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Effect of Enset (*Ensete ventricosum (wele) cheesman)* Variety and Fermentation on Nutritional Composition, Anti-nutritional Factors, Physicochemical Characteristics and Functional property of Bulla

By

Hiwot Bekele

Advisers: Dr Ashagrie Zewdu (Assistant Professor)

Mr Kelbesa Urga (Associate Professor)

A thesis submitted to Graduate Studies Program of Addis Ababa University in partial Fulfilment of the requirements for the Degree of Master of Science in Food Science and Nutrition

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Study on the effect of Enset (*Ensete ventricosum (wele), cheesman)* Variety and Fermentation on Nutritional Composition, Anti-nutritional Factors, and Physicochemical Characteristics and Functional property of Bulla: From Wolaita, Ethiopia.

Msc. Thesis

By: Hiwot Bekele Dagnachew

Under The Supervision of: -

Dr Ashagrie Zewdu (Assistant Professor)
Mr Kelbesa Urga (Associate Professor)

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June, 2015

Declaration

I, undersigned, declare that this is my original work and has never been presented in this or any other University and that all the source materials used for this thesis have been duly acknowledged;

Name: Hiwot Bekele

Signature _____

Place: Addis Ababa University, Food science and nutrition Centre, Addis Ababa, Ethiopia

Date of Submission: _June, 2015

The Research proposal has been submitted for examination with our approval as a University Advisors.

Name: Dr. Ashagrie Zewedu

Signature: _____

Date: _____

Name: - Mr Kelbesa Urga

Signature _____

Date: _____

Approved by the Examining Board:

1. Examiner ……..

2. Examiner ……..

3 .Advisor ……..

4. Chairman ……..

Dedication

I need to dedicate this work to my mother Etete and my father Akaye, who invested on me, on my thought, on my vision, on my personality, and on my work habit. I am very glad to you, you made me to be as what I am now thank you so much. And my soul mate Rediet Dejen, you were my best friend I ever had. We shared voluminous things together. I missed you a lot, let God put your soul in heaven.

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List of Abbreviations and Acronyms

AARC ……..… Areka Agricultural Research Centre

AAS …… Atomic Absorption Spectrophotometer

AAU ……..…….. Addis Ababa University

ADAP …….…..…Agricultural Development in the American Pacific

AHI ……..... African Highlands Initiative

ANOVA …..……....Analysis of Variances

AOAC …… Association of Official Analytic Chemists

BD …… Bulk Density

BDL …… Below the Detected Level

CRD …….……. Completely Randomized Design

Db …….. Dry based

ECAE ……..... Ethiopian Conformity Assessment Enterprise

EFMHACA ……..… Ethiopian Food, Medicine and Healthcare Administration and Control Authority

EHNRI……..…. Ethiopian Health and Nutrition Research Institute

EPHI ……..Ethiopian Public Health Institute

FAO ……. …..Food and Agricultural Organization

FCES ……..…… French Centre of Ethiopian Study

FDRE …… …… Federal Democratic Republic of Ethiopia

GDP ……..……. Growth and Development Plan

IBC ……. Institute of Biodiversity Conservation

LAB ……Lactic Acid Bacteria

LSD …….…... Least Significant Difference

MEq ……..…….. Milliequvalent

OAC …… Oil Absorption Capacity

PGRFA ……... Plant Genetic Resources for Food and Agriculture

Rpm ……..… Revolution per minutes

SNNPR ……..…… South Nation Nationality People Region

SP ……. Swelling Power

SPSS …….…….. Statistical Package System Software

TA ……..Titratable Acidity

UV …….……. Ultra Violet

V/V …….…… Volume by Volume

W/W …….. Weight by Weight

WAC ……...……...… Water Absorption Capacity

Wb ……. Wet based

ABSTRACT

This study was conducted to evaluate the effects of variety (yanbule, gewada, zereta, and messina) and fermentation time (0, 15, and 30 days) on the nutritional composition, antinutritional content, physicochemical properties, functional properties and sensory acceptance of bulla collected from Wolaita, Ethiopia. Bulla samples were prepared from newly improved enset varieties. The results revealed that bulla prepared from gewada variety had relatively higher values of fat (0.3g/100g), fiber (1.04g/100g), carbohydrate (97.71g/100g), energy (394.24Kcal), and Fe (2.54mg/100g). On the other hand, bulla prepared from zereta and messena varieties were found to be high in protein content (0.36g/100g). Yanbule had relatively higher ash content (1.05g/100g) and considerably higher Ca (317.98mg/100g) than bulla prepared from the rest of the varieties. Magnesium (56.86mg/100g) and Zn (2.3mg/100g) were relatively higher in bulla prepared from messena. A very low level of tannin was detected only for gewada (0.01mg/100g) and contents of phytate up to 112.56mg/100g were obtained. The pH and titratable acidity of the bullas ranged from 5.23-6.05 and 0.22-0.3%, respectively with messena being the highest in both pH (6.05) and titratable acidity (0.3%). With respect to the functional properties of bulla fermented for 30 days, no significant ($p>0.05$) differences were observed among the varieties except for water absorption capacity. Generally, bulla porridge and kofeme prepared from all varieties were extremely liked for their sensory attributes with a mean score of (7.6) and (8.6), respectively. In this study, fermentation time had resulted in tremendous reduction of the moisture, fibre, pH, phytate, and tannin contents of bulla. Moreover, it also enhanced the protein, fat, ash, carbohydrate, total energy, mineral, and titratable acidity of the raw sample. In conclusion, extending the fermentation time and different Enset variety had resulted in differences in chemical composition, physic-chemical characteristics, and sensory attributes of bulla. Bulla has advanced quantity of carbohydrate and calcium when it is compaired with cereals and other root and tubers.

Key words*: Enset variety, fermentation, Anti-nutritional factor, proximate composition, Functional property, Bulla*

1. INTRODUCTION

1.1. Background

Orphan or understudied crops are considered as the major staple food crops in many developing countries because of their particular role in food security, nutrition, and income generation to resource poor farmers and consumers. Like other crops, orphan crops are also categorized under cereals, legumes, root crops, and fruit crops. Orphan crops are in general more adapted to the extreme soil and climatic conditions prevalent in Africa than the major crops of the world (Tadele, 2013).

Root and tuber crops are widely cultivated in southern Ethiopia, and support a considerable portion of the country's population as source of food. Prominent among these are: potato (*Solanum tuberosum L.),* sweet potato (*Ipomoea batatas L.*), enset (*Ensete ventricosum* (Welw), Cheesman), godere (*Colacasia esculanta L.*), yams (*Dioscorea spp.*), Ethiopian dinch (*Coleus parviflorus*), koteharrie (*Diaspora bulbiferous*), and anchote (*Coccinia abyssinica*). Among these, enset, anchote, and, some yams are endemic to Ethiopia (Aregahegn *et al*., 2013).

Ensete ventricosum (Welw.) Cheesman, commonly known as enset, is a monocarpic perennial herb originated in Ethiopia. Geographically distributed as a wild species in many parts of Sub-Saharan Africa and Asia, enset is cultivated only in its native indigenous farming systems of South and South-Western Ethiopia. In fact in Ethiopia, *E. ventricosum* is arguably the most important crop contributing to food security and rural livelihoods for about 1/4 (20 million people) of the country's population (Olango *et al*., 2014). It is a multipurpose plant with a range of utilities including food, feed, construction and medicinal uses (Annual report, 2013). Moreover, enset cultivation improves soil by permanent soil tillage due to its high demands to soil fertility and soil structure (Zippel and Karina, 2002).

The crop is known for its tolerance to transient drought, high productivity, gender equity and environmental sustainability (Annual report, 2013). Research suggests "that populations depending on enset have never suffered from famine, even during Ethiopia's tragic drought and famine prone decades of the 1970s and 1980s" (Macentee *et al*., 2013).

Kocho, bulla and amicho are primary enset products. Kocho and bulla are obtained after processing, whereas the Amicho or corm is a cooking type boiled and eaten directly without any processing (Olango *et al*., 2014 and Forsido *et al*., 2013). Kocho and bulla foods are

extremely popular at restaurants that serve the Ethiopian delicacy of Kitfo (raw minced beef mixed with butter and spice) (Brand *et al*., 1997 and Forsido *et al*., 2013).

Ethiopia is a country rich in cultural diversity. The variety of food and beverage processed and consumed among the various ethnic groups are manifestation of this diversity. Although some of the food items may be consumed in their raw or processed forms such as salting, drying and fermentation. Fermented foods are essential part of diets in all region of the world. In Ethiopia, like in many developing countries fermented food products constitute the major staple food. Fermentation also reduce anti-nutritional factor such as tannins and trypsin inhibitors (Ashenafi, 2002).

Enset products (Kocho and bulla) are the foods consumed fermented (Ashenafi, 2002). The length of fermentation time varies from weeks to several months, depending on ambient temperatures of incubation. In the cooler regions, it is kept in a pit for years, and the quality is said to increase with increasing fermentation time. In warmer regions, fermentation is rapid and is therefore, terminated within 3 to 6 months. However, during the bridging period or otherwise in difficult situation, fermentation cannot afford to wait more than a month, even fifteen days, for fermentation (FCES, 2005). After the fermentation is completed, a portion is removed from the pit and the liquid is squeezed out, it resulting in a moist fibrous Kocho (Yirmaga, 2013). Roots and tubers generally are processed into various foods before consumption. Processing makes them digestible and palatable, extends the shelf-life and reduces post-harvest losses (Ugwu, 2009).

Bulla is one of the major products of enset "the best part" obtained from the transformation process of enset into kocho. The internal part of the pseudostem is scraped with a piece of split bamboo in order to separate the starch from the fiber. This pulp is then pressed with the feet to collect the juice, which is then left to decant. This decantation produces the bulla which is then left to ferment for several weeks, which is highly starchy food and best quality of food. It is consumed on specific occasions (religious, traditional feasts) or in the honour of guests (FCES, 2005). But the nutritive value of starchy foods depends mainly on their nutrient content, physicochemical properties of their starches and the existence of antinutritional factors and toxic substances (Atlabachew, 2007 and Treche, 1996).

All the roots including enset exhibit a very low lipid (0.12-2.7%) content and may affect the palatability of the crops (Ugwu, 2009). The protein content is low in almost all root crop,

sulphur-containing amino-acids are limiting in the proteins as in legume proteins and no vitamin at all (Atlabachew, 2007 and Ugwu, 2009).

1.2. Statement of the problem

There is a growing interest in the role that enset currently plays in regions of high consumption but it remains an understudied crop in relation to exploring its challenges and development potential (Macentee *et al*., 2013).There are some researches done on enset; (Forsido *et al*., 2013; Debebe *et al*., 2006; Mohammed *et al*., 2013), which determine the minerals (calcium, Potassium, selenium, manganese, copper, zinc, chromium, cobalt, copper, nickel, phosphorus, iron, cadmium and lead), crude protein, crude fiber, crude fat, and ash of Amicho, unfermented kocho, fermented kocho, unfermented bulla, fermented bulla and on different parts of enset. In another study Atlabachew, (2007) has determined the levels of major, minor and trace elements in commercially available enset food products from Wolkite and Wolliso towns.

In Ethiopia, like many other roots, and tuber crops, enset is rarely eaten raw. Traditionally, it is fermented prior to consumption. Such processing can have both detrimental and beneficial effects of the nutrient content of the food. In the case of bulla, however, no published information is available as to which traditional processing methods are optimized to reduce the effects of the inherent anti-nutritional factors and to increase availability of the nutrients. Indeed Yirmaga, (2013) investigate the effects of fermentation time, variety and processing methods on physicochemical qualities, chemical composition, microbial quality and sensory acceptance of kocho.

In addition, food composition data are needed in order to assess foods to promote for health purposes, evaluate the diet, establish locally relevant dietary guidelines, carry out research on the relationship between diet, health, and disease, and stimulate markets for new ethnic foods (Englberger, 2005). However, relatively few such data are available in relation to bulla. Enset feeds 15 million people in Ethiopia, but is arguably the least studied African crop (Harrison J. *et al*., 2014).The role of root and tubers in developing-country food systems also raises interest about the impact of these crops on the environment.

Among hundreds of enset varieties, Areka Agricultural Research Centre identified six enset varieties (*yanbule, gewada, zereta, kelisa, endale* and *messina*) based on their quality and kocho yield in 2009 and 2010 (Yeshitila and Yemataw, 2010). In previous studies the

samples of enset (bulla and kocho) were taken either from communities (who consume enset as staple food) or from the market, but major drawback of commercially available enset, it is a mixture of different variety of enset. Therefore there is few work on the nutritional composition, physicochemical and anti-nutritional characteristics of bulla of different enset varieties. Hence this study introduces a new knowledge in this area.

1.3. Significance of the study

Roots and tubers generally are one of the cheapest sources of dietary energy in the form of carbohydrates in developing countries. Therefore, there has been a tremendous increase in consumption of these roots and tubers over years as food for adults and young children (Ugwu, 2009). Since enset products such as Bulla and Kocho serve as the staple and co-staple food for many peoples of Ethiopia, knowledge towards its overall application is minimal. Thus this study contributes:-

- To increase understanding of the available food composition data among indigenous and newly released varieties of enset product (bulla).
- To provide information on health benefits or risks of consuming different varieties of bulla.
- Help to indicate nutrient that lacks in enset (bulla) that may suggest fortification opportunities to improve its nutritive value.
- The study will also use to determine which enset/ bulla variety have relatively better nutrients.
- This indigenous crop some lesser known than others that deserve wider attention, thus one of the importance of this research is to increase people attention.
- It will further prove whether the raw or the fermented bulla has provided better nutrition to improve the quality of bulla for human consumption.
- This paper will provide information for future researchers, students, consumers, producers, who are interested to work on bulla /enset.

1.4. Research question

- Does the difference in variety of enset have significant effect on nutritional composition of bulla?
- Does fermentation have improving effect on the nutritional composition of bulla?

- Do the four varieties of enset product (bulla flour) have significance difference in titratable "acidity, pH value, bulk density, swelling power, water solubility index, and water and oil absorption capacity?
- Are there anti-nutritional factors (phytate and tannin) in bulla? And the impact of fermentation on their concentration.
- Is there difference in the sensory perception of food prepared from bulla among each variety?

1.5. Objectives

1.5.1. General objective

The general objective of this study is to assess the effect of enset variety and fermentation time on nutritional composition, anti-nutritional factors, and physicochemical characteristics of bulla in Wolaita, Ethiopia.

1.5.2. Specific objective

- To assess the nutritional composition of bulla obtained from different varieties of enset in order to select bulla with better nutrient for human consumption.
- To determine the effect of traditional processing method (fermentation) on nutritional composition of bulla.
- To determine physicochemical characteristics of bulla obtained from the varieties
- To determine anti-nutritional factors (phytic acid and tannin) in bulla among the varieties.
- To evaluate the sensory properties of porridge made from selected time of fermentation from each variety.

2. LITERATURE REVIEW

2.1.Root and tubers crop

According to FAO estimates, the 5 major food and agricultural commodities produced in Ethiopia in 2004 were roots and tubers, maize, sorghum and other cereals and cow milk. All these productions were mainly for local human consumption. (FAO, 2008). Root crops are the edible energy-rich underground plant structures developed from modified roots while tuber crops are those crops in which the edible carbohydrate-rich storage organs develop wholly or partly from underground stems (Iyagba, 2010). There are several indigenous cultivated or semi-cultivated root and tuber crops in Ethiopia. These crops have an important place in the diet of the population (IBC, 2007). Enset /*Ensete ventricosum*/ is one of the common staple plants categorized under root and tubers (Aregahegn *et al*., 2013).

2.2.Taxonomy and classification of Enset (*Ensete ventricosum*)

Ensete ventricosum (Welw.) is an indigenous, perennial, herbaceous, monocarpic crop belonging to the family *Musaceae* (Annual Report, 2013). Morphologically it resembles a banana plant but bananas belong to the related genus Musa. Both Enset and Musa have a large underground corm, a bundle of leaf sheaths (pseudostem), and large paddle-shaped leaves. It reaches 4-8 meter or even up to 11 meter in height (Atlabachew, 2007) and one meter in diameter (Stone *et al*., 2011). Although further research still needs to be done on the taxonomy and distribution of enset species, current data reveal six species of genus *Ensete.* Enset (*Ensete ventricosum*) is one of the six commonly recognized species of the genus Ensete *(E. gilletti, E. homblei, E. perrieri, E. glaucum, E. superbum,* and E. ventricosum (Brand *et al*., 1997).

