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Screening food test parameters to detect adulteration of teff (*Eragrostis tef* (Zucc.) Trotter) flour and injera with non-edible adulterants

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This is to certify that the thesis prepared by Biruk Sileshi entitled: "Screening food test parameters to detect adulteration of teff (*Eragrostis tef* (Zucc.) Trotter) flour and injera with non-edible adulterants" submitted in partial fulfilment of the requirements for the Degree of Master of Science in Food Science and Nutrition complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abstract

Teff (*Eragrostis tef* (Zucc.) Trotter) is the main staple cereal in Ethiopia that is used to prepare a fermented flatbread with a unique slightly spongy texture named injera. Majority of Ethiopian diets consist of injera which accounts for about two-thirds of the daily protein intake of the Ethiopian population. Recently, adulteration of teff flour and injera with non-edible ingredients is becoming a serious problem putting the consumers' health at risk. Therefore, the main purpose of this study is to screen some of the common food analyses test parameters that could detect non-edible adulterants in teff flour and injera for potential use in future routine inspection analysis.
In the study, simulation of the adulteration was made on two widely distributed white teff varieties, namely Quncho Teff (DZ-Cr-387) and Magna Teff (DZ-01-196). Three adulterants were selected based on the information gathered from local health bureau, namely Sawdust (organic adulterant) and two inorganic adulterants, chalk powder (calcium carbonate) and gypsum (calcium sulphate dihydrate). Based on the information gathered, the teff to adulterant ratio was (7:3). Thus, the primary investigation was conducted on this adulteration ratio. Accordingly, the analytical tests at this adulteration ratio resulted in a significant mean value difference between the adulterated and control teff flour samples.

Secondary investigation was done by preparing injera using lower ratio of teff flour to adulterants (9.5:0.5), (9:1), (8.5:1.5), (8:2) and (7.5:2.5), while unadulterated (100%) teff flour injera was used as control. Ratios resulting in acceptable injera appearance were used as cut-off adulteration ratios to apply the selected test parameters. The final investigation was conducted using the cut-off adulteration ratio, on which the selected test parameters were applied to compare significant mean differences ($p < 0.05$) between control and adulterated teff flour and injera samples.

The overall results of the physicochemical analysis showed that some parameters like crude ash, and crude fibre, and mineral analysis of calcium could be used as detection test parameters for samples as low as 5% adulteration ratio. Qualitative tests such as effervescence and precipitation are also effective tests to detect adulteration. Therefore, this study provides test parameters for preliminary teff flour and injera adulteration detection, which can further be developed by concerned bodies to come up with standard analytical procedures for the detection teff and teff injera with the tested and other similar adulterants.

Dedication

I dedicate this work for my beloved family…

My mother Aster Atlaw for her God-given, unconditional love…

My sisters (Helen, Bethlehem and Eyerusalem) and my brothers (Mitiku and Fitsum)

for their unfailing love and support throughout my life...,

Which is constantly building in me a special character of love, kindness and respect

for the Creator and all of His creation

Acknowledgment

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Most of the work in this thesis, if not all, was accomplished with the provisions made by the Centre for Food Science and Nutrition (CFSN) of Addis Ababa University and therefore I would like to thank the Centre and all staff personnel for their contribution. Preliminary investigation of this study was carried out based on information obtained from Bole Sub-City Health Bureau for which I thank Mrs. Demissie M and all health officers in the bureau for their co-operation.

During the end of class courses, I faced unexpected illness and was hospitalized. My instructors at the time, Dr. Kaleab Baye and Dr. Zelalem Debebe kindly considered my situation and gave me enough time to write and submit my term papers. I thank them dearly. I also thank Pastor Wendessen Tadesse and all people from the local Apostolic Church of Ethiopia for their prayer and encouragement.

Others who deserve my gratitude are my dear friend Mrs. Tirsit Belay who helped me bake injera samples in a good traditional way and my brother Mr. Tariku Tesfaye, for transporting baked injera samples from our residence to the laboratory at CFSN. I am very grateful to my mother Wro Aster Atlaw and my brother Fitsum Seleshi for their dedicated support throughout the work.

For all of you (including those whom I have not mentioned by name), who have somehow contributed to this work in particular, and to my life in general, this is a result of your contribution. Thank you.

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List of Abbreviations

AAU	Addis Ababa University
AOAC	Association of Analytical Chemists
CC	Calcium Carbonate ($CaCO_3$) or Chalk Powder
CFSN	Centre for Food Science and Nutrition
CS	Calcium sulphate dihydrate ($CaSO_4.2H_2O$) or Gypsum (GY)
CSA	Central Statistical Agency of Ethiopia
DNA	Deoxyribonucleic acid
DZARC	Debrezeit Agricultural Research Centre
EFMHACA	Ethiopian Food, Medicines and Healthcare Administration and Control Authority
EMA	Economically Motivated Adulteration
EPHI	Ethiopian Public Health Institute
ESA	Ethiopian Statistical Agency
FAO	Food and Agriculture Organization
SD	Sawdust
SEM	Standard Error of Mean
TM	Teff Magna - DZ-01-196
TQ	Teff Quncho - DZ-Cr-387
UK	United Kingdom
USDA	United States Department of Agriculture
WHC	Water Holding capacity
WHO	World Health Organization

CHAPTER ONE

1. INTRODUCTION

1.1. Background

All human beings consume food on a daily basis and any process that does not work to ensure and deliver safe and sound foods threatens the wellbeing of the consumer. Food adulteration and counterfeiting refers to the addition or mixing of any substance to any food item to increase bulk and gain profit [1-3]. Though food adulteration is considered to be as old as food production itself, there has not been a time like the present century in which food demand and supply are unbalanced, giving rise to different kinds of strategic food frauds for economic gain [4-6]. Food adulteration also includes, the preparation of food products by adding harmful substances that affect food quality and human health [7], and also making food similar with another food product of different manufacturer by imitating packaging and trade mark. These are viewed as intentional adulteration and has legal consequences from concerned regulatory bodies [2].

Intentional food adulteration can be viewed in two ways. The first involves the addition of food items into another food product (such as food-to-food fortification) and is aimed at improving the nutritional content and sensory characteristics of a food product [8-10]. Another kind of food adulteration is aimed at making economic profits through the intentional mixing of edible or non-edible materials to marketed food items [11, 12]. The former is based on a pure motive for improving overall food quality and needs active collaboration, in contrary, the latter being a fraud posing risk to the consumer, needs to be detected, controlled and eliminated. Unintentional food adulteration refers to the mixing of food with other items during and after production, both at agricultural level and consequent handling of agricultural products in the market chain and preparation of food, and is usually considered as a contamination [12, 13].

Most widely adulterated food items include honey, milk and milk powders, edible oils, flour based foods, spices and beverages. There were typical food adulteration instances worldwide which surfaced and brought about renewed international awareness on the growing incidents in food adulteration and related systematic fraud [11]. On its official website, the International Police (Interpol) has published various incidences of food fraud which were being apprehended with collaboration of concerned authorities in the regions. Among the food items seized include nine tonnes of sugar adulterated with fertilizer in Sudan, more than 85 tonnes of olives painted with copper sulphate solution to enhance colour, and other counterfeit food and beverages were also seized and destroyed by the organization. Many other incidences have also been reported by Interpol to create awareness on the seriousness of the issue [14]. The 2007 WHO global report indicated both intentional and unintentional food contamination as one of the leading health threats for modern society [7, 15].

Food adulteration has been in practice for generations within individual households primarily for the improvements of sensory characteristics. Good enough, it has been done within the moral compass of the society [16]. In contrast, current food frauds in the country aimed at economic gain are done through direct violation of both the moral and legal compass of the society. With this regard, recent food adulteration incidents in Addis Ababa city on commercial injera preparations, which was broadcasted through different multimedia and state-owned broadcasting corporation, has rang a bell for concerned bodies and institutions to take efforts in preventing harmful food adulteration practices and to ensure the public at large is not exposed to unsafe foods [17, 18]. Teff injera is widely consumed throughout Ethiopia and contributes roughly 600 and 200 kcal energy per day in urban and rural areas respectively [8]. It is one of the most highly traded commodities in the food market and is highly demanded for both local consumption and export. Hence, any threat due to adulteration of teff flour should not be left unstudied [6].

As mentioned above, for quite some time, teff flour has been used in combination with other grain flours to improve nutritional and sensory characteristics [8]. In contrast, the recent adulteration incidences in teff injera preparation using non-edible adulterants gypsum and sawdust, are illegal acts. These adulteration acts were directly aimed at gaining profit by mixing non-edible and potentially harmful materials to teff flour used in injera preparation [19].

The techniques employed in these fraudulent acts are systematic. Based on the assumption that the fermentation might mask the presence of the adulterant, the adulterant paste and teff dough were mixed and fermented together before baking. This makes the adulterated bake indistinguishable from the normal bake (Demisse, M., personal communication, June 21, 2016). Therefore, studies to develop or screen existing food analyses techniques, which can detect the adulterants, is necessary. The problem is still aggravated, that recently there were new cases on criminating teff flour and injera in Addis Ababa city [18, 20]. Therefore, the main purpose of this study was to screen food composition test parameters that can easily detect or indicate the presence of non-edible adulterants in teff flour and injera.

1.2. Statement of the problem

Teff is used for human consumption as injera in many parts of Ethiopia. Due to population growth and subsequent increase in the demand for healthy foods, the price of teff is increasing [19, 21, 22]. Also, due to constant change in the lifestyle of individuals living in urban areas and busy life schedule, people are forced to buy injera commercially rather than preparing at home. This is most evident in the increased market demand for baked injera and the increasing number of injera producers and exporters [6]. This has made regulatory activities difficult with the growing demand and supply with a growing number of unaccounted teff injera suppliers [23, 24]. This has made teff flour and injera adulteration very serious.

In fact, the production, commerce and consumption of teff injera is not a new practice in Ethiopia, and it is usually accompanied by trust on behalf of both the producers and consumers [25]. The interpersonal trust is nowadays betrayed by some individuals primarily for economic gain through fraud. As a recent consequence, residents of Addis Ababa city were exposed to adulterated commercial injera products. The incidence was reported by state-owned media like Ethiopian Broadcasting Corporation (EBC) and other communication media such as Fana Broadcasting Corporation (FBC), and through radio and internet [20]. The adulterants were mixed with the flour and pasted ahead of fermentation. This will mask adulterant presence in the final baked injera by making them similar with normally baked injera. It is the Ethiopian Food, Medicines and Health Care Administration and Control Authority (EFMHACA) that is responsible for the issue under discussion [26]. The concerned authority (EFMHACA) has ceased and destroyed unsafe and adulterated food items with co-operation of residents. Though a design has been put for the control of food safety, it has not been implemented thoroughly due to lack of capacity in terms of resources and technical issues and the authority mostly depends on the information from residents and whistle-blowers [23, 27].

Once the news was disseminated, the investigator of this study gathered more information from sub-cities where the criminated food was exhibited. According to the report and statistics division of Addis Ababa Police Commission, the authorities have seized, in five sub-cities (Lideta, Kolfe-

Keranio, Yeka, Gulele and Bole Sub-cities), a large portion of adulterated injera baking stores, which produced and distributed more than one thousand injera pieces per day to different restaurants and hotels [17, 20]. As per the police information, the adulterants used in the injera production were of two kinds; edible and non-edible. The edible adulterant was cassava powder (occasionally known in Amharic as 'yeferenj inchet'), seized in Bole Sub-City. The non-edible adulterants recently seized by the authorities in Bole, Lideta and Sebeta sub-cities included sawdust (in Amharic known as "Sagatura"), chalk powder (in Amharic known as "nora") and gypsum (locally known as "jesso" (Demisse, M., personal communication, June 21, 2016).

One of the major challenges in adulterated food products is the difficulty to distinguish them from the non-adulterated products. Similarly, as it was observed in recent incidences, the adulterated injera products were indistinguishable from the normal bake. Unless, the criminals are caught red-handed, police investigation faces difficulties to detect the adulteration. This is also the major reason why food fraud in teff injera has not been detected by regulatory body. Instead, it is usually through the cooperation of city residents and whistle blowers that such incidences were brought to the surface and dealt by concerning government bodies [18-21]. Thus, intervention from both government bodies and the scientific community is necessary. It is straightforward to assume that if adulterant detection test parameters or methods are screened and applied in inspection process, it will reduce the incidences of criminating food items and will help to bring the criminals to justice. This will also help in the prevention of further food fraud acts that may spread and put consumers at risk.

Hence, screening basic food analysis tests that would indicate or detect the presence of the adulterants both in the flour and injera is necessary. So far, as to our knowledge, there are no studies conducted on screening teff flour and injera adulteration detection methods. Therefore, this study will help to screen some basic analytical techniques or test parameters that can be used in detecting adulterated teff flour and injera with non-edible adulterants including chalk powder, gypsum and sawdust. Regulatory bodies need reliable and accurate analytical tools to identify and eliminate food fraud. As part of this effort, the present study was planned to address the following research questions in general:-

i. Is it possible to use the non-edible adulterants (found in recent food fraud incidents) in injera preparation and produce acceptable quality injera to the market?

ii. What kind of physicochemical analytical techniques could be employed to identify teff flour and injera products prepared with non-edible adulterants?

iii. Which one of the chosen non-edible adulterants can be used at higher ratio to produce a closely related injera character with unadulterated injera?

iv. What are the lowest adulteration ratios that could be detected using the screened analytical techniques?

1.3. Objective

1.3.1. General objective

To screen common food composition analyses techniques that can detect adulterated teff flour and injera with non-edible adulterants.

1.3.2. Specific objectives

- To investigate the possibility of making injera using the reported non-edible adulterants
- To screen physico-chemical test parameters that can detect teff flour and injera adulteration with a maximum adulteration ratio of 30%.
- To select the non-edible adulterant that can be used at higher ratio to prepare indistinguishable injera from the normal bake
- To determine the minimum detectable concentration of adulterant with teff flour or injera based on the screened test parameters.

1.4. Scope, significance and limitations of the study

The current study focuses on food adulteration incidences observed in the last few years in Addis Ababa city and how to solve these issues by presenting reliable test parameters in the analytical procedures of adulterated samples. Among the wide range of adulterants that may be encountered, only three non-edible adulterants were selected based on information gathered from local health bureau. And only two widely consumed teff varieties were used.

There is lack of research articles on adulteration detection in teff flour and injera. This is the first research so far directly related to teff adulteration detection. Due to lack of previous work related to teff adulteration detection, the test parameters screened are the basic test parameters in food analysis.

The scope of this study is also limited to Addis Ababa city and its surroundings but the outcome can be applied as a reference for future incidences and as an alternative approach for any existing analytical technique in food science laboratories.

The scope of this study is also limited in many ways. The case of adulterated teff injera incidences are not few, but well documented studies in the detection techniques are very scarce.

However, this study has many limitations. Test samples were preparations made from two teff varieties only. Other widely consumed teff varieties could not be included in the study. Test parameters such as pH, fermentation kinetics, diameter and weight of injera and number of eyes of injera, food energy and microbial tests and toxicology were not performed. The main setback was economical reason and also the limited time which would not allow to entertain all of the tests listed. These and other tests could be performed by other researchers interested in the area.

Therefore this study should not be considered as comprehensive or inclusive. It doesn’t address all problems associated with teff injera adulteration nor the socio-economic implications and regulation process by concerned authorities. This work will remain open to suggestion, comments and improvements for better use.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. History of food adulteration

History of food adulteration throughout the modern world can be put in three different stages. From the time of ancient civilizations to the beginning of the early 1800, in which food adulteration was not a serious problem with little need for methods of detection. The main reason was because food was procured from small businesses or individuals, and transactions took place through interpersonal accountability [28].

The second stage of food adulteration began in the early 1800s, when deliberate food adulteration was amplified greatly in both occurrence and seriousness. This development was associated with increased centralization of food processing and distribution, with further weakening of interpersonal accountability and partly to the rise of modern chemistry [28-30].

The early 1800s was a time of deep public concern over quality and safety of food supply in Europe. This concern was aroused in England with the publication of two research articles “A Treatise on Adulteration of Food and Culinary Poisons” and “Death in the Pot” by the German chemist Frederick Accum (1769 – 1838) [31]. In these publications, Accum indicated the severity of adulterated foods in the market. He also outlined the role of chemistry as it was perverted into play for adulteration of food [30]. The main purpose of adulteration is to gain economical profit through the improvement of weight, colour, appearance and taste of a food product, usually by mixing it with non-edible materials and chemicals. Thus intentional food adulteration remained a serious problem until about 1920, after which regulatory pressures and effective methods of detection reduced the frequency and seriousness of deliberate food adulteration to tolerable levels, and the situation has gradually improved due to laws and regulations set up by authorities [28, 32].

Food adulteration, eventually remained systematic and hidden in many traded food items in the current modern world even through the past few decades. Melamine and cyanuric acid in pet-food were reported in 2004 and 2007 to have caused renal failure, sickness and death of many cats and dogs in Europe and the United States. The Food and Drug Administration of USA, after several inspections in its laboratories, banned foods contaminated with melamine. Consequently, the analysis of similar protein foods continued in major producing countries, and in 2008, Chinese authorities discovered milk and infant formula adulterated with melamine. There were six confirmed deaths with hundreds of thousands of victims. Also, there was also a mass recall of products in many countries [33, 34].

Food adulteration varies widely among hundreds of food items and in different factors including geographical origin, purity, economic and health impact, level of sophistication and difficulty of identification. Some kinds of food adulterations cause little harm while others can cause serious harm including loss of hundreds of lives [35]. The main motive behind food adulteration is economic profit, usually known as economically motivated adulteration (EMA), is the major kind of food fraud globally [36].

In Ethiopia, food adulteration for economic reasons are primarily spotted by consumers and dealt by the law enforcement. Recently, there have been a number of reports by government bodies and communication media about food frauds and subsequent measures taken by authorities [18]. In the past, the deliberate and unhygienic adulteration of edible oil with expired oils, the mixing of banana with butter and the adulteration of pepper flour with red brick powder were among the food frauds encountered (Demisse M, personal communication, June 21, 2016).

The most recent adulteration of teff flour and injera in Addis Ababa city was quiet new and exceptional in its kind. According to local health offices and communication media, regulatory bodies have apprehended teff flour adulterated with edible and non-edible materials (sawdust and gypsum) in Addis Ababa city [37]. Samples collected by sub-city regulatory workers have been studied by the concerned regulatory body and some individuals responsible for the adulteration act were subsequently tried and sentenced. According to Bole Sub-City Health Bureau worker, Demisse M. (personal communication, June 21, 2016), samples were analysed and toxicity studies have shown that the foods were unfit for human consumption.

2.2. Commonly adulterated food items

Among the widely distributed food products and agricultural commodities, there are common food items prone to recurrent adulteration duet to the ease and lesser possibility of being detected by consumers. Dairy products such as milk, cheese and butter and grain flour, honey, coffee and tea, olive oil, alcoholic drinks and beverages are among the most widely adulterated food stuffs (Fig.

1). According to the United States National Centre for Food Protection and Defense (NCFPD) database, EMA is the driving force for most of the food fraud incidences observed [12, 36].

