



# **Developing Calibration Model for Prediction of Malt Barley and Teff Genotypes Quality Traits Using Near Infrared Spectroscopy (NIRS)**

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**This Thesis is submitted to the Center for Food science and Nutrition, College of Natural and Computational sciences, Addis Ababa University; In Partial Fulfillment of the Requirements for Master of Science Degree in Food Science and Nutrition**

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I, the undersigned, declare that this thesis is my original work and all sources of materials used in this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements of MSc degree in Food Science and Nutrition to Addis Ababa University, College of Computational and Natural Science, Center for Food Science and Nutrition.

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## **LIST OF ABBREVIATIONS**

AOAC	European Brewery Convection
EBC	European Brewery Convection
FT	Fourier-Transform
MIR	Mid-Infrared
MSC	Multiplicative Scatter Correction
NIRS	Near-Infrared Spectroscopy
PCA	Principal Component Analysis
PLS	Partial Least Square
PLSR	Partial Least Square Regression
RMSEC	Root Mean Square Error of Calibration
RMSEP	Root Mean Square Error Prediction
RPD	Ratio of Prediction Deviation
SEC	Standard Error of Calibration
SEP	Standard Error of Prediction
SNV	Standard Normal Variate
USDA	United State Department of Agriculture

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## ABSTRACT

Barley is one of the most important cereal crop largely produced in Ethiopian central, mid and high altitude areas. Even if the country is the second largest barley producer in Africa next to Morocco, the quality traits are always influenced by the cultivar itself and growing environment. Teff is also cultivated in Ethiopia widely as first among common cereals in area coverage and 2/3 of the population daily protein intake. Similarly it is globally interesting crop because of its whole grain nutritional merits and gluten free protein. However, breeders need simple and fast methods to select good quality traits of these crops in their breeding program to meet the targeted end use. Thus the study was targeted on developing calibration model for predicting malt barley and teff quality traits for genotypes grown at different locations using near infra-red spectroscopy. For this purpose 60 barley samples were collected from Holeta, Debre-Birhan and Bekoji, in similar way 60 teff samples were also collected from Holeta, Debre-Zeit and Ginchi. These samples were chemically analyzed for barley traits extract(%), protein(%), friability(%), beta-glucan(mg/L) and moisture(%), for teff traits protein(%) and moisture(%) using international methods. For barley and teff traits mentioned above, calibration model was developed using 120 samples in duplicate based on the calibration software of the Fourier Transform Near Infrared Spectroscopy. The barley and teff Protein calibration model having ( $R^2_c= 0.97$ ; RPD=5.7 and  $R^2_c=0.94$ ; RPD=4.16) respectively, can be regarded as broadly applicable; Extract and Friability ( $R^2_c= 0.96$ ; RPD=4.54 and  $R^2_c=0.95$ ; RPD=4.36) respectively were accepted as useable with good prediction capability; whereas  $\beta$ -Glucan calibration model ( $R^2_c= 0.90$ ; RPD=3.18) allowed only for screening purpose in some applications, because it delivered less trusted model as its model statistical parameters reflected. Barley and teff grain dry matter with model parameters result( $R^2_c=0.86$ ; RPD=2.69 and  $R^2_c=0.87$ ; RPD=2.77) respectively shown less performed as compared to others, but were usable with caution for rough screening purposes. Therefore, since near infrared spectroscopy is fast and cost-efficient the breeding program can increase the intensity of variety selection using these models.

**Keywords:** Barley, Teff, Malt, Trait, Near Infrared Spectroscopy, Calibration, Validation

# 1. Introduction

## 1.1 Background

Barley (*Hordeum vulgare* L.) is the fourth most important cereal crop worldwide after wheat, corn and rice (Marwat et al, 2012). It is a crop of ancient origin in Ethiopia and the country is considered as a center of diversity for barley, because of the presence of great diversity in ecology (Birhane, 1991). In the country barley has a long history of cultivation in the highlands (Firdissa et al, 2010). Barley has the ability to adapt and survive in a wide range of environmental conditions, but the diversity of barley types found in Ethiopia is probably not expanded in any other region of comparable size (Bekele, 1983). Ongoing breeding processes, from as early as the 1800's in Europe and the 1900's in North America, have improved barley quality and productivity immensely (Nilan & Ullrich, 1993). Even though it is most important crop for food, it is used mostly for the production of malt (Ullrich, 2002). Barley malt is mainly used as a source of fermentable sugars for alcoholic fermentation for the production of beer (Kreisz, 2009).

Malted barley is a complex mixture of many organic components that include protein, starch, water, oil, fiber polysaccharides and sugars (Duffus et al., 1992). The amount of each of the constituents will vary due to both the genetic background and the environmental conditions during grain development. The malting process of barley in particular, modifies the grain components during the controlled steeping, germination and drying processes (Bamforth et al., 1993). Malt is an essential ingredient in beer production where soluble components from the malt are extracted into a liquid broth called wort and these provide the basis for yeast to continue the fermentation cycle (Briggs et al., 1981). Varying the malting process conditions influences the level to which the carbohydrate and protein constituents are modified, which in turn influences beer processing and product characteristics such as wort color, filtration performance, fermentation properties, flavor and stability (Coghe et al., 2005). However, the problem arises in the selection of suitable cultivars for each of these quality compositions that meet required quality specifications.

On the other hand, Teff (*Eragrostis tef*) is classified into the cereal group of *Gramineae* family, is cultivated in Ethiopia on an area of about 2.73 million hectares and cover about 22.6% of the

total grain crop area (CSA, 2012) making it the first among cereals in the country in area coverage. Furthermore, out of the total cereal grain produced, *teff* accounted for 16% (3.498 million tons) (CSA, 2012). There is a growing interest in teff grain utilization because of nutritional merits (whole grain); the protein is essentially free of gluten, about two-third of Ethiopian diet consists of *injera* and it accounts for about two-third of the daily protein intake of the Ethiopian population (Arogundade, 2006).

The ability to predict grain quality for different purposes in early generations would be of great benefit to breeders and maltsters, allowing for selection of suitable lines to deliver product of the highest quality. At later stages in the barley breeding programs, micro-malting can be carried which requires large sample sizes, destructive and requires experienced personnel (Marte et al, 2009). The fact the production of malt barley is restricted to these specific areas is advantageous with respect to transport, storage and research (Kotze, 2009). However, the problem arises in the selection of suitable cultivars for each of these regions that meet the required quality specifications. Breeding of new cultivars therefore requires the evaluation of many quality characteristics and the testing and selection of thousands of breeding lines, starting with early generation material; many tests require larger samples of barley than are available in earlier generations of the breeding programme. Near infrared spectroscopy is an ideal technique for this purpose as it is fast, reliable, and non-destructive and does not require large sample sizes (Osborne, 1981).

Teff is also currently the most expensive grain to purchase in Ethiopia, because injera made of teff is the favorite diet of the citizens and usually considered as a prestige in the community and also its flour is exported to USA. Besides this, the absence of gluten and its nutritional value made tef increasingly well-known and attractive in the United States, Europe and other regions and countries outside Ethiopia. Among the expanding segments of health-conscious consumers, tef is marketed by various sellers as a unique and healthy alternative to more common staples like wheat (BOSTID, 1996). Because of its nutritional quality advantage and being an indigenous crop to the country, at least protein content determination of the grain need calibrated model using NIRS for the future easy quantification of crude protein.

Thus, the need for effective quality specification evaluation is still essential as most analytical methods are time consuming and laborious. In current breeding programs, limited quality evaluation is carried out by means of near infrared (NIR) spectroscopy for evaluation of malt barley and tef quality traits. This method offers the potential to conduct rapid tests on a small sample of whole grain for quality evaluation in early generations where limited seed is available (Woodcock *et al.*, 2008).

Near infrared spectroscopy is a type of vibrational spectroscopy that employs photon energy in the range of  $7.96 \times 10^{-20}$  to  $2.65 \times 10^{-19}$  J. The range is higher than necessary to promote molecules to their lowest excited vibrational states and lower than typical values necessary for electron excitation in molecules (Pasquini, 2003). Because of that, the NIR spectra of food is comprised of broad spectral bands arising from overlapping absorptions corresponding mainly to overtones and combinations of vibrational modes involving C–H, O–H, N–H and SH chemical bonds (Huang *et al.*, 2008). As a consequence of this overlapping, strong collinearity exists in NIR spectral data, making their interpretation rather difficult and the interference of NIR spectral signals from various food components renders spectra with high noise levels and baseline drift (Wang and Paliwal, 2007). Thus, sophisticated mathematical techniques, termed chemometrics, are heavily employed to allow calibration for reliable extraction of relevant information encoded in the NIR spectral data. The symbiotic relation between NIR spectroscopy and chemometric methods, in which one poses new challenges to the other, has been one of the main motivations for many of the improvements done to these mathematical techniques over the last decades.

Therefore, the aim of this study is to determine constituents of particular interest to the brewing of barley criterion and teff grain important traits like its protein by using NIRS. This will be performed by developing calibrations model using Near Infra-Red Reflectance (NIR) technology which is a non-destructive, cost effective and rapid tool for the simultaneous prediction of multiple constituents in agricultural products.

## **1.2. Statement of the Problem**

Barley is one of the main inputs for malt and beer industry in our country (Ethiopia). Maltsters evaluate barley quality by producing malt from the grain on a small scale, known as "micro-malting" and then analyzing the malt for characters directly related to malting quality. Malt

protein, malt friability, malt beta-glucan and malt extract are key selection criteria in a malt barley breeding program. However, the time required for chemical malt analysis, labour-intensive as well as cost and seed requirement, limit selection progress for these traits. A number of screening techniques for these malt quality traits would be amenable to routine use in plant breeding programs. Their methods are not sufficiently rapid for assessing breeders' lines as selections must be made before the next growing season (Haslemore et al, 1982).

Teff is a dual purpose cereal, valued for both grain and forage production in dry areas with short rainy season. The teff grain is rich in protein, carbohydrates and fibre and is mainly used for human food, particularly in Ethiopia where it used for the production of the bread (*injera*) and beer (*tela*). Until recently, little was known about the nutritional composition and potential health benefits of teff. This, along with technological limitations in processing teff and nutritional profile, long prevented its widespread adoption as a cereal grain outside Ethiopia (Baye, 2015). However, since the late 1990s, the recognition of teff as a gluten-free cereal of good nutritional value has resulted in new found interest (Baye, 2015). The main thing what makes teff most important and healthy food is its protein composition which is gluten free unlike wheat and some other crops. Thus it is interesting to develop calibration model at least for its protein content that could make easy to predict protein content of teff genotypes in breeding lines to enhance breeding program nutritional information and to encourage the future teff processing technology

Therefore in regard to the determination of properties of agricultural and food products, NIR spectroscopy offers a number of advantages over traditional chemical methods (Paliwal, 2007). It is a physical, non-destructive, non-invasive method, fast, relatively inexpensive, precision is rather controllable, requires minimal or no sample preparation, more environmentally friendly than other analytical methods, i.e., no reagents are required and no hazardous wastes are produced. It is a multi-task analytical technique; several determinations related to both physical and chemical traits can be done simultaneously and suitable for in-line use (Morgan and Gothard, 1979). Because of these various screening techniques for barley malt and teff quality NIR appears the most promising.

### **1.3. Significance of the Study**

Because of the problems stated above it is very important to develop an easy and cheap method to determine traits of several genotypes. As we know in our country barley breeding programs in many agricultural research institutes are operating with so many genotypes in breeding lines that needs to be checked for its malt and food quality criteria characterization in many for the input of many malt and food industries. This forces us to shift our exhaustive chemical analysis method to simple, fast, cost effective and non-destructive like NIRS method to characterize a number of malt barley and teff samples quality traits within a short period of time, even though it needs developing calibration model for each parameter. It plays a key in the work of breeders and in controlling industry input raw materials. Also it enables the analyst to determine the quality traits of more than 100 samples per day and support the laboratory technician to work multi-disciplinary works rather tied to chemical analysis the whole working times. It is also safe to the environment and laboratory workers health. These important issues makes the study to focus on NIR model developing for some barley and teff quality traits.

