science Journal 16: 89-95.

Yirmaga, M. T. (2013). Improving the indigenous processing of kocho, an Ethiopian traditional fermented food. Journal of Nutrition and Food Sciences,3 (1),1-6.

Yemataw ,Z.Mohamed H, Diro M, Addis T, Blomme G (2014). Ethnic based diversity and distribution of enset (Enseteventricosum) clones in southern Ethiopia. Journal of Ecology and the Natural Environment 6: 244 – 251.

Yemataw, Z., Tesfaye, K., Zeberga, A. and Blomme, G. (2016), “Exploiting indigenous knowledge ofsubsistencefarmers’ forthemanagementandconservationofenset(Enseteventricosum(Welw.) cheesman) (musaceae family) diversity on-farm”, Journal of Ethnobiology and Ethnomedicine, Vol.12No.1,pp.1-25.

Y. Tsehaye and F. Kebebew, "Diversity and cultural use of enset (Enset Ventricosum (Welw.) Cheesman) in Bonga in situ conservation site, Ethiopia," Ethnobot. Res. Appl., vol. 4, pp. 147-157, 2006.

APPENDIX

Appendix 1 . Analysis of variance for pH, Moisture content, ash content of kocho

Parameter			**Ph**			**Mositur**			**ash**	
	Df	Mean Sq	F- value	Pr(>F)	Mean Sq	F- value	Pr(>F)	Mean Sq	F- value	Pr(>F)
Replication(R)	2	0.04185	275.196	4.055e-13 ***	114.554	268.86	4.860e-13 ***	0.001244	0.2655	0.7701488
Storing methed	2	0.011158	73.37	4.382e-10 ***	6.401	15.024	2.796e-05 ***	0.030669	6.5428	0.0025611 **
Fermntation time	3	0.02829	186.016	1.201e-12 ***	26.713	62.697	4.594e-09 ***	0.05022	10.7137	0.0004171 ***
Interaction	6	0.008601	56.557	1.472e-10 ***	15.403	36.151	4.421e-09 ***	0.046734	9.9698	3.924e-05 ***
Residuals	23	0.000152			0.426			0.004688		

Appendix 2 Analysis of variance for fat , fiber , carbohydrate , yeast and LAB content of kocho

		fat content		**fiber content**		**Carbohydrate content**		**Yeast**		**LAB**	
	Df	F- value	Pr(>F)	F- value	Pr(>F)	F- value	Pr(>F)	F- value	Pr(>F)	F- value	Pr(>F)
Replication(R)	2	82.997	3.569e-09 ***	30.1067	3.773e-06 ***	3.1887	0.06831	240.73	3.779e-16 ***	52.1013	4.517e-09 ***
Storing methed	2	64.594	1.141e-09 ***	3.5277	0.0301850 *	1.4525	0.26267	1288.25	< 2.2e-16 ***	1301.0267	< 2.2e-16 ***
Fermntation time	3	243.214	1.486e-13 ***	9.5133	0.0007636 ***	3.324	0.04648 *	17631.68	< 2.2e-16 ***	16663.6931	< 2.2e-16 ***
interaction	6	52.687	2.535e-10 ***	1.9851	0.1067999	2.2938	0.04648 *	111.7	7.749e-16 ***	7.0355	0.000280 ***
Residuals	23										