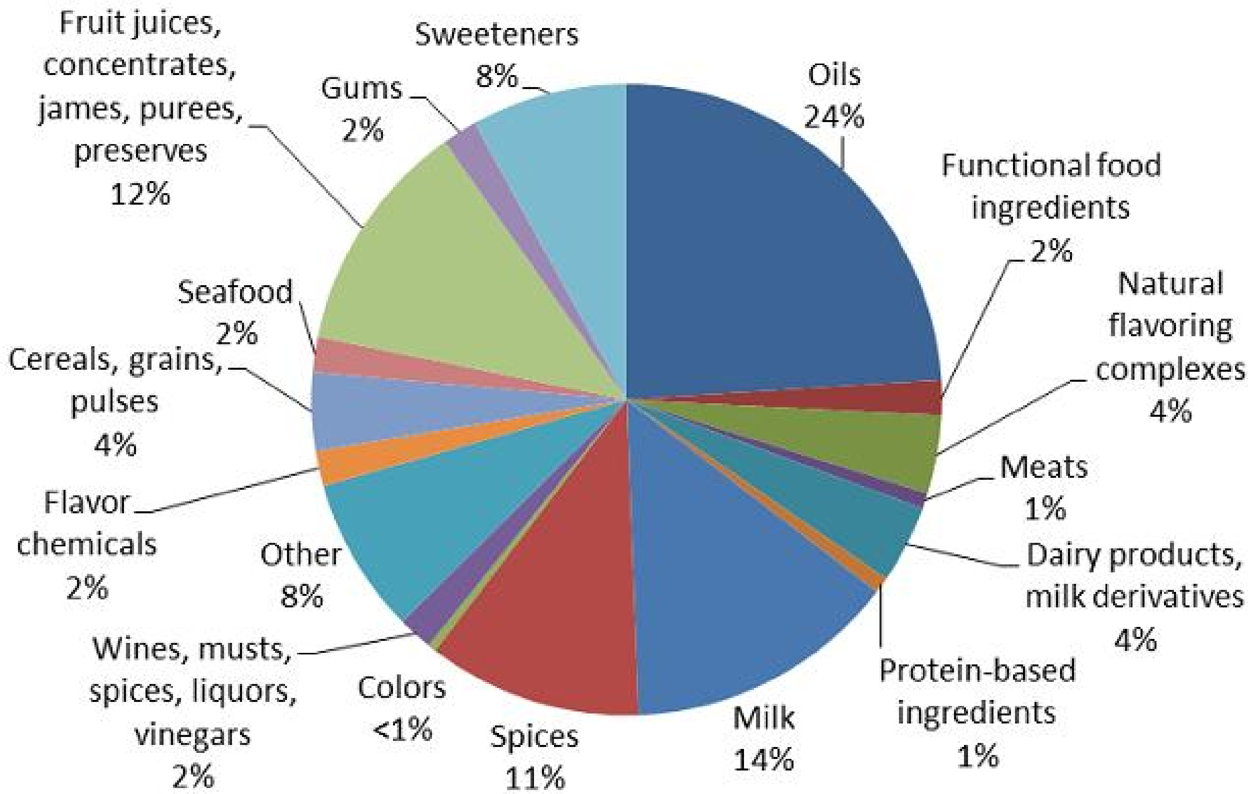

Figure 1: Leading reported types of food frauds (USP Food Fraud Database, 2010)

According to the annual report of incidents by Food Standards Agency of the United Kingdom, for two consecutive years of 2011 and 2012, India, China and Bangladesh had the highest records of food fraud incidents [38].

Similarly, food fraud in Ethiopia has become a challenging issue with growing number of incidents reported in different kinds of independent and government media outlets. In the duration of the present study, attempts have been made by the candidate to gather information on food fraud incidences in Ethiopia. Unfortunately, due to lack of co-ordination between concerned government bodies and lack of awareness within the community at large, information related to food fraud incidences and associated health issues have not been aggregated and presented in a meaningful manner, nor stored in databases for the purpose of communication and research.

Amongst the few food adulteration incidences studied in Ethiopia include the epidemic dropsy in Addis Ababa city during May – June 2008. The study published in 2013 was conducted by researchers at Ethiopian Health and Nutrition Research Institute (now EPHI), and it confirmed that edible oil contaminated with argemone oil was the primary cause of the epidemic dropsy in the city [39]. In another research conducted to evaluate the quality of milk in and around Bahir Dar city, published in the June 2015 edition of Ethiopian Journal of Science and Technology, it was found that milk consumed by rural and peri-urban children was not adulterated, whereas milk consumed by urban children was adulterated. The research concluded that adulteration reduced the quality of milk [40].

The adulteration of teff flour and injera is a serious problem as it is a majorly consumed food item in Ethiopia. The basic nutritional information on teff and the nature of some non-edible adulterants are discussed below.

2.3. Teff

Teff, scientifically known as *Eragrostis tef* (Zucc.) Trotter, is a member of the grass Family Poaceae (Graminae) and indigenous to Ethiopia [41]. The Ethiopian Standards Agency (ESA) has classified the major teff grain varieties and their specifications in four grades as very white (Magna teff), white (Nech teff), brown (Key teff) and mixed (Sergegna teff). Compared to many other cereals and crops, teff grain is very small in size, and the colour of teff grains vary widely [42].

2.3.1. Origin and geographic distribution of teff

Several endemic and indigenous species of Eragrostis exist in Ethiopia. The genetic diversity for teff exists nowhere in the world except in Ethiopia, proving that teff originated and was domesticated in Ethiopia and north-eastern parts of Africa [43]. Though the exact time and place of teff domestication is not known, some writers have indicated that teff has been domesticated in Ethiopia even long before the birth of Jesus Christ. Teff seeds were also in use in ancient Egypt such as in the Pyramid of Dashur and ancient Jewish city of Ramses in Egypt [41].

Teff is a highly adaptable grain species which can grow from sea level up to 2950 meters. Teff grows and performs well between an altitude of 1700 and 2400m. Teff can grow both in areas of high and law rainfalls. It has been introduced to different parts of the world through different occasions. The Royal Botanic Gardens, Kew, imported seed from Ethiopia in 1866 and then it was distributed to India, Australia, the USA and South Africa. In 1916, it was further introduced into other regions in Africa and Asia [21, 44].

In the exhaustive search and study made to trace the origin, domestication and species of teff and other cereals, the survey of collection of different Eragrostis species at the Royal Botanic Gardens, Kew, proves the differences in teff plant taxa in Africa, Asia and Europe. With the detailed explanation of the presence and diversity of teff species in these major continents, it is possible to give sufficient evidence that teff species were originally domesticate in north-eastern parts of Africa (Egypt, Sudan, Ethiopia, Uganda and Kenya) [41].

While teff is a widely cultivated indigenous crop in Ethiopia, it is also cultivated in other East African countries such as Sudan, Rwanda and Uganda, and in South Africa. It is also being cultivated in areas as diverse as the dry mountains of Idaho in USA and low wetlands of the Netherlands [45, 46].

2.3.2. Production and utilization of teff in Ethiopia

According to the Central Statistical Agency of Ethiopia (CSA), the total annual teff production for the year (2015/2016) in Ethiopia amounts to 44,713,787 quintals with a utilization of (54% for household consumption, 13% for seed and 28% for sale), making teff the most produced and utilized next only to maize [47].

Teff is not easily attacked by weevils and other pests during production and storage, resulting in lower cost of postharvest management cost. Teff has an excellent resistance to moisture stress [41, 43], which enables farmer to use it as a rescue crop, and is also suitable for multiple cropping. The straw is also a valuable feed for cattle in drought seasons, and cattle prefer teff straw over straw of other cereals. Teff can be grown in waterlogged areas and withstands anaerobic conditions better than most cereals including maize, wheat and sorghum [41]. Teff also has a great acceptance in the national diet with growing demand both domestically and abroad enabling farmers to earn more than with other crops. Compared to most cereals and crops teff is the least attacked by disease and pests making it a low risk crop than other cereals and crops in Ethiopia [6, 43].

With all the advantages it has, teff also has some limitation due to the small size of grain [43, 46] which brings difficulties during its production. It makes sowing and weeding difficult for traditional farming practice and even for mechanical farming, which sometimes leads to hand weeding or herbicide use and manual harvesting of the grains [43].

Teff grains are ground into flour which is mainly used for making the traditional Ethiopian bread called “injera” (sometimes spelled as enjera), which is a flat, round and soft bread baked after fermentation. Injera is usually consumed with “wot”, a traditionally prepared sauce, which is prepared from flour of ground pulses or beans which are usually prepared with meat and other vegetable sauces [48].

2.3.3. Chemical composition of teff

2.3.3.1. Protein

The protein content of teff grain ranges between 8.7% and 11.1%, which is comparable to other cereals like wheat (10.3%), sorghum (7.1%), maize (8.3%) and millet (7.2%) [46, 49, 50]. Teff is free of gluten, a protein composite found in cereals wheat, rye, barley and some oat varieties. Some people have gluten intolerance as an autoimmune response to the consumption of foods containing gluten. This hereditary disorder is called celiac disease and the current therapy for it is a gluten free diet. This makes teff products alternative therapeutic foods for people suffering from celiac disease [49, 51].

Teff flour has a favourable composition of amino acids and easily digestible protein compared to other cereals like maze and sorghum. This is because teff protein is composed of the most digestible types such as glutelin, albumin and globulin [50]. These proteins could be used as important regulators in defining texture of food, with ability to serve as binding, thickening, gelling and emulsifying agents [46].

2.3.3.2. Fat

The crude fat content of raw teff grains is 2.3% on average, which is similar to wheat (2.47%), sweet corn (1.18%), and barley (1.16%) but slightly lower than millet (4.22%). Specifically, the total saturated fatty acids in teff amount to 0.5%, total monounsaturated fatty acids (0.6%), total polyunsaturated fatty acids (1.07%). Among the unsaturated fatty acids, oleic acid takes the largest part (32.4%) followed and linoleic acid (23.8%) [52]. These unsaturated fatty acids are crucial in human nutrition as these are essential (cannot be synthesized by human body) and need to be provided in the diet [49].

2.3.3.3. Carbohydrate

Teff grain contains about 80% complex carbohydrates, which is enough source of energy in human nutrition. Starch (37%) is the main source of carbohydrate in teff (73%) out of which amylose content on average is 23%. Teff starch granules have been found to have lower in vitro digestibility than wheat starch, giving teff a lower glycaemic index than wheat. This along with high fiber content of teff is advantageous in controlling blood glucose levels to prevent diabetes [46, 49].

2.3.3.4. Fiber

According to the National Food and Nutrient Analysis Program of USDA (2005), teff contains a total dietary fiber of 8%, which is similar to millet (8.5%) but much lower than barley (15.6%) [53]. Since teff flour is whole containing bran and germ, it usually has higher fiber content. This brings many health benefits in that high fiber diet helps to prevent many serious illness such as coronary heart disease, diabetes and colon cancer [10, 46].

2.3.3.5. Amino acids and vitamins

Teff has a good balance of amino acid composition, including all 8 essential amino acids, making it an ideal source of food and starting material for malting and brewing. Teff contains higher amounts of the essential amino acids than wheat and thus is regarded more nutritious than wheat. The lysine content of teff (3.68%) is higher compared to other cereals [54]. Teff grains also contain some of the B vitamins such as thiamine, riboflavin, niacin and pyridoxine and a small amount of fat soluble vitamins such as vitamin A, vitamin K and vitamin E [46, 54].

2.3.3.6. Moisture, ash and minerals

The moisture content of teff grain is about 8.82%. Teff ash content differs between varieties, with an average value of 2.3 -2.8%, higher than most cereals such as wheat (1.7%), barley (1.1%), maize (1.4%), brown rice (1.4%), sorghum (1.6%) and pearl millet (1.7%). In general, teff is a richer source of minerals than most cereals [52].

There is a difference in mineral content of different *Eragrostis* species. The iron content of teff has been studied frequently with varying results. Some authors also suggested that the relatively higher iron content was due to contamination during the process of production and harvesting. But controlled studies have proven that teff has relatively higher iron content than most cereals and staple crops [10, 50, 55].

Table 1: Minerals in Teff, (Eragrostis tef (Zucc.) Trotter)

Constituent	Value (mg/100g)	Standard error	1 Cup = 193.0 g
Calcium, Ca	180.00	3.61	347.00
Iron, Fe	7.63	1.42	14.73
Magnesium, Mg	184.00	3.06	355.00
Phosphorus, P	429.00	18.00	828.00
Potassium, K	427.00	13.53	824.00
Sodium, Na	12.00	2.67	23.00
Zinc, Zn	3.63	0.19	7.01
Copper, Cu	0.81	0.08	1.56
Manganese, Mn	9.24	1.22	17.83
Selenium, Se	4.40*	1.11	8.50
*µg/100g Source: USDA National Nutrient Database for Standard Reference, May 2016			

2.3.3.7. Phytochemicals:

Teff also contains considerable amount of phytates, tannins and polyphenols including iron binding phenolic compounds and phenol acids [49]. The United States Department of Agriculture (USDA) National Nutrient Database lists in detail the overall constituents of teff studied and documented so far. Since teff is gluten free, it is being studied frequently for various food formulations which curb celiac disease [51]. And according to the United States Department of Agriculture National Nutrient Database (May 2016), uncooked teff contains most of the major minerals iron, zinc, copper and manganese and others, and also most of the B vitamins, selenium and all of the eight essential amino acids [52].

2.4. Types of adulterants

2.4.1. Edible adulterants

Food adulteration is usually aimed at gaining economic profit and the practice is not aimed at harming customers. So far there is no hard evidence for adulterated foods with the intention of harming consumers. Most edible adulterants are added to increase the bulk weight of products [11]. Thus adulteration usually involves the intentional mixing of edible but cheaper food items to expensive food items for economic gains. For example coffee is relatively expensive than wheat, barley and most cereals. These cereals may be roasted and powdered and used in some proportions to adulterate coffee flour [33, 56]. Most edible cereals such as flours of rice, maize and wheat are used as adulterants in other cereal based food preparations. Potato flour is also used as a bulk additive in different wheat bread preparations [57].

Previously used tea leaves along with dried leaves of various plants have been used as adulterants to expensive tea brands. Milk has also been adulterated with water in many occasions to increase volume. Water addition increases bulk or volume to the product but it causes degradation of nutritional content that the consumer ought to get from the unadulterated product. The overall effect of edible adulterants is the reduction of essential food components which sometimes may lead to malnutrition and even toxicity due to the different mixture of components [33, 57].

2.4.2. Non-edible adulterants

The non-edible adulterants employed in foods vary greatly. Some are chemicals usually employed to improve taste and appearance of foods for imitating the nature of unadulterated foods. Among the documented chemical adulterants used in colour enhancement include hydrated iron (III) oxide, copper acetate, copper arsenite and other chemicals. Most of these chemicals are toxic and unfit for human consumption. Other non-edible adulterants commonly used include plant woody materials such as sawdust, powdered sand and commercial calcium carbonate for increasing bulk and whitening of wheat based products [33, 57].

For the purpose of this thesis, three of the non-edible adulterants used, sawdust, chalk powder and gypsum are briefly described below.

2.4.2.1. Sawdust

Sawdust is the powdered form of plant woods. The chemical composition of wood is complex and varies from one plant species to another. The roots, stems and branches of a tree contain varying constituents due to the nature and progress of plant growth, soil conditions, climate and geographic location. The general composition of plant woods is characterized by two major chemical components namely lignin (18-35%) and carbohydrate (65-75%) [58].

The major classes of carbohydrates in wood are cellulose (40-50% of dry wood weight) and hemicelluloses (25-35%). Lignin is a complex phenolic compound bonded via hydroxy- and methoxy-substituted phenyl-propane units. The major building units of lignin biosynthesis are p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol. Other minor components in wood are found mostly in the form of extractable organics and as inorganic minerals (ash). These make up (4-10%) of wood composition. Elemental composition of wood can be summarized as 50% carbon, 44% oxygen, 6% hydrogen and trace amounts of several metal ions [58].

2.4.2.2. Chalk powder

Chalk powder is a common term used for calcium carbonate ($CaCO_3$). It is one of the most abundant compounds that make up to (7%) of the Earth's surface. It mainly occurs as an odourless and tasteless white wider. It is used in agriculture as a calcium source for plants and to maintain soil pH. It is also used in the paper industry for whitening and good finishing, and in the production of concrete within construction industry [59]. Chalk powder along with alum, is used as an adulterant in flour based products for bulk increment and as a whitening agent [60, 61]. Coloured chalk powder has also been used as an adulterant in turmeric powder [62, 63]. On the other hand, calcium carbonate is known to have been formulated in some antacids for neutralizing excess hydrochloric acid in the stomach and serves to relieve heartburn and indigestion [31, 59].

2.4.2.3. Gypsum

Gypsum naturally occurs in three forms, namely, anhydrous calcium sulphate ($CaSO_4$) as a soluble or insoluble anhydrite, calcium sulphate dihydrate ($CaSO_4.2H_2O$) also called native calcium sulphate and calcium sulphate hemihydrate ($CaSO_4.\frac{1}{2}H_2O$) known as plaster of Paris [59]. This bulky powder, other than its use in the production of calcium and sulphur compounds, is used primarily in areas of construction and agriculture [64]. It can irritate the respiratory tract if gasped and can also cause irritation to skin and the eyes. If ingested it can cause nausea, stomach ailment and vomiting [59]. Gypsum, along with chalk powder (calcium carbonate), was popular in food adulteration in the last century as a whitening and bulk additive in wheat flour products [31].

2.5. Adulteration detection strategies

Plant based foods and different agricultural products are being adulterated bringing in different calamities ranging from adverse health effects to economic losses. Adulteration involves wide range of materials from earthy materials and plant parts to synthetic chemicals. The conventional analytical tools in many laboratories are efficient to detect most food adulterations. However, some adulterations are not easily detected and quantified by these conventional methods. These are biological adulterants which require DNA based methods of analysis to fingerprint the origin of a certain product. Apart from the common analytical tools, different methods have been developed, mainly based on morphological or anatomical characterization and organoleptic markers (odor, colour, texture) along with physical parameters such as screening, bulk density testing, and chemical testing to verify the authenticity of foods and to check adulterants [33].

Generally, there are three basic strategies used for demonstrating adulteration in food or any agricultural commodity. The first is demonstrating the presence of a foreign substance, compound or marker, the second is demonstrating that a component is deviated from its normal level and the third is demonstrating that a profile is unlikely to occur [13, 35, 60]

Among the major analytical tools employed include physical analytical test procedures including macroscopic and microscopic, visual and structural assessment and texture, solubility and bulk density. Chemical and biochemical techniques of adulteration detection include application of modern instruments like high performance liquid chromatography (HPLC), thin layer chromatography (TLC), gas chromatography (GC), mass spectroscopy (MS), GC-MS, nuclear magnetic resonance (NMR) spectroscopy and immunological method like enzyme linked immune-sorbent assay (ELISA) [33].

Because of the cost and sophistication of the instrumental methods listed above, it may be difficult to use them in routine sample analysis with in the Ethiopian context. Therefore, simpler alternative analytical procedures or test parameters should be screened, as presented in this study, to come up with potentially applicable test parameters that can detect food adulteration in general and teff adulteration in particular, which will be easy to apply in the current capacity of laboratories in Ethiopia.

2.6. Food safety

Food safety is broadly associated with contamination of food in various stage of food production in farmlands, harvesting, storage, transportation, storage and preparation of food. Contamination from fertilizers, pesticides and herbicides is one of the increasing concerns in food safety. The other concern in food safety, other than contamination is adulteration. Food adulteration usually results in food contamination with harmful microbes and addition of toxic chemicals. Adulterated foods are therefore associated with high health risk on consumers [7, 56, 65].

The wide ranging health effects of adulterants differ in the type and degree of adulteration involved. Many food adulterants have been associated with vomiting, abdominal pain, allergy, asthma, and headache and even mental retardation, cardiac arrest, kidney failure and cancer. Food additives are also common adulterants which are primary causes of allergy and cancer in the developed world, though not considered by many as such [7, 66].

Scientists in the area have been studying the effect of toxic substances that enter the human body, types of toxicants, chemical carcinogenesis, their effect on different organs and physiological systems. Accordingly many of these studies have shown the negative effect of food contamination and adulteration on human and animal health [65].

2.7. Current food regulation laws in Ethiopia

According to the Ethiopian Council of Ministers Regulation No. 189/2010, the Ethiopian Food, Medicine and Health Care Administration and Control Authority (EFMHACA) has been established [26]. This is the highest government body which is responsible for the regulation, safety and quality of both local and imported food items [67].

Though there has been laid out routine regulation laws for inspection and control of both locally produced and imported food items, the authority still faces difficulty in the essential practical aspect to deal with the growing food fraud problems in the local informal market and depends on city residents and whistle blowers to uncover most food adulteration incidences. This has been proven as the recent incidences are being dealt with after information gathering from consumers and their complaints [37, 67].

The Ethiopian Standards Agency (ESA) has established standard requirements for teff grain (*Table 2*) and teff injera products (*Table 3-4*). These standards are upgraded and utilized to control and regulate the quality of teff and teff injera in the market [68, 69].

Table 2: Teff Grain Specification

Classes	**Characteristics**	**Maximum Limits of Impurities in %**				**Method of tests**
		Grades				
		1	**2**	**3**	**4**	
Very white (magna) teff	Foreign matter	1.5	2.5	3.5	5.0	ES ISO 5223
	Stone	0.6	0.6	0.6	0.6	ES ISO 5223
White (Nech teff)	Foreign matter	1.5	2.5	3.5	5.0	ES ISO 5223
	Stone	0.6	0.6	0.6	0.6	ES ISO 5223
Brown (key teff)	Foreign matter	1.5	2.5	3.5	5.0	ES ISO 5223
	Stone	0.6	0.6	0.6	0.6	ES ISO 5223
Mixed (Sergegna) teff	Foreign matter	1.5	2.5	3.5	5.0	ES ISO 5223
	Stone	0.6	0.6	0.6	0.6	ES ISO 5223
Ethiopian Standard ES 671:2001, ©ESA 2012						

According to ESA there are four major classes of teff which are graded based on the extent impurities present and the method of test are standard according to the current valid standards of the International Standard Organization (ISO) for cereal and cereal products [68].