Ensete ventricosum (Welw.) is endemic to Ethiopia and occurs throughout the country both cultivated and wild. It is an important staple to a large number of people in the south and southwest of the country. Although the plant is propagated vegetatively, there is tremendous variation in several characters, including colour of pseudostem and leaf midribs, earliness, disease resistance and product quality (IBC, 2007). Even though, the production is high, the utilization of Enset plant is insignificant. The main problem is the inability to produce at a commercial scale and the loss of its product during harvesting, processing and the improper storage of the final product before consumption and lack of knowledge about nutrition. Even though there is enough food, the people are not accustomed to vary their meal to fulfil the

nutritional requirement. This is due to the lack of knowledge of the people on the balanced diet, the lack of income to purchase foods, and to use different raw materials as a source of food (Ayele and Sahu, 2014).

Photo taken by the author,Angecha Kenbata.

Figure 1. Matured Enset plant which grows around house yard

It grows in house yards and consumed predominantly in the south and southwest regions of Ethiopia (Macentee *et al*., 2013). Although the exact age of Enset domestication is not yet established, it was practiced in Ethiopian highlands between 5000 and 10000 years ago (Amedea and Dirob ,2005). It is cultivated in areas extending from 1700 to 3300 meter altitude with annual average temperatures between 8°C and 22°C and annual precipitation between 900 and 1500 mm (Zippel and Karina, 2002).

Enset ranges from being a staple to secondary food crop in different regions of the country and in 1991, the Ethiopian government declared enset a national crop. It plays an important role in national food security (Macentee *et al*., 2013). It has probably given rise to better intensification of production systems involving year round cultivation of the land, with emphasis on root crops and close integration between livestock and crop production units. One plant of 5 years old. Enset could produce up to 21 kg of local food (Kocho, Bulla and Amcho) (Amedea and Dirob, 2005). Enset in the sub-system fulfils both production and protective functions (Kippe, 2002). Because of its very high yield it covers the food demand of household covering from 25% where it is a subsidiary crop to 85% where it is the major food crop (Tsegaye, 2002).

2.3.Origin of Enset in Ethiopia

Ethiopia is a centre of origin for many cultivated plants such as Teff (*Eragrostis teff*), noug (*Guizotia abyssinica*), Ethiopian mustard (*Brassica carinata*), enset (*Ensete ventricosum*), anchote (*Coccinia abyssinica*) and coffee (*Coffea arabica*) (FDRE, 2014). Even though, enset originated in Ethiopia it is geographically distributed as a wild species in many parts of Sub-Saharan Africa and Asia (Olango *et al*., 2014). *E. superbum and E. glaucum* grow in Asia, whereas *E. ventricosum, E. gilletti* and *E. homblei* occur in sub-Saharan Africa and *E. perrieri* in Madagascar. Enset *ventricosum* grows wild also in a number of countries in central and eastern Africa including Congo, Mozambique, Uganda, Tanzania, and Zambia (Debebe, 2006). It grows in a wide range of environmental conditions. Even though it grows in many administrative regions, the dwellers of the central and south western parts of Ethiopia are the only people that use enset as a staple and co staple crop. The majority of enset production is confined to Sidamo, Shoa, Keffa, Gamo Goffa and Illubabor administrative regions (Atlabachew, 2007).

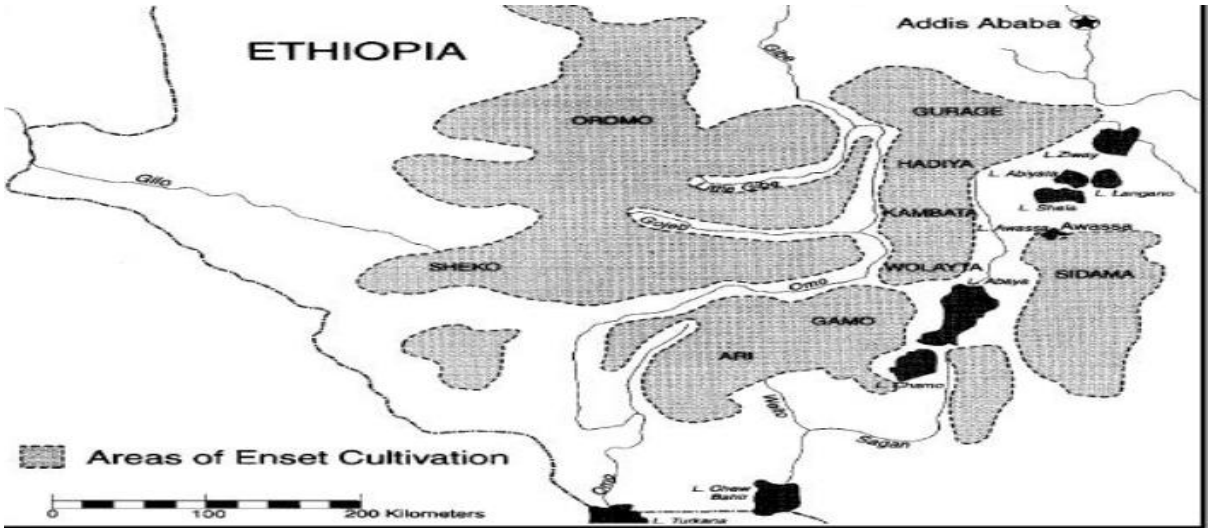

Figure 2. Major Enset growing areas in southern Ethiopia and ethnic group

2.4.Enset varieties

There are numerous varieties of enset, each variety playing a specific part and their combination expressing the priorities of each peasant some varieties are known to give a Kocho of pleasant flavour (Gimbo, Itine, Sabara, Argama, Tasa, Gobmorsa, Wochamada etc.) others are more resistant against the Alloya, the enset's bacterial disease (Dirbo, Wolantche) some others give stronger fibrous products arrived at during the transformation of enset into

Kocho. They are usually used for the fabrication of ropes (Siskela, Guichira).Others are cropped for their medicinal properties among these varieties, peasants distinguish two kinds, male and female. Male enset would give higher yields of kocho per enset, would reach maturation more quickly than female enset and would be more resistant to the enset's bacterial disease Alloya. Female enset would give a tastier kocho and would be the only one whose root can be consumed (FCES, 2005).

A total of 400 enset varieties were collected from the major enset growing zones (Kembata, Dawro, Gamogofa, Wolaita, Sidama and Gurage) of SNNPR. Then evaluated and characterized by Areka Agricultural Research Centre (AARC) with other 5 research centres for quality and kocho yield. The experiment resulted in six enset varieties (yanbule, gewada, endale, kelisa, zereta and messena) and registered by Ministry of Agriculture and Rural Development, Animal and Plant Health Regulatory Department in 2009 and 2010 (Yeshitila and Yemataw, 2010).

2.5.Indigenous uses of Enset in Ethiopia

Cultural identity crop: - The enset plant and its cultivation have special cultural meaning and value. The cultivation of enset also bears a cultural symbol for the communities and it is an expression of their identity. Enset being highly valued as a status symbol, people appreciate a hamlet by enset around it and give special respect and name for a man (Allawa) owing a large number of vigorously growing enset plants (Alla) in his home garden.

Staple and cultural food crop: - Enset foods are served both as staple daily diet as well as in occasions of cultural festivals; hence enset foods have both nutritional and cultural values for the society (Olango *et al*., 2014).

Multipurpose material culture crop: - Every part of enset plant has some sort of use in material culture of the Wolaita. The green enset leaf, yecha, is used as traditional plate to serve food, or as wrapping material for different products and baking breads, but it can be used also as an umbrella during the rainy seasons. Dried leaves and other pseudostem parts are also collected for making traditional seats (shill'a) and mattress (konashia hitta). Any dried part of the enset plant, mostly the dried lamina (gombila) and petiole, is used as a firewood. Susa, the dried and semi-dried long lamina, is used for fastening harvests of grasses, crops, and firewood. For construction of fences and traditional houses (Wolaita ketta) only dried, water-soaked and relatively strong lamina is used. The fiber of enset, gola,

produced as side product during food processing kocho is a very strong and high quality fiber. It is used for making ropes, strings, baskets, sacks, house floor carpets, filters, utensils' cleansers and many traditional house decors. Also, the fiber is used for fencing and construction of houses.

Medicinal plant:- it contribute to indigenous ethno-medicinal values traditional enset medicines include (i) porridge made of bulla from Agino and Gefetanuwa landraces, for strengthening women after delivery, and healing bone fractures in humans respectively; (ii) very highly fermented kocho from Maziya and Halla landraces, for curing stomach cramps; and (iii) boiled corm of Lochingia, for birth control and abortion in humans, and to feed cows to facilitate placental expulsion (Olango *et al*., 2014).

Health benefit of enset/bulla: - As the principles of good nutrition people are encouraged to eat more starches, especially those high in fiber, vitamins, and minerals. Fiber in foods may help to lower blood-glucose and blood-fat levels. Most people should increase the amount of carbohydrate and fiber they eat. This can be done by eating more bulla (swamp taro), giant taro, yams, sweet potato, cassava, bananas, dried beans, and peas; more whole grain breads, cereals, and crackers; and more fruit and vegetables (Shovic, 1999).

Other uses: - As described by the community, enset agriculture fulfils an ecological role, because it is an organic farming systems using only farmyard manures, with no external chemical fertilizers, herbicides and insecticides. Enset is considered a safety-shield cattle feed because it is available during the drought prevalent seasons of the year. The enset plantation in home gardens serves also as a wind break for enset and other crop nurseries. Enset farming also fulfils an aesthetic requirement for the home garden through colourful ornamental landraces (Olango *et al*., 2014).

2.6.Major Enset Based Foods

2.6.1. Kocho

Kocho is the bulk of the fermented starch obtained from the mixture of the decorticated (scarped) leaf sheaths and the grated corm (underground stem base). Kocho needs a lengthy period of processing and preparation, which is carried out by women. The first stage involves removing the leaf stalks and grading of the corm. Then the fibres are separated and the pulp is crushed to extract the starch. This is put in a pit about 1.5 m deep and 1 m diameter, wrapped

airtight with enset leaves before being weighed down with stones (Atlabachew, 2007). It is then allowed to ferment which requires weeks to months (Nigatu and Gashe, 1998), which may last from 4 months to three years. The pit is opened at intervals to allow aeration, and the enset leaves are replaced. This is repeated until the desired fermentation quality is reached or the food is needed. Finally, the fermented starch is dried and treated as flour. This can be used to prepare a pancake − like bread, which is eaten with milk and cabbage. Kocho is increasingly exported to urban markets, Kocho can be stored for a long period of time without spoiling. The quality of Kocho depends on the age of the harvested enset plant, the type of clone (variety), and harvesting season. Moreover, within one plant, the quality is influenced by the part of leaf sheath and corm processed (Atlabachew, 2007).

Photo taken by the author, Angecha Kembata

Figure 3. Long lasting storage form of bulla and kocho

2.6.2. Amicho

Amicho is the fleshy inner portion of the enset corm, which may be cooked and eaten separately, tasting similar to potato (Atlabachew, 2007), often served with other root crops and vegetables (Olango *et al*., 2014). Corm is also used as a source of kocho and it acts as a fermenting agent or starter (Yeshitila and Yemataw, 2010). Enset is mostly consumed in the form of kocho and bulla. However, the roots of enset (amicho), may be consumed separately, without being transformed or fermented before. It may also be consumed during the bridging period, from February to May. The families having an enset plantation smaller than 0.25

hectare consume an important quantity of boiled roots during the bridging period (FCES, 2005).

2.6.3. Bulla

Bulla is the small amount of water-insoluble starchy product that may be separated from kocho during processing by squeezing and decanting the liquid. After decanting, the bulla is left to dry or fermented in a way similar to Kocho. But mostly, instead of being fermented, it is dehydrated to make a flour that can be stored for extended periods of time (Stone A. *et al*., 2011) or can be directly cooked without fermentation. It is considered the best quality enset food and is mainly from fully matured enset plant (Atlabachew, 2007).

Photo taken by the author, Angecha Kembata

Figure 4. Fermented bulla powder, which is ready to eat

Season for Bulla Preparation

The best seasons to prepare kocho and bulla are the months of June, July and August during the rainy season, as well as December and January during the dry season. Bulla prepared during those months will give higher yield and much tastier respectively. The tastiest kocho or bulla, also the ones giving the best yield, would be prepared with enset cut just after flowering (8-9 years old), at complete maturation stage (FCES, 2005).

2.7.Fermentation and its Effect on nutritional and anti-nutritional composition

Fermentation is a widely practiced ancient technology and fermented foods are an essential part of diets in all regions of the world (Abegaz *et al*. 2002). Different types of food fermentations have been suggested and are classified based on the major products of the fermentation process. These include alcoholic fermentation, lactic acid (non-alcoholic) fermentation, acetic acid fermentation, alkaline fermentation and amino acid/peptide sauce fermentations. |Lactic acid is the main by- product of the fermentation process, the pH of the ferment is always lower than 5 (Chelule et al. 2010).

In all the foods and beverages examined, lactic acid bacteria (LAB) are the dominant microorganisms for fermentation, and therefore, lactic acid fermentation is considered as the major contributor to the beneficial characteristics observed in fermented foods (Chelule et al., 2010). Lactic acid is the predominant acid in enset products, which is responsible for the fermentation process of bulla and kocho.

2.7.1. What LAB fermentation does to food?

Flavour enhancement

Fermentation makes the food palatable by enhancing its aroma and flavour. These organoleptic properties make fermented food more popular than the unfermented one in terms of consumer acceptance (Karssa et al., 2014 and Sobowale, 2007).

Nutritional quality

LAB fermentation has been shown to improve the nutritional value and digestibility of these foods. The acidic nature of the fermentation products enhances the activity of microbial enzymes at a temperature range of 22-25 ºC. The enzymes, which include amylases, proteases, phytases and lipases, modify the primary food products through hydrolysis of polysaccharides, proteins, phytates and lipids respectively. Thus, in addition to enhancing the activity of enzymes, LAB fermentation also reduces the levels of anti- nutrients such as phytic acid and tannins in food leading to increased bioavailability of minerals such as iron, protein and simple sugars. The amount of vitamins is also increased in the ferment (Chelule et al., 2010).

Preservative properties

The lowering of the pH to below 4 through acid production, inhibits the growth of pathogenic microorganisms which can cause food spoilage, food poisoning and disease (Sobowale, 2007). For example, LAB have antifungal activities. By doing this, the shelf life of fermented food is prolonged. This is because the sheer overgrowth of desirable edible bacteria in food outcompetes the other non-desirable food spoilage bacteria. Thus LAB fermented foods have lactic acid as the main preservative since lactic acid bacterial growth is accompanied by the production of lactic and acetic acids with decrease in pH and increase in titratable acidity (Chelule et al., 2010 and Karssa et al., 2014).

Detoxification

Food and feeds are often contaminated with a number of toxins either naturally or through infestation by microorganisms such as moulds, bacteria and viruses. Certain moulds often produce secondary toxic metabolites called mycotoxins. Several methods are available for degrading toxins from contaminated food, for example, using alkaline ammonia treatment to remove mycotoxins from food. However, these methods are harsh to food as they involve use of chemicals which are potentially harmful to health or may impair/reduce the nutritional value of food. Cooking food does not remove mycotoxins either, as most of them are heat-stable. Detoxification of mycotoxins in food through LAB fermentation has been demonstrated over the years. Using LAB fermentation for detoxification is more advantageous in that it is a milder method which preserves the nutritive value and flavour of decontaminated food. In addition to this, LAB fermentation irreversibly degrades mycotoxins without leaving any toxic residues.

Antibiotic activities

LAB fermentation products have also been shown to have antitumor effects. One of the arguments supporting the use of LAB fermentation to prevent diarrheal diseases is because they inhibit the composition of intestinal microorganisms, LAB also produce fungal inhibitory metabolites. Also, fermentation has been demonstrated to be more effective in the removal of Gram- negative bacteria (Chelule *et al.*, 2010 and Sobowale, 2007).

In fermented food product; the bioavailability of minerals may be higher than in similar non fermented products due to the partial breakdown of the phytate. Fermentation also improves

protein quality because of bacterially produced lysine and by improving protein digestibility (Mckevith, 2004).

Extending of fermentation time and different Enset variety had resulted in differences in chemical composition, physical characteristics, and sensory attributes of kocho. Kocho fermented for 30 days, prepared from Astare variety and processed by modified processing method, resulted in best quality in physical characteristics quality, chemical composition, and sensory acceptance (Yirmaga, 2013).The study was done on Kinnare and Astare enset varieties.