### **1.4. Objective**

#### **1.4.1. General Objective**

- The overall objective of this study was to develop calibration model for predicting malt barley and teff quality traits for genotypes grown in different environmental variability using NIRS.

#### **1.4.2. Specific Objectives**

- ✓ To analyze chemical and NIRS measures of malt quality traits (malt protein, malt extract content, malt friability, malt beta-glucan)
- ✓ To analyze chemical and NIRS measures of teff protein and moisture content
- ✓ To develop calibration model for these quality traits of malt and tef protein and
- ✓ To investigate the models predictive ability through validation

## **2. Literature Review**

### **2.1. Barley**

Barley (*Hordeum vulgare*) is a specialized cereal crop with a long breeding, malting and brewing tradition. Barley has the capability to adapt and survive in a wide range of environmental conditions, but the diversity of barley types found in Ethiopia is probably not expanded in any other region of comparable size (Bekele, 1983). Continuing breeding program from as early as the 1800's in Europe and the 1900's in North America, have superior barley quality and productivity enormously (Nilan & Ullrich, 1993). Even if it is most important crop for food, is used mostly for the production of malt (Ullrich, 2002). Barley malt is mostly used as a source of fermentable sugars for alcoholic fermentation for the production of beer and whisky (Kreisz, 2009). Therefore barley malt is the main raw material for brewing worldwide, even though quality specifications for brewing barley are the most challenging specifications in comparison to other cereals in the food industry (Kreisz, 2009).

#### **2.1.1. Barley Biochemical Characteristics**

The barley kernel is a complex combination of carbohydrates, proteins, lipids, minerals and other compounds (Savin & Molina-Cano, 2002). Carbohydrates are the most important component (70-80%) of the barley grain (Duffus & Cochrane, 1993; Ullrich, 2002; Kreisz, 2009) with starch as the primary component (50-70% of dry weight (Kreisz, 2009)). Protein could account for 8-15% of dry weight and -glucans for 3-6%. In unmalted barley, -amylase may account for 1-2% of total protein in the starchy endosperm. Variations in proportions are due to differences in genotype and environmental conditions as well as measuring techniques (Duffus & Cochrane, 1993; Savin & Molina-Cano, 2002).

The perfect malt barley can be referred to as mature, plump kernels with a reticulated skin and comparatively low protein content and contains a white mealy endosperm from which starch granules can be readily removed (Poehlman, 1985). For maltsters and brewers, starch and protein contents are the most important constituents to consider. Structural differences may occur in the endosperm of the barley grain and can be visually classified as mealy or steely (Ferrari *et al.*, 2010). Unripe grain usually shows a smooth, unwrinkled skin, and when broken will exhibit a dark, steely endosperm surface (Briggs *et al.*, 1981). The latter grain modifies slowly during

malting and produces unsatisfactory malt due to a higher nitrogen content (Briggs *et al.*, 1981; Ferrari *et al.*, 2010).

### **2.1.2. Process of Malting**

Malting is a controlled germination process consisting of steeping or hydration of grains, a germination phase in moist conditions and finally the termination of the grain's physiological activities by heating during a phase called kilning. Fundamentally, the aim of malting is to unmask starch granules from the surrounding cell walls and protein matrix so that fermentable sugars can be optimally released from starch during the brewing process (Swanston *et al.* 2014). It makes a grain modification and is described as the "liberation of starch granules from the matrix of the cells of the endosperm in which they are embedded. Throughout the early stages of the malting process, the cell walls of the endosperm are dissolved through the action of hydrolytic enzymes, permitting the diastatic enzymes to come into contact with starch granules, which are then liberated from the protein matrix ( Bamforth & Barclay, 1993).

Barley should be clean, graded and free of extraneous matter before malting. The high-class demanded is specified by several quality parameters like germination capacity, protein content, kernel size, moisture content, kernel abnormalities and infestation (Kreisz, 2009). If the moisture content of the barley is more than 15%, it must be carefully dried before storage to inhibit the development of fungi and bacteria. The most important factors to consider when making malt are the quality of the finished malt, the yield of malt from a given quantity of barley and the efficiency of the process with respect to labour and energy. Commercial malting operations involve five basic steps: barley intake, drying and storage; steeping; germination; and kilning (Bamforth & Barclay, 1993).

#### **2.1.2.1. Steeping**

Steeping is the first and most important stage in malting. An even moisture content must be achieved across the grain bed to obtain homogenous malt (Meijering *et al.*, 2009). Water saturation of the starchy endosperm is also important before the food reserves of that tissue can be mobilised through enzyme action (Bamforth & Barclay, 1993), because high moisture content results in faster modification. Water uptake primarily depends on the steeping water temperature and the duration of the wet periods. Higher steeping water temperature and long wet periods

increase the water uptake, but the risk of drowning water-sensitive barley is higher and microbial growth is accelerated (Kreisz, 2009).

During steeping the nature of the barley must be considered including kernel size and nitrogen content. Moisture content required for germination varies with barley supply and steeping involves submerging grain in water for 43 hrs until the moisture content has reached a desired level of 46% (Bamforth & Barclay, 1993). A clean barley batch is immersed in water at approximately 14 to 17°C in a steep tank, forming a 3 m thick bed (Hough, 1991; Kreisz, 2009). Water enters the barley at the base (embryo end) of the kernel and spreads through the endosperm until evenly distributed. The rate of water absorption is rapid during the first few hours of steeping and slows down gradually as the saturation level is approached (Schuster, 1962). During steeping, the embryo and husk take up water rapidly while the starchy endosperm hydrates more slowly (Hough, 1991; Bamforth & Barclay, 1993; Kreisz, 2009).

When barley absorbs water, the embryo uses oxygen dissolved in the steeping water for respiratory purposes. In the process steeping is interrupted by draining after 12 to 24 hrs, for that this trend is known as air rest (Hough, 1991). Air rest removes carbon dioxide and ethanol, which may inhibit germination. Air is forced through the steep water using perforated pipes or is pulled through by suction (Hough, 1991). This adds oxygen which is needed by the kernels for respiration. A lack of oxygen may provoke CO<sub>2</sub> accumulation followed by fermentation and consequently poisoning of the germ (Kreisz, 2009). The alternation of steep water and air rest continues until the barley has reached a moisture content of 46% (Hough, 1991). A typical steeping process may involve an initial steep to 32% moisture; the start of germination is promoted by an air rest of 10 to 20 hours (Bamforth & Barclay, 1993). At this stage of the process, the kernel changes visually by developing a small white root at the base of the kernel called a chit (Hough, 1991; Kreisz, 2009). Chitting is encouraged with a second air rest of 10 to 15 hours before the final steep to raise the moisture to 46% (Bamforth & Barclay, 1993; Kreisz, 2009).

#### 2.1.2.2. **Germination**

Germination is the second stage of malting process that is involved until the endosperm has been degraded by enzymes to be mobilized for development of the germ (Briggs *et al.*, 1981).

Germination is targeted at generating the maximum available extractable material by promoting endosperm modification through the development, distribution and action of enzymes. The maltster is therefore concerned with both the degradation of the endosperm and the mobilization of the enzymes of the grain. The maltster makes use of the natural germination process but only allows it to proceed until enzyme activity is optimal and terminates the degradation of the endosperm and growth of the embryo by drying the grain (Bamforth & Barclay, 1993).

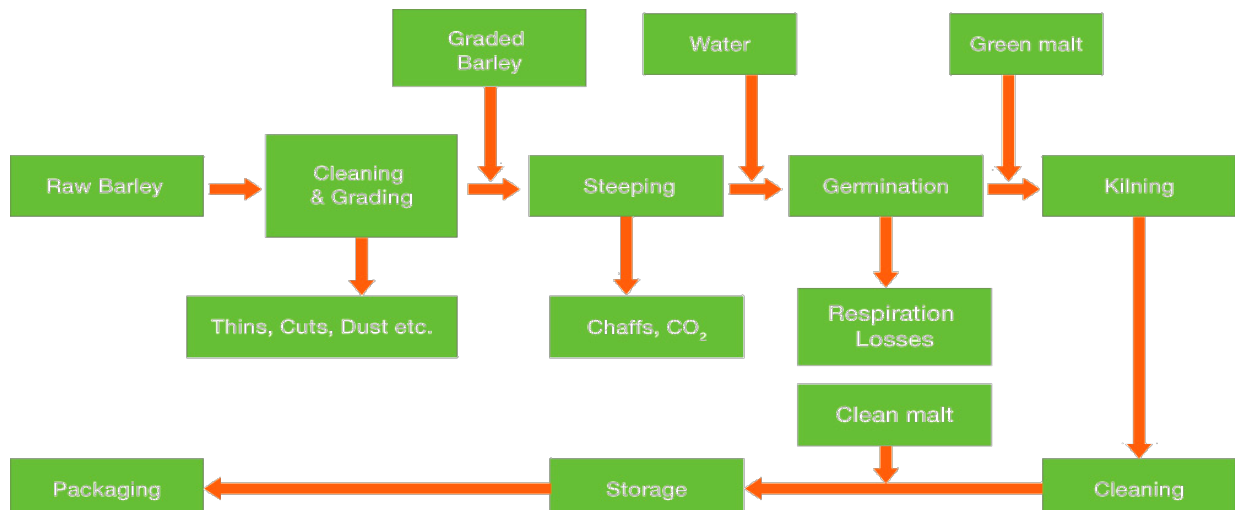
The role of germination is optimal production levels of hydrolytic enzymes, controlled breakdown of cell walls and matrix proteins, hydrolyzation of certain barley reserves (protein to form free amino nitrogen (FAN)), minimizing loss of potential extract from growth and respiration while achieving optimal modification, and produce balanced, well-modified green malt for kilning (Kreisz, 2009). After the steeping process, the water is drained off and the barley is spread out as a malt bed (approx 1.5 m deep) where it will germinate. Cool humidified air is pushed through the bed at 14 to 17°C (Meijering et al, 2009). Soon after germination begins, a synthesis/activation of hydrolytic enzymes occurs, allowing for the development of an extensive root system (Kreisz, 2009) at the base of the grain, beneath the husk. Gibberellic acid induces the production of many different hydrolytic enzymes in the aleuronic layer which covers the whole endosperm (Kreisz, 2009).

It is now a challenge for the maltster to control the hydrolysis of proteins (proteolysis), cell walls (cytolysis) and starch (amylolysis) (Kreisz, 2009). The germination process is controlled by maintaining a constant moisture level within the grain, supplying oxygen and removing carbon dioxide while also eliminating heat formed by respiration. The grain is turned mechanically every 8 to 12 hrs, as well as immediately before kilning (Bamforth & Barclay, 1993). Germination is performed at 14 to 17°C for four days (Meijering et al., 2009). During germination, moisture is transferred from the malt to the surrounding air and the embryo withdraws moisture from the starchy endosperm to sustain its growth, causing it to dry out (Bamforth & Barclay, 1993). This interferes with modification (Bamforth & Barclay, 1993) and the water content of the grain should remain constant during germination.

### 2.1.2.3. Kilning

Kilning is the last stage of malting process that terminate embryo growth and endosperm degradation (Kreisz, 2009) and reduce moisture levels to less than 5% (Bamforth & Barclay, 1993; Kreisz, 2009). The most important aim of kilning is to fix those sought-after properties obtained in the malt during germination, and in addition, allow for development of flavour and aroma characteristics in the malt (Kreisz, 2009). This process must be carefully regulated to conserve enzyme complexes developed during malting that will hydrolyze the malt starch into fermentable sugars during brewing (Bamforth & Barclay, 1993; Kreisz, 2009).