Teff injera standard set by ESA contains general requirements along with detail of microbiological and nutritional composition requirements. In 2012, the agency, with its continued effort to standardize teff and teff products, published an standard edict as “Teff Enjera Specification” [70], which has been revised and improved in the 2013 as “Teff Injera Specifications” which contains several nutrient and microbial requirements and has been summarized in a table below [69].

Table 3: Teff injera general requirements (ES 3788:2013),

General requirements	
Injera	A slightly fermented, leavened product made from teff flour.
Irsho (or ersho) (traditional yeast)	Thin yellow liquid, Ethiopian version of sourdough starter which is a remnant of fermentation from previous batch that accumulates on the surface of the batter
Ye injera ayin (Bubbly eye)	Small hole formed on the top surface of injera when moisture has evaporated
Absit	Hot, thick dough fluid resulted from a mixture of small portion of fermented dough with boiling water
Ingredients for injera preparation	Teff flour Irsho (traditional yeast) Potable water as specified in ES 261
Injera characteristics	
Texture	Upper side of injera should have bubbly eye texture, backside shall be smooth. Should be free from mould and any other evidence of incomplete mixing of dough or baking. Shall be free from non-porous mass, from splits and large holes
Hygienic conditions	Injera shall be manufactured and handled in premises maintained in hygienic conditions
Serving size	Shall be 51 cm in diameter
Net mass	310 g (minimum)
Moisture	58 – 63%
Ethiopian Standards Agency, ES 3788:2013, ©ESA	

The nutrient composition of teff injera has also been one of the developments in the preparation of standard requirements associated with teff products. Among the basic food nutrients, moisture, crude protein, ash and fiber are specified for teff injera, *Table 4,* along with minerals like iron and calcium.

Table 4: Teff injera nutrient requirement (ES 3788:2013)

Nutrient	**Value on dry basis of 100g of edible portion**
Energy, *cal*	145.0 – 155.9
Protein, *g*	3.0 – 3.8
Gluten	Not detectable
Total carbohydrate, *g*	31.9 – 34.0
Crude fiber, *g*	1.0 - 1.8
Fat, *g*	0.6 – 0.7
Total ash, *g*	0.7 – 1.7
Calcium, *mg*	50.0 – 68.0
Phosphorus, *mg*	100.0 – 115.0
Iron, *mg*	7.0 – 14.7
Zinc, *mg*	1.5 – 1.7
Teff Injera Specification, ES 3788:2013, Ethiopian Standards Agency, ©ESA	

Common sensory evaluation is among the most important test parameters used to evaluate the quality of any food product both by individuals and food industries. However, the food quality evaluation can only be complete with proper investigation of nutritive values [3]. Due to the primary objective of this study, sensory evaluation was limited to the mere appearance and texture of injera samples in which control and adulterated injera samples were compared. This examination was done based on the standard appearance of injera explained using the "bubbly eye" and "texture" as outlined by ESA [69].

CHAPTER THREE

3. MATERIALS AND METHODS

3.1. Materials

Two white teff varieties DZ-Cr-387 (Quncho) and DZ-01-196 (Magna), 5.0 kg of each, were collected from Debrezeit Agricultural Research Centre (DZARC). These two varieties are among the widely cultivated white teff varieties in Ethiopia [22].

A 3.0 kg of sawdust sample was purchased from three local providers and wood workshops located in three areas in Addis Ababa city. Commercial grade gypsum (calcium sulphate dihydrate), 3.0 kg, and analytical grade chalk powder (calcium carbonate), 3.0 kg, were purchased from local construction stores.

Teff varieties and adulterants were coded throughout the experiment as follows:

Quncho teff (DZ-Cr-387) coded TQ

Magna teff (DZ-01-196) coded TM

Commercial Sawdust coded SD

Analytical grade Chalk powder ($CaCO_3$) coded CC

Commercial grade Gypsum ($CaSO_4.2H_2O$) coded CS / or GY

3.1.1. Teff flour and adulterant preparation

Teff grain samples were cleaned in a step by step process. First the grains were cleaned manually to remove impurities such as straw and bulky materials. Then winnowing was followed according to traditional method in which small size impurities were removed by wind blowing to separate grains from tares. Then, teff grains were milled using small scale grinder (High Speed Universal Disintegrator, FW 100, India) and sieved with a stainless steel (mesh size 0.425mm). Finally, the fine flour of the two varieties were transferred into polyethylene bags and kept at room temperature until analysis.

For mineral analysis of control flour samples, a portion of the teff grain samples of each variety were further cleaned using cold, running de-ionized water and dried rapidly to remove any remaining soil dust that could result in erroneous results on mineral analysis. After washing by the running water, grains were dried rapidly at 100 – 105 °C to constant weight using electrical oven (CHINCAN, DHG-9055A, China). After drying, the grains were ground using small scale grinder (High Speed Universal Disintegrator, FW 100, India). Flour was further dried to assure complete removal of moisture. Dried flour was sieved to ensure uniformity of flour composition (mesh size 0.425mm).

Sawdust samples were also manually cleaned to remove any foreign matter and sieved initially with stainless steel sieve (mesh size 1.00mm) to eliminate larger size sawdust particles. After weighing equal amount (1 kg) from each of three cleaned sawdust samples, a composite sample was prepared by using an automatic digital mixer (Ultra-Turrax IKA, T 25 D, Germany), to ensure uniformity of the mixture. The sawdust mixture was milled to make a fine powder using small scale grinder (High Speed Universal Disintegrator, FW 100, India). The fine sawdust powder was further sieved (0.425mm) and kept in polyethylene at room temperature until analysis. The gypsum and calcium carbonate samples were sieved (mesh size 0.425) to ensure particle size uniformity with teff flour.

3.1.2. Blending ratio

Selection of initial adulteration ratio was based on the original information gathered from Bole Sub-City Health Bureau (i.e. one of the sub-cities where the crime was reported) and sub-city police report. This was a 30% foreign matter adulteration in teff injera preparation [71]. Therefore a teff to adulterant blending ratio (7:3) was used to prepare blended samples for primary investigation (Demisse M, personal communication, June 21, 2016).

For the secondary investigation, each of the two teff varieties were blended with each of the three adulterants with 5, 10, 15, 20 and 25% adulterant ratio for injera preparation. The appearance of these injera preparations was used to determine cut-off adulteration ratios that result in similar injera with control injera samples from unadulterated teff flours of the two varieties.

Tertiary investigation was carried out on blends and injera preparations from the secondary investigation using the cut-off adulteration ratio obtained from secondary investigation.

3.1.3. Injera preparation

Teff injera was prepared by considering the minimum weight of injera set by ESA. According to the standard, the minimum weight of injera for commercial purposes should not be less than 310 g, with a minimum moisture content of 52% [69].

Injera preparation was carried at home, with some modification, using the traditional techniques as outlined in [72]. A flour to water ratio of 1:2 (w/w) was used, where 350 g of flour was mixed with 700 ml of tap water. After adding about 50 ml of starter or "ersho", from previous household preparation, mixture was kneaded by hand in mixing bowl. (Mixing bowls for this study were prepared from large plastics which were large enough to accommodate up to 2 litres of liquid). The resulting dough was allowed to ferment at room temperature for three days. After this initial stage of fermentation, the upper water layer was decanted.

About 250 ml of water was added to thin the dough and mixed well. The resulting thin dough was allowed to stand for two hours. Meanwhile, a traditional starter for secondary fermentation called "absit" was prepared by boiling about 70 ml of unadulterated teff flour ferment in about 150 ml of water.

The warm abist preparation was then added to the dough, mixed and allowed to stand for half hour to ferment. The resulting batter was then mixed again and checked for any need of further thinning. Baking was done on conventional baking electric oven called 'mitad' usually used for baking injera in the city households. Baking took few minutes for each injera, usually from 2 to 4 minutes depending on the thickness of batter of each preparation. Careful observation was taken during each critical step of the preparation and recorded accordingly.

Baked injera was transported to the Centre for Food Science and Nutrition laboratory of Addis Ababa University for further analysis within the same day of preparation. The appearance of each adulterated injera was compared against control injera samples. The moisture content was also determined using moisture analyser (AND ML-50, WAGTECH, Japan).

All injera samples were dried to constant weight and crushed with mortar and pestle to reduce size. The dried and crushed injera was further grounded using electrical miller (High Speed Universal Disintegrator, FW 100, India) to produce fine powder for analysis. The powder was passed through sieve (mesh 0.425 mm) to ensure uniformity.

3.2. Study Design

The experiment was designed to screen physico-chemical test parameters which can be used to identify teff flour and injera prepared with some non-edible adulterants. The effect of adulteration ratio on teff flour and injera composition was studied using completely randomized design (CRD) consisting of two teff varieties and three non-edible adulterants at adulteration ratios of (30, 5, 10, 15, 20 and 25%) respectively. Mean comparison was made between adulterated and control samples using one way analysis of variance (ANOVA) to determine test parameters with significant differences at $p < 0.05$.

The study was conducted in three stages of investigation. The first stage of the investigation was conducted using the maximum adulteration ratio of teff flour based on the information from the interview made with concerned government body (i.e. adulteration of teff flour and injera with 30% of each of the adulterants). Physical and chemical tests were made on the blends of each teff variety with adulterants. The resulting mean values of control and adulterated teff flour were compared. Test parameters resulting in significant mean difference ($p < 0.05$) were selected for use in further analysis. Injera preparation was also made for both the control and blends at 30% adulteration ratio.

In secondary investigation, injera was prepared in triplicates from both teff varieties blended with 5, 10, 15, 20 and 25% adulterant to see the effect of adulteration on injera appearance. Injera preparation was a very critical stage of the study because it reveals which adulteration ratios show no visible sign of adulteration when compared with control injera samples. This observation indicates the adulteration ratios that give rise to non-suspicious adulterated injera that may be distributed in the food market.

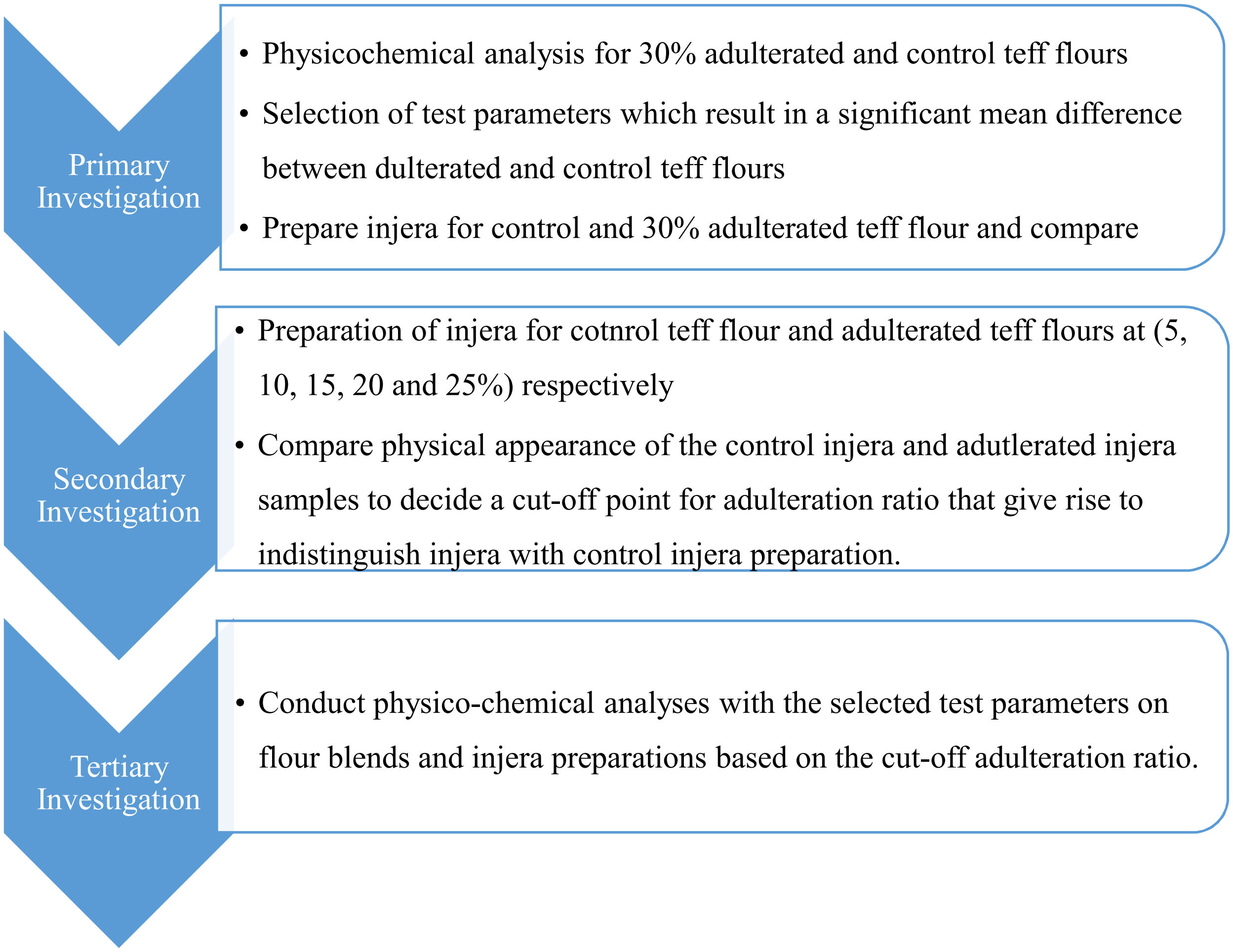

Figure 2: Stages of investigation

In the tertiary investigation, test parameters selected from the initial investigation were used to test flour and injera samples based on the cut-off adulteration ratio from the secondary investigation. This was necessary to reduce the number of samples and test parameters in the final stage of investigation.

3.2.1. Primary investigation

A 100% of teff flour from two varieties, Quncho and Manga, were used as controls. The physicochemical characteristics of the corresponding 30% adulterated blends were investigated in the primary stage. The adulterants (sawdust, calcium carbonate and gypsum) were previously prepared in the mentioned ratios with flours of each teff variety. Injera was prepared from both the control teff flours and blends at the 30% adulteration ratio to observe injera appearance. The physicochemical analyses were conducted according to standard AOAC (2000) official methods, in triplicates [73].

3.2.2. Secondary investigation

Based on the results of the primary investigation, test parameters with significant mean difference at the 30% adulteration level, were further determined at a minimum adulteration ratio of 5%. For test parameters not resulting in a significant mean difference at the 5% adulteration ratio, a 10%, 15%, 20% and 25% adulteration ratio were used successively to distinguish adulteration ratio at which the test parameter result in injera appearance similar to the control injera samples prepared from unadulterated teff flour. This was the threshold ratio or cut-off adulteration ratio used in further investigation.

3.2.3. Tertiary investigation

The third stage of the study is preparation of injera samples from teff blends adulterated at different ratios. A 30% adulterated teff injera was prepared for all adulterants in the primary investigation, which was followed by preparation of injera with decreasing ratio of adulterants in the secondary investigation (i.e.25, 20, 15, 10 and 5%).

3.1.Test parameters

A range of analytical testing procedures were applied to examine the physico-chemical composition of the blends and injeras. The detailed procedures used for the determination are listed in the sub-sections below. Among the several conventional techniques employed in food analysis, the most common test parameters were used and screened.

Analyses of iron, zinc, calcium and magnesium were only conducted at the primary investigation. Based on this initial result, mineral with highest significant mean difference between control and adulterated flour sample was used for further investigation.

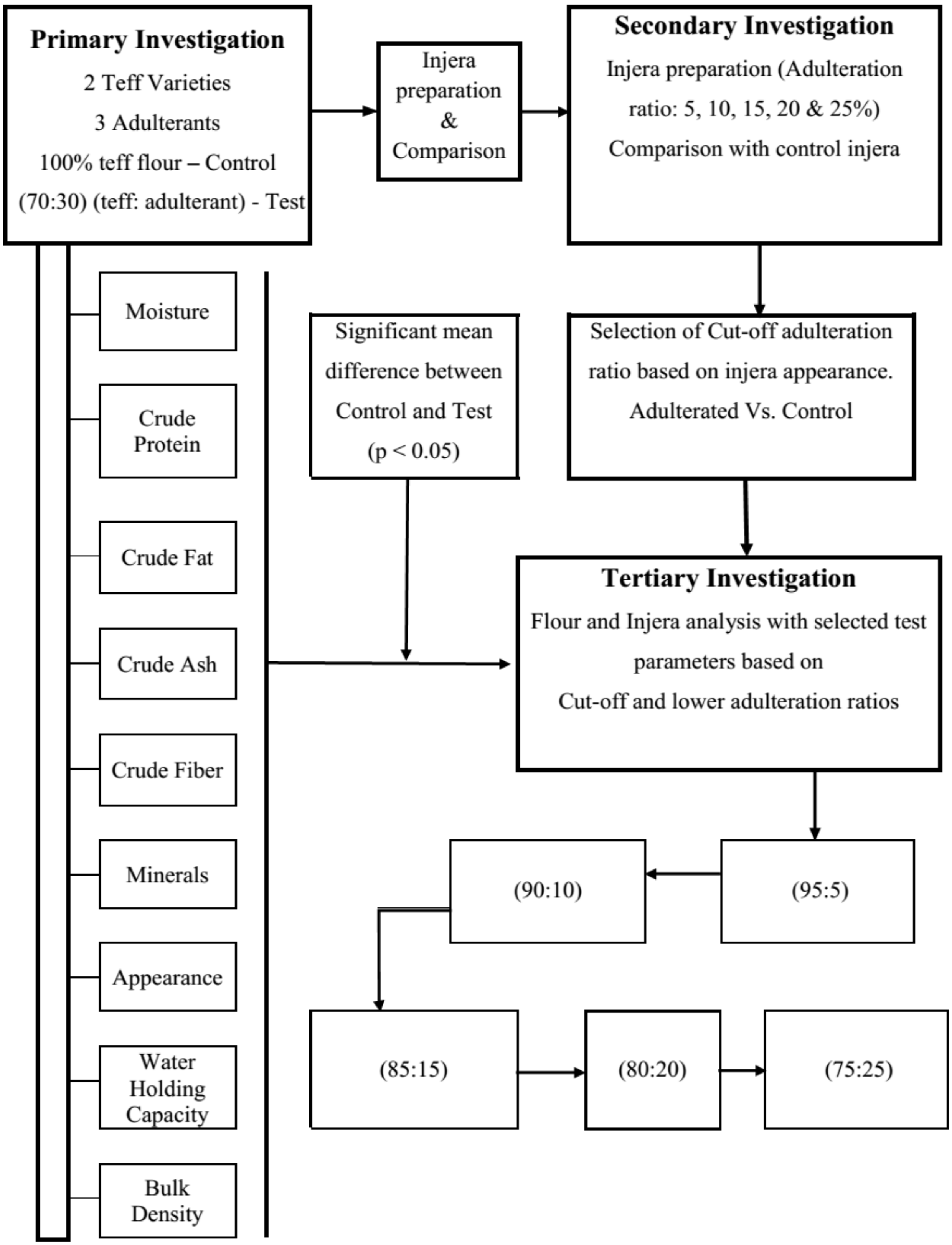

Figure 3: Study Flow Chart

3.1.1. Appearance

The two teff varieties and each of the three adulterants exhibit unique physical appearance like colour and uniformity. Thus it was logical to compare the external visual character of the blended teff flour with control flours.

Briefly, about 15 g of pure and adulterated teff flour were placed on petridish and observed by the naked eye for any difference in appearance. Similarly the appearances of the control and adulterated injera samples were also examined.

In this study, physical appearance of injera plays a key role in determining the cut off point for a range of adulteration ratios used to blend teff flour with the non-edible adulterants. Consequently, physico-chemical analysis was conducted only for adulterated injera samples which have similar appearance with control injera samples (based on the cut-off point).

3.1.2. Bulk Density

The bulk density of control teff flour and blends were determined [74]. Previously cleaned and dried measuring cylinders of 100 mL capacity were used. Measuring cylinders were weighed (W_1) and 15 g of flour samples were carefully transferred into the cylinders (W_2) and tapped to reduce air space within flour, until there was no visible decrease in volume. The final volume (V) was then recorded in cubic centimetres.