Table 1:- Effect of fermentation and variety on the chemical composition, pH and TA of kocho

Parameters	**Raw**		**Fermented**		
	Kinnare	Astare	10^{th} day	20^{th} day	30^{th} day
Moisture content (%wb)	57.15	60.20	46.78	46.66	44.55
Crude fiber (% db)	3.37	4.42	2.67	2.29	1.89
Ash (% db)	2.18	2.33	3.41	3.39	3.52
Crude fat (% db)	1.27	1.04	1.60	1.52	1.50
Crude protein (% db)	3.43	3.17	4.42	4.78	5.09
CHO (% db)	35.53	32.75	44.08	39.63	33.80
Ph	4.78	4.86	4.64	4.28	3.79
TA (%)	0.61	0.55	0.27	0.49	0.87

Where: - Wb – Wet based and Db – Dry based

Source Yirmaga, 2013

2.8. Nutritional and Anti- nutritional Composition of Enset

2.8.1. Proximate composition of Bulla and Kocho

The main feature of enset foods is their high energy, derived almost entirely from carbohydrate. Carbohydrates are the main sources of nutritive energy, the principal constituent of edible carbohydrate is starch together with some sugars, the proportion depending on the root crop. All the roots contain a very low lipid (Ugwu, 2009) and also protein content is low in almost all root crop, sulphur-containing amino-acids are limiting in the proteins as in legume proteins. Most root crops contain a reasonable amount of lysine though less than in legumes (Atlabachew, 2007 and Ugwu, 2009). The corm contained 17 of 20 amino acids, in concentrations from 1.2 to 8.7 g per 100 g dry protein and from 25.6 to 186.6 mg per 100 g fresh corm. The amino acids not present in enset corm were asparagine, glutamine and tryptophan (Mohammed *et al*., 2013). Atlabachew, 2007 done study on the nutritional composition of bulla and kocho and obtain the following results.

Table 2. Nutritional Composition of bulla and Kocho

Parameters	**Dry Kocho/100 g**	**Dry Bulla/100g**
energy value	1410–1950 KJ	1580–1850 KJ
Carbohydrate	95–98 g	93–98 g
Protein	1.1–2.8 g	0.4–0.8 g
Fat	0.2–0.5 g	0.2–0.4 g
Ash	1.7 g	0.2 g
Fiber	-	0.6– 0.8 g
Moisture/fresh based	47–62 g	44–55 g

Source Atlabachew, 2007

2.8.2. Mineral composition of kocho and bulla

Kocho and Bulla are free of heavy metals (Cd and Pb) contaminations. (Atlabachew, 2007). Enset products provide more calcium and iron than most cereals, tubers and root crops (Mulualem and kifle, 2014). Atlabachew, 2007 done study on the mineral content of bulla and kocho on dry weight basis of bulla and kocho and obtain the following results.

Table 3. Mineral content of bulla and kocho on dry weight basis

Types of mineral	Kocho (µg/g)	Bulla (µg/g)
Potassium	2753 – 4380	708 – 875
Sodium	462 – 688	402 – 442
Calcium	498 – 584	385 – 446
Magnesium	180 – 290	58.4 - 89.5
Iron	92.5 -135	36.5 - 59.8
Zinc	31 - 32.08	22- 44.3
Copper	3.4 – 4.3	2.01 - 3.53
Manganese	8.58 -10.13	1.0 - 4.98
Nickel	≤ 5.61	<4.0
Chromium	5.96 - 6.42	≤ 5.38
Cobalt	5.5 - 6.1	5.0 - 5.01

Source Atlabachew, 2007

2.8.3. Anti-nutritional factors in root and tubers

Anti-nutrients have been defined as substances, which by themselves, or through their metabolic products arising in living systems, interfere with food utilization and affect the health and production of animals (Gobezie,2010). These chemicals have been evolved by plants for their own defence, among other biological functions and reduce the maximum utilization of nutrients especially proteins, vitamins, and minerals, thus preventing optimal exploitation of the nutrients present in a food and decreasing the nutritive value (Gemede and Ratta, 2014).

Several anti-nutritional factors are present in root and tuber crops and are partially neutralized during ordinary cooking and fermentation. Among various anti-nutrients, and plant toxins, Phytate, Oxalate, Tannin, Cyanide and Trypsin inhibitors are found in root and tuber crops, which may have adverse effects on health through inhibition of digestion, absorption, and growth (Gemede, 2014). Apart from cassava, cultivated varieties of most edible root and tuber crops do not contain serious toxic substances. But wild species, occasionally used at the

times of food scarcity, may contain lethal levels of toxic principles (Treche, 1996). Enset starchy food had the lowest phytic acid content (Abebe *et al*., 2007)

Phytate

Phytate is common in plant seeds, they can chelate with mono, di and trivalent mineral ions such as Na^+, K^+,Ca_2^+, Mg_2^+, Zn_2^+, Cu_3^+ and Fe_3^+ resulting in these ions becoming unavailable for consumers or reduce the bioavailability. With particular reference to zinc bioavailability, the phytate zinc molar ratio is considered as a better predictor of zinc bioavailability than total phytate levels alone. Phytate also forms sparingly digestible phytate protein complexes, thus reducing the availability of dietary protein (Olayiwola, and Okhiria, 2012).

Phytic acid is hydrolysed, enzymatically by phytases, or chemically to lower inositol phosphates such as inositol pent phosphate (IP5), inositol tetra phosphate (IP4), inositol triphosphate (IP3) and possibly the inositol di- and monophosphate during storage, fermentation, germination, food processing and digestion in the human gut (Estepa et al. 1999). LAB fermentation also reduces the levels of anti- nutrients such as phytic acid in food leading to increased bioavailability of minerals such as iron, protein and simple sugars (Chelule et al., 2010).

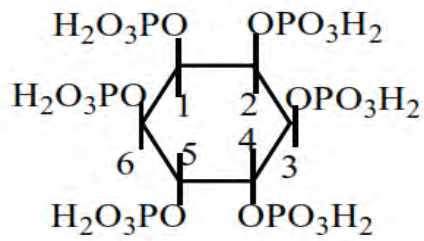

Figure 5. Chemical structure of phytate (Abera, 2009)

Tannins

Tannins are secondary metabolites of various chemical structures widely occurring in plant kingdom. They are defined as high-molecular-weight polyphenolic compounds that have the ability to bind with protein and preserve animal hides. However, the term “tannin” is commonly used to refer polyphenolic compounds. Their anti-nutritional effects include interference with the digestive processes either by binding the enzymes or by binding to food components like proteins or minerals (Gobezie, 2010). LAB fermentation also reduces the

levels of anti- nutrients such as tannins in food leading to increased bioavailability of minerals such as iron, protein and simple sugars (Chelule et al., 2010).

Tannins are polyphenolic compounds of plant origin, which bind with proteins. There are two types of tannins (hydrolysable and condensed tannin)

Figure 6. Base structures of hydrolysable and condensed tannins (Abera, 2009)

2.8.4. Functional properties of flours

Functional properties are the fundamental physicochemical properties that reflect the complex interaction between the composition, structure, molecular conformation and physicochemical properties of food components together with the nature of environment in which these are associated and measured. Functional characteristics are required to evaluate and possibly help to predict how new proteins, fat, fibre and carbohydrates may behave in specific systems as well as demonstrate whether or not such protein can be used to stimulate or replace conventional protein (Chandra and Samsher, 2013).

The food property is characterized by the structure, quality, nutritional value and /or acceptability of a food product. A functional property of food is determined by physical, chemical, and/or organoleptic properties of a food. Example of functional properties may include solubility, swelling power, bulk density, water absorption capacity, and oil absorption capacity, water retention, frothing ability, elasticity and absorptive capacity for fat and foreign particulars. Typical functional properties include emulsification, hydration (water binding), viscosity, foaming, solubility, gelation, cohesion and adhesion. Knowledge of the functional properties will provided useful information to industry purpose and other alike on the subsequent incorporation of the different flours to produce natural, cheap and acceptable functional foods (Chandra and Samsher, 2013).

2.8.5. pH and Titratable acidity

Titratable acidity deals with measurement of total acid concentration within a food. It is a better predictor of acid's impact on flavour than pH. Food acids are usually organic acids

with citric, malic, lactic, tartaric and acetic acid being the most common. The organic acid present in the food influence flavour, colour, microbial stability, keeping quality. Organic acid may be naturally presented in the food, they also may be formed through fermentation Sadler and Murphy, 2010).

2.9. Processing method of bulla and kocho

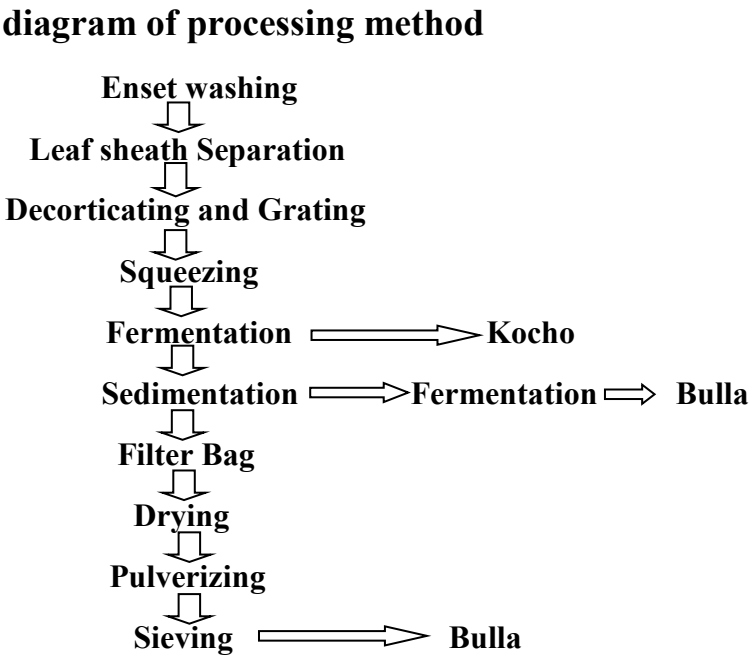

Figure 7. Flow diagram for the processing of bulla and kocho (Ayele, and Sahu, 2014)

Harvesting and transportation: - Enset is usually harvested just before flowering, the preferred harvesting time is just when the plant flowers. The time duration required to flower depends upon climatic conditions, clone type, and management. Hence, the flowering time varies from 3 to 15 years but is optimally around 6 or 7 years.

Raw material storage: - This helps to fasten the processing eliminating transportation delay and efficient use of employer's energy without time wastage by waiting raw material and also to control deterioration of the raw material by storing for long time. It stored at room temperature.

Enset washing: - The enset is washed to remove the soils, insects, dusts and any unwanted impurities which may decrease the quality of the product.

Leaf sheath Separation: - After harvesting leaves and older leaf sheaths are first removed from the designated plants. The internal leaf sheaths (commonly up to two meters in length)

are separated from the pseudostem down to the true stem, which is about a 20 centimetre section between corm and pseudostem. Then the true stem is separated or stumped from the underground corm. The concave side of the leaf sheath is peeled and cut into pieces of about one meter length and split lengthwise in order to shorten the leaf sheath to a workable size.

Decorticating and Grating: - Then the leaf sheath is decorticated using a decorticating machine which helps to reduce the size. There is variation in the way that the corm is grated in different places. One practice is to uproot the corm and remove any soil from its surface.at this stage the pseudostem and corm reduced to small pieces and ready for squeezing.

Squeezing: - Squeezer is used to separate Kocho and bulla from decorticated and chopped Enset by adding water, which added to aid the extraction of bulla, with the application of some force using presser. Then the Kocho is send to the fermenter and Bulla is collected in received tank.

Fermentation: - After the completion of decorticating and grating, the leaf sheath pulp is spread on fresh enset leaves covering the tank in the ground, after which the grated corm is spread on the processed pulp. A starter is added to aid in fermentation. This starter consists either of already fermented Kocho to which various spices and herbs are added or fermenting agents are prepared from the inner portion of the corm and then mixed with the decorticated pulp and grated corm after some weeks. Turning, mixing, rinsing, and chopping continue over a period of time until the mixture partially ferments, when it is then referred to as Kocho. The total time period for this fermentation to occur ranges from 15 to 20 days. Then the fermented Kocho is stored in fermenter tank that placed in the ground. The Kocho must be left in a storage tank for a minimum of a month, but it can be stored for many months and even for several years. The fermenter tank is opened at intervals to allow aeration. This is repeated until the desired fermentation quality is reached or the food is needed.

Sedimentation: - This helps to separate bulla from impurities before it left for fermentation and drying.by density difference the water becomes at the top and bulla settles down. The bulla easily collected at the cone shape of the cylinder and the water left either for further process or drainage system.

Filter Bag: - It is used to remove fine fibres and other impurities by filtering Kocho to get purified product and also increasing the efficiency of the dryer by removing some water.

Drying; - The moisture contained in bulla and Kocho is removed by using drying process. The drying performed by either in open air (sun drying) or using industrial drying equipment like that of rotary drum dryer in order to conduct the process in short period of time.

Pulverizing: - It used for cutting to reduce the size of dried Kocho and bulla prepared for sieving of flavoured product in the standard size.

Sieving: - This separates big size products from specified sizes. The oversize left at the top and the size that we need to use only left at end of the sieve passing through series of sieves of different size, finally the products stored in the form of flour **(**Ayele, and Sahu, 2014).

2.10. Nutrients that Lack in Bulla and Kocho

Even though, enset is a very good source of carbohydrate and mineral especially calcium, it lacks many other nutrients. The deficient nutrients in enset are protein, fiber, fat, and minerals like iron and zinc. Enset do not have vitamin at all.

3. MATERIALS AND METHODS

3.1. Study setting

The experiments were conducted at Addis Ababa University (AAU) centre for Food Science and Nutrition department Central Laboratory, Ethiopian Food, Medicine and Healthcare Administration and Control Authority (EFMHACA) and Ethiopian public health institute (EPHI).

3.2. Location of the study

Welayita is located in the southern part of Ethiopia with in the area of about 4,400 square kilo meters. The northern tip of the boundary of Wolaita is at about 360 km to the south of Addis Ababa, at 6° 49' N latitude and 39° 47' E longitude and at an altitude of about 1900 m. The average monthly temperature in the area ranges between 11.9°C (August) and 26.2°C (January) with a mean annual temperature of 18.9°C. Rainfall averages 1100 mm a year and is bimodal, with the short rains from February or March until April and the long rains from June until September or October. Areka is one of the districts of welayita Zone which are known for enset production and consumption.

At the community level, combinations of different enset are used for preparing bulla. For this reason, the varieties of enset were not collected from market, but from Areka agricultural research centre (AARC), which is found about 30 km from Sodo in SNNPR.

Figure 8. Map of welayita zone and its boundaries.

3.3. Chemicals and apparatus

Table 4. Summary of chemicals and apparatus for each parameter

Ser. no	Parameters	Chemicals required	Equipment's required
1.	Moisture	-	Moisture dish, balance, tong, hot air oven, desiccator
2.	Crude protein	Sulphuric acid, H_2O_2, catalyst, NaOH, boric acid, HCl, methyl blue	Kjeldahl, Tecator tube and rack, bird/oxford pipette, conical flask, balance
3.	Crude fat	diethyl ether /petroleum ether	Soxhlet extraction, fume hood, drying oven, desiccator, extraction cylinder and timble
4.	Total ash	HCl **,** Nitric acid	Muffle furnace, hot plate /Bunsen burner, porcelain crucible
5.	Crude fiber	H_2SO_4, NaOH, ethanol	Oven, muffle furnace, Fibertec, fritted crucible
6.	Phytate	HCl, sulphosalsalic acid$FeCl_3.6H_2O$, and standard	centrifuge, centrifuge tube, UV/v spectrometer
7.	Tannin	HCl, methanol, vanillin HCl, D-catechin	Centrifuge, centrifuge tube, UV/v spectrometer
8.	PH value	Buffer solution	glass electrode attached to digital pH meter
9.	TA	NaOH, phenolphthalein	
10.	Minerals Ca, Mg, Zn, Fe	($LaCl_3$), standard Ca, Mg, Zn, Fe, HNO_3, HSO_4, HCl	instrument for digestion, AAS
11.	Functional property	Centrifuge,centrifuge tube, graduated cylinder, oven shaking water bath,	Distilled water

Asean manual of food analysis, 2012

3.4. Sample collection

4 kilograms of fresh bulla was collected purposively from the four varieties of enset (*Yanbule, Zereta, Messena* and *Gewada*). The samples were packed in polyethylene bags, kept in an ice box (to prevent moisture loss and fermentation), and transported to the centre for Food Science and Nutrition Laboratory of the Addis Ababa University. Once the samples arrived at the laboratory, they were apportioned into three lots for fermentation (0, 15, and 30 days).