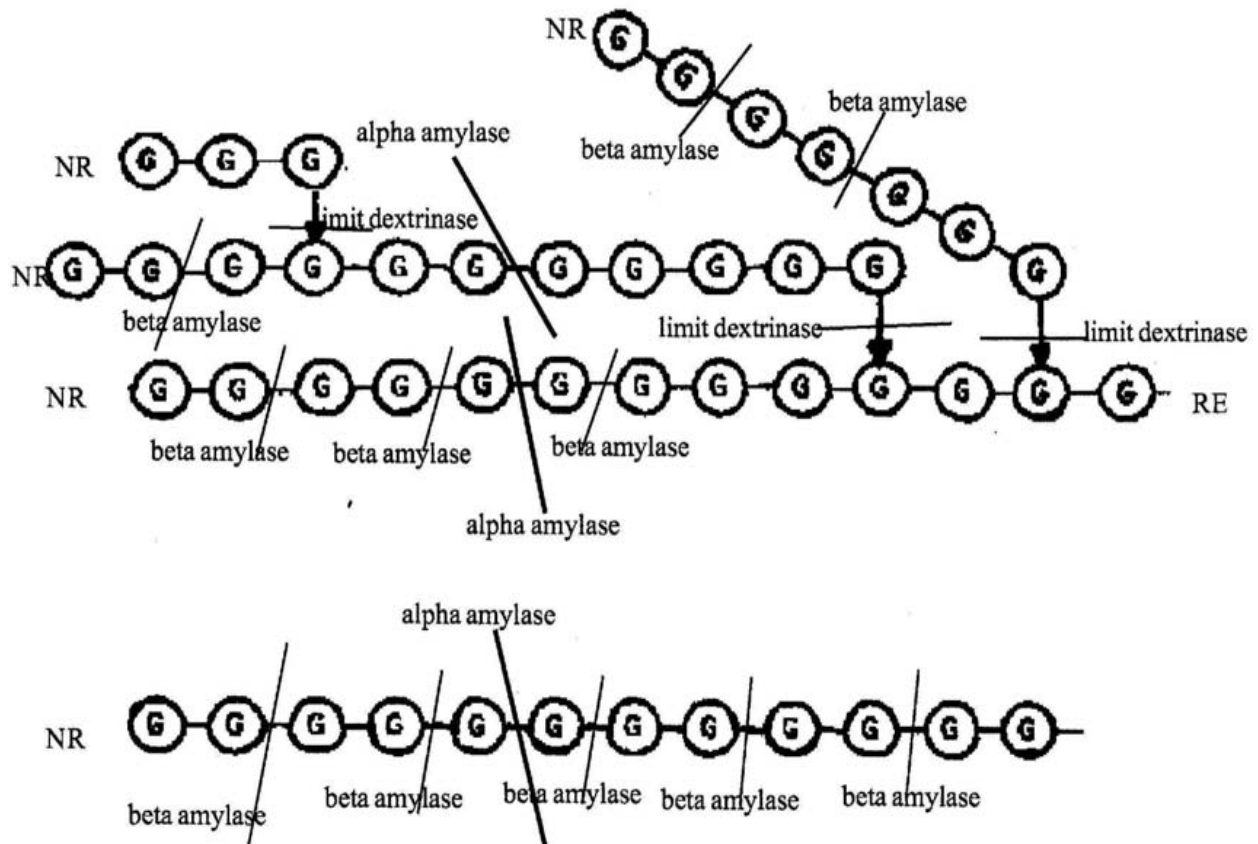
Drying is divided into four main phases: free drying to 23% moisture; an intermediate stage of drying to 12% moisture; the bound water stage from 12 to 6%; and curing, in which the moisture is taken to 3 to 5% (Bamforth & Barclay, 1993). The basic principles of kilning are that drying should commence at a relatively low temperature to ensure survival of the most heat-sensitive enzymes (limit-dextrinase, -glucanase) followed by an increase of temperature to ensure flavour and colour changes and complete drying in less than 24 hrs (Bamforth & Barclay, 1993). There is firstly a drying period where water is removed from the malt at 50 to 60°C (Hough, 1991). Then follows the curing process carried out at higher temperatures of approximately 80°C, during which a further 3 to 4% of water is removed. The grains are then screened allowing the shriveled and brittle rootlets to fall off. The remaining product is malt.



**Figure 1:** Flow chart diagram of malting process for barley malt

### 2.1.3. Biochemistry of Malted Grain

Many changes occur in barley during the various malting processes. During steeping, the grain swells and increases in volume. The initial indication of germination is the appearance of the white chit (the root sheath) which protrudes from the base of the kernel, beyond the husk. After this, seminal roots (rootlets) break through the root sheath and form a tuft at the end of the grain. Meanwhile, the coleoptile, with its enclosed first leaf (acrosipire), penetrates the testa on the dorsal side of the grain and grows towards the apex between the testa and the pericarp. This leads to the breakdown of the starchy endosperm and degradation of the cell walls in the intact grain. The growth of the acrosipire is often used as a guide to indicate the progress of the malting process (Briggs *et al.*, 1981).



**Figure 2.** Barley biochemical changes and enzyme activities during malting (Shewry, 1993)

Following cell wall breakdown, the proteins of the endosperm undergo degradation and the starch granules are partially degraded. All these changes are termed 'modification' and are catalysed by hydrolytic enzymes (Briggs *et al.*, 1981). During this stage, changes occur in the

cells of the aleurone layer and scutellar epithelium, which are associated with the mobilization of the cell's reserves, the synthesis and release of hydrolytic enzymes and the uptake of soluble substances (sugars, amino acids and minerals that serve as nutrients for the growing embryo) from the starchy endosperm and aleurone layer (Briggs *et al.*, 1981). The endosperm of ungerminated barley is tough, but after germination, the moist endosperm is friable, because the process of modification reduces the strength of the endosperm and changes its character. During malting, it is the synthesis of hydrolytic enzymes and the breakdown of the structural components of the starchy endosperm which constitute modification. The material remaining at the end of malting delivers the brewers' extract (Briggs *et al.*, 1981).



**Figure 3:** Image of raw and malted barley grain

#### **2.1.4. Malt Quality Factors**

Breeding malt barley cultivars requires the assessment of many grain characteristics that contribute to malt quality (Henry, 1985; Savin & Molina-Cano, 2002). Malt quality is affected by the source of raw barley grain and as the result quality evaluation of the barley grain is imperative to breeders. Since malt is one of the main raw materials in the brewing process its quality must be rigidly assessed in order to satisfy the requirements of the brewing process (Meredith *et al.*, 1962; Savin & Molina-Cano, 2002).

Malt properties are determined on malted barley that has been ground to form flour with many components but mostly rich in starch, and enzymes (diastase) capable of acting rapidly when hot water is added. The resultant liquid is known as wort, the sweet liquor from which beer is made (Hunter, 1962; Briggs *et al.*, 1981c). In South Africa, malt is mashed to produce wort in accordance with the method in Analytical European Brewery Convention (EBC) Methods Manual, Section 4.9.1 (European Brewery Convention, 1998).

## **2.1.5. Malt Barley Quality Traits**

### **2.1.5.1. Friability**

Friability measures the percentage breakdown of malt endosperm cell wall components. Measuring the friability of marketable malt has been used as an indicator to malting and brewing quality as well as trouble shooting on samples of poor malt quality. The association between malt quality parameters and friability has been well documented since (Chapon et al., 1978) first reported the use of the Friabilimeter. The relationship between friability and key malt traits wort  $\beta$ -glucan, Kolbach Index, wort viscosity along with other malt quality parameters have been studied through detailed experiment examining malt quality (Brennan et al., 1996). Most of these studies reported the strong negative relationship between friability and wort viscosity. However, further studies on the residual malt in the friabilimeter indicated where malts were poorly modified then the malt had a high percentage of nonfriable material (Brennan et al., 1996). The non-friable material produced higher wort viscosity than the friable malt fraction. Friability is now an important selection parameter for malt barley breeding.

### **2.1.5.2. Hot water Extract**

Hot water extract is the most frequently analyzed trait in malt to measure the maximum percentage of soluble yield obtained from specific malt (Anger *et al.*, 2009). The superior the extract the more soluble the material, less husk and protein is present (Kotze, 2009). The ability of barley grain to synthesize enzymes that degrade the cell walls of the starchy endosperm is an important determinant of malt extract values. Cell walls act as barriers to the diffusion of starch- and proteindegrading enzymes during germination. Inadequate degradation of the cell wall will result in diminished degradation of starch and proteins, and therefore lower malt extract values (Fincher & Stone, 1993).

The extract is a mixture of soluble malt components, like mainly sugars, dextrin, nitrogenous compounds and minerals. As it is very laborious to analyze each component, the physical property, density, is analyzed. Density must then be converted to the weight of extract in solution or Plato. The conversion is based on sucrose solutions because sucrose has a similar density to maltose, the main ingredient in extract (Anger *et al.*, 2009). A value of > 81% extract is required in South Africa (F. Smit, Caledon, South Africa, Personal Communication, 2009) and this

property is determined according to the method in the Analytica EBC Methods Manual, Section 4.5.1 (European Brewery Convention, 1998).

#### **2.1.5.3. Nitrogen Content**

The total nitrogen content (TN) of malt is directly influenced by the protein content of the barley it was obtained from (Bamforth & Barclay, 1993) and the presence of low nitrogen content in a barley sample indicates its potential to provide high extract malt. Brewers demand the percentage nitrogen (dry basis) in malt to be  $1.76\% \pm 0.06\%$  (Smit et al., 2009) which is determined in accordance with EBC methods 4.3.1 or 4.3.2 (European Brewery Convention, 1998). Total protein also determined using the method of (AOAC,2005).

#### **2.1.5.4. Beta-Glucan content**

Beta-glucans are major component of the endosperm cell wall (Fincher & Stone, 1993), are unwanted in malting and brewing processes. High wort -glucan levels indicate incomplete cell wall degradation and diminished mobilization of the starch-protein matrix. This results in lower malt extract values and lower nutrient availability for fermentative growth by yeast during brewing (Duffus & Cochrane, 1993). In addition, high wort -glucan levels could contribute to negative beer characteristics such as beer haze or instability in shelf life.  $\beta$ -glucan levels in wort are measured with the Skalar segmented flow analysis method according to EBC method 8.13.2 (European Brewery Convention, 1998). High wort -glucan content results in high wort viscosity, which is unwanted in the brewing process. A -glucan value of  $<100$  mg/L is required in South Africa (Smit et al., 2009).

#### **2.1.5.5. Moisture Content (%)**

The moisture content of barley is 14-15% on average. The moisture content can vary between 12% in very dry harvesting conditions and over 20% in wet conditions. More precisely, it is less than 13% in the South region of the European Brewery Convention (EBC) barley and malt committee, and it is more than the 16% in the North region, where consequently the barley should be dried before a long term storage. In fact, barley must have moisture content below 15% for long term storage. Moreover, the determination of the moisture content is important

because the amounts of the other components are related to the dry weight (Raghavan et al, 2003).

### **2.1.6. Malt Quality Analysis**

Malt quality traits are assessed by chemical analyses after processed by micro-malting (Briggs *et al.*, 1981). Micro-malting techniques date back to as early as 1895, with small scale tests used by many maltsters through the ages (Meredith *et al.*, 1962). Micromalting probably originated due to the fact plant breeders wanted to obtain malting results with the simple apparatus at their disposal (Meredith *et al.*, 1962). Various methods exist, each using different sample sizes (Gothard *et al.*, 1980) and attempt to obtain a reliable estimate of malt extract, enzymatic properties and wort quality (Meredith *et al.*, 1962).