Equation 1: Bulk Density

$$Bulk\ Density\ \left(\frac{g}{cc}\right) = \frac{W2 - W1}{V} = \frac{sample\ weight}{tapped\ volume}$$

Where

W_1 = Weight of empty tapping cylinders, *g*

W_2 = Weight of cylinders with sample, *g*

V = Final volume of flour in cylinder after tapping, *cc*

3.1.3. Water Holding Capacity (WHC)

The water holding capacity (WHC) or water absorption index (WAI) was determined using previously approved methods with some modification [10, 73, 75]. A digital electronic balance

(Shimadzu, AX120, Japan) was used to weigh all mass measurements. In a cleaned, dried and tarred centrifuge tube, 1 g sample (W_1) was placed and mixed with 10 g of distilled water (W_2) and shaken vigorously for 1 minute to make homogenous suspension using vortex mixer (K Gemmy, VM-300P, USA), and allowed to stand for 2 hours. Samples were then centrifuged (Zhengji, 800D, China) at 2200 rpm for 10 minutes. The supernatant was decanted and weighed (W_3). Water holding capacity was determined by calculating the difference between the weights of water added and decanted, expressed as (g of water absorbed/100g of dry sample) on dry basis.

Equation 2: Water holding capacity (WHC)

$$WHC\left(\frac{g}{100g}\right) = \frac{W2 - W3}{W1} x\ 100$$

Where

W_1 = Weight of sample on dry basis in g

W_2 = Weight of initial water added to the sample in g

W_3 = Weight of unabsorbed, decanted water in g

3.1.4. Moisture content

Moisture content of both control and adulterated flours were analysed using AOAC (2000), official method 925.09.[73] A previously cleaned measuring crucible is dried in oven at 105°C and cooled to room temperature in a desiccator and weighed (W_1) using a digital electronic balance (Shimadzu, AX120, Japan). About 5 g of sample was weighed in the crucible and weighed (W_2). Drying was carried out at 105°C in a drying oven (CHINCAN, DHG-9055A, China) for five hours. The crucible with dried sample was cooled in desiccator and weighed. Drying was repeated for another half hour and final weight (constant dry weight, i.e. successive dried weights do not differ by more than 1mg) was recorded (W_3). Moisture content was calculated using the formula

Equation 3: Moisture

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

Where

W_1 = Weight of empty drying dish in g

W_2 = Weight of drying dish and sample (W1 + Sample weight)

W_3 = Final weight of W_2 after 3 hours of drying at 105 °C to constant weight

The moisture content of all injera samples was carried out using a rapid moisture analyser (AND ML-50, WAGTECH, Japan). This technique is employed within the food industry for quick and reliable data during the analysis of large number of samples. Rapid moisture analysers give data with a very good accuracy and precision in small amount of time [76]. A target weight of 2.0g was set and approximately a 2.00g of injera sample was placed and rapid drying applied to obtain the moisture content of all baked injera samples. An optional, pre-set drying rate of 5-10% per minute was used for all injera samples analysed using the instrument.

3.1.5. Crude Protein

The crude protein content of flour and injera samples were determined by using the AOAC (2000) official method 955.04 following the Kjeldahl procedure [73].

Digestion: A 0.5 g of previously dried and ground sample (W) was placed in tecator tube which was placed in tecator rack. Samples were transferred into fume hood for the next procedure. Six ml of concentrated sulfuric acid was added to the tubes using standard pipette and mixed well. Blank sample was prepared similarly without adding sample. With extreme caution, 3.5 ml of hydrogen peroxide was added drop-wise, in which violent reaction resulted. After the violent reaction stopped, tubes were manually shaken to mix components well. Then a 3 g of catalytic mixture of copper sulphate and potassium sulphate was added and mixed by swirling carefully.

The mixture in the tube was allowed to stand for 15 minutes. Samples in the tubes were then digested, in fume hood at a working temperature of 370 °C. Digestion was completed when a clear solution was formed which took about 3 hours. After removing and cooling to room temperature, tubes were placed in racks and 50 ml of distilled water was added to prevent precipitation of sulphate.

Distillation: 25 ml of 35 % sodium hydroxide was added to the digested sample and placed in distiller. The distillate was then collected into a 250 ml conical flask containing 25 ml of 2% boric acid, 25 ml of distilled water and methyl red indicator solution. The tips of tubes of distiller were immersed into the bottom of the flask. Distiller was checked for continuous running water for proper condensation. After 200 ml of distillate was collected, the tips were rinsed into distillate received, the tips were rinsed into distillate solution with few ml of distilled water before removal.

Titration: the above distillate solutions of each sample being tested, which contain the indicator, ammonium and borate ions was titrated with a 0.1 N hydrochloric acid solution to a reddish end point. The initial and final reading of titrant was recorded for each titration, taking the difference as the volume of titrant consumed (V). The blank was also titrated (B). The net volume consumption by each sample under test was determined by subtracting the blank volume from each of the volume of titrant consumed by sample titration (V - B).

Equation 4: Total Nitrogen (%)

$$\% \text{ Nitrogen} = \frac{(\text{V} - \text{ B}) \times \text{N} \times 14.02}{1000 \times W} \times 100$$

Where

V = Volume of titrant (HCl) consumed to the end point of titration

N = The Normality of titrant used (HCl) in moles per liter

W = Weight of sample calculated on dry matter basis in g

Equation 5: Crude Protein

$$\% \, Protein = 6.25 \times \% Nitrogen$$

3.1.6. Crude Fat

The crude fat content of each flour and injera sample was determined using AOAC (2000), official method 4.5.01 of the Soxhlet extraction procedure [73].

Previously cleaned flasks (extracting thimbles) and boiling chips were dried in oven at 100 °C for 1 hour, cooled in a desiccator to room temperature (30 minutes) and weighed (W_1). About 2 g of accurately weighed sample (W) was placed in the thimble containing fat free cotton. Thimbles were placed in thimble holders, 50 ml of petroleum ether poured in the flask, and thimble immersed in the petroleum ether containing flask and attached to extraction unit, Fat Determinator (SZC-D, Shanghai Qianjian Ins. China).

Samples were subjected to extraction with solvent for 2 hours at 60°C (with rinsing) followed by solvent recovery for 15 minutes. The extracting cylinders were then dried in an oven (CHINCAN, DHG-9055A, China), at 105 °C for half an hour. After cooling too room temperature, final weight of extracting cylinder with extracted crude fat was weighed (W_2).

Equation 6: Crude Fat

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

Where,

W = Weight of sample flour used in g

W_1 = Weight of extraction flask with boiling chips in g with boiling chips

W_2 = W1 + weight of extracted dried crude fat in g

3.1.7. Crude Ash

The crude ash content of flour and dried injera samples was determined using AOAC (2000), official method 923.03 [73].

Cleaned porcelain crucibles were placed an electric Muffle Furnace (Carbolite CSF 1200, Aston Lane, England) at 550 °C for 30 minutes, and cooled in desiccator to room temperature and weighed (W1). A dried and ground sample of about 2.5 g was weighed into crucibles and weighed (W2). The crucibles were then placed on hot plate for charring in fume hood, with progressive increasing temperature, until the samples were completely carbonized. The crucibles were then transferred into the furnace for complete ashing at 550 °C. Upon completion of ashing, temperature of furnace was lowered to cool to about 200 °C and crucibles were removed and placed in a desiccator for cooling to room temperature. The final weight of residue and ash was weighed (W_3).

The total ash expressed as percentage is calculated as

Equation 7: Total Ash

$$\% \, Total \, Ash = \frac{W3 - W1}{W2 - W1} \times 100$$

Where,

W_1 = Weight of empty crucible in g

W_2 = Weight of crucible and sample in g

W_3 = Weight of crucible and ash in g

3.1.8. Crude Fibre

All samples to be tested for crude fibre were dried and ground prior to packaging and labelling. Crude fibre determination was carried out at using AOAC (2000) official method 962.09 [73].

Crucibles were cleaned and dried with 1 g celite in an oven at 105 °C for 1 hour and cooled. To the cooled crucible 1 g of sample was accurately weighed and added (W1). Crucibles were then placed in digester holder and 200 ml of a 1.25% sulfuric acid solution was added and boiled for half an hour. Acid was drained by using vacuum pump. After cooling the sample it was washed by distilled water, and a 1.25% solution of sodium hydroxide was added for further treatment. After treatment was completed at the set time and temperature, the basic solution was drained by using vacuum pump.

Crucibles containing the residue were dried at 130 °C for 2 hours and weighed (W2) after cooling to room temperature for half an hour. The crucibles were then transferred into an electric muffle furnace and ignited at 550 °C for 3 hours. After cooling to below 200 °C, crucibles containing the ashes were removed and cooled to room temperature. The final weight of ash and crucible was weighed and recorded as (W3).

Equation 8: Crude fiber

$$\%\,Crude\ fiber = \frac{W2 - W3}{W1} \times 100$$

Where,

W1 = Weight of sample in g

W2 = Weight of residue and crucible in g

W3 = Weight of ash and crucible in g

3.1.9. Mineral Analysis

Among the major minerals in teff, calcium, iron, zinc and magnesium were investigated in the flour and injera preparations using flame Atomic Absorption Spectroscopy (AAS) [77].

The crude ash obtained (from 2.5 g sample) after dry ashing at 550 °C was treated with 6 ml of 6N HCl solution to wet it completely and was carefully dried on a low temperature hot plate not exceeding 100 °C. Using graduated pipette, 10 ml of 3N HCl solution was added to the dried ash and heated again on the hot plate until the solution just boiled. After cooling to room temperature in a fume hood preparations were filtered into a 50 ml volumetric flask using 125 mm Whatman (No. 42) filter paper. Again 10 ml of 3N HCl solution was added into the crucible and heated until the solution started to boil and cooled to room temperature and filtered into the first filtrate within the volumetric flask. The crucibles were rinsed finally using deionized water three times and filtered into the flask. A 2.5 ml of 10% lanthanum chloride solution was added into the flasks. Each solution was finally cooled and diluted to 50 ml using deionized water.

A blank which contains 25 ml of 3 N HCl and 5 ml of 10% lanthanum chloride was added into 100 ml volumetric flask. Four series of working standard metal solutions were prepared for each of the major cationic minerals being analysed, by dilution of metal stock solutions (nitrates of the metals) with deionized water containing 2.4 ml 3 N HCl in a 10 ml volumetric flask. This gave a series of working standard solutions for iron (0.00, 2.00, 6.00, and 10.00), zinc (0.00, 0.60, 1.00, 1.40, and 1.80) and calcium (0.00, 1.00, 1.50, 2.50, and 3.00) in (µg/mL). Calibration graphs of each standard series were determined using (Concentration vs. Absorbance) in mg per 100 g of sample for each element by using the prepared standard solutions (Appendix I - IV).

All standard and sample concentrations were then determined using appropriate flame atomic absorption spectrophotometer (Shimadzu, AA-7000, Japan). A single mineral hollow cathode lamp was used for each element. Reading was taken from the prepared calibration graphs for the metal concentrations that correspond to the absorption values of the samples, and the blank. The mineral contents were calculated using the following formula:

Equation 9: Metallic content

$$Metallic\ content\ in \left(\frac{mg}{100g}\right) = \frac{(A-B)}{W} \times \frac{V}{1000} \times 100 \times D$$

Where,

A = Concentration (µg/ml) of the sample solution

B = Concentration (µg/ml) of the blank solution

W = Weight of the sample (g) on dry matter basis

V = Volume of the solution/extract (ml)

D = Dilution factor

3.2. Qualitative tests

3.2.1. Effervescence test

Based on the universal test to detect carbonate with hydrochloric acid, flour blends were tested along with control samples for effervescence formation. Dried and powdered injera samples were also tested. Test procedure specified in Indian Standards (IS 15642) of quick test parameters or methods for detection of adulterants/contaminants in common food products [78] was used with some modification.

Initially, control teff flour of the two teff varieties and their respective blends with the highest adulterant ratio were tested. For samples responding to effervescence test, lower adulteration ratios were also tested.

Briefly, 0.5 g of each test sample was spread over watch-glass. Few drops of distilled water was added to wet the sample and two to three drops of concentrated hydrochloric acid solution was added with careful observation. Samples that resulted in effervescence reaction were recorded as positive for the presence of calcium carbonate.

3.2.2. Precipitation of insoluble salt

The presence of excess sulphate ions was also tested using concentrated barium salt solution prepared from barium chloride dihydrate ($BaCl_2 2H_2O$). With suitable solvent, barium cations bind with sulphate anions in acidic aqueous solution to form barium sulphate precipitate which is an insoluble salt in aqueous solutions. The solutions are made acidic to prevent the precipitation of other anions such as carbonates in the form of barium carbonate. Based on this principle teff flour and dried and ground injera samples were tested for excess sulphate anions. Since there are no standard test procedures related to the determination of excess sulphate in teff flour or injera, a combined and modified test procedure was used to determine the amount of sulphate precipitated as barium sulphate [79-82]. Therefore the procedure followed was controlled using blank solutions and control solutions prepared from control samples.

Approximately 1.0 g of test sample was transferred into a 50 mL volumetric flask and 20 mL of deionized water was added and mixed well. Then 20 ml of 2M hydrochloric acid solution was added and mixed well by shaking. Finally the mixture was diluted to 50 mL with deionized water, mixed and allowed to stand. Control samples were prepared by taking 1 g unadulterated teff flour and 1 g of dried and ground injera sample from each of the two varieties. A blank was prepared with the same procedure without sample.

After allowing the mixture in the flasks to stand for an hour, the supernatant was filtered using Whatman (No.42) filter paper into a test tube. A 5 mL filtrate was then transferred to a second test tube and a 10% w/v barium chloride solution was added drop wise until there was no further turbidity formation in the test solution. The samples were centrifuged at 1600 rpm for 10 minutes using electrical centrifuge (Zhengji, 800D, China) and supernatant discarded. Precipitate was washed with 5 ml of 60% ethanol. Precipitate was further washed using two portions of 5ml distilled water. Precipitate was transferred into a previously weighed evaporating dish (W_1) and dried in oven (CHINCAN, DHG-9055A, China), at 100 °C to constant weight. Final weight of evaporating dish and residue were recorded (W_2). Weight of residue was obtained by subtracting the empty weight of dish. Weight of precipitate (W) was determined after cooling to room temperature and subtracting weight of empty dish ($W = W_2 - W_1$).

3.3. Statistical Analysis

Data from quantitative treatments was analysed using the software IBM SPSS (Statistical Package for the Social Sciences) version 20.0 for Windows. Tests were performed in triplicates and data was expressed as mean ± SE (Standard Error). For the purpose of comparing significant mean differences, independent sample t-test was used. Analysis of variance (ANOVA) was also used in the primary investigation to evaluate significant mean differences at $p < 0.05$.

CHAPTER FOUR

4. RESULTS AND DISCUSSION

One of the major staple cereal in Ethiopia is teff. For quite some time teff flour has been used in combination with other grain flours to make better nutritional and sensory characteristics Also this kind of adulteration is linked with increased cost of teff but presents no harm since the added items are similar edible foods [8]. In contrast, the recent adulteration incidences in teff injera preparation with non-edible adulterants such as gypsum and sawdust, which were directly aimed at gaining profit were potentially harmful [83]. To prevent the distribution of such adulterated teff injera products, systematic and cost effective test procedures need to be implemented as routine analysis procedures to analyse as many marketed injera samples as possible. This can help in detecting adulterated injera samples in the market.

Teff adulteration is now among the major public health concerns that should be tackled because of its wide consumption by the majority of our population [6]. If there are standards for food products, there should also be routine sampling and analytical procedure easily implementable by all concerned bodies both in academic research and government offices to detect and protect food frauds. With this regard, the main purpose of this study was to screen food composition test parameters that can easily detect or indicate some of the non-edible adulterants that are encountered in teff flour and injera.

Accordingly, the adulteration was simulated in laboratory, based on the information obtained from concerned bodies. For this simulation, two widely distributed teff varieties, Magna and Quncho teff flours were selected [22]. The adulterants for this study were also selected based on the recent investigation and findings of concerned government bodies (i.e. local health bureaus). The adulterants seized include saw dust called "Sagatura", chalk powder and gypsum (Demisse M., personal communication, June 21, 2016).

In this study, possibility of making injera using the reported non-edible adulterants was investigated. Also, physico-chemical test parameters that can detect teff flour and injera adulteration with a maximum adulteration ratio of (30%) were screened. The minimum detectable concentration of adulterants that can be detected using the screened test parameters was also studied. The results of the three phase investigations is reported as follows.

4.1. Primary investigation

The primary investigation was conducted to screen analytical test parameters that can detect the adulteration of teff flour with the selected adulterants at the maximum adulteration ratio (30%). Physico-chemical properties were tested (proximate composition, mineral analyses, qualitative tests and functional properties). Mineral analysis was conducted on both washed and unwashed teff grain samples. However, for the purpose of meeting the objective of the present study, only the results of washed teff grain mineral content were used as controls in both teff varieties for the initial investigation.

Based on the result of the investigation, test parameters which resulted in highest mean differences between control and adulterated samples were selected for the secondary investigation. Also, the possibility of making indistinguishable injera with this maximum adulteration ratio was evaluated.

4.1.1. Physico-chemical analysis of control and 30% adulterated Quncho teff flour

The physicochemical analysis of control and 30% adulterated Quncho teff flour was carried out. The results are presented in *Table 5*.

As can be seen from the mean comparison of sawdust adulterated and control teff flour analysis, there is a significant mean difference at $p < 0.05$ for all tested parameters except test of crude ash.

All mean values of proximate analyses in the calcium carbonate adulterated samples have shown significant mean difference with control sample mean values, while the water holding capacity and bulk density mean values showed no significant differences.

Similarly, the gypsum adulterated teff flour resulted in significant mean difference with control sample except for Zinc, water holding capacity and bulk density mean values, which showed no significant mean difference with the control.

As can be seen from *Table 5*, the calcium content of Quncho teff flour adulterated with 30% of chalk powder is twenty times higher than the calcium in Quncho teff flour. Similarly, the calcium content in the gypsum adulterated flour is 15 times higher than the level of calcium in the control Quncho teff flour. There is also a significantly different mean value between the iron, magnesium and zinc content of control teff and 30% adulterated teff flour. Because of the elemental composition of the two inorganic adulterants, the difference in mineral content between adulterated teff flour and unadulterated (control) teff flour is highlighted strongly by the mineral calcium than the other minerals.

Table 5: Proximate and micronutrient composition of control and 30% adulterated Quncho teff flour

Test parameter	Flour Composition			
	Control (100%)	(Flour: Adulterant) (70:30)		
	TQ	TQ:SD	TQ:CC	TQ:CS
Moisture (g/100g)	$11.65^{c} \pm 0.24$	$8.52^{a} \pm 0.13$	$8.41^{a} \pm 0.09$	$9.44^{b} \pm 0.18$
Protein (g/100g)	$10.92^{b} \pm 0.05$	$7.25^{a} \pm 0.20$	$7.28^{a} \pm 0.09$	$7.38^{a} \pm 0.21$
Fat (g/100g)	$1.95^{c} \pm 0.02$	$0.43^{a} \pm 0.02$	$1.44^{b} \pm 0.01$	$1.43^{b} \pm 0.01$
Ash (g/100g)	$2.01^{a} \pm 0.02$	$1.95^{a} \pm 0.02$	$30.20^{c} \pm 0.17$	$28.57^{b} \pm 0.07$
Fiber (g/100g)	$2.07^{b} \pm 0.02$	$19.83^{c} \pm 0.26$	$1.42^{a} \pm 0.02$	$1.36^{a} \pm 0.03$
Calcium (mg/100g)	$170.60^{ab} \pm 0.14$	$171.52^{b} \pm 0.82$	$4117.00^{d} \pm 2.31$	$3419.00^{c} \pm 1.53$
Iron (mg/100g)	$7.64^{d} \pm 0.04$	$5.40^{a} \pm 0.02$	$5.85^{b} \pm 0.04$	$8.25^{c} \pm 0.07$
Magnesium (mg/100g)	$6.36^{c} \pm 0.03$	$4.04^{a} \pm 0.04$	$6.02^{b} \pm 0.03$	$6.58^{cd} \pm 0.02$
Zinc (mg/100g)	$5.87^{b} \pm 0.02$	$4.77^{a} \pm 0.03$	$4.68^{a} \pm 0.02$	$6.25^{c} \pm 0.03$
WHC (g/100g)	$137.00^{a} \pm 3.21$	$535.67^{c} \pm 2.03$	$142.67^{b} \pm 2.40$	$140.33^{b} \pm 1.33$
BD (g/cc)	$0.83^{b} \pm 0.00$	$0.56^{a} \pm 0.00$	$0.83^{b} \pm 0.00$	$0.84^{b} \pm 0.01$

Data are expressed as mean ± SEM. Means in the same raw not sharing the same superscripts are significantly different at ($p < 0.05$). Tests assume equal variances (N =3); WHC = water holding capacity; BD = bulk density; TQ = Quncho teff flour; SD = Sawdust; CC = Calcium carbonate (Chalk powder); CS = Calcium sulphate dihydrate (gypsum); all values are on dry matter basis except Moisture test

Hence, all test parameters are further used in secondary investigation with the following arrangement. For sawdust adulterated Quncho teff flour, water holding capacity and bulk density tests are further used in the secondary investigation (for a 5% and above sawdust adulterated Quncho teff flour). Test of calcium is not used for further analysis in sawdust adulterated sample in tertiary investigation.