In figure 9:- **A** - *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*. **B**- *Decortication of the pseudostem by using a locally made wooden scraper.* **C**- *The decorticated and grated leaf sheath pulp was spread on fresh Enset*

leaves covering the ground, after which the grated corm was mixed on the decorticated pulp. **D**- *Addition of water on chopped enset to aid the extraction of bulla.* **E-** *Separation of Kocho and bulla from decorticated and chopped enset.* **F**- *Sedimented bulla after the supernatant was discarded.* **G**- *Packaging of the sample with polyethylene bag.*

A B C

D E

F **G**

Figure 9. Pictorial presentation of sample collection

3.5. Sample preparation and Fermentation

The first lot were used for analysis without fermentation and the remaining two lots were fermented for 15 and 30 days and analysed for their nutritional and anti-nutritional content. The latter lots were allowed to ferment in air tight plastic cupped container at room temperature on the same condition until the required time. The moisture content of each lot were determined before drying at their specified time of fermentation. For other nutritional and anti-nutritional analysis, each of the three lots dried in the oven at 60 ºC for 72 hours prior to analysis. Each dried samples were milled into fine powder using an electric grinder to pass through 0.425 mm sieve mesh size and then packed in polyethylene bags until required for analysis (Fekadu *et al*., 2013).

3.6. Chemical Analysis

3.6.1. Proximate Analysis

3.6.1.1. Determination of moisture content

Moisture content of the sample were analysed by drying air oven (DHG 9055A) method according to the official method 925.09 of the AOAC (2000). A drying dish was dried in an oven at 105°C for 1 hr and placed in desiccator to cool. The weight of the drying dish (W_1) was determined. 5 g of bulla samples were weighed in the dry dish (W_2), oven dried at 105°C for 3 hrs and after cooling in a desiccators to room temperature, it was again weighed (W_3). And brought to a constant weight by put it again in an oven for extra one hour.

$$Moisture\ (\%) = \frac{W2 - W3}{W2 - W1} \times 100$$

3.6.1.2. Determination of crude protein

Protein content were determined by the Kjeldahl method according to the official method 979.09 of AOAC (2000). The general procedure included the following steps of digestion at 370^{o}c for 4 hrs. 0.5 g sample was digested by adding 6 ml of concentrated sulfuric acid and then, 3.5 ml of (30%) H_2O_2 was added step by step. When violent reaction stopped 3gm of catalyst (mixture of potassium sulphate and copper sulphate) was added and left for 30 minutes. The mixture was digested in digestion stove for 4hrs at 370^{0}c. After digestion was completed, the content in the flask was diluted by 50 ml of water and neutralized using 35% of NaOH. Upon addition of NaOH, the ammonium was distilled off and trapped into a boric acid and solution containing methyl blue and methyl red indicators. Finally, titration of the ammonium attached to borate anion was titrated with standardized 0.1 HCl, and the amount

of HCL consumed was recorded and total crude protein of bulla was calculated as total nitrogen.

$$\%N = \frac{S - B \times 14 \times NHCl}{10 \times W}$$

Crude Protein (%) = %N x 6.25

Where, S- is sample titration reading V- is volume taken for titration

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

N is normality of HCl

3.6.1.3. Determination of crude fat

Crude fat test were carried out on soxilate extraction (SZC-D fat determination meter YLC 2000) method utilizing Diethyl ether according to official method 920.39 of the AOAC (2000). A 2 g dried sample of bulla was extracted with 50 ml diethyl ether for about hrs in the soxhlet extractor. The cylinder containing the extracted fat was dried in an oven until constant mass was obtained. The total crude fat was calculated as percentage by weight: -

$$\%\ crude\ fat = \frac{W2 - W1}{W} \times 100$$

Where, W1= weight of the extraction flask

W_2= weight of the extraction flask plus the dried crude fat (g)

W= weight of the sample

3.6.1.4. Determination of crude fiber

Crude fiber content was determining by using Fibertec (Fibertec 2010). The sample were analysed by the steps of digestion, filtration, washing, drying and combustion according to official method 962.09 of the AOAC (2000). About 1 g (M_3) sample was transferred to pre-dried crucible which contains 1 g of celite sand for the purpose of simplifying filtration. Then the crucible with its content was placed in the Fibertec and the sample was digested. After digestion with 1.25% sulfuric acid for 37 minutes and washed with distilled water, and then digested by 1.25% NaOH for 37 minutes, the sample was filtered in coarse porosity crucible in apparatus at a vacuum of about 25 mm. The residue was then dried in an oven at 130°C for 2 hrs, cooled in desiccators, and weighed (M_1). Finally the crucible were placed in muffle furnace and the sample was ashed at 525°C for 3 hrs, it was cooled in desiccators, and weighed again (M_2). The total crude fiber was expressed in percentage as:

$$Total\ crude\ fiber = \frac{M1 - M2}{M3} 100$$

3.6.1.5. Determination of ash

The ash content was measured according to dry ashing procedure using official method 923.03 of the AOAC (2000). Clean drying dish was dried at 105°C in hot air oven, cooled in a desiccator and weighed using analytical balance (M_1). Then, 2.5 gram of bulla powder sample was put and weighed (M_2). The sample was charred on a hot plate until the contents turn black. The dish with its contents was transferred to a muffle furnace (S30 2RR England) and ignited at 550°C for 5hrs. Finally, the residue was weighed (M_3) and the total ash was expressed as percentage on dry basis as follow:

$$Total\ ash\ \ \% \ = \frac{M3 - M1}{M2 - M1} \times 10$$

3.6.1.6. Determination of total carbohydrate

The content of carbohydrate in the sample was determined by difference method that is done by subtracting the sum of the percentages of moisture, crude protein, crude fat, crude fiber and ash content from 100%.

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

Where, M=Moisture content in percent %

P=Crude protein content in percent %

F=Crude Fat content in percent %

FI=Fiber content in percent %

A=Ash content in percent %

3.6.2. Determination of mineral (Ca, Mg, Fe, Zn)

Mineral analysis for levels of calcium, magnesium, zinc and iron in the samples was determined by the method described by (Dickman and Bray, 1940) by atomic absorption spectrophotometry (model AA-6800 Shimazdu) after digestion with concentrated nitric acid (AOAC, 2000). Ash obtained after dry at 550^0C for 5 hrs was treated with 7 ml of 6N HCl to wet it completely and carefully dried at a low temperature on hot plate. 15 ml of 3N HCl was added and the dish was heated on the hot plate until the solution just boils. Then allowed to be cooled and filter through a filter paper in to a 50ml volumetric flask. Again 10 ml of 3N HCl was added to the dish and heated until the solution just boils. Finally, cool and filter into

the volumetric flask. For the determination of calcium 2.5ml of lanthanum chloride (10% w/v) was added to both standards and samples. Using atomic absorption spectrophotometer a calibration curve was prepared by plotting the absorption or emission values against the metal concentration in mg/kg by using a serious of standard solution (0, 0.5, 2, 4, 6, and 8) for calcium, (0, 0.05, 0.1, 0.2, 0.3 and 0.4) for Mg and (o, 1, 2, 3, 4, and 5) was for Fe and Zn. Reading was taken from the graph, which depicted the metal concentrations that correspond to the absorption or emission values of the samples and the blank. The metal contents were calculated by using the formula.

$$Mineral \frac{mg}{100g} = \frac{[(Cs - Cb) \times V}{10 \times W}$$

Where: - Cs - Concentration of the sample W – Weight of the sample

Cb - Concentration of the blank V - Volume factor

3.6.3. Anti-nutritional Analysis

3.6.3.1. Phytic acid

Phytic acid was determined by using (Latta and Eskin, 1980) as modified by (Vantraub and Lapteva, 1988). About 1g of sample extracted with 10 ml 0.2NHCl for 1hr at an ambient temperature and centrifuged (3000 rpm/30min). The supernatant was used for Phytate estimation. About 2 ml of Wade reagent (0.03% solution of $FeCl_3.6H_2O$ and 0.3%sulfosalicylic acid in distilled water) were added to 3 ml of the supernatant solution and centrifuged. The absorbance at 500 nm was measured by using Ultra Violet spectrophotometer (Lambda 950). A series of standard solution were prepared containing 0, 5, 9, 18, 27 and 36 µg/ml of phytic acid (analytical grade sodium Phytate) in 0.2N HCl (Y= -0.0083x + 0.4905). A 3ml of standard was added into 15ml of centrifuge tubes and 3ml of water which were used as a blank. A 2ml of the Wade reagent was added to each test tube and the solution was mixed on a Vortex mixer for 5 seconds. The absorbance of the solutions (both the sample and standard) were measured at 500nm by using de ionized water as a blank. A standard curve was made from absorbance versus concentration and the slope and intercept were used for calculation.

$$phytic\ acid \quad \frac{\mu g}{g} = \frac{[Absorbance - Intercept] \times 10}{Slope \times Wt\ of\ sample \times 3}$$

3.6.3.2. Tannins

The amount of condensed tannin was determined by the (Vanillin assay of Burns, 1971 as modified by Maxson and Rooney, 1972). About 3g of sample weighed and extracted with 10mL of 1% HCl in methanol, at room temperature in mechanical shaker for 24 h. The mixture was centrifuged at 1000G for 10 minutes. 1mL supernatant were mixed with 5mL of vanillin-HCl (prepared by combining equal volume of 8% concentrated HCl in methanol and 4% Vanillin in methanol) reagent in another test tube. When the reaction is completed (after 20 minute), the absorbance read at 500nm using Ultra Violet spectrophotometer (Lambda 950). D-catechin was used as standard value of tannin in mg D-catechin per gram of sample. 40mg D-catechin was dissolved in 100mL of 1%HCl in methanol and from this 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml and 100µg/ml was taken in a test tube and the volume was adjusted to 1mL with 1% HCl in methanol. 5mL of vanillin-HCl reagent in each test tube was added. After 20 minutes the absorbance was measured at 500nm.The absorbance of the blank was subtracted from the absorbance of the corresponding vanillin-containing sample(y=0.1437x+0.0273). A standard curve was made from absorbance versus concentration and the slope and intercept were used for calculation.

$$condesed\ Tannin\ \ \frac{mg}{100g} = \frac{As - Ab\ \ - Intercept}{slope \times d \times w}$$

Where: - W = Weight of sample in gram

d = Density of solution (0.791g/ml)

Ab = blank absorbance and: As = sample absorbance

3.6.4. Determination of functional property of bulla flours

3.6.4.1. Determination of bulk density

The flour sample was filled to 5 ml into an already weighed measuring graduated cylinder (W_1). For the packed bulk density determination, the flour sample was gently tapped to eliminate spaces between the flour and the level was noted to be the volume of the sample (V) and then weighed (W_2). No tapping was made in the case of loosed bulk density and the level is also noted to be the volume of the sample and then weighed. The study was conducted in triplicate (Adebowale *et al*, 2005 and Narayana and Narasinga, 1984).

$$Bulk\ density\ \ \frac{g}{cm3} = \frac{W2 - W1}{V}$$

3.6.4.2. Determination of water absorption capacity (WAC)

WAC was determined with the method reported by (sosulski, 1988). 25 ml of distilled water was added to a sample of 3g flour (W_1) in a weighed centrifuge tube (W_2) and stirred six times for 1 minutes at 10 minutes intervals. The mixture were centrifuged at 3000 rpm for 25 minutes and the clear supernatant was decanted and discarded. Pellets were dried at 50^{o}c for 25 minutes and the adhered drops of water removed and reweighed (W_3). The amount of water retained in the sample was recorded as weight gain and was taken as water absorbed. Water absorbed capacity was expressed as the weight of water bounded by 100g dried flour

$$WAC \quad \frac{g}{100g} = \frac{W3 - (W1 + W2)}{W1} \times 100$$

3.6.4.3. Oil absorption capacity (OAC)

10 mL of refined corn oil with the density of 0.92 g/cm3 was added to 1.0 g of the sample in a 25ml of centrifuge tube. The suspension was stirred using a magnetic stirrer (Model MS-12B, MS-17B, and MS- 22B) for 5 min. The suspension obtained was centrifuged at 3555 rpm for 30 min and the supernatant was measured in a 10 ml of graduated cylinder. Oil absorbed will be calculated as the difference between the initial volume of oil added to the sample and the volume of the supernatant (Adebowale *et al*, 2005, Adeleke and Odedeji, 2010, Buchat 1997 and Ocloo1 *et al*., 2010).

3.6.4.4. Swelling power and solubility

Swelling power and solubility determination were carried in the temperature range of 60-90^{o}c (using the method of Leach et al., 1959). About 1g of bulla flour sample was accurately weighed and transferred in a clear dried test tube and weighed (w_1). About 10 ml of distilled water were added and mixed gently at low speed for 5 minutes. The slurry was heated in a thermo stated water bath (YCW-0125) at 80^{o}c for 30 minutes and was stirred gently to prevent lumps forming in the flour. Then the cooled test tube (20^{o}c) was centrifuged at 2200rpm for 15 minutes. The supernatant was decanted immediately after centrifuging into a pre weighed evaporating can and at 100^{o}c to a constant weight approximately for 4 hrs. The weight of the sediment was taken and recorded as (W_2) or the swollen.

$$Swelling\ power = \frac{wt\ of\ sediment}{sample\ wt - wt\ of\ soluble}$$

3.6.5. Titratable acidity and pH analysis

3.6.5.1. Titratable acidity value

The titratable acidity of bulla was determined by titrating the sample with a standard base (NaOH) to the phenolphthalein endpoint. A 5g bulla sample was blended with 15 ml distilled water and waited for 30 minutes. Then titrated with 0.1N standard solution of NaOH, after addition of 3 drops of 1% of alcoholic phenolphthalein indicator. As the colour of the sample changed to pink, the volume of NaOH consumed until end point reached was used to calculate the titratable acidity expressed in terms of the predominant organic acid.

$$\%\ of\ acid\ \ \frac{wt}{wt} = \frac{N \times V \times Eq.wt}{w \times 10}$$

Where; N = Normality of NaOH (mEq/ml)

V = Volume of titrant (ml)

Eq.Wt. = Equivalent weight of predominant acid (mg /mEq)

W = weight of sample

3.6.5.2. pH value

The pH of the sample measured by dipping the electrode of a digital Lab PH meter (MP 511) in to the sample after a proper calibration of the meter with buffer solution. The pH of bulla sample were determined from 1/10 dilution of sample by glass electrode attached to digital pH meter before and after fermentation time. 10 g of bulla sample mixed with 90 ml of distilled water. After calibrating the pH meter at 7.00, 4.00 and 9.2 with buffer solution, the pH sample was measured.

3.7. Preparation of Genfo and Kofeme

Genfo (stiff porridge): - The dried and milled bulla flour was used for the preparation of genfo, while mixing the flour in boiling water and was cooked until it retains the right consistency and flavour (Asrat, 2011).

Kofeme: - To prepare Kofeme (traditional food around Wolaita), which looks like enjera firfir. The semi-dry bulla paste was spread on the hot girdle and mixed with a spoon until uniform pearls are formed. The kofeme was baked for about 15 minutes (Urga *et al*., 1993).

3.8. Sensory analysis

The sensory analysis were done on y*anbule*, g*ewada*, *zereta* and *messena* varieties of bulla which were fermented for 30 day, because bulla fermented for 30 days had better nutritional value when it is compaired with unfermented and 15 days fermented bulla.

The experiments were performed in Ethiopian public health institute experimental kitchen. Bulla porridge and kofeme from each variety was prepared by adding same amount of salt (half tea spoon), presented to 10 trained panellists to evaluate the taste, colour, appearance, flavour, and overall acceptability, the panellist were assigned to score their preference for the various attributes using nine (9) hedonic scale. With nine being like extremely and one dislike extremely (Urga and Umeta, 1993).

3.9. Experimental design and statistical analysis

The product was produced in triplicate and evaluated under completely randomized design (CRD). Data was analysed by the analysis of variance (ANOVA) procedures using SPSS/20.0 software for windows. Least significant differences (LSD) were used for multiple mean comparison tests. Significance was accepted at ($p< 0.05$) level of probability.

4. RESULTS AND DISCUSSION

The effect of variety and fermentation time (raw, 15 days, 30 days) on proximate, antinutritional factors, physicochemical and functional properties of four improved enset samples (*yanbule*, *gewada*, *zereta* and *messena*) obtained from Areka Agricultural Research Centre were studied.