Micro-malting remains a time consuming and laborious method. It is also a destructive technique requiring larger samples (200 g) than are available in the earlier generations of breeding programmes, and can only be applied for quality evaluation at later stages in the programme, from year 8 onwards (Smit et al., 2009). If sample sizes are too small, more water is taken up during steeping which produces malt with higher moisture content. This leads to a lower malt yield and higher malt extract for small samples which does not allow accurate prediction of malting performance on a commercial scale (Henry & McLean, 1984).

The main problem is that breeding materials are limited in amount and are grown under one set of conditions in earlier generations (F1 to F4). When material has reached the stage of small scale field trials, replicated samples become available, but such replication is also limited since it is generally confined to a single trial. As further selection and elimination proceeds, the field trials are usually conducted at more than one site in a year and a better picture can be obtained regarding the malting potential of selections and information can be accumulated on environmental adaptability and adaptation to particular conditions. Nonetheless, it is not until the final selections are under full-scale field trials that really comprehensive quality testing can be applied (Kroonenberg, 1995). This quality evaluation is expensive and requires large quantities of grain not available in early generation breeding lines. Therefore, breeders need a rapid, objective technique to be applied to small sample sizes, which would allow the prediction of malt

quality properties from whole grain barley. This would ultimately allow for the elimination of poor malting lines in early stages of the breeding programme.

At present, in Ethiopia Holeta Agricultural Research Center has introduced NIRS for malt quality traits rapid prediction to enhance malt barley technology both in breeding and quality. This is given also special attention to expand the range of NIR application. This studies about prediction of barley quality as well as malt quality characteristics with NIR spectroscopy in reflectance mode, both on whole grain, ground barley and malt, as well as on wort, have delivered suitable prediction models in a number of cases. Studies performed with instruments only operating in transmittance mode include the determination of nitrogen and moisture contents on whole grain barley (Williams *et al.*, 1985) as well as on malt (Angelino, 1996) and the determination of Free Amino Nitrogen (FAN), extract and fermentability on wort (Halsey, 1986).

The reality the production of malting barley is limited to these specific areas is advantageous with admiration to transport, storage and research (Kotze, 2009). However, the problem arises in the selection of suitable cultivars for each of these regions that meet required quality specifications. Breeding of new cultivars therefore requires the evaluation of many quality characteristics and the testing and selection of thousands of breeding lines, starting with early generation material; many tests require larger samples of barley than are available in earlier generations of the breeding programme. Near infrared (NIR) spectroscopy is an ideal technique for this purpose as it is fast, reliable, non-destructive and does not require large sample sizes (Osborne, 1981).

The purpose of a NIR instrument is to estimate the concentration of chemical properties (such as protein content), quickly and precisely from spectrophotometric measurements. This allows to replace slower, expensive or tedious methods for assessing the desired chemical constituents. The small cereal grains were among the first commodities used in the development of NIR instruments and methods (Delwiche, 2004) and cereal chemists in breeding programmes were among the first to identify the potential of NIR spectroscopy to replace conventional laboratory quality tests. NIR spectroscopy has been used for quality testing in cereal breeding programs

since the late 1970's (Osborne, 2006). The NIR technique is very simple and, once suitable calibrations have been developed, sample analysis takes no longer than a minute.

This literature study will review malting barley, barley breeding and the malting process. The biochemistry of malting barley and important barley and malt quality factors will be discussed, followed by a review of NIR spectroscopy. Studies regarding the prediction of malting barley quality with NIR spectroscopy will also be reviewed.

## **2.2. Teff and Its Nutritional Quality**

In Ethiopia, tef (*Eragrostis tef*) is cultivated on an area of about 2.73 million hectares and cover about 22.6% of the total grain crop area (CSA, 2012) making it the first among cereals in the country in area coverage. Furthermore, out of the total cereal grain produced, teff accounts for 16% (3.498 million tons) (CSA, 2012). Tef is considered to have an excellent amino acid composition, with lysine levels higher than wheat or barley, as well as very high calcium, phosphorous, iron, copper, aluminum, barium, and thiamine (Mengesha, 1966).

The principal use of tef grain for human food is the Ethiopian bread (*injera*). *Injera* is a major food staple and provides approximately two thirds of the diet in Ethiopia (Stewart and Getachew, 1962). It can also be used in many other food products such as *kitta* (unleavened bread), *anebaberro* (double layered injera), porridge, gruel and local alcoholic beverages such as *tella* and *katikala* (Hailu *et al.*, 2003). Tef protein essentially lacks gluten, the type found in wheat, so it is alternative food for consumers who suffer from wheat gluten allergies (Hopman *et al.*, 2008). The grain proteins are also presumed easily digestible because prolamins are very small (Twidwell *et al.*, 2002).

Teff is currently the most expensive grain in Ethiopia, because injera made of tef is the favorite diet of the citizens and usually considered as a prestige in the community and also its flour is exported to USA. Besides this, the absence of gluten and its nutritional value made tef increasingly well-known and attractive in the United States, Europe and other regions and countries outside Ethiopia. Among the expanding segments of health-conscious consumers, tef is marketed by various sellers as a unique and healthy alternative to more common staples like wheat (BOSTID, 1996).

### **2.2.1. Teff Protein**

The protein content of teff is between the ranges of 12 – 17%, within the same range with wheat (Pomeranz, 1988). Solely looking at the protein content, it appears as if the behavior of teff flour would be quite comparative to the behavior of other grain flour. This is of course not the case and the difference in behavior is largely attributed to the protein quality. Protein quality is where the characteristics of teff differ significantly from others. Although teff has high protein content and concentrations of essential amino acids, it is the protein quality that sets teff apart from wheat.

In the previous section, the word quality was used to describe the nutritional merits of teff compared to wheat. Unfortunately, the proteins that make teff high in nutritional quality offer very little when it comes to their role in producing a satisfactory baked good. However, since the late 1990s, the recognition of teff as a gluten-free cereal of good nutritional value has resulted in new found interest (Baye, 2015). The main thing what makes teff most important and healthy food is its protein composition which is gluten free unlike wheat and some other crops.

Evaluating protein quality based on the end-use product rather than the nutritional aspects is what makes wheat so much better to bake with when compared to teff. Even among different wheat varieties, flours may have the same protein content but behave much differently in baking operations (Pomeranz, 1988). This is why it is important to select appropriate flour that has been processed from a wheat variety bred for a specific end-use in mind. Wheat flours used for cakes and breads, for example, would not produce an appealing cookie (Pomeranz, 1988). So what is the protein responsible for such differences in flour quality? Qualitative differences in types of flours are the result of gluten proteins which wheat contains and teff does not. Because of its nutritional quality advantage and indigenous crop to Ethiopia at least protein content determination of the grain need calibrated model using NIRS for the future easy quantification of crude protein trait.

### **2.3. Near infrared spectroscopy (NIRS)**

The NIR region was discovered in 1800 by Sir William Herschel, a musician who also discovered the planet Uranus (Pasquini, 2003). He was searching for the colour of glass that would pass the maximum amount of light with the least amount of heat, to be used in a telescope

(Butler, 1983). While experimenting with colours of light carrying the warmth of the sun (Osborne, 1981; Butler, 1983), he observed light was diffracted through a prism. He measured the temperature of these different colours. As the thermometers were moved from the blue to the red end of the rainbow, the temperature increased and continued increasing even past the visible region (Butler, 1983; Davies, 1998). He concluded the warmth of the sun was carried by waves that are not visible to the human eye and that there is energy beyond the red light. He gave these waves the term 'infrared' (IR), meaning 'beyond the red' (Osborne, 1981; Butler, 1983; Pasquini, 2003).

The function of NIR spectroscopy reflects the general accessibility of computers and expansion of chemometric techniques (mathematical techniques) (Davies, 1998). The contributions of Karl Norris (named the 'First Fellow of Near Infrared Spectroscopy' by the NIR community (Davies, 1998) and generally regarded as 'The Father of NIR' (Butler, 1983)) have been instrumental to the everyday applicability of NIR spectroscopy. Norris, of the Beltsville United States Department of Agriculture (USDA) laboratory, recognized the potential of diffuse reflectance measurements in the NIR for the quantitative analysis of major components in agricultural commodities (Wetzel, 1983). Other important contributions were made by Phil Williams, Fred McClure and John Shenk (Davies, 1998).

### **2.3.1. Near Infrared spectroscopy Principles**

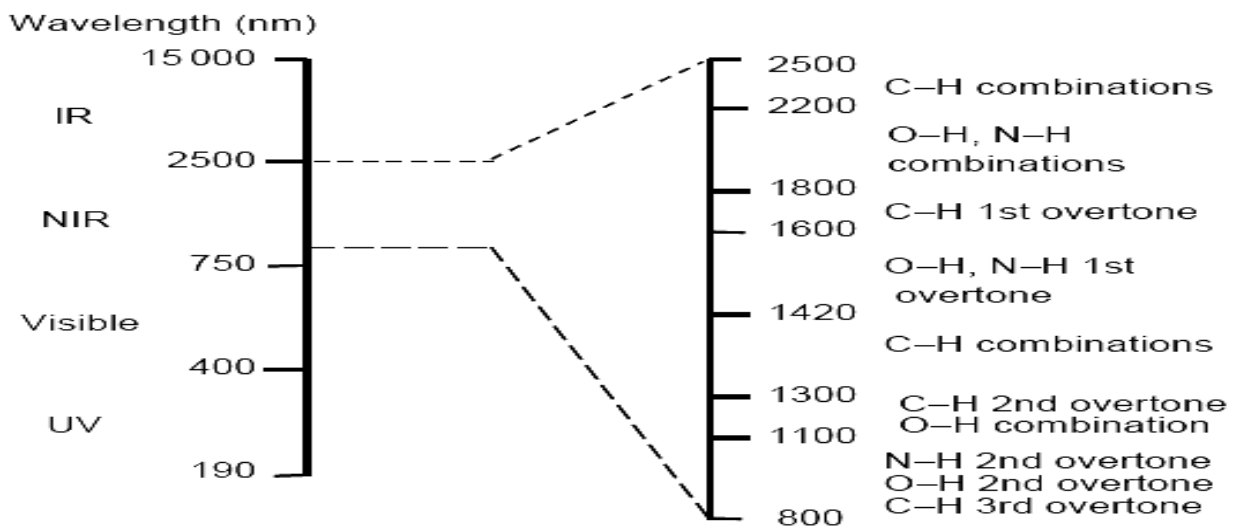
NIR spectroscopy can be described as the interactions of NIR energy with matter (McClure & Tsuchikawa, 2007). The part of the electromagnetic spectrum, observed by the human eye, termed the visible region, extends from 400 to 750 nm, while the IR region is located from 2500 to 15 000 nm. The NIR region is situated between the IR and visible region (Osborne, 1981), from 750 to 2500 nm (Butler, 1983). NIR spectroscopy is a type of vibrational spectroscopy that employs photon energy in the range  $2.65 \times 10^{-19}$  to  $7.96 \times 10^{-20}$  J (Pasquini, 2003) and is based on absorption of electromagnetic radiation in the NIR wavelength range (Osborne, 2000).

Like radiation, NIR behaves as a wave with the properties of a simple harmonic motion. Chemical bonds between atoms in molecules are oscillators which vibrate constantly. This vibration is approximately a simple harmonic motion (Osborne, 1981; Osborne, 2000). When molecular vibrations occur at the same frequency as that of the radiation wave, a net transfer of

energy from the radiation to the molecule will occur (Osborne, 2000). Vibrations can only occur at fixed frequencies and radiation is absorbed in discrete packets. A molecule can therefore only absorb at specific fixed frequencies (Osborne, 1981). This energy transfer can be measured as a plot of energy versus wavelength and is called a spectrum (Osborne, 2000).