For chalk powder and gypsum adulterated Quncho teff flour, calcium mean value showed greater mean difference compared with control (15 times higher) than iron, magnesium and zinc. Therefore calcium is selected as a target analyte in tertiary investigation.

4.1.2. Physico-chemical analysis of control and 30% adulterated Magna teff flour

The mean value determinations of the physico-chemical tests on control and 30% adulterated Magna teff flour are presented in *Table 6*.

There is a significant mean difference (at $p < 0.05$) for all tests in the sawdust adulterated Magna teff flour except the test of ash.

All mean values of proximate analyses in the chalk powder and gypsum adulterated samples show significant difference with control sample, except test of water holding capacity and bulk density, which are not significantly different between adulterated and control sample.

Therefore, all test parameters were further used in secondary investigation with the following arrangement. Water holding capacity and bulk density tests are further used in secondary investigation. Since the calcium mean value in chalk powder and gypsum adulterated Magna teff flour is significantly higher than the other tested minerals, it was further used for secondary investigation.

As can be seen from the result of *Table 6*, the mean calcium content of Magna teff flour blended with 30% of the two inorganic adulterants is significantly different from the mean calcium content of the control (100%) Magna teff flour. The mean calcium content of the flour blend prepared with 30% chalk powder is twenty times higher than the mean calcium content of control Magna teff flour and that prepared with 30% gypsum is more than 15 times higher. The mean iron, magnesium and zinc contents of the control teff flour are also significantly different from the corresponding mean values within the adulterated samples. But compared with calcium, the other mineral tests have lower mean differences with control sample.

Table 6: Proximate and micronutrient composition of control and 30% adulterated Magna teff flour

Test parameter	Flour Composition			
	Control (100%)	(Flour: Adulterant) (70:30)		
	TM	TM:SD	TM:CC	TM:CS
Moisture (g/100g)	$12.18^{b} \pm 0.13$	$8.66^{a} \pm 0.06$	$8.29^{a} \pm 0.43$	$8.66^{a} \pm 0.11$
Protein (g/100g)	$10.47^{d} \pm 0.04$	$7.13^{c} \pm 0.10$	$6.65^{b} \pm 0.14$	$6.31^{a} \pm 0.08$
Fat (g/100g)	$1.98^{c} \pm 0.04$	$1.52^{b} \pm 0.04$	$1.39^{a} \pm 0.02$	$1.46^{ab} \pm 0.02$
Ash (g/100g)	$1.97^{a} \pm 0.02$	$1.93^{a} \pm 0.02$	$30.30^{b} \pm 0.08$	$30.00^{b} \pm 0.12$
Fiber (g/100g)	$2.01^{b} \pm 0.01$	$19.50^{c} \pm 0.46$	$1.19^{a} \pm 0.02$	$1.22^{a} \pm 0.02$
Calcium (mg/100g)	$188.40^{a} \pm 0.4$	$197.87^{b} \pm 0.98$	$4214.33^{d} \pm 1.76$	$3510.63^{c} \pm 1.08$
Iron (mg/100g)	$7.72^{d} \pm 0.06$	$5.72^{b} \pm 0.02$	$5.87^{c} \pm 0.03$	$8.54^{a} \pm 0.05$
Magnesium (mg/100g)	$4.83^{b} \pm 0.04$	$4.25^{a} \pm 0.04$	$5.85^{c} \pm 0.05$	$7.62^{d} \pm 0.06$
Zinc (mg/100g)	$5.95^{c} \pm 0.05$	$4.73^{b} \pm 0.04$	$4.30^{a} \pm 0.04$	$6.25^{d} \pm 0.03$
WHC (g/100g)	$151.67^{a} \pm 0.88$	$546.67^{b} \pm 3.76$	$152.00^{a} \pm 0.58$	$151.33^{a} \pm 0.33$
BD (g/cc)	$0.85^{a} \pm 0.02$	$0.55^{b} \pm 0.00$	$0.80^{a} \pm 0.00$	$0.81^{a} \pm 0.02$

Data are expressed as mean ± SEM. Means in the same raw not sharing the same superscripts are significantly different at ($p < 0.05$). Tests assume equal variances (N =3); WHC = water holding capacity; BD = bulk density; TM = Magna teff flour; SD = Sawdust; CC = Calcium carbonate (Chalk powder); CS = Calcium sulphate dihydrate (gypsum)

On both teff varieties, calcium concentration showed most significant mean value difference with control mean values. In Quncho and Magna teff flour adulterated with 30% chalk powder (calcium carbonate), the calcium content was increased by more than twenty folds. With the same amount of adulteration with gypsum (calcium sulphate dihydrate), the calcium content increased by more than fifteen folds. These associations are also true when compared to mean calcium content of other teff varieties in other researches, in which the reported calcium contents are comparable with control teff calcium content in this study [46, 84, 85].

4.1.3. Appearance of teff flour and adulterants

Tests of physical characters are the initial steps in most physico-chemical analysis procedures. Within any food market, the first characteristics of any food item that is perceived by consumers is appearance, which is the first and critical part of the sensory evaluation. Within the framework of this study, other tools of sensory evaluation (smell, flavour and taste) are not included. Since the intention of the study was to screen test parameters for rapid and routine laboratory test procedures, most sensory analysis tests were not covered.

Initially, the appearance of control teff flours and adulterants were observed for visual difference. Then, the appearance of 30% adulterated flour samples were compared against the appearance of control. Since the adulterants mimic the physical property of flour, flour appearance could be used as indicator of purity of flour, particularly during sampling procedures. Some of the relevant photographic images of flour and adulterants are shown and discussed in the following sections. The control teff flour and adulterants exhibit unique appearances. Upon careful examination with naked eye and feel of the touch, one can distinguish the dissimilarities.

The appearance test, similar to the identification of teff grains (as white, brown and mixed), is a way to initially perceive the nature of flour purity. It is not an essential indicator of adulteration but can be used as an aid during flour sampling procedure.

4.1.3.1. Appearance of Quncho teff flour and adulterants

Unadulterated Quncho teff flour and adulterants were observed. As can be seen from *Figure 4*, Quncho teff flour (TQ), sawdust (SD) and gypsum (GY) powder exhibit some similarity in appearance, while the chalk powder or calcium carbonate (CC) appears whiter. To observe the effect of adulteration on teff flour appearance, a blend of Quncho teff flour and chalk powder was used in the ratio (70:30) and was compared to the control teff flour and chalk powder. The adulterated flour takes an intermediary appearance between the control teff flour and the chalk powder *Figure 5*.

As can be seen from *Figure 5* the appearance of control Quncho teff flour and blend with 30% calcium carbonate were easily distinguishable by the naked eye. Calcium carbonate is pure white and is easily distinguished than the other two adulterants.

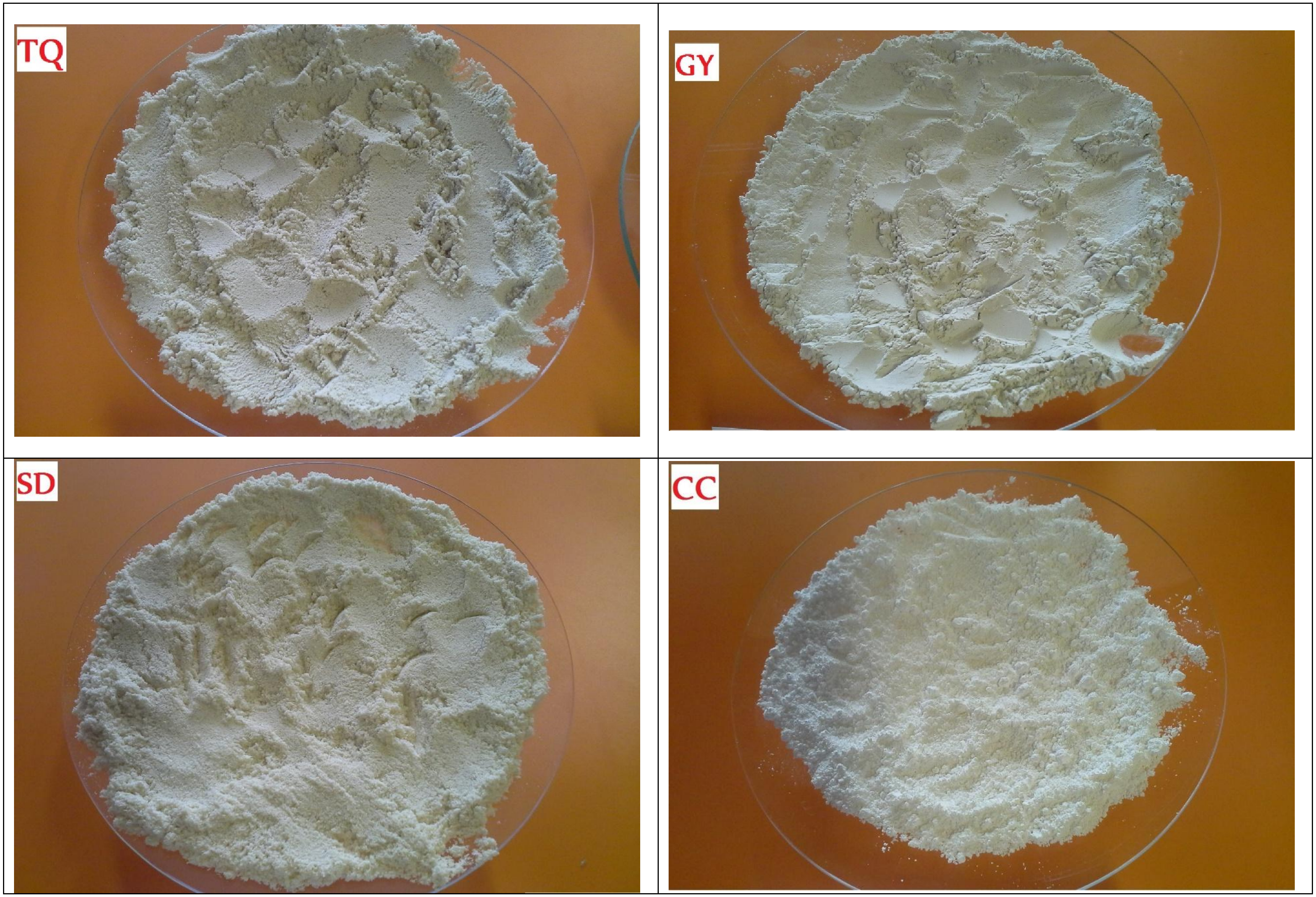

Figure 4: Appearance of Quncho teff flour -TQ, Sawdust -SD, Calcium Carbonate -CC and Gypsum -GY

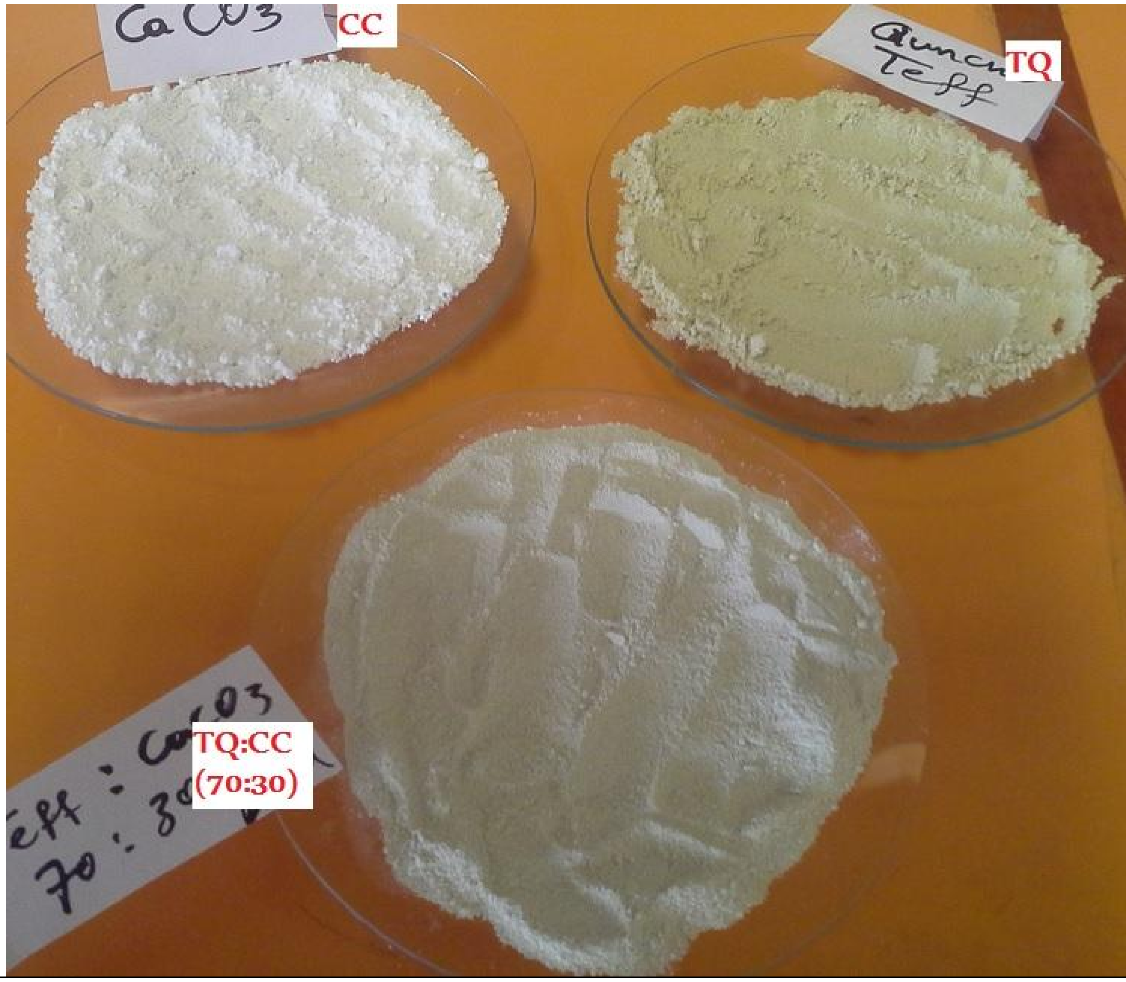

Figure 5: Appearance of Quncho teff flour, calcium carbonate and 30% blend

4.1.3.2. Appearance of Magna teff flour and adulterants

Unadulterated Magna teff flour, sawdust, chalk powder and gypsum were observed for any difference in appearance. As can be seen from *Figure6,* chalk powder was easily distinguishable between the flours, while sawdust and gypsum resemble the teff flour in appearance. Magna teff flour blended with chalk powder at 30% exhibits intermediary appearance between the control flour and chalk powder *Figure 7*.

Figure 6: Appearance - Magna teff flour -TQ, Sawdust -SD, Calcium Carbonate -CC and Gypsum -GY

As can be observed from *Figure 7*, Magna teff flour exhibits the same degree of adulteration effect on its appearance, similar to Quncho teff flour, due to a 30% adulteration with calcium carbonate. The images show that the external appearance of teff flour is different from adulterants. However, upon adulteration, the blend takes intermediary appearance.

Figure 7: Appearance of Magna teff flour (TM), Calcium carbonate (CC) and 30% adulterated teff flour

In both teff varieties, control teff flour was easily distinguishable from calcium carbonate, very slightly distinguishable with gypsum and nearly indistinguishable with sawdust.

The external appearance of flour can be used in initial inspection and sampling process to distinguish the quality of different kinds of food items in the food market.

4.1.4. Appearance of injera

The appearance of prepared injera samples of each variety at the (30%) level of adulteration were compared with the appearance of control teff injera prepared from unadulterated (100% teff flour). The Ethiopian Standard Agency has also set requirements for appearance of teff and injera standard *Table 3*, which was used as reference standard for both control and adulterated teff injera samples in this study.

External appearance may not necessarily indicate the purity of baked injera, since it is a result of many processing factors such as fermentation time, baking temperature, pH and preparation method used [86]. Therefore the appearance test is not a conclusive test to detect adulterated injera products, but should be regarded as an essential starting criteria for injera quality assessment.

4.1.4.1. Appearance of control and 30% adulterated Quncho teff injera

The results showed, *Figure 8*, that injera from unadulterated Quncho teff flour (TQ 100%) exhibit suitable appearance, such as texture, uniformity and distribution of bubbly eyes and pass the appearance requirement of a standard injera set by ESA. Whereas injera samples from adulterated flour showed diminished character based on the degree of adulteration. Among the adulterated injera samples, that prepared with 30% gypsum [(TQ:GY) (70:30)] showed a better appearance than the similarly adulterated injera with sawdust and chalk powder.

According to the results obtained *Figure 8*, the 30% adulterated injera samples from sawdust [(TQ:SD) (70:30)] and calcium carbonate [(TQ:CC) (70:30)] gave no injera appearance. Instead, as observed during the baking process, the 30% sawdust adulterated injera preparation resulted in a shapeless mass and the calcium carbonated adulterated preparation could not even be removed from the baking plate 'mitad'. In both cases there were no acceptable formation of uniform texture, uniformly distributed bubbly eyes nor satisfactory appearance required by ESA.

4.1.4.2. Appearance of control and 30% adulterated Magna teff injera

As can be seen from *Figure 9*, appearance of control Magna teff injera (TM 100%) passes the requirements set by ESA with uniformly distributed bubbly eyes, uniform texture and appearance. As has been shown with the previous teff variety, similarities were observed as to the effect of the adulterants used on injera appearance.

Figure 8: Control and 30% adulterated Quncho teff injera

Figure 9: Control and 30% adulterated Magna teff injera

As can be seen from *Figure 9*, injera prepared from 30% gypsum adulterated Magna teff flour [(TM:GY) (70:30)], showed better resemblance to the control than the 30% chalk powder [(TM:CC) (70:30)] and 30% sawdust [(TM:SD) (70:30)] adulterated injera samples.

In both teff varieties, a 30% adulteration with sawdust and chalk powder resulted in unacceptable injera quality. The baked samples could hardly be called injera. The colour, the distorted bubbly eyes and the offensive odour observed during baking indicated that what is shown in the picture is not a teff injera but a contaminated or spoiled stuff. From both *Figure 8* and *Figure 9*, the adulterated Quncho and Magna teff injeras showed diminished and fine bubbly eyes which are dissimilar to bubbly eyes in the control injera sample, in which the bubbly eyes are larger and defined. This is also observed for the lower adulteration ratios, *Figure 10*. As can be seen from the prepared injera samples, adulterated injera has appearance differences with control injera samples. At higher adulteration ratio, injera appearance of adulterated teff takes much lesser similarity with control injera preparation.

As can be seen from the pictures (Appendix 5) and as observed directly during laboratory analysis, there is an inverse relationship between the quality of injera appearance and adulteration ratio. Highly adulterated injera samples have less similarity with control injera samples (hence less acceptable quality) compared to the unadulterated injera sample. The acceptance is determined based on the injera quality requirements put by the ESA. Customers usually accept or reject a certain food product based on the outward appearance. This is quite helpful in determining food products in the market that may have been adulterated or contaminated.

As observed during the baking process, it was nearly difficult to remove the 30% calcium carbonate adulterated injera baked sample from the baking plate or the mitad as the bottom of the baked was stuck with the smooth surface of the baking mitad.

But the injera prepared from 30% gypsum adulterated Quncho and Magna teff shows the characters of injera, though upon close examination, one can easily behold that the appearance does not convince the observer to perceive characters of a genuine teff injera in that the impure nature and dissimilarity with control injera is seen clearly.

Figure 10: Injera appearance – lower adulteration ratios; TQ (100% Quncho flour injera, Sawdust (SD); Chalk powder (CC); Gypsum (GY)

The difference between the 30% adulterated teff flour injera samples for the two adulterants gypsum and chalk powder may be explained due to the difference in the structure of the two compounds. Chalk powder (calcium carbonate) is in a dehydrated (anhydrous) form whereas the gypsum is in the dihydrate form of calcium sulphate, the latter having a more bonding ability than the former due to hydrogen bonding [82]. Due to this reason, one may expect gypsum particles to have a more homogenizing ability with teff flour during fermentation than the calcium carbonate and hence results in a better injera appearance at higher (30%) adulteration ratio.