4.1. Proximate composition of bulla

The proximate composition of bulla among the four enset varieties (*yanbule*, *gewada*, *zereta* and *messena*) are presented in Table 5 and also the effect of fermentation time (0, 15, and 30 days of fermentation) on the proximate composition of bulla is presented in Table 6.

4.1.1. Moisture Content

The moisture content of bulla sample significantly varied with the varieties of enset (Table 5). Bulla prepared from *gewada* (54.1g/100g) had the highest moisture content and the least moisture content was recorded for bulla prepared from *messena* (48.6 g/100g). The moisture content of bulla prepared from *yanbule*, *gewada*, and *zereta* were significantly ($p< 0.05$) affected by fermentation time (Table 6). The moisture content of bulla prepared from *yanbule* significantly varied from raw, fermented for 15 days and for 30 days. However, for bulla derived from *gewada* and *zereta*, moisture content was significantly reduced only after 15 days and 30 days of fermentation. The significant variation in moisture content among the bulla samples greatly reflects the genetic difference among the varieties (Tobiaw and Bekele, 2011) and the variation in the degree of sedimentation during bulla preparation. The reason that the moisture content of fermented bulla was reduced could be because the microorganisms responsible for bulla fermentation might have utilized some moisture for their metabolic activities (Igbabul *et al*., 2014). The moisture contents of bulla reported in this study were in close agreement with the moisture content of commercially available bulla reported by Atlabachew (2007), which ranged from 44-55 g/100g The moisture results also agreed with values reported for bulla (54.9 g/100g) in the Ethiopian Food Composition Table (EHNRI, 1997).

The moisture content of foods varies greatly. Water is the major constitute of most food products. The moisture content of food is important to food processors and consumers for a variety of reasons (Bradeley, 2010).

Table 5. Proximate composition of bulla (g/100g) prepared from different varieties of enset

Variety	Moisture	Protein	Fat	Ash	Fibre	Carbohydrate	Energy kcal/100g
Yanbule	52.5±0.08[b]	0.19±0.003b	0.29±0.027a	1.05±0.19a	1.02±0.01a	97.45±0.21a	393.19±0.6a
Gewada	54.1±0.45[a]	0.18±0.005b	0.3±0.027a	0.77±0.06a	1.04±0.005a	97.71±0.03a	394.24±0.37a
Zereta	49.57±0.29[c]	0.36±0.005a	0.23±0.026a	0.91±0.06a	1.04±0.005a	97.45±0.1a	393.35±0.14a
Messina	48.55±0.11[d]	0.36±0.011a	0.29±0.026a	0.77±0.1a	1.04±0.01a	97.54±0.1a	394.21±0.22a

Value in each cell is mean ± standard deviation duplicate analysis

Means in the same column followed by different letters superscripts are significantly different ($p < 0.05$).

4.1.2. Protein content

The value of protein for the enset varieties is given in Table 5. The protein content of bulla significantly differed ($p<0.05$) among the varieties. Bulla derived from *zereta* (0.36g/100g) and *messena* (0.36g/100g) had significantly higher protein contents than bulla from the rest of the varieties. The fermentation time brought significant increment in the protein content of bulla prepared from all varieties. The longer fermentation time (30) resulted in highest protein content for bulla. The protein content increased from 0.19 to 0.56g/100g for *yanbule*, 0.18-0.72g/100g for *gewada*, 0.36-0.73g/100g for *zereta*, and 0.36-0.90g/100g for *messena* (Table 6). The protein contents obtained in this study were in agreement with the result of study done by Atlabachew (2007) that ranged from 0.4 – 0.8g/100g, but were higher than the values for bulla included in Ethiopian Food Composition Table (0.2g/100g on dry matter basis) (EHNRI, 1997). Research done on seven yam species in Ghana revealed that the protein contents ranges from 4.03 to 6.52% on dry matter basis (Ezeocha and Ojimelukwe 2012; Ezeocha *et al.* 2012), which is much higher than the protein content of bulla.

The observed increase in protein content of fermented bulla samples could be attributed to the increase in microbial mass during fermentation, causing extensive hydrolysis of protein molecules to amino acid and other simple peptides. Secondly, the enzymatic hydrolysis of some protein inhibitors during fermentation, for instance, the degradation of antinutritional factors especially phytate may contribute to the increase in protein content due to the breakage of phytate-protein complexes. The increase may also be due to the structural proteins that are an integral part of the microbial cell (Igbabul *et al.*, 2014). On the other hand, the variation in protein content of bulla could be a result of the differences in the genetic makeup of the enset varieties. According to Elyas *et al.*, (2002) and Abdeheleen *et al*., (2008), the observed increase in protein content after fermentation can probably be due to the shift in dry matter content through depletion.

Protein is a larger compound with numerous functions such as synthesis of enzyme and hormone, growth and maintain of cell, regulation of body fluid and acid base balance, transportation lipid and oxygen source of energy and for formation of antibody and the like (Chang, 2010).

4.1.3. Total Ash

The total ash, which is an indirect indicator of mineral content of food stuff, varied between 0.77 and 1.05g/100g (Table 5), Although there was no considerable variations in ash contents of the bulla prepared from the four varieties, bulla from yanbule variety (1.05g/100g) had the highest ash content and the lowest ash value was recorded for bulla prepared from *gewada* (0.77g/100g) and *messina* (0.77g/100g). The relative variation in the ash content may largely be due to the differences in genetic composition and/or maturity level.

Fermentation on the other hand did not result in significant change of ash content of bulla prepared from all the four varieties of enset (Table 6). But the ash content was high in bulla fermented for 30 days. The values of ash content in the current study were higher than the results reported by Atlabachew (2007), which was 0.2g/100g and also were higher than the values presented in Ethiopian Food Composition Table, 0.1g/100g (EHNRI, 1997).

Ash refers to the inorganic reside remaining after either ignition or complete oxidation of organic matter in a food stuff. Ash content represents the total mineral content in food. Ashing is the first step in preparing a food sample for specific elementary analysis (Marshall, 2010).

4.1.4. Crude Fat

The values for crude fat content of raw bulla prepared from the four varieties were not significantly different ($p>0.05$). Bulla prepared from *gewada* (0.30g/100g) had the highest fat content and was followed by bulla derived from *yanbule* (0.29g/100g) and *messena* (0.29g/100g) (Table 5). Fermentation time did not significantly ($p>0.05$) changed the fat content for bulla prepared from *yanbule* and *messena* (Table 6). But the fat content of bulla derived from *gewada* and *zereta* significantly varied with the fermentation time. The fat content of bulla prepared from *gewada* and *zereta* increased from 0.3g/100g to 0.45g/100g and 0.23g/100g to 0.7g/100g, respectively. The obtained result was comparable with the study by Atlabachew (2007) (0.2-0.4 g/100g) and the study done on seven yam spp. Which ranges (0.46-0.76%) (Ezeocha and Ojimelukwe 2012; Ezeocha *et al.*2012). However, it was higher than the figure reported (0.1 g/100g) in Ethiopian Food Composition Table (EHNRI, 1997). The increase in the fat content of bulla as the fermentation time extends could be attributed to the possibility that the fermenting microorganism which could secrete microbial oil as a result of extensive break down of large fat molecule to simpler fatty acid units due to the high activity of the lipolytic enzymes (Igbabul *et al.*, 2014). The decreased fat content of

bulla may help to extend its shelf life because food products containing high fat are susceptible to both hydrolytic and oxidative or enzymatic rancidity and responsible for both the general acceptability and storage stability of the product. Lipids are a group of substance that in general are soluble in either, chloroform or other organic solvent but are sparingly soluble in water. Foods contain much type of lipids, but those which tend to be greatest importance are the triacylglycerol and phospholipids. The lipids contents of foods varies widely, but quantitation is important because of regulatory requirement, nutritive value and functional properties (Min and Ellefson, 2010).

4.1.5. Crude Fibre

The crude fibre content of the bulla derived from the four varieties seemed to be the same except for *yanbule* (1.02g/100g) which was slightly lower than others (1.04g/100g) (Table 5). The fibre values reported in the present study were slightly higher than the value reported by Atlabachew (2007) (0.6 – 0.8 g/100g) and fibre content of 0.3 g/100g reported in Ethiopian Food Composition Table (EHNRI, 1997).

The fibre contents of the fermented bulla samples were substantially ($P<0.05$) reduced for all varieties as the fermentation time became longer (Table 6). Fermentation decreased the fibre content of bulla by 40.4% (*gewada*)-60.5% (*messena*). The least fibre content (0.41g/100g) was recorded for bulla prepared from *yanbule* and *messena* that was fermented for 30 days and fermented bulla derived from *gewada* had the highest fibre content (0.62g/100g). The observed decrease in the fibre content of bulla during fermentation could be attributed to the partial solubilisation of cellulose and hemicellulose type of materials by microbial enzymes (Inyang and Zakari, 2008). Dietary fiber are structural part of plants, some can dissolve in water (soluble fiber) are easily digested by bacteria in the colon and often associated with protecting against heart disease and DM by lowering blood cholesterol and glucose level respectively, whereas insoluble fiber are less readily fermented thus promote bowel movement and alleviate constipation (Whitney and Rolfes, 2008).

Table 6. The effect of fermentation on the proximate composition of bulla (g/100g)

Proximate composition	Varieties	Duration of fermentation		
		Raw	15 days	30 days
Moisture	Yanbule	52.5±0.08[a]	51.1±0.36[b]	49.87±0.41[c]
	Gewada	54.1±0.45[a]	50.97±0.31[b]	50.27±0.34[b]
	Zereta	49.57±0.29[a]	48.38±0.52[b]	47.88±0.32[b]
	Messina	48.55±0.11[a]	48.07±0.17[a]	47.76±0.91[a]
Crude protein	Yanbule	0.19±0.003[c]	0.37±0.005[b]	0.56±0.01[a]
	Gewada	0.18±0.005[c]	0.54±0.01[b]	0.72±0.016[a]
	Zereta	0.36±0.005[c]	0.54±0.013[b]	0.73±0.005[a]
	Messina	0.36±0.011[c]	0.58±0.011[b]	0.91±0.01[a]
Crude fiber	Yanbule	1.02±0.01[a]	0.79±0.029[b]	0.41±0.006[c]
	Gewada	1.04±0.005[a]	0.88±0.03[b]	0.62±0.012[c]
	Zereta	1.04±0.005[a]	0.81±0.012[b]	0.56±0.056[c]
	Messina	1.04±0.01[a]	0.85±0.017[b]	0.42±0.004[c]
Ash	Yanbule	1.05±0.19[a]	1.24±0.06[a]	1.27±0.06[a]
	Gewada	0.77±0.06[a]	0.78±0.06[a]	0.88±0.08[a]
	Zereta	0.91±0.06[a]	0.92±0.08[a]	1.14±0.08[a]
	Messina	0.77±0.1[a]	1.05±0.25[a]	1.25±0.04[a]
Crude fat	Yanbule	0.29±0.027[a]	0.34±0.026[a]	0.42±0.052[a]
	Gewada	0.3±0.027[b]	0.35±0.027[ab]	0.45±0.026[a]
	Zereta	0.23±0.026[b]	0.4±0.026[a]	0.47±0.05[a]
	Messina	0.29±0.026[a]	0.39±0.027[a]	0.39±0.027[a]
Carbohydrate	Yanbule	97.45±0.21[a]	97.25±0.002[a]	97.34±0.01[a]
	Gewada	97.71±0.03[a]	97.46±0.11[ab]	97.34±0.03[b]
	Zereta	97.45±0.1[a]	97.33±0.03[a]	97.11±0.18[a]
	Messina	97.54±0.1[a]	97.13±0.28[a]	97.03±0.004[a]
Energy kcal/100g	Yanbule	393.19±0.6[b]	393.57±0.27[ab]	395.36±0.48[a]
	Gewada	394.24±0.37[b]	395.13±0.23[ab]	396.23±0.41[a]
	Zereta	393.35±0.14[b]	395.05±0.42[a]	395.58±0.27[a]
	Messina	394.21±0.22[a]	394.38±0.94[a]	395.29±0.3[a]

Value in each cell is mean ± standard deviation duplicate analysis

Means in the same row followed by different letters superscripts are significantly different ($p < 0.05$).

4.1.6. Utilizable carbohydrate

Utilizable carbohydrate content was determined by difference method that means there was no analysis conducted for utilizable carbohydrate determination. Instead it was determined using the formula (i.e. utilizable carbohydrate = 100-(fat+ fibre+ protein+ ash+ moisture). The multiple comparison test had showed no significant difference ($p>0.05$) among bulla derived from the four varieties of enset in its carbohydrate content (Table 5). Bulla prepared from *gewada* had the highest carbohydrate content (97.71 g/100g) and *zereta* and *yanbule* had the lowest carbohydrate content (97.45g/100g). The values were not different from the finding reported by Atlabachew (2007), which ranged from 93–98g/100g, but they were much higher than the value (44g/100g) reported in the Ethiopian food composition data table (EHNRI, 1997). The carbohydrate content of cereals are composed of approximately 75%, which is still lower than carbohydrate content in bulla sample (Mckevith, 2004). The current study indicated a decreasing pattern in the carbohydrate content during fermentation process in all the varieties. However, the reduction was not significant for all bulla products (Table 6). The reduction of the value as fermentation extends could be due to the fact that soluble starch and sugar are principal substance for fermenter microorganisms. Therefore, degradation and subsequent decrease in starch content are expected to occur. Fermentation will activate enzymes which act on polysaccharides. These enzymes degrade polysaccharides and latter leads to reduction of utilizable carbohydrate. Carbohydrate (which is exclusively of plant origin) is important in foods as a major source of carbohydrates, which are converted in to monosaccharide, which are absorbed, provide metabolic energy. Worldwide carbohydrate account for more than 70% of the caloric value of the human diet. It is recommended that all person should limit calories from fat to not more than 30%. Carbohydrates also contribute other attributes, including bulk, viscosity, and water holding capacity, flavour, aroma and range of desirable texture (Bemiller, 2010 and Whitney, 2008).

4.1.7. Gross Energy

The total energy in (kcal) was calculated using the equation, total energy (kcal) = (9×fat) + (4×carbohydrate) + (4×protein). All the bulla samples prepared from the four varieties of enset did not significantly ($p>0.05$) differ in the total energy content. As presented in Table 5, the total energy value ranged from 394.24 to 393.19kcal/100g. The highest and the lowest

energy values were obtained from bulla prepared from *gewada* and *yanbule* varieties, respectively. The energy values reported in this research were in agreement with the findings of Atlabachew (2008), 378 to 442.6kcal/100g and much higher than value of 180.5kcal/100g reported in the Ethiopian Food Composition Table (EHNRI, 1997). On the other hand, the duration of fermentation did not result in considerable ($p<0.05$) variations in the amount of energy (Table 6).

4.2. Mineral composition of bulla

Calcium, magnesium, iron and zinc contents of the four enset varieties (*yanbule*, *gewada*, *zereta* and *messina*) are presented in Table 7 and also the effect of fermentation time (0, 15, and 30 days of fermentation) on the mineral composition of bulla is presented in Figures 10-13.

Table 7. Mineral composition of bulla prepared from enset varieties

Varieties	Ca	Mg	Fe	Zn
Yanbule	317.98±2.54^{a}	46.41±1.13^{a}	1.5±0.026^{b}	1.55±0.39^{ab}
Gewada	194.18±6.42^{c}	55.86±0.14^{a}	2.54±0.117^{a}	1.57±0.25^{ab}
Zereta	266.38±2.79^{b}	31.51±4.23^{b}	1.55±0.154^{b}	0.76±0.05^{b}
Messina	168.15±7.94^{d}	56.05±2.26^{a}	2.1±0.184^{a}	2.34±0.05^{a}

- Values are mean of duplicate ± SD.
- Means not sharing a common superscript letters in a column are significantly different at ($p<0.05$).

4.2.1. Calcium

The calcium contents of the unfermented bulla prepared from *yanbule* (317.98mg/100g) and *messena* (168.15mg/100g) had the highest and the least calcium content, respectively (Table 7). Bulla prepared from all varieties differed significantly ($p<0.05$) from one another. These significant variation in the calcium content of bulla could be because of genetic differences among the enset varieties. Atlabachew (2007) reported the calcium content of bulla to be varied between 38.5 and 44.6 mg/100g which was much lower than the value in this study. The amount of calcium (77 mg/100g) which was presented in the Ethiopian food composition data table (EHNIR, 1997) and the calcium content of yam spp. (*D. abyssinica*) ranged from 17.2 t0 44.8 mg/100g (Aregahegn *et al.* 2013) was also much lower than the value in present study. Bulla had significantly higher amount of calcium as the result is compaired with the calcium content of some fortified cereals flours, these are white wheat flour, whole wheat

flour, brown rice, white rice, and barley with the value of 140mg/100g, 38mg/100g, 51mg/100g, 10mg/100g, and 52mg/100g (Mckevith, 2004).