Various foods chemical bonds absorb MIR wavelengths strongly and the weaker overtone and combination band absorptions of the NIR region enable spectra to be measured with greater ease. The absorption bands due to constituents such as protein, oil and moisture are strong enough in the NIR to be measured accurately (Osborne, 1981). Correlation charts showing where absorption bands of O-H, C-H, N-H and S-H bonds of certain compounds are located in the NIR region can be used for qualitative analysis (Osborne *et al.*, 1993; Siesler *et al.*, 2002). Determination of the concentrations of constituents such as water, protein, fat and carbohydrate using absorption spectroscopy is also possible (Osborne, 2000). There is, for example, a prominent peak at 1930 nm in the spectrum of water and measurement of the magnitude of this peak can be related to the amount of water present in a sample (Osborne, 1981).

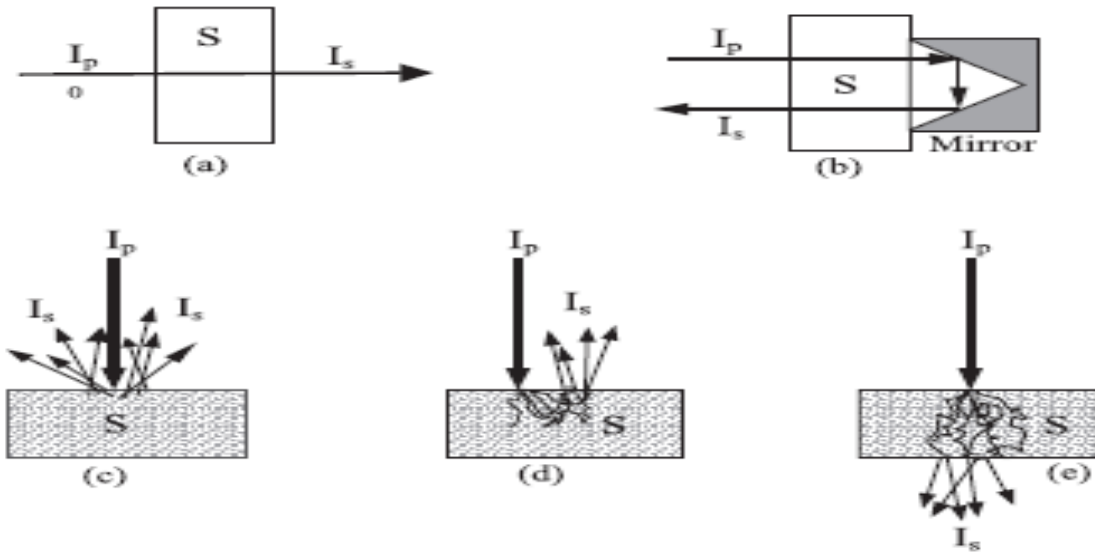
NIRS requires calibration against a reference method for the constituent of interest (Osborne, 2000). The application of NIR spectroscopy is based on the empirical relationship between reference data obtained by conventional analytical methods and spectral data measured with a spectrometer (Pasquini, 2003). When computing a set of calibration constants in NIR reflectance technology, the reference values are the dependent variables (y) and the optical data (log 1/R or absorbance values) are the independent variables (x).



**Figure 4.** Principal types of NIR absorption bands and their locations (Mark, 1992)

### 2.3.2. Measurement Modes

Light directed onto a sample may be transmitted or reflected and NIR instruments can operate in reflectance or transmittance mode. In reflectance mode, the light source and the detector are on the same side of the sample (above or below). As light illuminates the surface of the sample, only some of the light is absorbed and the remainder is diffusely reflected from the surface. The study of the reflected light can be used to measure the amount of light absorbed (Osborne, 1981; Kawano, 2002). In this case, the sample should be opaque, for example a powdered sample. Usually, light cannot reach a deeper position in a sample due to high absorption or multiple scattering.



**Figure 5.** Modes of Measurements Employed in NIR spectroscopy; (a) Transmittance, (b) Transflectance, (c) Diffuse Reflectance, (d) Interflectance and (e) Scattering Transmittance (visoottiviseth, 2002)

If samples have sufficient thickness, the optical sample thickness should be regarded as infinite and attention may only be paid to the absorption coefficient. This is very useful for analyzing NIR spectra of powdered and solid samples and a sample of more than 1 cm depth is recommended (Tsuchikawa, 2007). In transmittance mode, the light from the light source passes through the sample and is received by the detector on the other side of the sample (Kawano, 2002). This mode is widely applicable for liquids without scattering or in low scattering conditions where a cuvette is used (Tsuchikawa, 2007).

Development of NIR spectroscopy as a unique analytical technique began when Karl Norris proposed a spectral measurement could be obtained by analysing the portion of radiation diffusely reflected by solid samples instead of the weaker signal of transmittance. Today, diffuse reflectance is widely employed in the NIR spectral region (Pasquini, 2003).

### **2.3.3. Advantages and Disadvantages of NIR Spectroscopy**

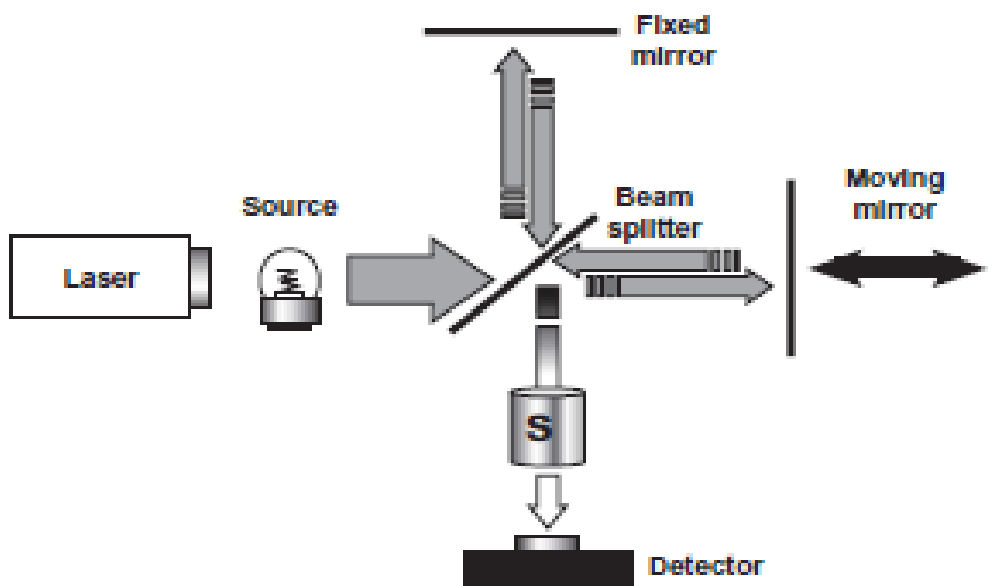
NIR spectroscopy is advantageous since it offers the potential to conduct rapid tests on small samples of ground grain or non-destructively on whole grain (Woodcock *et al.*, 2008). It is predominantly known for its accuracy, simplicity and safety (Osborne, 1981). Reproducibility is equal to and at times better than reference measurements (Williams, 2007). It allows for the simultaneous measurement of multiple quality properties (Sissons *et al.*, 2006) since it is not necessary to repeat scans for each constituent (McClure & Tsuchikawa, 2007).

NIR spectroscopy is fast, taking no more than one minute per sample, it is non-invasive and requires no sample preparation (Pasquini, 2003). No sample dilution is needed as absorptions in the NIR region are much weaker than in the IR region (Davies, 1998). A major disadvantage of NIR is the dependence on less precise reference methods (Osborne, 2000). NIR instrumentation must be calibrated by scanning a set of samples with known qualitative or quantitative properties, involving expensive and complicated reference methods that require highly skilled personnel. Modern day calibrations are dependent on sophisticated chemometric techniques, which also require the expertise of trained personnel (McClure & Tsuchikawa, 2007). The most apparent disadvantage has always been that separate calibrations are needed for each constituent. There is also the need to monitor accuracy and precision regularly and it is expensive to purchase NIR instruments (Williams, 2007).

### **2.3.4. Near Infrared Spectrometer Instrumentation**

Successful use of NIR spectroscopy depends on determining the most appropriate instrument for the application and three different types are available (Osborne *et al.*, 1993; Osborne, 2000). Instrument selection must be considered for the end application be it for research, in-line monitoring or laboratory/in-field applications (Pasquini, 2003). Ideally, the instrument should be able to accommodate at least 100 g of sample, to minimize sampling error (Williams, 2001).

Grating monochromators are the most versatile instruments and are used to measure the full visible and NIR spectrum in transmittance or reflectance mode. Such equipment is used when a wide range of different applications is required or when spectral information from a wide range of wavelengths is necessary for the development of an accurate and stable calibration (Osborne, 2000). Interferometers are modulators that do not produce angular dispersion and fall into two groups, double-beam and multiple beams (Osborne *et al.*, 1993). Fourier-transform (FT) instruments are based on the use of interferometers and FT to recover the intensities of individual wavelengths in the NIR region and combine the best characteristics in terms of wavelength precision and accuracy, high signal-to-noise ratio (S/N) and scan speed (Pasquini, 2003).

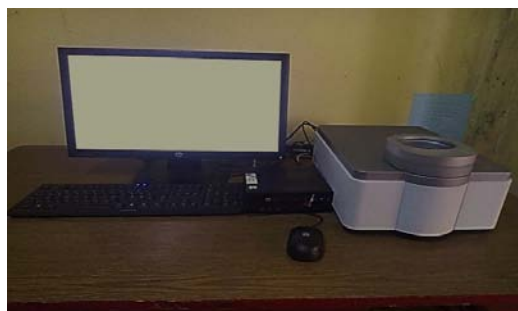


**Figure 6:** Schematic representation of an interferometer in a FT-NIR system (Visoottiviseth, 2002)

An interferometer offers excellent resolution and wavelength reproduction in comparison to a grating monochromator (Wetzel, 2001). Filter instruments are the simplest and cheapest NIR instruments and are based on a limited number (between six and twenty) of interference filters. These filters are chosen as wavelength selectors to represent the absorptions used for the most popular applications, e.g. protein, moisture and oil in agricultural samples (Osborne, 2000; Pasquini, 2003). Filter instruments are designed for a limited range of routine analysis, either in

the laboratory or on-line (Osborne, 2000) and remain economical alternatives for NIR analysis (McClure, 2003).

The most frequently employed detectors for the NIR spectral region are based on silicon, lead sulphide (PbS) and indium gallium arsenide (InGaAs) photoconductive materials (Osborne, 2000; Pasquini, 2003). A silicon detector covers the range 400 to 1100 nm, an InGaAs detector covers the range 800 to 1700 nm and a PbS detector covers the range 1100 to 2500 nm (Osborne, 2000). High powered radiation sources such as a tungsten coil or halogen lamp are employed by the majority of manufacturers and can impart a very high S/N for NIR measurements, which compensates for lower intensities of NIR absorption bands (Pasquini, 2003). Modern-day NIR instruments are continually changing as additional features and flexibilities are added with every new instrument. Portable and hand held instruments remain a keen interest in this emerging line of work (McClure, 2003).