As observed during baking, a 20% calcium carbonate adulterated teff injera shows a better appearance than the 25% adulterated injera. This is only logical as the adulteration ratio is decreased the appearance of the baked injera gets a closer resemblance with the control (100%) teff flour injera sample. This was observed in both teff varieties and with all the three adulterants tried at every decreasing adulteration ratio with few exceptions due to fermentation process. Magna teff injera adulterated at a 10% closely imitates the nature of a control injera sample than the 15% or 20% adulterated injera (Appendix 5).

4.2. Physico-chemical characteristics of control and adulterated teff flours

The comparison of mean values for the proximate composition of control (unadulterated) teff flour and adulterated teff flour assists in the detection of food adulteration or contamination. The standard requirements or specifications of teff flour and injera set by the ESA were used as an additional tool to evaluate the effect of adulteration at different adulteration ratios with different adulterants.

As can be observed from literature review, next to the total carbohydrate content of teff flour, the moisture and crude protein content constitute higher portions of the proximate composition which is an advantage for adulteration detection using conventional test procedures. Therefore protein and moisture content were determined across all range of adulteration.

4.2.1. Composition of Quncho teff flour blended with different ratio of adulterants

As can be seen from the mean comparison table 7, adulteration with sawdust significantly reduced the moisture, crude protein and fat content of teff flour significantly at higher adulteration ratio and the significance is lower as adulteration ratio is decreased. For example, moisture content, crude ash and crude fat contents have mean values not significantly different from control teff flour mean values at reduced adulteration ratios (5%).

Results of the primary investigation and the selected test parameters tested at the lowest adulteration ratio (5%) are given on the next pages. A 15% adulterated teff flour was also included as part of the secondary investigation due to the corresponding injera preparations with (15% adulteration) which showed better similar appearance with control injera samples than injera prepared with higher adulteration ratios.

Table 7: Physicochemical properties of Quncho teff flour and blends with different ratios of sawdust

Test Parameter	TQ	SD	TQ:SD (7:3)	TQ:SD (8:2)	TQ:SD (8.5:1.5)	TQ:SD (9:1)	TQ:SD (9.5:0.5)
Moisture	$11.65^{d} \pm 0.24$	$5.03^{a} \pm 0.17$	$8.52^{b} \pm 0.13$	$9.58^{c} \pm 0.12$	$11.59^{d} \pm 0.08$	$11.66^{d} \pm 0.27$	$11.72^{de} \pm 0.05$
Crude Protein	$10.92^{e} \pm 0.05$	$0.37^{a} \pm 0.00$	$7.25^{b} \pm 0.20$	$7.18^{b} \pm 0.13$	$8.68^{c} \pm 0.10$	$8.86^{c} \pm 0.08$	$9.97^{d} \pm 0.15$
Crude Fat	$1.95^{c} \pm 0.02$	ND	$0.43^{a} \pm 0.02$	ND	$0.67^{b} \pm 0.01$	$1.88^{c} \pm 0.03$	$1.89^{c} \pm 0.01$
Crude Ash	$2.01^{a} \pm 0.02$	$1.95^{a} \pm 0.02$	$1.95^{a} \pm 0.02$	ND	$2.00^{a} \pm 0.01$	ND	$1.99^{a} \pm 0.01$
Crude Fiber	$2.07^{a} \pm 0.02$	$63.10^{f} \pm 1.33$	$19.83^{e} \pm 0.26$	ND	$12.87^{d} \pm 0.15$	$8.03^{bc} \pm 0.12$	$6.07^{b} \pm 0.15$
Calcium	$170.60^{b} \pm 0.14$	$127.70^{a} \pm 0.72$	$171.50^{b} \pm 0.82$	ND	ND	ND	$170.10^{b} \pm 0.70$
WHC	$137.00^{a} \pm 3.21$	$536.00^{c} \pm 1.84$	$536.00^{c} \pm 2.03$	ND	ND	ND	$249.00^{b} \pm 1.15$
Bulk Density	$0.83^{d} \pm 0.00$	$0.17^{a} \pm 0.01$	$0.56^{b} \pm 0.00$	ND	ND	ND	$0.74^{c} \pm 0.01$

Data are expressed as mean $\pm$ SEM for N = 3. Means in the same row not sharing the same superscripts are significantly different at ($p < 0.05$). Unit of measurement is in g/100g for all parameters with exception of Calcium in mg/100g and Bulk Density in g/cc. WHC= Water Holding Capacity; TQ = a 100% Quncho Teff Flour; SD = Sawdust; ND = not determined

Table 8 Physicochemical properties of Quncho teff flour and blends with different ratios of Calcium Carbonate

Test Parameter	TQ	TQ:CC (7:3)	TQ:CC (8.5:1.5)	TQ:CC (9:1)	TQ:CC (9.5:0.5)
Moisture	$11.65^{cd} \pm 0.24$	$8.41^{a} \pm 0.09$	$9.84^{b} \pm 0.25$	$11.08^{c} \pm 0.08$	$11.17^{c} \pm 0.42$
Crude Protein	$10.92^{b} \pm 0.05$	$7.28^{a} \pm 0.09$	ND	ND	$9.51^{c} \pm 0.05$
Crude Fat	$1.95^{c} \pm 0.02$	$0.44^{a} \pm 0.01$	ND	ND	$1.88^{b} \pm 0.02$
Crude Ash	$2.01^{a} \pm 0.02$	$30.20^{d} \pm 0.17$	$14.71^{c} \pm 0.19$	ND	$5.07^{b} \pm 0.04$
Crude Fiber	$2.07^{d} \pm 0.02$	$1.42^{a} \pm 0.02$	$1.65^{b} \pm 0.04$	ND	$1.89^{c} \pm 0.02$
Calcium	$170.60^{a} \pm 0.14$	$4113.00^{d} \pm 2.31$	$1976^{c} \pm 0.82$	ND	$833.00^{b} \pm 2.03$
WHC	$137.00^{a} \pm 3.21$	$142.67^{a} \pm 2.40$	ND	ND	$140.36^{a} \pm 1.03$
Bulk Density	$0.83^{a} \pm 0.00$	$0.83^{a} \pm 0.00$	ND	ND	$0.84^{a} \pm 0.01$

Data are expressed as mean ± SEM for N = 3. Means in the same row not sharing the same superscripts are significantly different at ($p < 0.05$). Unit of measurement is in g/100g for all parameters with exception of Calcium in mg/100g and Bulk Density in g/cc. WHC= Water Holding Capacity, TQ = a 100% Quncho Teff Flour, CC = Calcium Carbonate (chalk powder); ND = not determined

Table 9: Physicochemical properties of Quncho teff flour and blends with different ratios of Calcium sulphate dihydrate

Test Parameter	TQ	TQ:CS (7:3)	TQ:CS (8:2)	TQ:CS (8.5:1.5)	TQ:CS (9:1)	TQ:CS (9.5:0.5)
Moisture	$11.65^{bc} \pm 0.24$	$9.44^{a} \pm 0.18$	ND	$11.04^{b} \pm 0.27$	ND	$11.21^{b} \pm 0.09$
Crude Protein	$10.92^{cd} \pm 0.05$	$7.38^{b} \pm 0.21$	$7.07^{a} \pm 0.07$	$8.57^{b} \pm 0.07$	$8.77^{b} \pm 0.18$	$10.24^{c} \pm 0.12$
Crude Fat	$1.95^{c} \pm 0.02$	$1.03^{a} \pm 0.01$	ND	$1.23^{ab} \pm 0.01$	$1.67^{bc} \pm 0.03$	$1.85^{c} \pm 0.07$
Crude Ash	$2.01^{a} \pm 0.02$	$28.57^{d} \pm 0.07$	ND	$14.09^{c} \pm 0.09$	ND	$4.98^{b} \pm 0.06$
Crude Fiber	$2.07^{d} \pm 0.02$	$1.36^{a} \pm 0.03$	ND	$1.62^{b} \pm 0.04$	ND	$1.80^{c} \pm 0.02$
Calcium	$170.6^{a} \pm 1.4$	$3419^{b} \pm 1.53$	ND	$1710^{c} \pm 1.45$	ND	$698^{d} \pm 1.49$
WHC	$137.00^{a} \pm 3.21$	$140.33^{a} \pm 1.33$	ND	ND	ND	$137.34^{a} \pm 1.06$
Bulk Density	$0.83^{a} \pm 0.00$	$0.84^{a} \pm 0.01$	ND	ND	ND	$0.83^{a} \pm 0.01$

Data are expressed as mean $\pm$ SEM for N =3. Means in the same row not sharing the same superscripts are significantly different at ($p < 0.05$). Unit of measurement is in g/100g for all parameters with exception of Calcium in mg/100g and Bulk Density in g/cc. WHC= Water Holding Capacity, TQ = a 100% Quncho Teff Flour, CS = Calcium Sulphate Dihydrate (Gypsum); ND = note determined

The water holding capacity (WHC), bulk density and crude fiber content of Quncho teff flour have been significantly affected by adulteration with sawdust even at the lowest adulteration ratio. This significance is observed in direct proportion with amount or ratio of adulterant throughout the adulteration range.

Tests of crude protein, crude fiber, water holding capacity and bulk density may be used as tools in teff flour samples which are in question due to possible adulteration with similar non-edible materials.

Sawdust flour is bulky in nature and can easily be spotted using the bulk density and water holding capacity tests. Upon examining the mean difference of all measured parameters, the crude ash test brings a clearly visible and easily distinguishable different mean value. This is obvious due to the nature of calcium carbonate. It is an inorganic salt and is easily converted to ash upon ignition.

From the mean comparison table, water holding capacity and bulk density mean values at a higher (30%) teff adulteration ratio with chalk powder is not significantly different from mean value of control teff flour sample. Calcium content of teff flour is significantly different from sawdust. But calcium content of sawdust blends contain similar amount as the unadulterated teff flour sample. The mean moisture, crude protein, fat, ash and fiber content of teff flour is significantly affected due to chalk powder throughout the adulteration ratio.

The water holding capacity and bulk density of Quncho teff flour is not significantly affected by gypsum adulteration. The crude fat test could only be used to see adulteration effect at higher adulteration ratio only. The crude protein, ash and fiber contents are significantly lowered due to adulteration with gypsum.

The crude ash test, as can be expected with samples of inorganic compounds, has shown a highly significant mean difference between the adulterated and non-adulterated teff flour samples. The crude ash test is therefore a very good test for any sample that may have been adulterated or contaminated with inorganic calcium salts. The ash value is also directly proportional to the amount of adulterant ratio in the flour blends, assuring the efficacy of the test in similar test procedures. Consequently, mean calcium content of tested blend ratios resulted in a significantly different mean value from unadulterated teff flour at $p < 0.05$, which infers the test could be used in detecting adulterants composed of calcium compounds.

4.2.2. Composition of Magna Teff Flour blended with different ratios of adulterants

From the mean values of proximate composition of Magna teff flour and blends, the moisture, crude fat, ash and fiber mean values are comparable with control sample mean values of control Magna teff flour and these may not be reliable tests of adulteration especially at lower adulteration ratios. As has been seen with the previous teff variety, the water holding capacity and bulk density tests stand out as reliable indicative tests for adulteration with sawdust. The crude protein test could also be used as adulteration indicator in this particular case for as low as 10% of adulteration with sawdust. The mean calcium content of unadulterated magna teff flour is significantly different with a blend from 30% sawdust but not significant in a 15% and 5% adulterated flour.

The crude protein test show significant mean difference from the control sample up to a 10% adulteration ratio. The water holding capacity and bulk density tests are of little value to be used as adulteration indicators in this case.

The most significant mean value difference is observed for the test of crude ash. The crude ash value is directly proportional to the ratio of adulteration. Inorganic calcium salts are stable and easily converted to ash. This test could be used as a good indicator for flour samples adulterated or contaminated with salts of calcium and other similar elements.

Table 10: Physicochemical properties of Magna teff flour and blends with different ratios of Sawdust

Test Parameter	TM	SD	TM:SD (7:3)	TM:SD (8.5:1.5)	TM:SD (9:1)	TM:SD (9.5:0.5)
Moisture	$12.18^{d} \pm 0.13$	$5.03^{a} \pm 0.17$	$8.66^{b} \pm 0.06$	$11.16^{c} \pm 0.16$	$11.15^{c} \pm 0.12$	$11.72^{cd} \pm 0.12$
Crude Protein	$10.47^{d} \pm 0.04$	$0.37^{a} \pm 0.00$	$7.13^{b} \pm 0.10$	$8.64^{c} \pm 0.12$	$8.40^{c} \pm 0.28$	$10.63^{d} \pm 0.17$
Crude Fat	$1.98^{c} \pm 0.04$	ND	$1.52^{a} \pm 0.04$	$1.63^{b} \pm 0.01$	$1.78^{b} \pm 0.04$	$1.89^{c} \pm 0.04$
Crude Ash	$1.97^{a} \pm 0.02$	$1.95^{a} \pm 0.02$	$1.93^{a} \pm 0.02$	$1.93^{a} \pm 0.02$	ND	$1.94^{a} \pm 0.03$
Crude Fiber	$2.01^{a} \pm 0.01$	$63.10^{f} \pm 1.33$	$19.50^{e} \pm 0.46$	$11.93^{d} \pm 0.15$	$8.03^{c} \pm 0.12$	$3.87^{b} \pm 0.15$
Calcium	$188.4^{b} \pm 0.4$	$127.7^{a} \pm 0.72$	$197.9^{c} \pm 0.98$	$188.60^{b} \pm 0.41$	ND	$188.30^{a} \pm 0.49$
WHC	$151.67^{a} \pm 0.88$	$535.67^{c} \pm 3.84$	$546.67^{cd} \pm 3.76$	ND	ND	$249.33^{b} \pm 2.73$
Bulk Density	$0.85^{d} \pm 0.02$	$0.17^{a} \pm 0.00$	$0.55^{c} \pm 0.00$	ND	ND	$0.69^{b} \pm 0.00$

Data expressed as mean ± standard error of mean (where N = 3). Mean values in the same row not sharing the same superscripts are significantly different at ($p < 0.05$). Unit of measurement is in g/100g with the exception of Calcium expressed in mg/100g and Bulk Density in g/cc; WHC= Water Holding Capacity; TM = a 100% Magna Teff Flour, SD = Sawdust; ND = not done

Table 11: Physicochemical properties of Magna teff flour and blends with different ratios of Calcium Carbonate

Test Parameter	TM	TM:CC (7:3)	TM:CC (8.5:1.5)	TM:CC (9:1)	TM:CC (9.5:0.5)
Moisture	$12.18^{cd} \pm 0.13$	$8.29^{a} \pm 0.43$	$9.84^{b} \pm 0.25$	$11.30^{c} \pm 0.03$	$11.34^{c} \pm 0.04$
Crude Protein	$10.47^{c} \pm 0.04$	$6.65^{a} \pm 0.14$	$8.31^{b} \pm 0.07$	$8.77^{b} \pm 0.06$	$10.05^{c} \pm 0.06$
Crude Fat	$1.98^{d} \pm 0.04$	$0.39^{a} \pm 0.02$	$0.76^{b} \pm 0.02$	ND	$1.38^{c} \pm 0.03$
Crude Ash	$1.97^{a} \pm 0.02$	$30.30^{d} \pm 0.08$	$15.41^{c} \pm 0.21$	ND	$4.97^{b} \pm 0.02$
Crude Fiber	$2.01^{d} \pm 0.01$	$1.19^{a} \pm 0.02$	$1.32^{b} \pm 0.03$	$1.61^{c} \pm 0.07$	$1.76^{c} \pm 0.03$
Calcium	$184.40^{a} \pm 0.4$	$4214.00^{d} \pm 1.76$	$1986^{c} \pm 1.15$	ND	$870.00^{b} \pm 1.45$
WHC	$151.67^{a} \pm 0.88$	$152.00^{a} \pm 0.58$	ND	ND	$151.41^{a} \pm 0.33$
Bulk Density	$0.852^{a} \pm 0.23$	$0.802^{a} \pm 0.003$	ND	ND	$0.837^{a} \pm 0.004$

Data are expressed as mean ± SEM for N = 3. Means in the same row not sharing the same superscripts are significantly different at ($p < 0.05$). Unit of measurement is in g/100g for all parameters with exception of Calcium in mg/100g and Bulk Density in g/cc. WHC= Water Holding Capacity, TM = a 100% Quncho Teff Flour, CC = Calcium Carbonate (chalk powder); ND = not determined

Table 12: Physicochemical properties of Magna teff flour and blends with different ratios of Calcium sulphate dihydrate

Test Parameter	TM	TM:CS (7:3)	TM:CS (8:2)	TM:CS (8.5:1.5)	TM:CS (9:1)	TM:CS (9.5:0.5)
Moisture	$12.18^{cd} \pm 0.13$	$8.66^{a} \pm 0.11$	ND	$11.11^{c} \pm 0.21$	$9.73^{b} \pm 0.24$	$11.21^{c} \pm 0.09$
Crude Protein	$10.47^{d} \pm 0.04$	$6.31^{a} \pm 0.08$	$7.27^{b} \pm 0.02$	$8.36^{c} \pm 0.10$	$8.53^{c} \pm 0.12$	$10.78^{d} \pm 0.04$
Crude Fat	$1.98^{c} \pm 0.04$	$1.46^{a} \pm 0.02$	ND	$1.82^{bc} \pm 0.04$	$1.89^{c} \pm 0.01$	$1.74^{b} \pm 0.03$
Crude Ash	$1.97^{a} \pm 0.02$	$30.01^{e} \pm 0.12$	ND	$14.13^{d} \pm 0.10$	$9.77^{c} \pm 0.16$	$5.05^{b} \pm 0.03$
Crude Fiber	$2.01^{d} \pm 0.01$	$1.22^{a} \pm 0.02$	ND	$1.43^{b} \pm 0.02$	$1.79^{c} \pm 0.02$	$1.72^{c} \pm 0.01$
Calcium	$188.4^{a} \pm 0.40$	$3510^{d} \pm 1.08$	ND	$1765^{c} \pm 2.6$	ND	$718.5^{b} \pm 1.32$
WHC	$152.00^{a} \pm .09$	$151^{a} \pm 0$	ND	ND	ND	$151^{a} \pm 0.11$
Bulk Density	$0.852^{a} \pm 0.02$	$0.814^{a} \pm 0.02$	ND	ND	ND	$0.836^{a} \pm 0.49$

Data are expressed as mean $\pm$ SEM where N =3. Means in the same row not sharing the same superscripts are significantly different at ($p < 0.05$). Unit of measurement is in g/100g for all Physico-Chemical parameters with exception of Calcium expressed in mg/100g and Bulk Density in g/cc; WHC= Water Holding Capacity; TM = a 100% Magna Teff Flour, CS = Calcium Sulphate Dihydrate (Gypsum); ND = not determined

The water holding capacity and bulk density mean values of gypsum adulterated Magna teff flour are not significantly different from the unadulterated teff flour. The crude fat test could only be used to see adulteration effect at higher adulteration ratio only. The crude protein, ash and fiber contents mean values are significantly lowered due to adulteration with gypsum. The crude ash test, as can be expected with samples of inorganic compounds, has shown a highly significant mean difference between the adulterated and non-adulterated teff flour samples. The crude ash test is therefore a very good test for any sample that may have been adulterated or contaminated with inorganic calcium salts. The ash value is also directly proportional to the amount of adulterant ratio in the flour blends, assuring the efficacy of the test in similar test procedures. Consequently, the mean calcium content of unadulterated magna teff flour is significantly different from flour blend ($p < 0.05$) of magna flour and gypsum.

4.3. Physicochemical composition of injera

The comparison of mean values determinations of the proximate composition of control (unadulterated) teff injera and adulterated teff injera reveals the test parameters which may be used in the detection of adulterated/contaminated teff injera. There are standard requirements and specifications for teff injera quality set by ESA which gives basic food analyses test parameters for effective identification of adulteration in similar cases.

4.3.1. Composition of Quncho teff injera blended with different ratios of adulterants

The mean moisture content of injera from sawdust adulterated teff flour is significantly different from unadulterated (100%) Quncho teff injera (control) in all of the three adulteration ratios given. Likewise, injera from chalk powder adulterated flour showed significant difference at the 15% adulteration ratio.