Fermentation time resulted in significant ($P<0.05$) improvement of the calcium content of the bulla prepared from the four varieties. The highest calcium content for bulla fermented for 30 days was about 567.6mg/100g (*yanbule*) and the lowest calcium content for bulla fermented for 30 days was 239.95mg/100g (*messena*) (Figure 10).

The level of mineral depends on a number of factors including genetic property of the crop species, climatic condition, soil characteristics, soil contamination and the degree of maturity of the plant at the moment of harvesting. Moreover, the varieties were improved for their quality of product, yield of product and shorting of the harvesting time (Yeshitila and Yemataw, 2010). Calcium is the most abundant mineral in the body (1150 g), 99 % of the calcium is found in bone where it act as integral part of bone structure and calcium store. The rest 1% circulate in the extracellular and intercellular fluids were it play vital role of life like maintaining normal blood pressure and muscle contraction (Whitney and Rolfes, 2008).

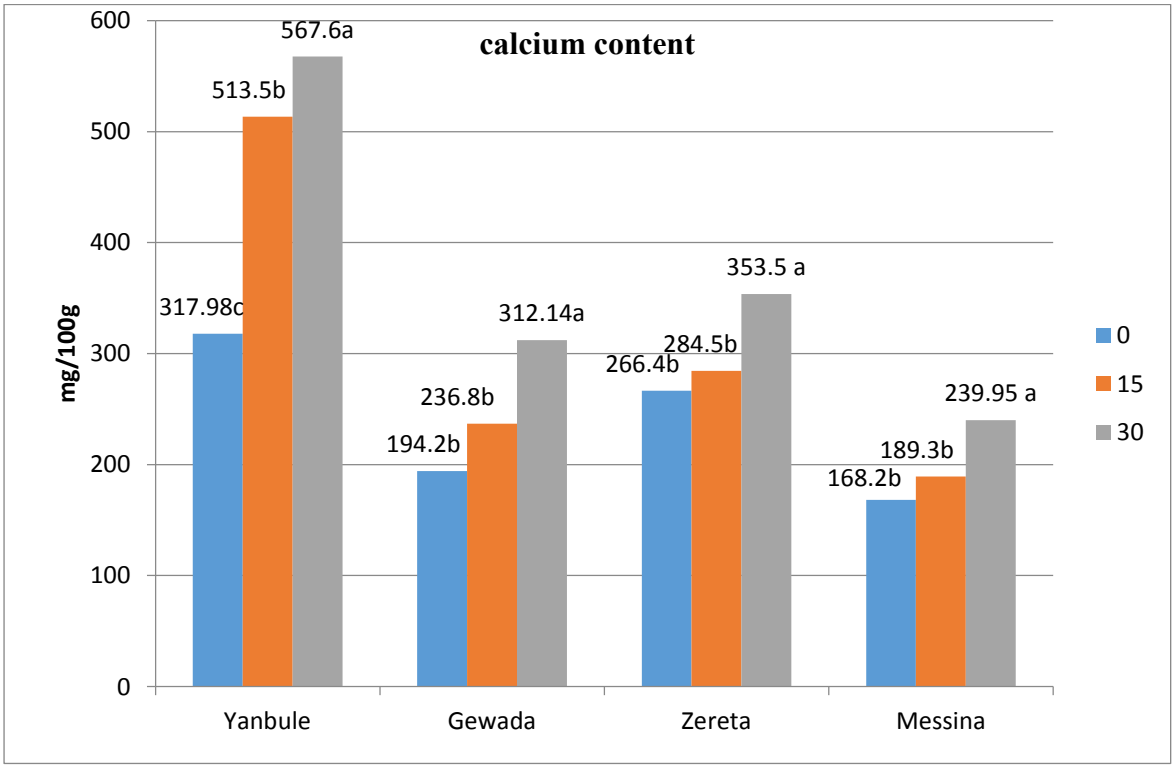

Figure 10. The calcium content of 0, 15, and 30 days fermented bulla

4.2.2. Magnesium

The values for thee level of magnesium content of raw bulla prepared from the four varieties were significantly different ($p<0.05$). Bulla prepared from *messena* (56.05g/100g) had the highest magnesium content and was followed by bulla derived from *zereta* (31.51g/100g) (Table 7). Fermentation time did significantly ($p>0.05$) change the magnesium content for bulla prepared from *yanbule* and *zereta* (figure 11). But the magnesium content of bulla derived from *gewada* and *messena* were not significantly varied with the fermentation time. The magnesium content of bulla prepared from *yanbule* and *zereta* increased from 46.41g/100g to 68.01g/100g and 31.51g/100g to 57.54g/100g, respectively. The obtained result was higher than the study done by Atlabachew (2007) (58.4 – 89.5µg/g).

The difference in the magnesium content of the current study and the previous one may be due to genetic makeup variation, although the plant have grown in the same environmental condition. Moreover this four varieties of enset covered by the current study and the additional varieties (endale and kelisa), are the improved varieties for quality and kocho yield (Yeshitila and Yemataw, 2010). It directly influences the mineral content of bulla.

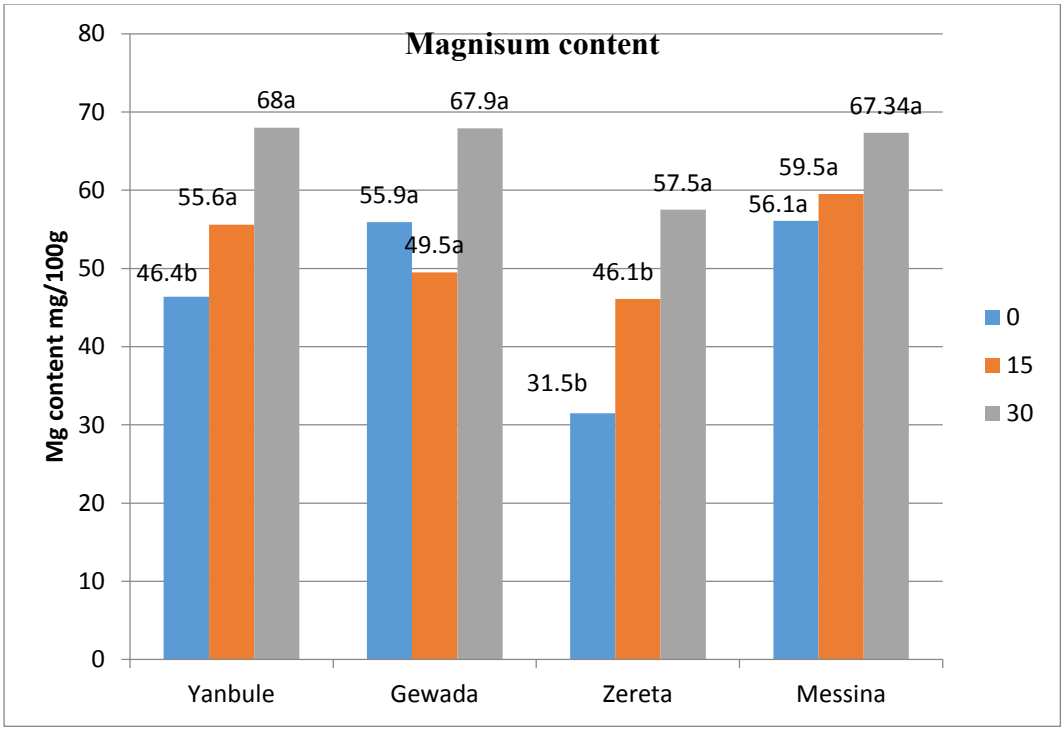

Figure 11. The magnesium content of 0, 15, and 30 days fermented bulla

4.2.3. Iron

Iron was another mineral analysed in this study. The level of iron contents of the unfermented bulla prepared from *gewada* (2.54mg/100g) and *yanbule* (1.5mg/100g) had the highest and

the least iron content, respectively (Table 7). Bulla prepared from *yanbule* and *zereta* varieties differed significantly ($p<0.05$) from bulla prepared from *gewada* and *messena* varieties. These significant variation in the iron content of bulla could be because of genetic differences among the enset varieties and degree of maturity of the harvested enset. Atlabachew (2007) reported the iron content of bulla to be varied between 3.65 – 5.98 mg/100g on dry matter base, which was much higher than the value in this study. The amount of iron (2.6 mg/100g) which was presented in the Ethiopian Food Composition Table (EHNIR, 1997) was in line with the present value. When we compare the level of iron for bulla with certain cereals some may be higher, lower or same range. For instance the iron content of white wheat flour (2.0mg/100g), and brown rice flour (1.4mg/100g) had similarity. Whereas, whole wheat flour (3.9mg/100g), and barley flour (3.0mg/100g) had higher iron content than bulla (Mckevith, 2004).

Even if there is no significant different, fermentation time resulted slight improvement in the iron content of the bulla prepared from the *yanbule* varieties. Whereas bulla prepared from *gewada*, *zereta*, and *messena* were not significantly ($P>0.05$) affected by duration of fermentation. The highest iron content for bulla fermented for 30 days was about 3.01mg/100g (*yanbule*) and the lowest iron content for bulla fermented for 30 days was 1.85mg/100g (*zereta*) (Figure 12). Iron is an essential nutrient categorized under trace mineral. It is vital to many of the cell's activity. Most of the body iron found into two protein, haemoglobin in red blood cell and myoglobin in muscle cells. In both, iron helps accept carry and release oxygen. Iron deficiency is the major type of malnutrition in developing country. The amount of iron found in bulla was very low when it compared with the RDA 8 -18 mg/day for healthier adult (Whitney and Rolfes, 2008).

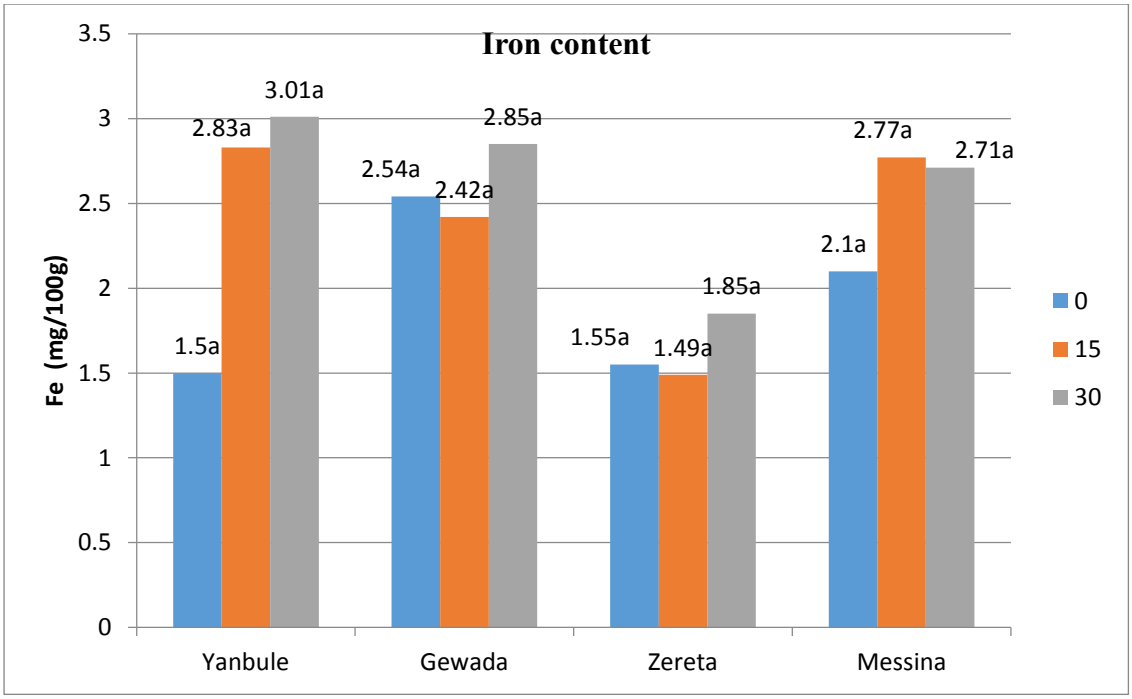

Figure 12. The Iron content of 0, 15, and 30 days fermented bulla

4.2.4. Zinc

The value of zinc content for the studied enset varieties is given in Table 7. The level of zinc content of bulla significantly differed ($p<0.05$) between *zereta* and *gewada* varieties. Bulla derived from *messena* (2.34mg/100g) and *zereta* (0.76mg/100g) had significantly higher and least zinc contents, respectively. The fermentation time brought significant increment in the zinc content of bulla prepared from all varieties. There was a significant difference ($p<0.05$) in the zinc content of fermented bulla sample among the varieties. The longer fermentation time (30) resulted in highest level of zinc content for bulla. The zinc content increased from 1.55-3.81mg/100g for *yanbule*, 1.57-3.19mg/100g for *gewada*, 0.76-1.41mg/100g for *zereta*, and 2.34-5.43mg/100g for *messena* (Figure 13). The zinc contents obtained in this study were comparable with the result of study done by Atlabachew (2007) that ranged from 2.2mg/100g-4.43mg/100g. The possible explanation for the increment of zinc content of bulla obtained from *messina* varieties might be contamination from the soil, water used for squeezing or contamination of sample from enset leaves. Zinc is a versatile trace element required as cofactors by more than 100 enzymes, which are involved in regulation of gene expression; stabilize cell membrane by helping to strengthen their defence against free radical attack and the like (Whitney and Rolfes, 2008).

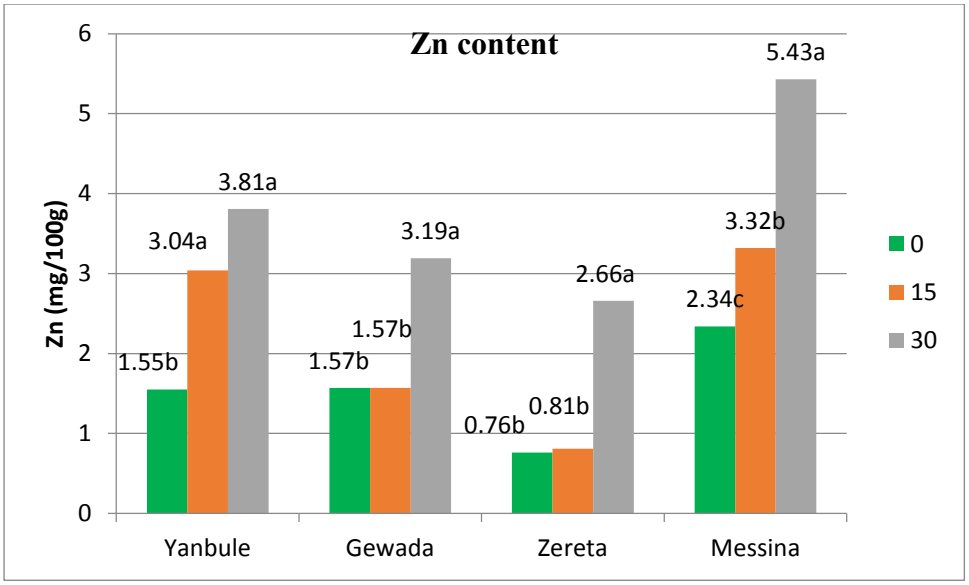

Figure 13. The zinc content of 0, 15, and 30 days fermented bulla

4.3. Anti-nutritional (phytate and tannin) factors content of bulla

The phytate and tannin contents of the four enset varieties (*yanbule*, *gewada*, *zereta* and *messena*) are presented in Table 8 and also the effect of fermentation time (0, 15, and 30 days of fermentation) on the phytate and tannin contents of bulla is presented in Figure 14.