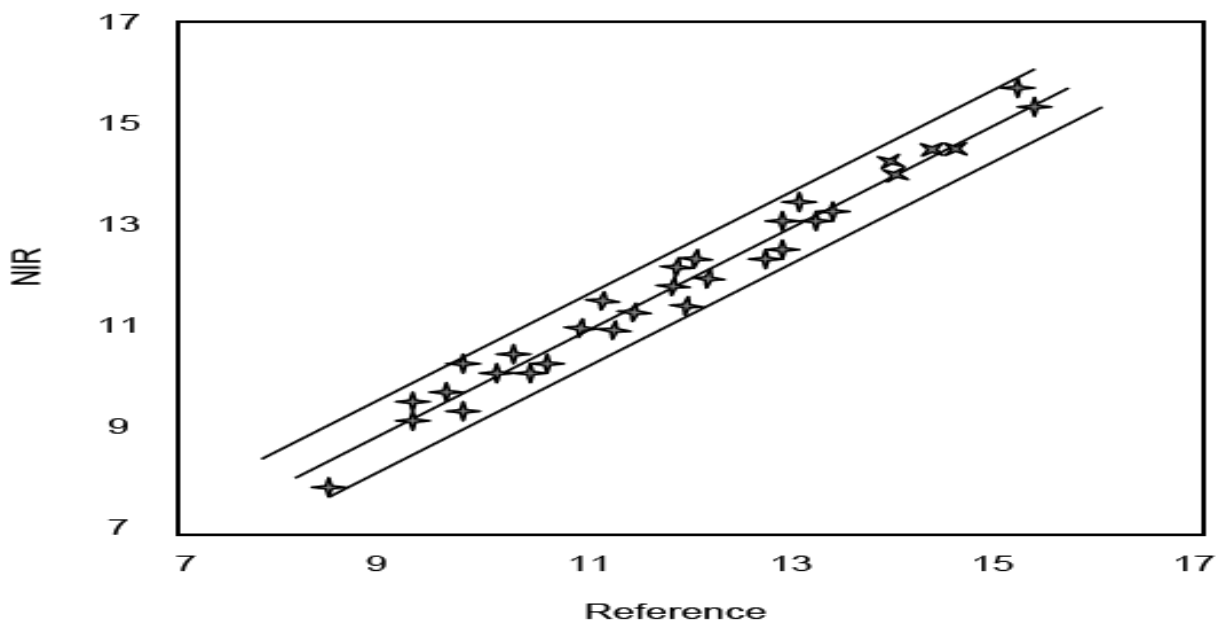


**Figure 7:** FT-Near Infrared Spectrometer (Tango, Bruker Optics GmbH, Germany).

### **2.3.5. Developing NIR Calibration Model**

NIR calibration model is developed by selecting a set of reference or calibration samples with known analyzed concentration (obtained by reference methods). The set of calibration samples should contain the range of chemical and physical variations expected in the samples the calibration model will be applied to. The purpose of this calibration experiment is to establish a mathematical relationship between the NIR spectrum and physical/chemical properties determined by reference methods. The accuracy of this mathematical relationship may be tested using the NIR spectra of independent samples (validation/test set) to predict the chemical or physical properties of interest (Bokobza, 1998; Cen & He, 2007); this is known as test set

validation. It is imperative that samples contain all possible sources of variation found in the independent test set due to physical or chemical presentations.



**Figure 8.** A typical Scatter plot of NIR versus reference data (Mark, 1992)

A good knowledge of the sample set enables the optimal use of NIR spectroscopy for quantitative purposes (Pasquini, 2003). Sample sets should be set up so the ratio of calibration to validation samples is 3:1. If calibration sets are too small, the calibration may be sample sensitive and analysis of fresh batches of samples may show significant differences in accuracy. In a perfect world, the calibration and validation sets should not be related to one another, but both should embrace the same variation dimensions (Williams, 2001).

Calibration is best evaluated with cross-validation, if the available sample set consists of up to only 60 samples. Cross-validation involves the division of the sample population into blocks consisting of one (leave-one-out cross-validation) or more (segmented cross-validation) samples. For both cross-validation procedures, the same steps are followed; samples are eliminated one at a time, or one block at a time, from the 'training set' and a calibration model is calculated using the remaining samples. This calibration model is used to predict the property of interest for the removed samples. The eliminated samples are put back into the sample set, another block is removed and the calculation procedure is repeated until all blocks have been removed. This procedure suffers from criticism since samples used for validation are selected from the original

sample set, whereas ideally samples used in the evaluation should be obtained independently like in test set validation method (Williams, 2001). The number of samples included in the calibration sample set is important.

Factors affecting the samples such as environment and seasonal variations must be taken into consideration, and all factors must be represented in the sample set. Some samples may be classified as outliers; samples that do not belong to the majority of the sample population. These samples may differ due to spectral characteristics or an error in its reference values obtained by conventional methods (Pasquini, 2003). Accuracy of the reference method is extremely important, as errors in the reference methods will be inherent in the calibrations developed. High quality calibrations can only be developed if reference methods are precise and accurate (Edney *et al.*, 1994; Williams, 2001).

The jack-knife based method (also referred to as ‘uncertainty testing’ (Davies, 2001)) is based on significance testing of model parameters, applied to regression coefficients, thereby eliminating useless or unreliable variables in order to simplify the final model and make it more reliable. The approximate uncertainty variance of PLS regression coefficient is estimated by significance tests, where a t-test is performed for each element in the regression coefficient relative to the square root of its estimated uncertainty variance, giving the significance level for each parameter. The uncertainties for the regression coefficients are estimated for a specific number of components, preferably the optimum number. The useless variables are removed as long as the root mean square error of performance (RMSEP) decreases. This method has several desired properties as it is computationally simple, has significance tests of model parameters and is robust toward crossvalidation schemes (Westad & Martens, 2000; Esbensen, 2002).

Kennard and Stone algorithm can be applied for selection of a representative test set (or sub set) from a population of samples. This algorithm selects an object which is closest to the data mean and adds it to the subset; calculates the dissimilarity between the object in the subset and the remaining samples and the object which is most dissimilar to the one already included to the subset is added to the subset. This is repeated until the desired number of objects in the subset is reached (Daszykowski *et al.*, 2002).

The general rule is that if a model with fewer variables is as good as or better with respect to predictability as the full model, the simpler model is preferred. There are a large number of applications that utilize only two or three wavelengths in routine prediction and these applications have shown that the full PLS model is sometimes inferior to a model based on a relatively small number of variables; probably due to redundancy and large amount of noisy, irrelevant variables in the NIR spectra. Results have shown variable selection, based on jack-knife estimates as it is a fast and reliable method with low risk of over fitting (Esbensen, 2002).

#### **2.3.5.1. Multivariate Calibration**

The practice of extracting chemical and physical information from relevant NIR spectra with the application of mathematical and statistical tools is known as chemometrics (Wold, 1995; Bokobza, 1998; Pasquini, 2003). It is used to relate the physical or chemical properties of a sample to the absorption of radiation in the NIR wavelength range. NIR spectral data contain a great deal of physical and chemical information. This cannot always be extracted, seeing as the NIR spectra consist of a number of overlapping bands which cannot be interpreted as easily as MIR spectra, which exhibit sharp and narrow peaks. Statistical methods have allowed for the extraction of qualitative as well as quantitative information from these complex NIR spectra (Bokobza, 1998). NIR spectral data can be pre-treated before it is used for quantitative and qualitative analysis. Pre-treatment is used to overcome problems associated with radiation scattering by a solid sample (Beebe *et al.*, 1998).

#### **2.3.5.2. Raw spectral Data Pre-processing**

Mathematical pre-processing techniques allow for the extraction of relevant information from the raw spectral data, which might contain defects, prior to analysis (Bokobza, 1998). Pre-treatment can be used for several purposes, i.e. removal of random noise; reduction of the physical effect of sample variation in scatter caused by particle size differences; and enhancement of weak absorption bands. The best pre-treatment is not known beforehand and the analyst must manually search for the technique that delivers the best results (Delwiche & Reeves, 2004). Pre-treatment techniques include derivatives (Massart *et al.*, 1988), normalisation (Næs *et al.*, 2002), multiplicative scatter correction (MSC) (Geladi *et al.*, 1985), standard normal variate (SNV) (Barnes *et al.*, 1989).

### **A) Derivatives**

As a widely-used pretreatment method, first and second derivatives are applied to eliminate drifting and scattering, respectively. They can remove background interference, distinguish superposed peaks and enhance the spectral resolution and sensitivity. Two commonly-used spectral derivative approaches are direct finite difference and Savitzky-Golay (S-G) derivatives. Before derivatization, smoothing should be applied because derivatives may extract differences of adjacent wavelength points and amplify spectral noise. Pissard *et al.* [6] proved S-G 1st derivative processing was the best pretreatment method, while Liu *et al.* [18] claimed that the 2nd derivative was the best. This second derivative is particularly useful if the absorbing species present sharp spectral bands (Næs *et al.*, 2002).

### **B) Multiplicative Scatter Correction (MSC)**

First proposed by (Ilari *et al.*, 1988), MSC was used to compensate the effect of non-uniform scattering induced by diverse particle sizes, uneven distribution and other physical effects in the spectral data. MSC is performed by linearizing each spectrum to an “ideal” spectrum, which corresponds to the average spectrum of the calibration set. The linear relationship between each spectrum and the average spectrum is fitted through the method of least squares. This suggests that MSC is feasible for removing the ‘ideal’ linear scattering and effects well when the linear relationship between absorbance and sample concentration is good. The feasibility of MSC was already confirmed by (Shao *et al.*, 2009).

### **C) Standard Normal Variate (SNV)**

Normalization involves changing spectra so that resultant spectra have more features in common or unwanted sources of variability are suppressed, which helps the visual understanding of the spectra (Hruschka, 2001). Standard Normal Variate (SNV) transforms spectral data by subtracting the mean of the spectra from each spectrum, and scaling all spectra by the standard deviation of the spectrum. SNV removes multiplicative interferences of scatter and particle size (Barnes *et al.*, 1989). Multiplicative Scatter Correction (MSC) is also a normalization procedure which separates chemical light absorption from physical light scatter (Geladi *et al.*, 1985).

### **2.3.5.3. NIR Calibration Model Statistics**

During developing NIR calibration model for the specified traits, common OPUS software statistical tools are used. For exploratory analysis such as principal component analysis can provide insight into the variation in the data. It can be applied to spectral data to give indications of relationships between samples during the early stages of data analysis (Cowe & McNicol, 1985). But quantitative chemometric techniques such as regression methods are commonly used directly in calibration model development.

#### **2.3.5.3.1. Principal Component Analysis (PCA)**

Principal component analysis is a variable compression method that reduces the data set of matrix  $X$  ( $K \times N$ ) to a much smaller number of variables are called principal components (PCs). The aim of PCA is to identify the directions, allowing the original data matrix to be reduced to a simpler one while deleting useless information. The mathematical algorithm used simply calculates the eigenvectors and eigenvalues of a matrix; as can be easily demonstrated, if the variables  $X$  are centered, then the vectors of the loadings  $p$  a (with a 1, 2, ... , A PCs) are the eigenvectors of the matrix  $(XTX)$  and those of the scores  $t$  a the eigenvalues of the matrix  $(XXT)$ . The most common among the computational algorithms available for this purpose calculate PCs in a sequential manner via an iterative least-squares process followed by subtraction of the contribution of each component. This means that the first PC extracted explained the maximum variation and so on (Martens and Naes, 1989).

#### **2.3.5.3.2. Partial least square (PLS)**

PLS is common regression statistical tool to establish a linear relationship between spectral data and the property value that needs to be determined (Wold *et al.*, 2001). The result is a calibration equation from which the property of interest can be predicted. The equation is evaluated by statistics which define the difference between the actual and predicted values (Osborne, 2000). During the optimization process the maximum number of PLS Components will be restricted to 10. The PLSR algorithm uses the information contained in both the spectroscopic data matrix,  $X$ , and the concentration matrix,  $Y$ , during calibration and compresses data in such a way that the most variance in both  $X$  and  $Y$  is explained. In this way, PLSR reduces the potential impact of large, though irrelevant, variations in  $X$  during calibration (ASTM, 2001).