There was no significant mean difference in the gypsum adulterated injera samples except at the 15% ratio where there is significant mean difference in moisture content with control sample. The moisture content requirement set by the Ethiopian Standard Agency (ESA) for injera is between 58 - 63 g per 100 g. Sawdust adulterated injera samples resulted in out of specification, whereas the chalk powder and gypsum adulterated samples resulted in out of specification values only at the 15% adulteration ratio.

Table 13: Composition of injera from Quncho teff flour and blends with different ratios of adulterants

Injera preparation	Test Parameter					
	Moisture	Protein	Fat	Ash	Fiber	Calcium
QE	$61.60^{ab} \pm 0.7$	$4.11^{c} \pm 0.14$	$0.98^{c} \pm 0.06$	$1.99^{b} \pm 0.01$	$1.96^{b} \pm 0.02$	$66.43^{a} \pm 0.62$
TQ:SD 15%	$68.37^{e} \pm 0.24$	ND	ND	$1.68^{a} \pm 0.01$	$7.87^{d} \pm 0.03$	$62.47^{a} \pm 0.41$
TQ:SD 10%	$66.77^{de} \pm 0.09$	ND	ND	ND	ND	ND
TQ:SD 5%	$65.17^{d} \pm 0.15$	$3.35^{b} \pm 0.14$	$0.44^{b} \pm 0.01$	ND	$4.90^{c} \pm 0.12$	$64.04^{a} \pm 0.43$
TQ:CC 15%	$65.03^{d} \pm 0.19$	$2.87^{a} \pm 0.39$	ND	$14.13^{d} \pm 0.44$	ND	$707.04^{d} \pm 0.70$
TQ:CC 10%	$62.47^{a} \pm 0.75$	ND	ND	ND	ND	ND
TQ:CC 5%	$61.47^{ab} \pm 0.38$	$3.46^{b} \pm 0.11$	$0.35^{a} \pm 0.01$	$6.20^{c} \pm 0.12$	$1.34^{a} \pm 0.03$	$295.00^{c} \pm 0.92$
TQ:CS 15%	$64.73^{c} \pm 0.26$	$2.93^{a} \pm 0.26$	ND	$13.61^{d} \pm 0.38$	ND	$697.29^{cd} \pm 0.32$
TQ:CS 10%	$62.73^{b} \pm 0.79$	ND	ND	ND	ND	ND
TQ:CS 5%	$60.67^{a} \pm 0.78$	$3.59^{b} \pm 0.11$	$0.41^{b} \pm 0.02$	$5.92^{c} \pm 0.12$	$1.31^{a} \pm 0.03$	$268.00^{b} \pm 1.47$

Data are expressed as mean ± SEM. Means in the same column not sharing the same superscripts are significantly different at ($p < 0.05$). Unit of measurement is in g/100g for all test parameters with exception of Calcium expressed in mg/100g;

QE = a 100% Quncho teff injera (Control); TQ = Injera from Quncho teff flour with blends in which SD = Sawdust (at 15%, 10% and 5%) blend ratio with Quncho teff flour; CC = Calcium Carbonate (at 15%, 10% and 5%) blend ratio with Quncho teff flour; CS = Calcium Sulphate (gypsum) at 15%, 10% and 5% blend ratio with Quncho teff flour used for injera preparation; ND = not determined.

The test of crude protein content showed a significant mean difference between the unadulterated and adulterated injera samples at the 15 and 10% adulteration ratios. The specified requirement of (3-3.8 g/100g) set by ESA has been met, except at the 15% adulteration ratio where the experimentally obtained values are less than 3g per 100g. However, the protein value of the unadulterated teff injera protein was slightly higher than the range given by the standard agency. As the standard pertains to "Teff Injera" with no specific variety within the teff specification, there is no need to assume the protein value of the control sample is out of specification.

The mean crude fat content of all injera samples differs significantly with control injera sample and is not within the limits set by ESA, 0.6-0.7g/100g. The mean crude ash value of sawdust adulterated injera samples showed no significant difference compared with control injera sample. On the contrary, the chalk powder and gypsum adulterated injera samples showed a significant mean difference with crude ash content of control injera sample. The mean crude fiber values of all adulterated samples are significantly different with control mean value. The sawdust adulterated samples showed a very high amount of fiber content than the specification put by the ESA. The mean fiber value of the control sample lies just above the upper limit set by the agency. This may be due to differences within teff varieties and other differences in processing techniques which may result in slightly differing mean values for some physico-chemical tests.

The mean calcium content of injera from unadulterated Quncho teff flour is significantly different with injera prepared from chalk powder and gypsum adulterated flours at the tested ratios whereas there is no significant mean difference with injera from 15 and 5% sawdust blended Quncho teff flour. The significant mean differences in ash and mineral (calcium) mean values proves the obvious and logical consideration of these tests as good indicators for adulteration with inorganic materials.

4.3.2. Composition of Magna teff injera blended with different ratios of adulterants

From the results presented (*Table 14*), the mean moisture content of injera samples from blends (5 and 10%) of sawdust and calcium carbonate are not significantly different from mean moisture content of control magna teff injera. The 15% adulterated teff injera showed significant difference with control injera preparation.

Table 14: Composition of injera from Magna teff flour and blends with different ratios of adulterants

	Test Parameters					
	Moisture	Protein	Fat	Ash	Fiber	Calcium
ME	$62.20^{b} \pm 0.35$	$3.98^{c} \pm 0.09$	$1.05^{b} \pm 0.06$	$1.67^{b} \pm 0.03$	$1.46^{b} \pm 0.1$	$68.10^{b} \pm 0.12$
TM-SD 15%	$65.04^{e} \pm 0.02$	ND	ND	ND	$6.85^{d} \pm 0.32$	$66.53^{a} \pm 0.52$
TM-SD 10%	$64.93^{e} \pm 0.20$	$2.77^{b} \pm 0.11$	ND	$0.88^{a} \pm 0.04$	ND	ND
TM-SD 5%	$62.83^{b} \pm 0.24$	$3.35^{c} \pm 0.16$	$0.49^{a} \pm 0.01$	$1.04^{a} \pm 0.04$	$4.73^{c} \pm 0.17$	$67.39^{b} \pm 0.52$
TM-CC 15%	$64.70^{de} \pm 0.35$	$1.98^{a} \pm 0.22$	ND	$16.02^{d} \pm 0.76$	ND	$711.00^{f} \pm 0.81$
TM-CC 10%	$64.13^{cd} \pm 0.18$	ND	ND	ND	ND	ND
TM-CC 5%	$63.73^{c} \pm 0.48$	$2.12^{ab} \pm 0.16$	$0.44^{a} \pm 0.04$	$5.14^{c} \pm 0.06$	$0.96^{a} \pm 0.05$	$298.00^{d} \pm 0.68$
TM-CS 15%	$61.22^{a} \pm 0.30$	$2.38^{b} \pm 0.17$	ND	$15.59^{d} \pm 0.49$	ND	$699.00^{e} \pm 0.62$
TM-CS 10%	$61.15^{a} \pm 0.46$	ND	ND	ND	ND	ND
TM-CS 5%	$61.50^{a} \pm 0.4$	$2.37^{b} \pm 0.14$	$0.45^{a} \pm 0.03$	$4.59^{c} \pm 0.15$	$1.05^{a} \pm 0.04$	$276.00^{c} \pm 0.74$

Data are expressed as mean ± SEM. Means in the same column not sharing the same superscripts are significantly different at ($p < 0.05$). Unit of measurement is in g/100g for all Physico-Chemical parameters with exception of Calcium expressed in mg/100g

ME = a 100% Magna teff injera (Control); TM = Injera from Magna teff with blends in which SD = Sawdust (at 15%, 10% and 5%) blend ratio with Magna teff flour; CC = Calcium Carbonate (chalk powder) (at 15%, 10% and 5%) blend ratio with Magna teff flour; CS = Calcium Sulphate (gypsum) at 15%, 10% and 5% blend ratio with Magna teff flour used for injera preparation; ND = not done

Except the 5% sawdust and calcium carbonate blends, all adulterated injera samples are out of the specification laid out by the ESA (58-63g/100g). The moisture test can be used as an effective tool to evaluate the quality of injera samples.

The mean moisture content of injera samples from blends (5 and 10%) are not significantly different from mean moisture content of control magna teff injera. The 15% adulterated teff injera showed significant difference with control injera preparation.

There is a significant difference between adulterated injera samples and control sample mean protein value except for a 5% sawdust adulterated injera sample, in which all adulterated injera samples have a mean protein value less than the minimum requirement (3.0g/100g) set by the ESA. Therefore, the crude protein test could be used to identify injera preparations that may have been adulterated with foreign matter.

The mean crude fat value of all adulterated injera samples are significantly different from the mean crude fat value of control injera preparation. These mean values are also below the minimum required value (0.6-0.7g/100g) as set by the ESA indicating crude fat test may be used to distinguish adulterated injera samples.

The mean crude ash value of all adulterated injera preparations are significantly different from mean crude ash value of control injera sample with the exception of 5% sawdust adulterated injera preparation. Crude ash test, as shown in previous data sets, is a very crucial test that may be used to expose injera preparations which may have been adulterated with inorganic materials. This is demonstrated in the test result from the mean calcium value of adulterated magna flour injera, which are significantly different across all adulterants and blending ratios for $p < 0.05$ except the 5% sawdust adulterated flour which has relatively closer value to the control sample.

4.4. Qualitative Tests

Based on the nature of adulterant suspected to be present within teff flour or injera, some additional tests may be performed to demonstrate presence of foreign matter.

4.4.1. Test of Effervescence

For teff flour that may have been adulterated with chalk powder or calcium carbonate, test of effervescence could be employed. In this test procedure, a positive effervesce test indicates the presence of excess carbonate in general and calcium carbonate in particular to this study [78].

Initially flour from the two teff varieties adulterated with 30% of each of sawdust, calcium carbonate and calcium sulphate were tested for effervescence.

Table 15: Primary test of Effervescence

Flour/Blend	30% SD	30% CC	30% CS
TQ	–	+	–
TM	–	+	–

Based on the result from the primary test of effervescence, teff flour blended with calcium carbonate at each adulteration ratio was tested for each of the teff varieties. The test was done on both blends and also on the dried and powdered form of the injera preparations. All injera test samples are finely ground after drying. Injera was not obtained from the 30% adulteration ratio, and the test was not applicable (NA). Effervescence test results are summarized below.

Table 16: Test of Effervescence for control teff and calcium carbonate adulterated samples

Sample Preparation	Calcium Carbonate (%)						
	30%	25%	20%	15%	10%	5%	0%
TQ Flour/Blend	+	+	+	+	+	-	-
TQ Injera	NA	+	+	+	+	-	-
TM Flour/Blend	+	+	+	+	+	-	-
TM Injera	NA	+	+	+	+	-	-

TQ = Quncho teff; TM = Magna teff; + positive for effervescence; (-) no effervescence; NA = Not Applicable (no injera was obtained for this ratio); 0% = 100% teff flour/injera (control)

As can be seen from the result summary above, teff flour or teff injera adulterated up to a 10% of calcium carbonate could be identified using the effervescence test. A 5% adulteration with chalk powder cannot be detected using effervescence test. This test could also be used as an initial test prior to mineral analysis like calcium and magnesium, which upon determination can prove the presence of foreign matter in the samples analysed.

4.4.2. Test for excess sulphate anions by precipitation with barium

One simple test that may be used as a tool to detect and quantify excess ions such as sulphate ions due to adulteration or contamination is the use of precipitation by barium salt. Barium sulphate is an insoluble salt in aqueous solutions and the precipitation requires free sulphate anions in a very slightly acidic medium [79].

Gypsum or calcium sulphate dihydrate ($CaSO_4.2H_2O$) mixed in teff flour can easily be solubilized to give its sulphate anions (SO_4^{2-}) into aqueous solution which can be precipitated with barium cations (Ba^{2+}) to give barium sulphate ($BaSO_4$) precipitate, which is uniquely insoluble and a specific test for sulphate anions in solutions [79].

The test procedure results in turbid solutions if sulphate ions are present, otherwise solutions remain clear. Though there are different analytical procedures to determine the concentration of precipitates, simple way would be to determine the amount of barium sulphate gravimetrically.

Control samples were prepared similarly from a 100% teff flour using the two varieties. Blank solutions for each were prepared without test samples.

A test sample of 1 g from a teff flour/blend or dried injera sample prepared by adulterating with 30% gypsum ideally contains 0.3 g of $CaSO_4.2H_2O$ (MW = 172 g/mol), which contains about 55.8% of SO_4^{-2} (about 0.1674 g). When sulphate anion is precipitated as $BaSO_4$ (MW = 233.4 g/mol), it gives about 0.4 g of precipitate theoretically, which would be enough to detect and quantify excess sulphate in tested samples [78, 82].

Table 17: Precipitation of sulphate anion using barium

Sample	Calcium sulphate dihydrate precipitation						
	30%	25%	20%	15%	10%	5%	0% (Control)
TQ Blend	+G	ND	ND	+	+	+G	-
TQ Injera	+G	+	+	+G	+	+G	-
TM Flour/Blend	+G	ND	ND	+	+	+G	-
TM Injera	+G	+	+	+G	+	+G	-

TQ = Quncho teff; TM = Magna teff; (+) positive for precipitate formation or opalescence; (+G) gravimetrically measured in triplicates; (-) clear solution or no precipitation; 0% = 100% teff flour/injera sample used as control; ND = not determined

To test the above theoretical assumption and consider this test procedure as a success, some of the opalescent solutions obtained with addition of barium chloride solution were allowed to settle and gravimetric determination made (+G). These measurements were made to show that gravimetric analysis could be employed to detect and quantify food adulterants in the form of sulphates using barium solution as a reagent. The control samples and corresponding blank solutions showed no precipitation and are not included in the table.

Table 18: Sulphate determination as Barium Sulphate

Residue (Weight of precipitate) as $BaSO_4$				
% Gypsum	**TQF**	**TQI**	**TMF**	**TMI**
30%	0.355 ± 0.024	0.286 ± 0.006	0.326 ± 0.006	0.268 ± 0.015
15%	ND	0.161 ± 0.005	ND	0.167 ± 0.010
5%	0.154 ± 0.008	0.108 ± 0.003	0.148 ± 0.004	0.118 ± 0.004

Data are expressed as Mean ± Standard Deviation (N =3); TQF = Teff Quncho Flour; TQI = Teff Quncho Injera; TMF = Teff Magna Flour; TMI = Teff Magna Injera; ND = not done

Test for excess sulphate using barium is a very effective tool for quick determination of the presence of foreign matter (containing excess SO_4^{-2} anion) in teff flour and teff injera. Control samples prepared from unadulterated teff injera and flour showed no precipitate or cloudiness (opalescence). Blank solution also showed no opalescence and remained clear (Appendix 6). This is a good indication that the test procedure could further be developed into a standard test procedure to test the sulphate concentration in teff samples and similar food items.

Table 19: Selected test parameters that indicate adulteration

Test	Sample type	*Mean		Limit	Adulterant implicated
		Control	Adulterated (5%)		
Ash (g/100g)	Grain Flour	1.97	5.02	[&]2.3 - 2.8	Chalk powder / Gypsum
	Injera	1.83	5.46	[α]0.7 - 1.7	Chalk powder / Gypsum
Bulk Density (g/cc)	Grain Flour	0.84	0.72	NA	Sawdust
	Injera	NA	NA	NA	NA
Calcium (mg/100g)	Grain Flour	179.5	779.89	[&]180	Chalk powder / Gypsum
	Injera	67.27	284.25	[α] 50.0 - 68.0	Chalk powder / Gypsum
Crude Fiber (g/100g)	Grain Flour	2.04	4.97	NA	Sawdust
	Injera	1.71	4.82	[α]1.0 -1.8	Sawdust
WHC (g/100g)	Grain Flour	144.34	249.17	NA	Sawdust
	Injera	NA	NA	NA	Sawdust
Qualitative tests		**Detection**			
Effervescence using HCl solution	Grain Flour	No Effervescence	No effervescence	NA	Chalk powder
	Injera	No Effervescence	Effervescence	NA	Chalk powder
Precipitation of excess SO_4^{-2} anion using Ba^{2+} solution	Grain Flour	No precipitate	Precipitate	NA	Gypsum
	Injera	No precipitate	Precipitate	NA	Gypsum

*Mean values obtained from the two teff varieties; Injera samples (dried, powder); NA = not applicable

[α] Ethiopian Standard Agency (ESA)

[&] United States Department of Agriculture (USDA)

CHAPTER FIVE

5. CONCLUSIONS AND RECOMMENDATION

5.1. Conclusion

In this study, attempts have been made to screen out common physico-chemical test parameters that ban be employed to detect teff flour and ultimately injera adulteration. The study was made by adulterating two white teff varieties (Quncho and Magna) with three non-edible adulterants (sawdust, calcium carbonate and calcium sulphate di-hydrate). The screened physico-chemical test parameters that are commonly used in food composition analysis included proximate analysis, mineral analysis, functional properties and specific qualitative tests.

Based on the findings of the study, the following conclusions can be drawn:

- There is a significant mean difference in all physicochemical properties of control teff flour samples (DZ-Cr-387 and DZ-01-196) and 30% adulterated teff flours ($p < 0.05$).
- It is possible to use non-edible adulterants (found in recent food fraud incidents) in injera preparation and produce acceptable quality injera to the market (i.e. up to 20% using sawdust, 15% using calcium carbonate and up to 25% using gypsum)
- Water holding capacity and bulk density tests can be used to detect teff flour adulterated with non-edible, organic adulterant like sawdust up to adulteration ratio of 5%.
- Crude fibre analysis can be used to detect teff flour and injera adulterated with sawdust up to 5%.
- Crude ash analysis can be used to detect inorganic adulterants like calcium carbonate (chalk powder) and calcium sulphate dihydrate (gypsum) in teff flour and injera up 5% adulteration.
- Calcium in mineral analysis can be used to detect inorganic adulterants such as chalk powder and gypsum as low as 5% of adulteration in teff flour and injera.

The flour blend and injera analysis data have shown that there are specific tests that reveal the presence of specific adulterant in teff flour. Depending on the nature of adulterant and nature of sample obtained (flour or injera), the analyst may perform some specific tests to check if there is an adulterant in the sample.

From the simulated flour and injera samples adulterated with sawdust, chalk powder and gypsum, the following test parameters were effective for indicating adulteration.

Inorganic adulterants could be detected and quantified at as low as 5% adulteration using crude ash test both in the teff flour and injera preparations. Non edible organic adulterants like sawdust could be detected using physical test like crude fiber, bulk density and water holding capacity (for flour analysis) and crude fiber test for injera samples at as low as a 5% adulteration ratio. Depending on the capacity of analysing laboratory and personnel, all test parameters could be used to detect and quantify adulterants in higher adulteration ratios.

Moisture: The moisture content of teff flour is affected at higher adulteration ratios. The effect of adulteration on moisture content depends on the kind of adulterant used. As has been shown, test of moisture is more valuable on the injera than on the flour. Though moisture analysis may indicate higher adulteration ratio in flours/blends, its efficacy is more evident on injera analysis and specifically on injera prepared with sawdust adulteration. Though further study is required to observe the effect of moisture due to adulteration on teff flour varieties, mean moisture content of Magna teff flour is more affected due to adulteration with sawdust than the Quncho teff flour. And in injera samples of the two teff flours, mean moisture content of injera from Quncho teff flour are affected due to adulteration than the similar preparation made with Magna teff flour.

Crude Protein: The crude protein test is a very essential test parameter that may be used as a very good indicator of adulteration in teff flour and injera throughout the ratios of adulteration. In this study, test of crude protein has been shown to be a good indicator of quality deviation between control and test samples in both the flour and injera analysis. Due to lower protein mean value in injera preparations, test of crude protein may be effective as quality indicator for adulteration ratio of 10% and higher.

Crude Ash: The test of crude ash is perhaps the most crucial test that distinguished between control samples and preparations adulterated with inorganic materials. Most inorganic materials or adulterants have a very low carbon and higher mineral content. Because of this obvious reason, teff flour samples and injera preparations adulterated with chalk powder and gypsum have resulted in a very high mean crude ash value. This gives food analysts an immense advantage in tackling the issues of food adulteration with non-edible inorganic materials. There are also other inorganic compounds that may be used as adulterant in flour based food products. The best approach to solve these kind of issues in laboratory testing is to properly analyse the crude ash content of food items in question.

Teff flour has an average of not more than 3% crude ash among its varieties. Crude ash values of teff injera are also expected to be between 0.7-1.7 g/100g for a given injera sample to be labelled as meeting the specification set by ESA. Therefore, any crude ash value that is significantly higher than the specified limit values clearly indicate the presence of foreign matter, either as an adulterant or as a contaminant.