Table 8. Phytate and tannin content of bulla prepared from enset varieties

Varieties	Anti-nutrient	
	Phytate	Tannin
Yanbule	112.56±1.95[a]	BDL
Gewada	86.16±0.63[b]	0.01±0.003
Zereta	84.25±0.23[b]	BDL
Messina	104.35±3.31[a]	BDL

- Mean value ± standard deviation n=3
- Means with in the same column followed by same letters or not statistically significant ($p<0.05$).
- BLD:- below the detected level

4.3.1. Phytic acid (phytate)

The values for the phytate content of raw bulla prepared from *yanbule* and *messena* varieties were significantly different ($p<0.05$) from *gewada* and *zereta*. Bulla prepared from *yanbule* (112.56mg/100g) had the highest phytate content and was followed by bulla derived from *messena* (104.35mg/100g) (Table 8). Fermentation time had significantly ($p<0.05$) changed

the phytate content for bulla prepared from all the varieties (Figure 14). The phytate content of bulla prepared from *yanbule, gewada, zereta* and *messena* decreased from 112.56mg/100g to 11.22mg/100g, 86.16mg/100g to 10.66mg/100g, 84.25 mg/100g-14.7 mg/100g, and 104.35 mg/100g-9.27 mg/100g respectively. Fermentation reduced the phytate by 88% in the current study. This in agreement with the previous studies on cassava varieties (Abera, 2009), which resulted as fermentation reduces phytate content by 88.4 %. Reduction in the phytate content of cereals with processing treatment has been frequently reported by (Ibrahim et al., 2002). This has been attributed to an increase of phytase activity on the phytate that make the phytate soluble and release bound protein and minerals. The reduction may be attributed to the activity of the endogenous phytase enzyme from the raw ingredient and inherent microorganisms which are capable of hydrolysing phytic acid in the fermented food preparations into inositol and orthophosphate (Igbabul et al., 2014).

The phytic acid content reported on cereals shows that the values for wheat were 4 mg/g in white, 22.2 mg/g for whole wheat flours and sorghum flours contained approximately 10 mg/g of phytic acid (Estepa et al. 1999). On a dry weight basis, corn contains 0.89% phytate, soft wheat 1.13%, brown rice 0.89%, barley 0.99% and oats 0.77% (Mckevith, 2004).

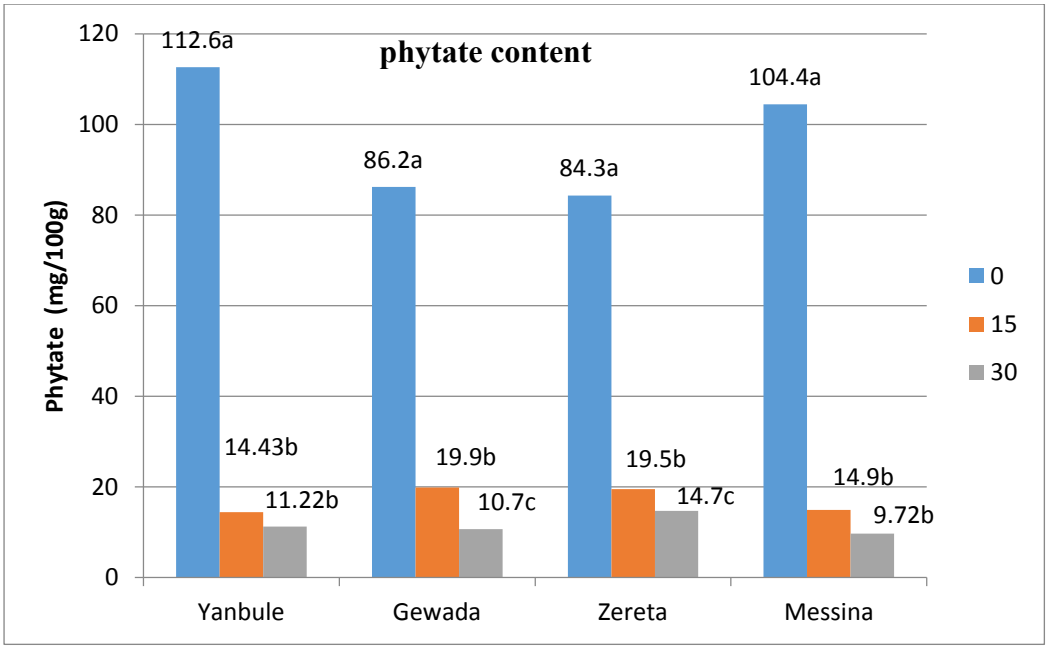

Figure 14. The phytate content of 0, 15, and 30 days fermented bulla

4.3.2. Condensed Tannin

The tannin content of *yanbule*, *zereta* and *messena* in all fermentation time were below the detected level. However, raw bulla prepared from gewada has 0.01 mg/100g of tannin (Table

8). As reported by Adane *et al* (2013) other root and tubers such as taro contain significant amount of tannin (26.94mg/100g-47.69 mg/100g). Fermentation significantly reduce the level of tannin according to Adane. The decrease in the tannin content of bulla is acceptable, because the higher content of tannins is known to inhibit the activity of the digestive enzymes and thus, interfere with the digestion and absorption of dietary protein, carbohydrates, minerals and other nutrients. They may also cause damage to the mucosa of the digestive tract and hence, they are undesirable for human consumption from a nutritional point of view (Vijayakumari et al., 2007).

4.4. Functional Property of Bulla Flour

The functional property of bulla flour from the four varieties of enset were shown in Table 9. The analysis were done on *Yanbule, Gewada, Zereta and Messina* varieties of bulla which is fermented for 30 days. Because bulla fermented for 30 days had better nutritional value when it is compaired with unfermented and 15 days fermented bulla. The functional property covered by the current study are bulk density, water absorption capacity, oil absorption capacity and swelling power.

Table 9: - Effect of variety on functional property of bulla fermented for 30 days

Variety	OAC (ml/g)	WAC (%)	BD (g/ml)	SP (%)
Yanbule	0.85 ± 0.15^{a}	118.75 ± 0.42^{c}	1.01 ± 0.01^{a}	1.65 ± 0.01^{a}
Gewada	0.9 ± 0.1^{a}	114.17 ± 1.67^{c}	1.01 ± 0.01^{a}	1.62 ± 0.004^{a}
Zereta	0.6 ± 0.1^{a}	130 ± 4.17^{b}	0.99 ± 0.01^{a}	1.62 ± 0.05^{a}
Messina	0.7 ± 0.1^{a}	141.67 ± 0.83^{a}	1.01 ± 0.01^{a}	1.6 ± 0.01^{a}

- Results are mean value of triplicate ± standard deviation
- Means with different superscript letters within the column are significantly different ($p<0.05$)
- Where
    - OAC: - oil absorption capacity
    - WAC: - water
    - BD: - bulk density
    - SP: - swelling power

4.4.1. Bulk density

The bulk density of bulla flour is presented in (Table 9). The result shows that the bulk density of bulla flour for *Yanbule*, *Gewada*, *Zereta* and *Messina* were 1.01, 1.01, 0.99 and

1.01 g/ml respectively. There was no significant difference between the four varieties ($p>0.05$) in their bulk density.

The value of bulk density determined in this study is close to the bulk density of cassava flour reported by Abera et al., (2009) that ranged from 0.65 to 1.70g/ml. As compared to the bulk density of potato flour reported by Chandra and Samsher, (2013), which is 0.72 g/ml, the value obtained from the current study is higher and can be chosen as good quality of flour. Bulk density of flour is related to the textural characteristics and eases of rehydration. Bulk density is an indication of the porosity of a product, which influences package design and could be used in determining the type of packaging material required for the product (Solsuki, 1962).

4.4.2. Water absorption capacity

The water absorption capacity of the bulla flour among the varieties is presented in (Table 9). The water absorption capacity of bulla flour for *yanbule*, *gewada*, *zereta* and *messena* was 118.78, 114.17, 130 and 141.67 % respectively. The water absorption of each variety has significant difference ($p<0.05$) except for yanbule and Gewada no significant difference between them. The result obtained by this study was much lower than the water absorption capacity of potato flour (752%) as reported by Chandra and Samsher, (2013). Water absorption capacity describes flour-water association ability under limited water supply. It has been observed to be dependent on the starch and protein concentration in the material coupled with the size of the particle. Generally, the water absorption characteristics of root flours is very important depending on the ultimate product to which the flour is intended to be converted, such as snack foods and bakery products.

4.4.3. Oil Absorption Capacity

The oil absorption capacity of *yanbule*, *gewada*, *zereta* and *messena* was 0.85, 0.9, 0.6 and 0.7 g/g, respectively (Table 9). It ranges from 0.6 – 0.9 ml/g, where the highest value is recorded for *gewada* and the lowest for *zereta*. In contrast the oil absorption capacity of cassava flour is 0.65 to 1.9 ml/g (Abebe et al., 2009).

4.4.4. Swelling Power

The swelling power of bulla flour, which is fermented for 30 days among the varieties were 1.65, 1.62, 1.62, and 1.6% for *yanbule*, *gewada*, *zereta* and *messena*, respectively (Table 9). The result obtained from the current study is lower than the value 42.9% reported for potato (Chandra and Samsher, 2013).

As swelling refers to absorption of water, it may be inhibited by the lipid content of the flour. Swelling power of the flour depends on the capacity of the flour to hold water through hydrogen bonding. After fermentation, the hydrogen bond of the starch molecules in the flour will be broken and are replaced by hydrogen bonds with water (Abera *et al*., 2009)

4.5. pH and Titratable Acidity of Bulla

The pH and titratable acidity of the four enset varieties (*yanbule*, *gewada*, *zereta* and *messena*) are presented in Table 10 and also the effect of fermentation time (0, 15, and 30 days of fermentation) on the pH and titratable acidity of bulla is presented in Figure 15-16.

Table 10. The pH and titratable acidity of bulla prepared from different varieties of enset

Varieties	Chemical Property	
	pH	Titratable Acidity
Yanbule	5.61±0.03[b]	0.25±0.02b[a]
Gewada	5.23±0.08[c]	0.22±0.03[b]
Zereta	5.55±0.04[b]	0.22±0.01[b]
Messina	6.05±0.02[a]	0.3±0.01[a]

- Mean value ± standard deviation n=3
- Means with in the same column followed by same letters or not statistically significant ($p<0.05$).

4.5.1. Titratable Acidity (TTA)

In the current study, there was significant difference in titratable acidity among the varieties ($p< 0.05$). The maximum titratable acidity among the four Enset varieties was found in bulla prepared from messena (0.3%) and the lowest value was (0.22) for bulla prepared from zereta as indicated in (Table 10). Fermentation significantly ($p< 0.05$) increased the titratable acidity for bulla prepared from yanbule, gewada, zereta, and messena from 0.25-1.05%, 0.22-1.01%, 0.22-0.9%, and 0.25-1.03%, respectively (Figure 15).

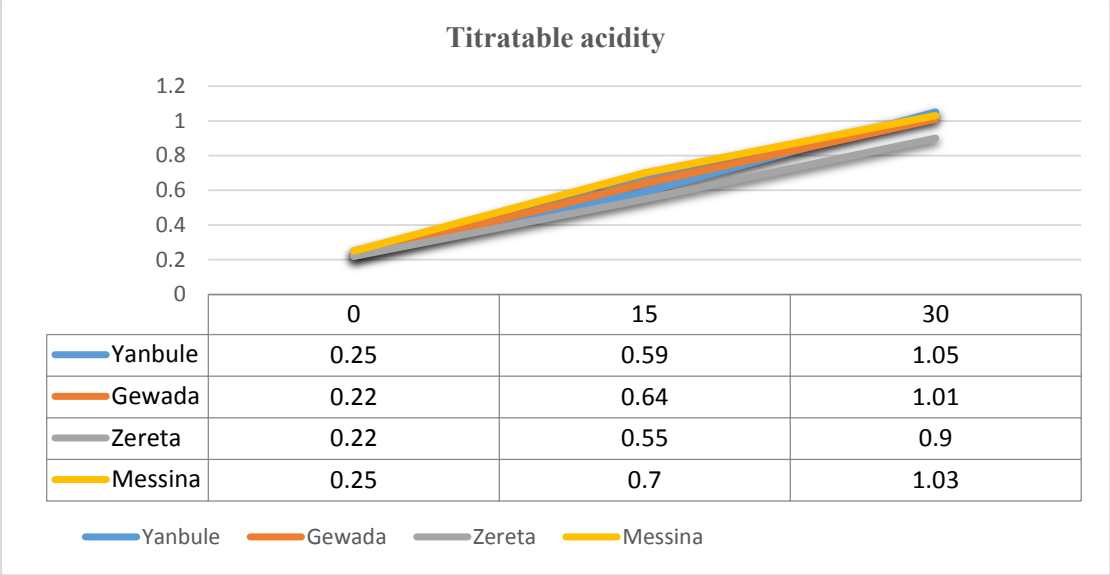

Figure 15. The titratable acidity of 0, 15, and 30 days fermented bulla

4.5.2. pH value

As the TA content of bulla increased, the pH value dropped pH value of bulla samples would be affected by enset varieties ($p<0.05$). As described in (Table 10) among enset varieties, the highest pH value (6.05) of bulla samples prepared from *messina* and the lowest pH value(5.23) was for bulla prepared from *gewada* variety. As fermentation time increased, the pH value of bulla samples declined (Figure 16). Therefore, the highest pH value was (3.42) obtained for bulla prepared from *messina* variety at the time of 30th days of fermentation time and the lowest value (2.8) was found for bulla derived from *gewada* variety, which is fermented for 30 days.

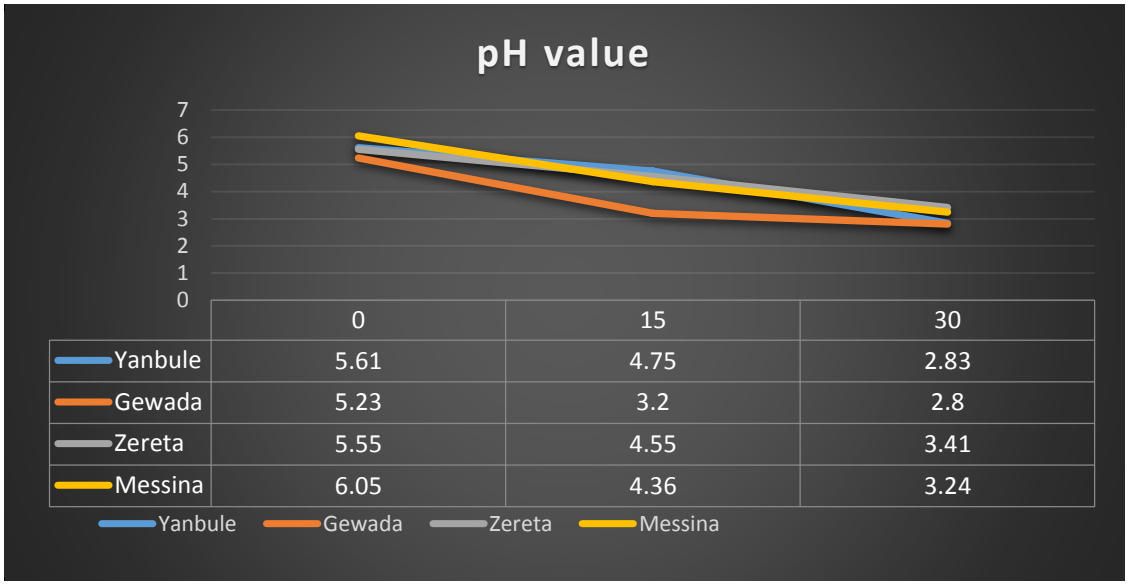

Figure 16. The pH value of 0, 15, and 30 days fermented bulla

4.6. Sensory Quality

The data from this experiment indicate that there was relationship between the bulla quality parameters and sensory evaluation results.

4.6.1. Sensory Quality for porridge

The prepared bulla porridge had good taste, colour, texture, aroma and overall acceptability. The highest mean score for porridge colour (8.6), texture (8.9), taste (8.4), aroma (7.2) and overall acceptability (7.6), was for *yanbule* except the aroma of *messena*. The lowest mean for colour (7.1), texture (7.2), taste (6.6), aroma (5.7) and overall acceptability (6.9) was obtained for *zereta* except texture to *messena*. As the fermentation time of bulla increased, its colour acceptance also increased. The fermenting microorganisms degrade some of the complex carbohydrates, pigments from the cellular structure of pulp, by producing active enzymes. With regards to the taste, as the fermentation time of bulla increased, its flavour level also increased. Bulla porridge prepared from *yanbule* variety showed good sensory profile and overall acceptability and the reverse is true for *zereta* variety.