. Unlike PCA, the loadings do not coincide fully with the direction of maximum variation since they have been corrected in order to maximize the predictive ability of matrix Y. If only the concentration of one of the components in Y is to be determined, even if all others are known, then the algorithm, PLS1, is a simplified version of the complete algorithm and is designated as PLS2. For calibration, the regressors matrix allowing a sample to be predicted without the need to resolve it into scores and loadings matrices is calculated. Thus, if the spectrum for a given sample is defined by vector  $x_i$ , the concentrations of the analytes  $y$  can be calculated. Accordingly the model performance will be assessed by the following statistical parameters (Conzen 2005).

#### **A) Coefficient of determination ( $R^2$ )**

This indicates the proportion of the accounted for the model. This parameter could be both for calibration and validation. It is best describer of the model performance as higher the value for  $R^2$  as approaches to 1, the better the positive correlation between the actual of the concentration and the spectral data (Conzen, 2003).

#### **B) Root Mean Square Error (RMSE)**

Root Mean Square Error of Estimation or prediction (RMSEE/RMSEP) which indicates the square root of the mean square error of the calibration and validation (Test Set Validation). This indicates how precise the value of the samples is presumed during the calibration or internal validation as it approaches to zero (Conzen, 2003).

#### **C) BIAS**

It is another important parameter which indicates the ordinate of a regression line. The closer the bias gets to 0, the better the calibration. which is a systematic deviation of the measured (predicted) values from the true value (Conzen, 2003). This bias could occur because of spectral noises and low instrumental condition as well as the biases that occurred in the chemistry data of the samples.

#### D) Ratio of Prediction Performance to Deviation (RPD)

Ratio of Prediction Performance to Deviation (RPD) is the most important parameter in evaluating the performance of the calibration model. Meaning its value indicates the suitability of the calibration for the prediction. With a higher RPD value up to 10, the calibration will more likely be able to predict the right sample values (Conzen, 2003; Cruciani et al, 1989).

#### E) Offset

The model also uses offset parameter which is the log of the time period under study with a regression coefficient of 1. In addition to other parameters Slope is again an important parameter that indicates the steepness of a calibration curve line. The greater the magnitude shows the steeper the line of calibration curve. It is also considered as the b value of calibration equation in the formula of regression equation (Concen, 2003).

**Table1.** Guidelines for the interpretation of  $r^2$  (Williams, 2001)

Coefficient of determination	Interpretation
Up to 0.25	Not usable
0.26 - 0.49	Poor correlation
0.50 - 0.64*	Acceptable for rough screening
0.66 - 0.81	Can be used for screening and approximate calibrations
0.83 - 0.90	Usable with caution
0.92 - 0.96	Usable in most applications and quality assurance
0.98+	Can be used in any application

\* Due to rounding off, no values of 0.65, 0.82, etc. are included in this table

**Table2.** Guidelines for the interpretation of the RPD (Williams)

RPD	Classification	Application
0.0 – 2.3	Very poor	Not recommended
2.4 – 3.0	Poor	Rough screening
3.1 – 4.9	Fair	Screening
5.0 – 6.4	Good	Quality control
6.5 – 8.0	Very good	Process control
8.1+	Excellent	Any application

RPD=Ratio of standard error of Prediction Validation to standard Deviation

### 3. Materials and Methods

#### 3.1. Sample Source and collection

The samples used in this study were from barley and teff breeding program trials of Holeta, Kulumsa, Debre Birhan and Debre Zeit Agricultural Research Centers. Accordingly the barley samples were collected representing a range of breeding generations grown at different environments throughout barley growing highland areas of Ethiopia specifically from (Holeta, Debre Birhan and Bekoji). About 60 samples from 2018 year trials of malt barley were collected from the pre-specified growing areas for the study. About 60 teff samples were collected from Holeta, Ginchi and Debre-Zeit directly from the controlled trials of breeding program. After pre-selection and collection was finished, the whole barley and teff samples were kept at Holeta Agricultural Research Center (Food science and Nutrition Laboratory) for sample preparation and analysis.



**Figure 9:** Barley and Teff genotypes Trials picture on field

**Table 3:** Selected Barley Sample Genotypes from Different Location, Source and Type

<b>Genotypes</b>	<b>Location</b>	<b>Source</b>	<b>Type</b>	<b>Qnty</b>
NPPT	Holeta, Bekoji & Debre-Birhan	Exotic, ICARDA, Elite	Malt, Food	10
MBNVT N	Holeta, Bekoji & Debre-Birhan	ICARDA	Food	8
MBNVT OG	Holeta, Bekoji & Debre-Birhan	Exotic, Cross	Malt	10
MBPVT	Holeta, Bekoji & Debre-Birhan	Cross, Exotic	Malt	6
FBPVT	Holeta, Bekoji & Debre-Birhan	Cross, ICARDA	Food	9
FBNVT OG	Holeta, Bekoji & Debre-Birhan	Cross, Elite	Food	8
FBNVT N	Bekoji, Bekoji & Debre-Birhan	Elite, ICARDA	Food	9

*NPPT=National parental performance Trial, MBNVT N=Malt Barley National Variety Trial New, Malt Barley National Variety Trial On Going, MBPVT=Malt Barley Preliminary Variety Trial, FBPVT=Food Barley Preliminary Variety Trial, FBNVT OG=Food Barley National Variety Trial On Going, FBNVT N=Food Barley National Variety Trial New.*

Teff samples were also taken directly from the research breeding program of controlled trials depending on genotypes, location, source from they were obtained, type (red or white teff) and quantity variability. These genotypes were different types which were under the breeding process that have been selected for their best performed characteristics to the end of the breeding program.

**Table 4:** Selected Teff Sample Genotypes from Different Location, Source and Type

<b>Genotypes</b>	<b>Location</b>	<b>Source</b>	<b>Type</b>	<b>Qnty</b>
PVT	Holeta, Debre-Zeit & Ginchi	Elite, Land race	Red and white	10
NVT	Holeta, Debre-Zeit & Ginchi	Cross	white	10
NVT-N	Holeta, Debre-Zeit & Ginchi	Cross & Elite	white	10
PON	Holeta, Debre-Zeit & Ginchi	Cross, land race	Red	8
NPPT	Holeta, Debre-Zeit & Ginchi	Cross	White and Red	10
VVT	Holeta, Debre-Zeit & Ginchi	Cross, Elite	White teff	12

*PVT=Preliminary Variety Trial, NVT=National Variety Trial, NVTN= National Variety Trial New, PON=Preliminary Observation Nursery, NPPT= National Parental Performance Trial.*

### 3.2. Sample preparation

Barley and teff samples which were collected from different locations and different genotypes of breeding trials were selected purposively from different plots depending on agronomical data and source of genotypes from where they originated as well as the history of their quality data. For barley reference and spectral data analysis 300g per sample was taken after manually cleaned and graded. Then the samples were packed into low density polyethylene bag (plastic bag). Again for teff samples analysis, 100g per each selected samples was taken from manually cleaned and graded samples, then packed into the same plastic bag as for barley samples. After that, prior to reference samples chemical analysis, malt(after malted) and teff samples were ground using a Laboratory Sample Mill3100 (Perten Instruments, Hagersten, Sweden) to pass through 0.5mm sieve for calibration reference data. But for malt friability determination the malt sample was not grounded, because the friabilimeter machine itself grounds the sample for the ratio of friability measurement.



**Figure 10:** Sample preparation, labeling and arranging picture

#### 3.2.1. Malt Processing

Before starting the other malt quality parameters analysis, the malt barley samples were malted according to Phoenix Automated Micro malting system (Phoenix Bios stems, Adelaide, Australia) designed to process 300g of 24 barley samples per batch. Samples were steeped with alternating periods of water immersion and air rest for thirty two hour (32-48h) at temperature of sixty degree centigrade (16°C) up to 45% moisture content, germination for ninety six hour (96) at 90% RH at a temperature sixteen degree centigrade (16°C) and kilning fifteen hours (15h) at a temperature ninety degree centigrade (90 °C) (Nilsen and Panozzo, 1995). After kilning the rootlets were removed from the malted samples by using a purpose built mechanical malt cleaner

that had been reconfigured to simultaneously process eight 250 g samples (Fraser Fabrications Pty Ltd, Malaga Western Australia).

### 3.3. Wet Chemistry Analysis

Malt and teff quality traits of malt barley were chemically analyzed for reference data at Holeta Food science and Nutrition Laboratory, EIAR (Ethiopian Institute of Agricultural Research) in collaboration with VLB Institute in Berlin (Germany) for the traits mentioned below. But simple quality traits were analyzed at Holeta, EIAR cereal quality laboratory. As an analysis control malt standard sample (MB3012) purchased from KWS breeding company was used. The standard sample has full malt quality traits with known concentration including protein content and it was also used as control in teff protein content determination. Each traits of control sample recovery after analysis and similar sample analyzed at Berlin (VLB) and Holeta laboratory difference are presented in annexes part in table form (see annex). The samples also duplicated in analysis to reduce the reproduced errors in each sample chemical analysis.



**Figure 11:** Reference Sample Laboratory Analysis

#### 3.3.1. Malt Quality Traits Analysis

After the malt sample flour was prepared, targeted malt quality traits for which the calibration model to be developed were analyzed using wet chemistry methods. Malt total protein content, malt  $\beta$ -glucan content, malt total extract content and malt friability were malt quality parameters that were analyzed using the following international standard methods.

### 3.3.1.1. Malt Extract Content

Malt extract content was determined according to a small-scale version of the European Brewery Convention (EBC) Methods Manual, Section 4.9.1 (European Brewery Convention, 1998) fine grind method with a grist liquor ratio of 10g: 70mL (Macleod *et al.*, 1991). This fine grind malt was extracted using a hot water mashing bath (SIEMENS Mashing Machine, Germany). For extraction, 50 g of finely ground malt was mixed with 200 mL of distilled water and mash at 45°C with continuous stirring. After 30 minutes of mashing, the temperature was increased by 1°C/min until 70°C. As temperature reaches 70°C, there was added of 100 mL distilled water. After 1 hour, the mash was cooled to 30°C and adjusted to a volume of 515 mL or a weight of 450g. The extract was filtered using whatman 12cm filter paper into 500ml cylinder and specific gravity was measured at 20°C using a DMA5000 density meter (Anton Paar GmbH, Graz, Austria). Therefore the following formula was used to put the end result.  $E = P(800+M)/(100-P)$ ; where, E= Extract content, P= Wort Density (°Plato), M= Malt Moisture content.

### 3.3.1.2. Malt Total Protein

The malt protein content was determined using kjeldhal method (Digester SBS 2000, Distillation Unit 5000DL, FoodALYT GmbH, Germany) according to (AOAC, 2005). For analysis one gram ground sample of malt barley was measured and transferred into completely dry kjeldhal flask. Ten gram of kjeldhal tablet was added to the sample inside the flask. Twenty milliliter of 98% concentrated sulphuric acid was mixed with the sample. The sample digestion was started by connecting the kjeldhal flasks with the digestion rock. The digestion was completed when the brown color of the sample completely disappeared. After the digested sample was cooled, 100 ml of distilled water and 80 ml of sodium hydroxide (32%) were added and distilled into 25 ml of excess boric acid containing 0.5 ml of screened indicator. The distillate was titrated with 0.1N hydrochloric acid to the methyl red end point. The protein was calculated by using this formula:  $CP\% = [(T-B) \cdot 14 \cdot 6.25] / [W(100-MC)]$ ; where CP=Crude Protein, T= Volume of HCl used in Titration, B= Blank used as control and W= Weight of sample taken for analysis.

### 3.3.1.3. Malt Friability

Unground malt grain samples were analyzed using a friability measuring machine (Pfeuffer Friabilimeter GmbH, Germany) which used a pressure roller to grind the sample against a

rotating screen. Low, medium and high friability malts were tested according to EBC method 4.15 (EBC, 1998). 50g malt sample was run in the friabilimeter for 8 min and the non-friable fraction was weighed to get the final result.