Crude Fibre: The crude fibre test is one of the most effective tools to determine the genuineness of teff flour and injera. Teff injera is expected to contain up to 2% of crude fibre as a requirement set by the ESA. If during preparation of the teff flour, other fibre rich adulterants are added, the crude fibre test will give an out of specification result as shown with the two teff varieties adulterated with sawdust (*Tables 13 & 14*). Sawdust contains a very high amount of non-edible fibre [87] and is indicated with a significant mean difference between adulterated and control samples for both the flour and injera samples upon analysis of crude fibre.

Purified cellulose powder is used in the pharmaceutical industry and as well as the food industry as a source of fibre additive. This additives are mainly obtained from wood through pulping followed by purification [88]. This may raise the issue that teff powder adulterated with sawdust may be of little concern as a food fraud. But care should be taken upon declaring such opinions. The food additives from wood pulp are purified and are added to food items and pharmaceutical products, but with adulteration acts, there are no purification stages to remove harmful substances or microorganisms. This is a threat to consumer health. In addition, teff has a naturally balanced amount of fibre and there is no need for arbitrary amount of sawdust addition.

Calcium: As has been shown with the test results from specific blending ratios, the test for mineral calcium is one of the effective ways to show the significant mean difference between control and test sample contaminated or mixed with inorganic matter.

5.2. Recommendation

Due to the increased demand for teff [6], possibilities have arisen within the less censored food suppliers in the midst of city of Addis Ababa to take advantage of the high demand and use it for improper gain and profit through adulteration.

For centuries teff has been produced and consumed on a large scale with its benefits largely unknown and undermined. Now, different researches and growing techniques in the food industry are allowing teff to be known and utilized for its great health benefits. Alone or with other cereals, teff is now used in the production of different kinds of food production [89, 90]. The demand is rising in many places and as opportunities are higher, so are the food fraud risks associated with it.

Therefore, the concerned government bodies, along with the scientific community should gather together to create a platform and organize necessary efforts that will eventually create a research community dedicated to indigenous crop preservation such as teff and other special crops which have special meaning to Ethiopia and its people.

Food adulteration with non-edible adulterants is becoming a recurrent situation in Addis Ababa city. So far, the concerned government bodies have shown reliable capacity to deal with food fraud incidents. To strengthen the fight against food adulteration, the following points are recommended based on the findings of this study.

- The Ethiopian Standards Agency (ESA) should increase efforts to establish additional teff grain and injera requirements. If possible, at the very least, all of the widely consumed teff varieties should be considered in the preparation of teff standard or teff grain and injera specifications.
- The Ethiopian Food, Medicine, Health Care and Administration Authority (EFMHACA) should expand the regulatory activities into more markets (both formal and informal markets) and perform regular or periodical sampling for routine analysis. The authority should be the singular body to sample, analyse and detect any kind of food frauds. It should not entirely be dependent on customer complaints and whistle-blowers to prevent food frauds.
- The City Police, (i.e. Addis Ababa Police Commission) should be able to rely on scientific evidence to seize and charge illegal food production and distribution by employing known standard procedures and enforcing regulatory activities conducted by other concerned bodies.

- Government bodies such as Ministry of Health, EFMHACA, ESA, Research Institutions such as EPHI should work together to address the issue properly. This can be done primarily by creating a national database for food fraud control.
- Universities should advocate the necessity of researches directed at solving food adulteration issues in effective and systematic ways.

There should be collaboration in the areas of teff research and development within concerned government and private sectors in which existing studies related to teff should be developed and maximized to the point where the full potentials of the crop are used and associated risks such as health hazards due to adulteration, food insecurity and malnutrition are faced and tackled effectively.

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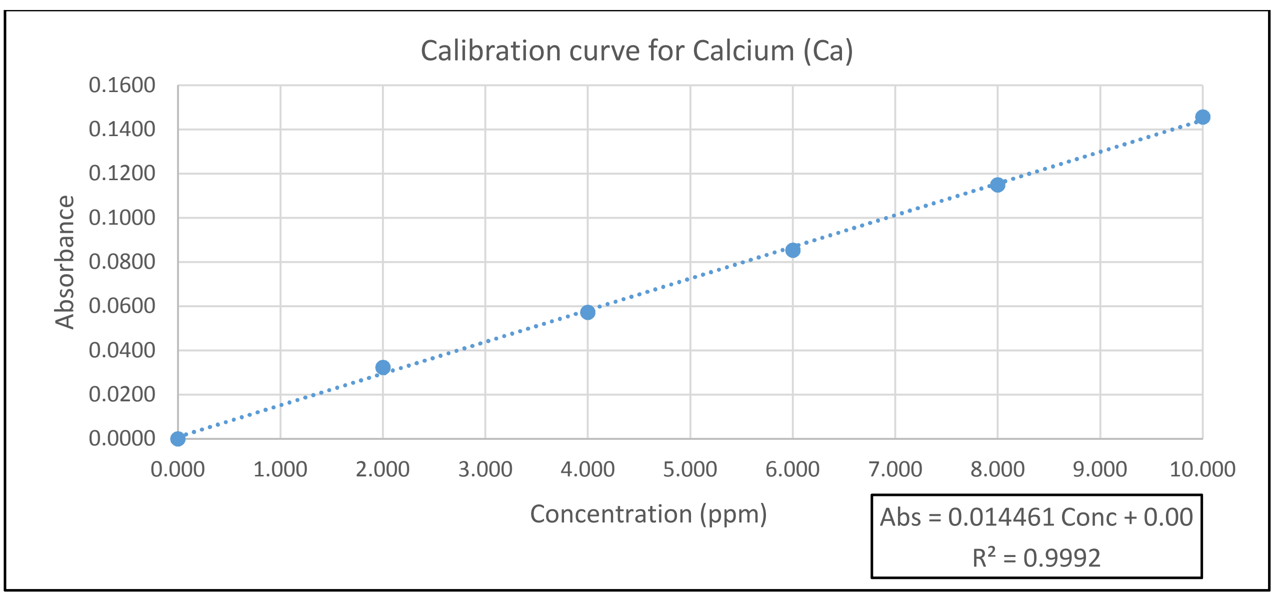
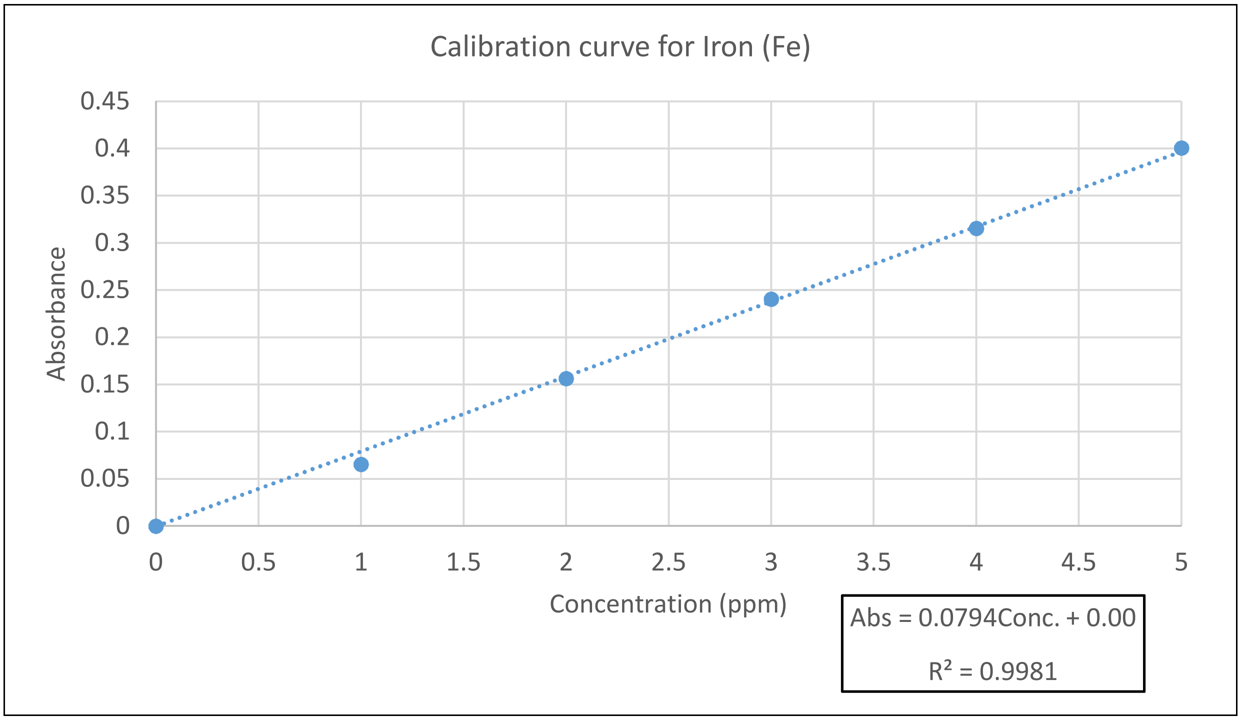
Appendices

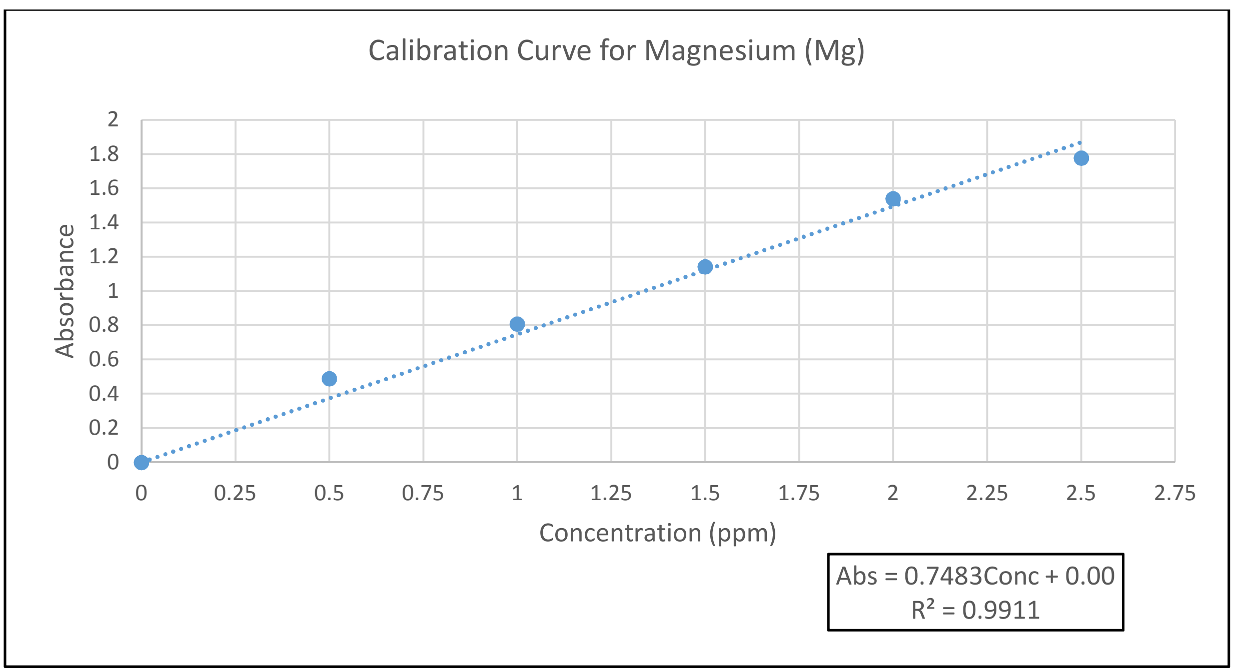
Appendix 1: Calibration curve for Calcium (Ca)



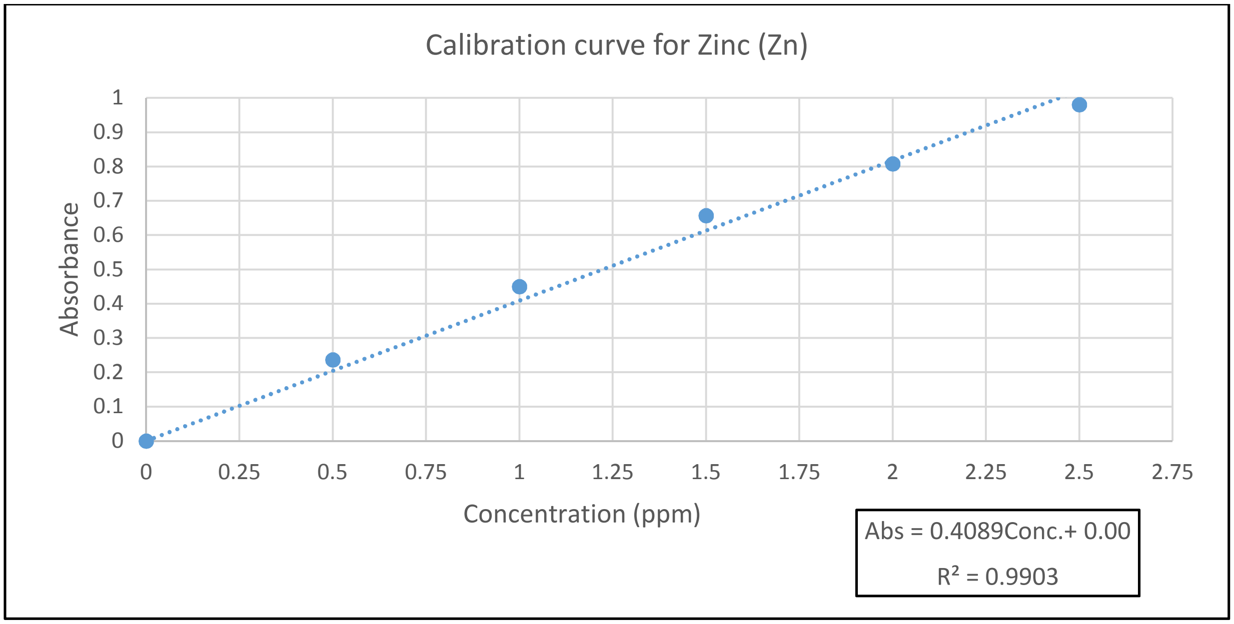
Appendix 2: Calibration curve for Iron (Fe)



Appendix 3: Calibration curve for Magnesium (Mg)



Appendix 4: Calibration curve for Zinc (Zn)



Appendix 5: Injera appearance at different adulteration ratios

Appendix 6: Precipitation of insoluble salt of calcium using barium solution

Appendix 7: In the laboratory

Appendix 8: Letter from Addis Ababa Police Commission

በአዲስ አበባ ፖሊስ ኮሚሽን

ADDIS ABABA POLICE COMMISSION

የወንጀልና ትራፊክ አደጋ ምርመራ ም/ኮሚሽነር ፅ/ቤት

CRIME &TRAFFICACCIDENTINVESTIGATIOND/COMMISSIONEROFFICE

ቁጥር አፖ /ወም አስ 12 / 8263
Ref.No
ቀን 08/06/10
Date

ለልደታ ክፍለ ከተማ ፖሊስ መምሪያ

ለኮልፌ/ቀራኒዮ ክፍለ ከተማ ፖሊስ መምሪያ

ለየካ ክፍለ ከተማ ፖሊስ መምሪያ

ለጉለሌ ክፍለ ከተማ ፖሊስ መምሪያ

ለቦሌ ክፍለ ከተማ ፖሊስ መምሪያ

አ/አበባ

በአዲስ አበባ ዩኒቨርስቲ የተፈጥሮና ኮምፒቲሽናል ሳይንስ ኮሌጅ የሁለተኛ ዲግሪ ተማሪ ለሆኑት ተማሪ ብሩክ ለሰሺ የተባሉት በጤፍ ዱቄት እና በጤፍ እንጀራ ላይ በሚቀየጡ ነገሮችን በላቦራቶሪ ፍተሽለማድረግ በሚያስችሉ ዘዴዎች ዙሪያ ጥናታዊ ፅሁፍ ለሰሚሰሩ አስፈላጊ የሆነ መረጃ ማሰባሰብ እንዲችሉ ዩኒቨርስቲው በቁጥር ምሳኒማ/186/10/18 በ05/06/2010 በተፃፈ ደብዳቤ የጠየቀ በመሆኑ አስፈላጊውን መረጃ ከክፍለከተማችሁ ለሰሚያሰባስቡ ተገቢው ትብብር እንዲደረግላቸው የሳከናቸው መሆኑን እንገልፃለን፡፡

ከሰላምታ ጋር

ዘውድነህ ከበደ
ZEWDNEH KEBEDE
ኮማንደር
Commander
የወ/ት/አ/ም/ም/ኮሚሽነር ጽ/ቤት ኃላፊ
Head of Executive S...retary

ማሳሰቢያ፦ ለደብዳቤያችን መልስ ሲሰጡ ቁጥራችንን ይጥቀሱ
NOTE Quote our Ref in Response to our Letter
ህገ-መንግስታችን የተግባር መመሪያችን ነው!

✉ 5647 ☎ 0118612393 0111185007

Appendix 9: Letter from AAU to Addis Ababa Police Commission

በአዲስ አበባ ዩኒቨርስቲ
የተፈጥሮ ሳይንስ ኮሌጅ

ADDIS ABABA UNIVERSITY
College of Natural Science

የምግብ ሳይንስና ኒውትሪሽን ማዕከል

Center for Food Science and Nutrition

ቀን: ነሀሴ 24/ 2008ዓ.ም
ቁጥር: ምሳኒማ/572/08/16

ለ፡ ቦሌ ክ/ከተማ ወረዳ 01 የምግብ መድሀኒት ጤና ክብካቤ አስተዳደር ቁጥጥር
ጽ/ቤት
<u>አዲስ አበባ</u>

ጉዳዩ፡- <u>ለጥናትና ምርምር የሚውል የጤፍ ዱቄት ናሙና ስለመጠየቅ</u>

እንደሚታወቀው በአሁኑ ወቅት በገበያ ላይ ከሚውሉት የምግብ አይነቶች ውስጥ አንዳንዶቹ ከተለያዩ ባዕድ ነገሮች ጋር ተቀላቅለው ሃላፊነት በጎደላቸው ግለሰቦች ተዘጋጅተው በገበያ ላይ እየቀረቡ ነው፡፡ ጉዳዩ የሚመለከተው የመንግስት አካልም ይህንን ጉዳይ በተመለከተ ጥብቅ ምርመራ፣ ክትትል እና እርምጃ እየወሠደ ነው፡፡

በተመሳሳይ በአዲስ አበባ ዩኒቨርሲቲ የምግብ ሳይንስና ኒውትሪሽን ማዕከል ውስጥ በተመሳሳይ ጉዳዮች ላይ ጥናትና ምርምር ለማድረግ አንዳንድ እንቅስቃሴዎች ተጀምረዋል፡፡ ከነዚህም አንዱ ለእንጀራ መጋገሪያ የሚውል የጤፍ ዱቄት ከተለያዩ ባዕድ ነገሮች ጋር ተደባልቆ በሚገኝበት ጊዜ እና ለዘለቄታውም የሚሆን ፈጣን ወጪ ቆጣቢ እና ቀላል የላቦራቶሪ ምርመራ ለማድረግ የሚያስችሉ ኬሚካላዊ መመሪያዎችን ለማዘጋጀት ምርምር ተጀምሯል፡፡

ይህንንም ምርምር ለማካሄድ በጉዳዩ ዙሪያ እውቅና ባለው የመንግስት አካል የተሰበሰበ የጤፍ ዱቄት እና ተመሳሳይ ናሙናዎች ያስፈልጋሉ፡፡ ቢሮአችሁም ከላይ ለተጠቀሰው ጥናትና ምርምር እነዲያግዘን የተቻለውን ያህል መረጃ እና ናሙና በመስጠት ትብብር እንዲያደርግልን ስንል በትህተና እንጠይቃለን፡፡ የጥናትና ምርመሩ ውጤት እንደአስፈላጊነቱ ከሚመለከተው የመንግስት አካል እና ከ አዲስ አበባ ዩኒቨርሲቲ የምግብ ሳይንስና ኒውትሪሽን ማዕከል ትምህርት ክፍል እውቅና ውጪ ለሌላ አካል እነደማይሰጥ ከወዲሁ እናረጋግጣለን፡፡ ስለሚደረግልን ትብብር በቅድሚያ እናመሰግናለን፡፡

ከሰላምታ ጋር

ቃልአብ ባዬ (ዶ/ር)
የምግብ ሳይንስና ኒውትሪሽን ማዕከል ዳይሬክተር

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