Table 11: - Sensory quality of fermented bulla for 30 days among varieties

Porridge					
Variety	**Sensory attributes'**				
	Taste	Colour	Aroma	Texture	Overall acceptability
Yanbule	8.4±0.52[a]	8.6±0.52[a]	7.1±2.13[a]	8.9±0.32[a]	7.6±2.27[a]
Gewada	6.7±2.12[a]	8±1.89[a]	6.1±2.42[a]	7.9±1.85[a]	7.4±1.26[a]
Zereta	6.6±2.32[a]	7.1±1.91[a]	5.7±2.67[a]	7.7±2.26[a]	6.9±2.32[a]
Messina	8±1.33a	8.5±0.97[a]	7.2±2.15[a]	7.2±1.99[a]	7.5±2.42[a]
Kofeme					
Yanbule	9.0±0.00[a]	8.7±0.95[a]	8.2±1.23[a]	8.4±1.26[a]	8.6±0.52[a]
Gewada	8.9±0.32[a]	8.8±0.42[a]	8.1±0.74[a]	8.2±1.03[a]	8.6±0.52[a]
Zereta	8.4±0.97[a]	8.7±0.67[a]	8.1±1.52[a]	8.6±1.26[a]	8.5±0.97[a]
Messina	8.7±0.48[a]	8.5±0.97[a]	8.3±1.25[a]	8.4±1.26[a]	8.6±0.97[a]

4.6.2. Sensory Quality for Kofeme

Kofeme prepared from the fermented bulla varieties had good taste, colour, flavour, texture, and overall acceptability. As the result indicated in table 11, the highest value was recorded (9.0) taste, (8.8) colour, (8.3) aroma, and (8.6) texture for bulla prepared from yanbule, gewada, messena, and zereta respectively. The overall acceptability of kofeme prepared from yanbule gewada and messena had the highest mean score (8.6). When the overall acceptability of kofeme is compared from overall acceptability of porridge, kofeme had better acceptance and yanbule had better quality than the rest of the varieties in case of both foods.

5. Conclusion and Recommendations

5.1. Conclusion

In this nutritional study, bulla prepared from four varieties of enset were analysed for six parameters. Four newly introduced cultivars of Enset (Enset ventricosum) grown in Ethiopia were collected and analysed for the parameters listed below. Moreover, the effect of fermentation time (0, 15, and 30 days) on the parameters of interest for all bulla was observed. The parameters analysed were proximate composition, anti-nutritional factors, mineral composition analysis, physicochemical characteristics, functional properties, and sensory analysis.

Bulla can be a good source of dietary energy and essential minerals, but it contains some antinutritional factors particularly phytate. In this study, tannin was below the detection level especially after fermentation. The presence of phytate in bulla may have deleterious effects on the absorption of nutrients and micronutrients. The presence of antinutritional factors in bulla products may limit its utilization for human consumption.

Among the varieties studied, *gewada* had better nutritional value in fat, fiber, carbohydrate, energy, and in iron content than the rest of the varieties. On the other hand bulla prepared from *zereta* and *messena* were found to be high in protein content. Calcium was better obtained from bulla prepared from *yanbule*. Combining all the results of this study, the most commonly used fermentation had resulted in tremendous reduction of the moisture, fibre, pH, phytate, and tannin contents of bulla. Moreover, it also enhanced the protein, fat, ash, carbohydrate, total energy, mineral, and titratable acidity of the raw sample.

In conclusion, extending the fermentation time and different Enset variety had resulted in differences in chemical composition, physic-chemical characteristics, and sensory attributes of bulla. Bulla fermented for 30 days had showed better nutritional quality and lower anti-nutritional factors. Bulla prepared from *yanbule* and *messena* varieties were better in physic-chemical properties, chemical composition, and sensory acceptability. Generally, the selection of appropriate varieties and use of recommended fermentation time were found to be good practice for the processing of tradition enset products such as bulla which is a traditional fermented food in Ethiopian.

Even if there is a slight difference among the varieties concerning the studied parameters, in most of the parameters the difference is not significant. Therefore according to this study the

varieties are not as such different in their nutritional, antinutritional, physicochemical and functional property of bulla.

5.2. Recommendations

- Mostly, commercially available bulla flour are prepared in their raw form, which may have phytate. Therefore it is recommended to ferment prior to drying
- Further study should be conducted to evaluate the remaining anti-nutritional factor such as oxalate, cyanide, alkaloids, and the rest.
- Among the improved enset varieties *kelisa* and *endale* were not included in this study, therefore same investigation should conducted for better information.
- As indicated it this research the protein content of bulla was very low, so it is healthier to consume blended with other protein rich food stuff such as legumes and cereals.
- Enset is drought resistant, energy and mineral (K, Na, and Ca) dense food stuff, and has multidirectional purpose. But, nowadays its availability is reduced due to civilization and people starts to cultivate another crops. Therefore, the Ministry of Agriculture and Rural Development should encourage and advocate production and utilization of enset plant.

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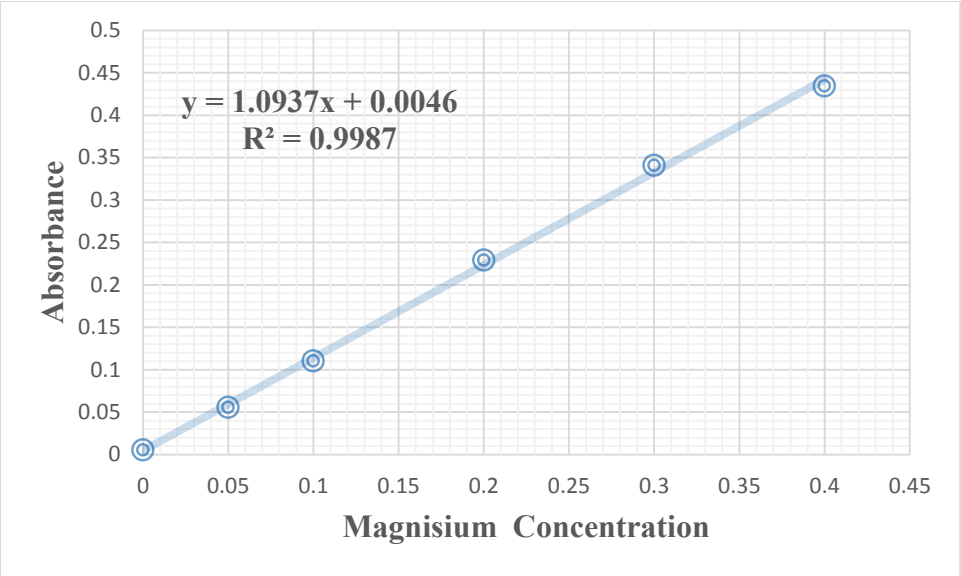
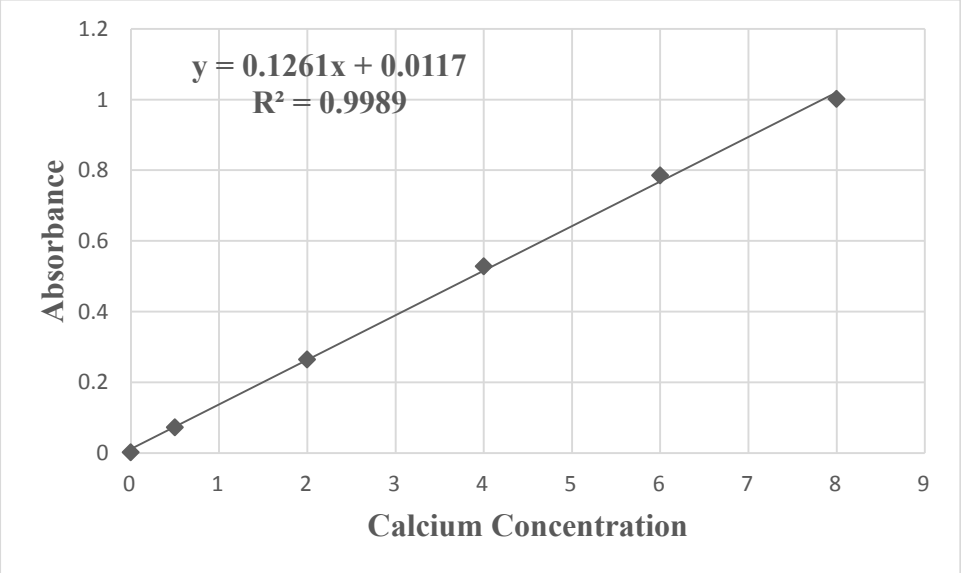
ANNEXES

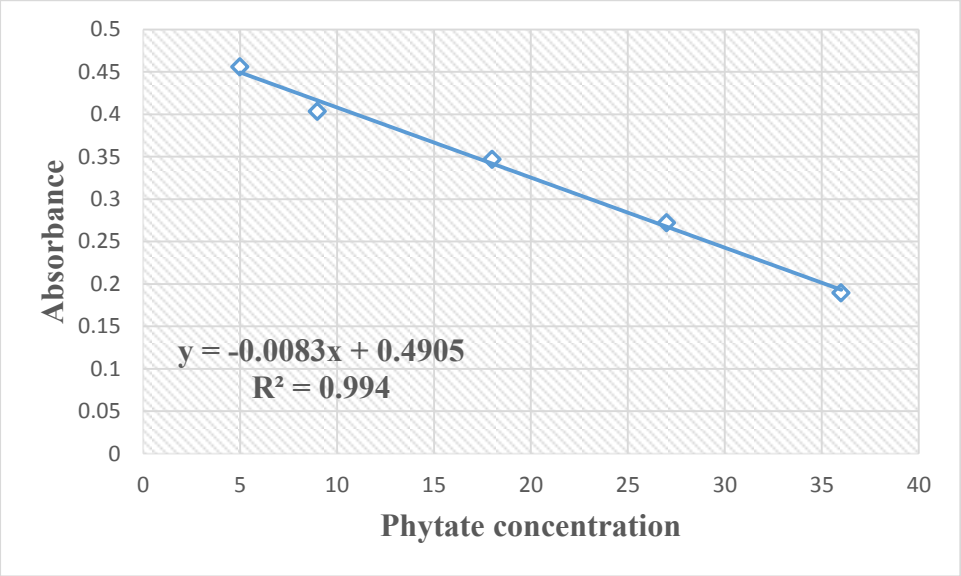
Annex 1. Calibration curve for magnesium



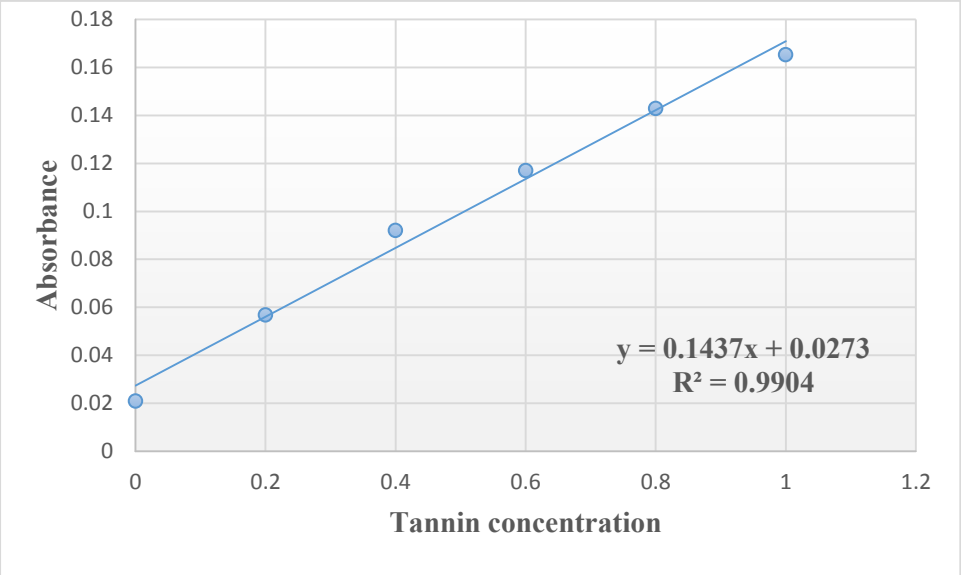
Annex 2. Calibration curve for Calcium



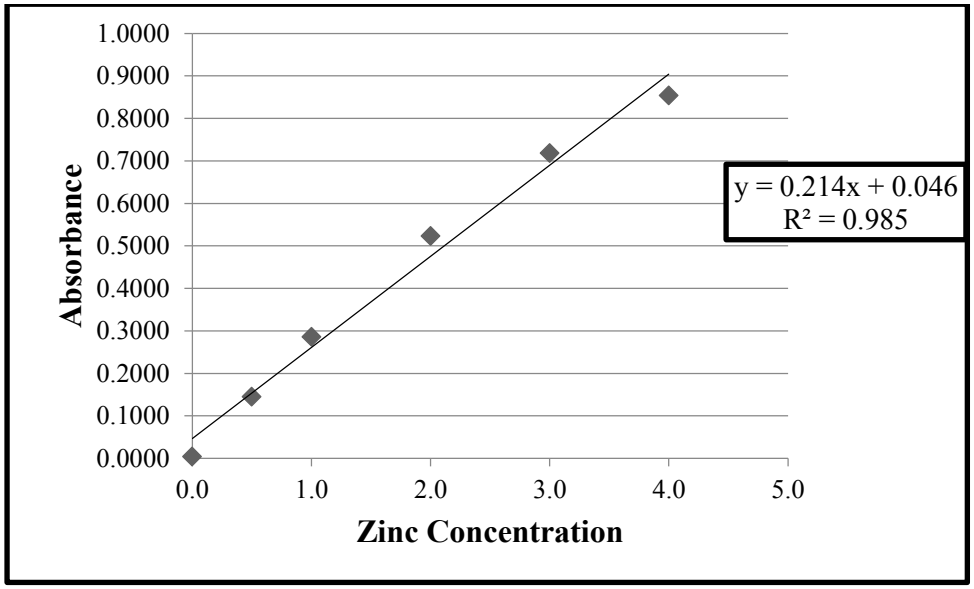
Annex 3. Calibration curve for phytate



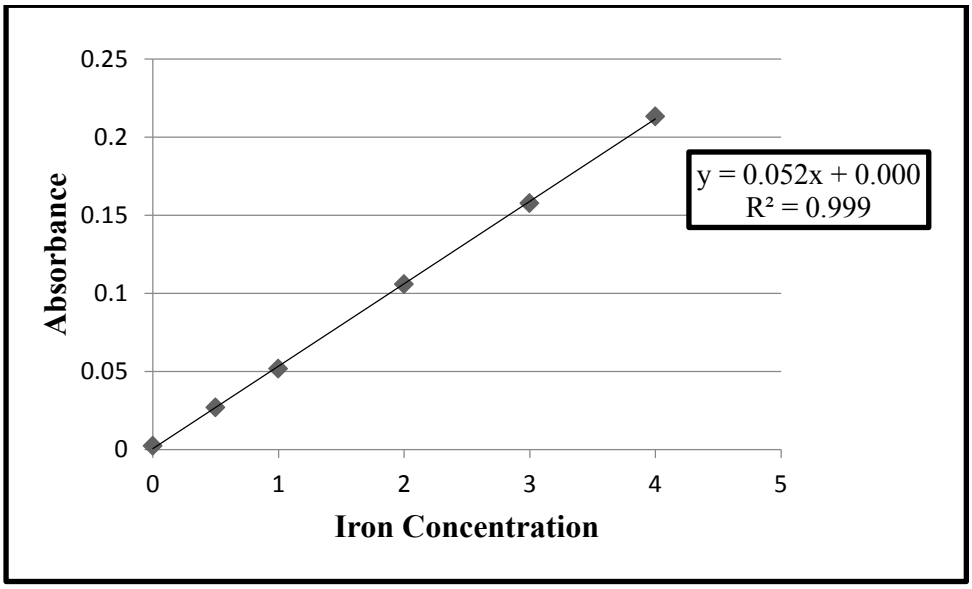
Annex 4. Calibration curve for Tannin



Annex 5. Calibration curve for Zinc



Annex 6. Calibration curve for Iron



Annex 7. Form for Sensory analysis of bulla of different varieties

Personal information

Recipe name: _____

Category: _____

Age: _____

Instruction

Direction: You have been provided with four coded samples, check one rating for each of the following: Colour, Taste, Texture, Aroma, and Overall acceptability.

Rating scale	Color	Taste	Texture	Aroma	Overall acceptability
9. Like Extremely					
8. Like very much					
7. Like Moderately					
6. Like Slightly					
5. Neither Like nor Dislike					
4.Dislike Slightly					
3. Dislike Moderately					
2. Dislike very much					
1. Dislike Extremely					
Office use only					
Panelist code: Date:					

Annex 8. Diagram of *yanbule* variety

Annex 9. Diagram of *messina* variet

Annex 10. Diagram of *zereta* variety

Annex 11. Diagram of *gewada* variety

Annex 12. Diagram of bulla bread

Annex 11. Diagram of kofeme