#### **3.3.1.4. Malt $\beta$ -glucan**

The malt  $\beta$ -glucan content was determined using the Megazyme kit method (Megazyme, Bray, Ireland) according to EBC,1998 Method 4.16.1. For the analysis 80-120 mg sample was suspended and hydrated in a buffer solution of pH 6.5 and incubated with purified lichenase enzyme and filtered. An aliquot of the filtrate was then hydrolyzed to complete with purified  $\beta$ -glucosidase. The D-glucose produced was assayed using a glucose oxidase/peroxidase reagent. The final prepared aliquot was measured by spectrophotometer at absorbance 510 nm against reagent blank within one hour. Finally the beta-glucan was calculated using the formula; B-glucan (%W/W) =  $\Delta A * (F/W) * 27$ ; Where,  $\Delta A$ = Absorbance after  $\beta$ -glucosidase treatment (reaction) minus reaction blank absorbance, F = Factor for the conversion of absorbance values to  $\mu\text{g}$  of glucose, W = The calculated dry weight of the sample analyzed in mg.

#### **3.3.1.5. Barley Dry Matter Content Analysis**

Barley grain dry matter content was determined according to AOAC international standard method from grain flour prepared using the above sample preparation method. 5g of barley flour was taken using a sensitive analytical balance and oven dried at  $105^{\circ}\text{C}$  temperature for 3 hours. After the dried sample was cooled in a desiccator, the final measurement was taken using the same analytical balance to get the result using the following known formula for moisture content.  $\text{MC}\% = (W_i - W_f) / W_i * 100$ ; where  $W_i$  is initial weight,  $W_f$  is Final Weight

#### **3.3.2. Teff Protein content Analysis**

The teff flour sample prepared according to the above method was analyzed for its crude protein content by using AOAC official method (AOAC, 2005). Thus 1g of the malt flour was taken using a sensitive analytical balance and transferred into completely dry kjeldhal flask. Ten gram of kjeldhal tablet was added to the sample inside the flask. Twenty milliliter of 98% concentrated sulphuric acid was mixed with the sample. The sample digestion was started by connecting the kjeldhal flasks with the digestion rock. The digestion was completed when the brown color of the

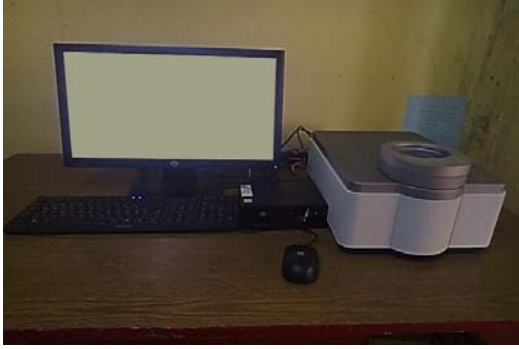
sample was completely disappeared. After the digested sample was cooled, 250 ml of distilled water and 70 ml of sodium hydroxide (32%) were added and distilled into 25 ml of excess boric acid containing 0.5 ml of screened indicator. The distillate was titrated with 0.1N hydrochloric acid to the methyl red end point. The end result was calculated by using the formula:  $CP\% = [(T - B) * 14 * 6.25] / [W(100 - MC)]$ ; where CP=Crude Protein, T= Volume of HCl used in Titration, B= Blank used as control and W= Weight of sample taken for analysis.

### **3.3.3. Teff Dry Matter Content Analysis**

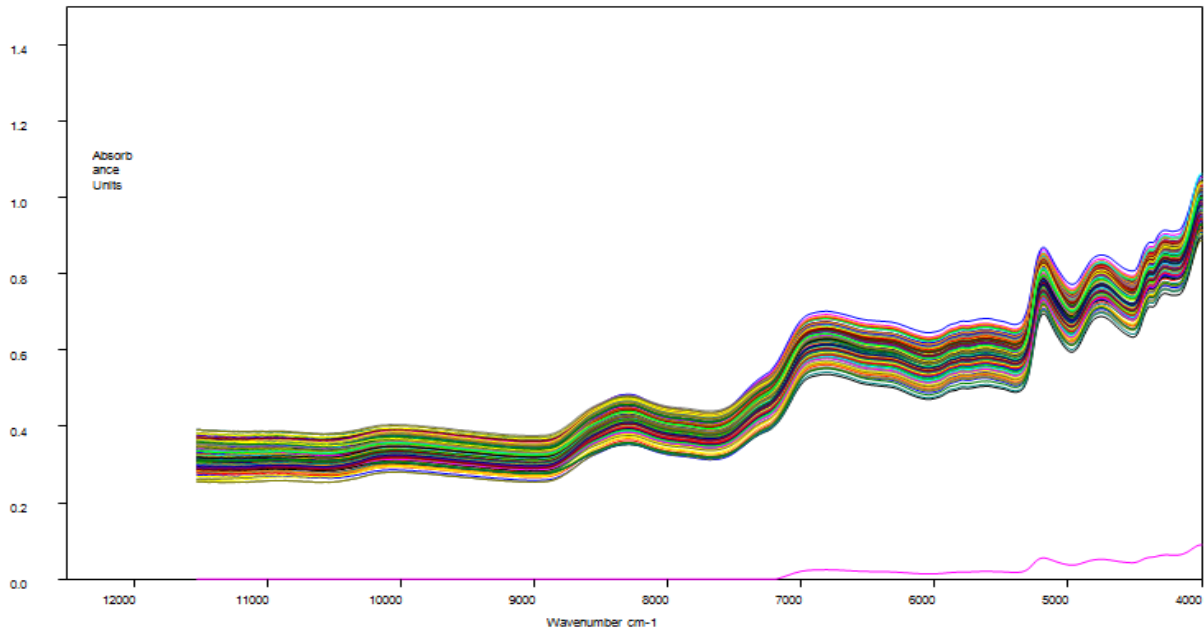
Teff dry matter content was determined according to AOAC international standard method depending on the teff flour prepared. Thus, 5g of teff flour was measured with a sensitive analytical balance and oven dried at 105<sup>o</sup>C temperature for 3 hours. After the dried sample cooled in a desiccators, the final measurement was taken using the same analytical balance with initial measurement to get the result using the known formula for moisture content.  $MC\% = (W_i - W_f) / W_i * 100$ ; where  $W_i$  is initial weight,  $W_f$  is Final Weight.

### **3.4. Samples Spectral Data Acquisition**

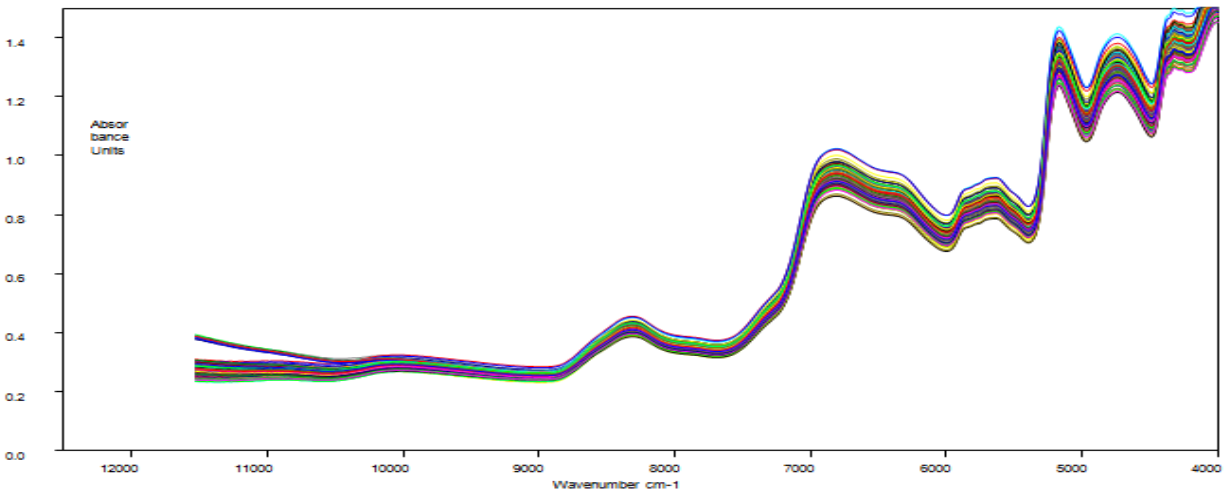
Before scanning the samples for spectral data, the spectroscopic performance of the device was checked using gold standard (1.038) and light trap standard (0.00065) provided by Bruker company of Germany. Then next without destructing the nature of the sample, 60 barley and teff sample were scanned for spectral data using near infrared spectrometer (Tango2017, Bruker Optics GmbH, Germany) which uses scan and rotating mode within 16 seconds sample presentation with rotating accessory having 600 mL sample cuvette. It used 32 amounts of scans between 867 –2535 nm wave length ranges with 16 cm<sup>-1</sup> wave number band resolution. The NIR sensor used in this experiment is capable of recording spectra by diffusion reflection measurements at the mentioned spectral wave length range. Spectra were assessed from untreated grains for barley before malting using a Bruker Tango. The device recorded spectra in diffusive reflectance by using an integrating sphere. For each sample 32 spectra were recorded in the pre-specified wavelength range. Measurements were done in triplicate for each sample, leading to a total number of 180 spectral data.



**Figure 12:** Scanning samples for spectral Data Using Tango FT-NIR Spectrometer



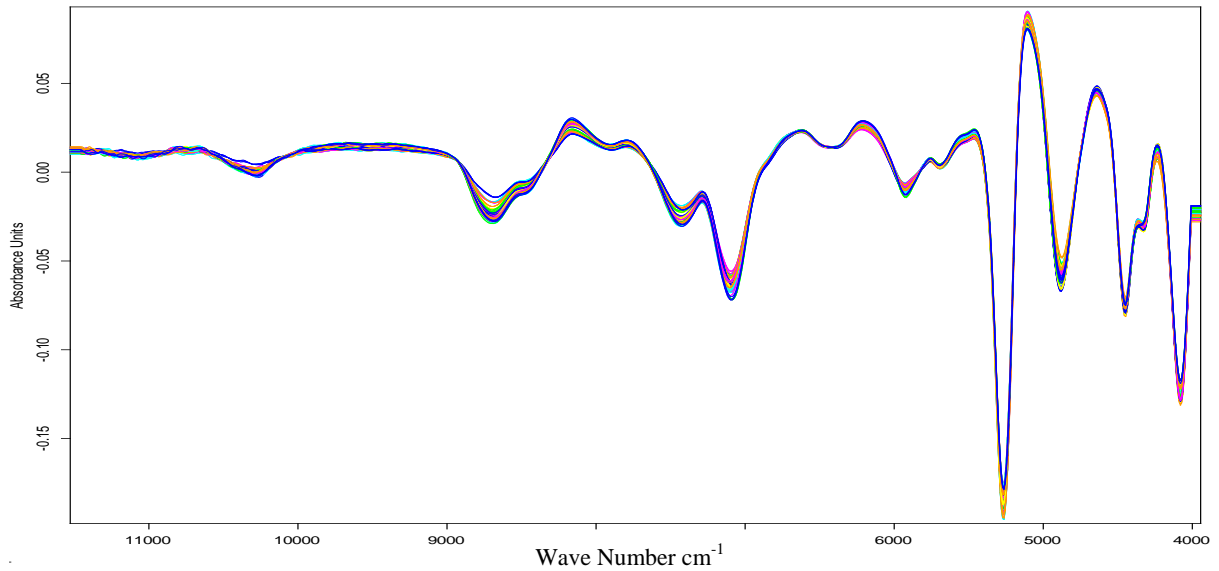
**Figure 13:** Barley samples component matrix raw spectral data collected by NIR



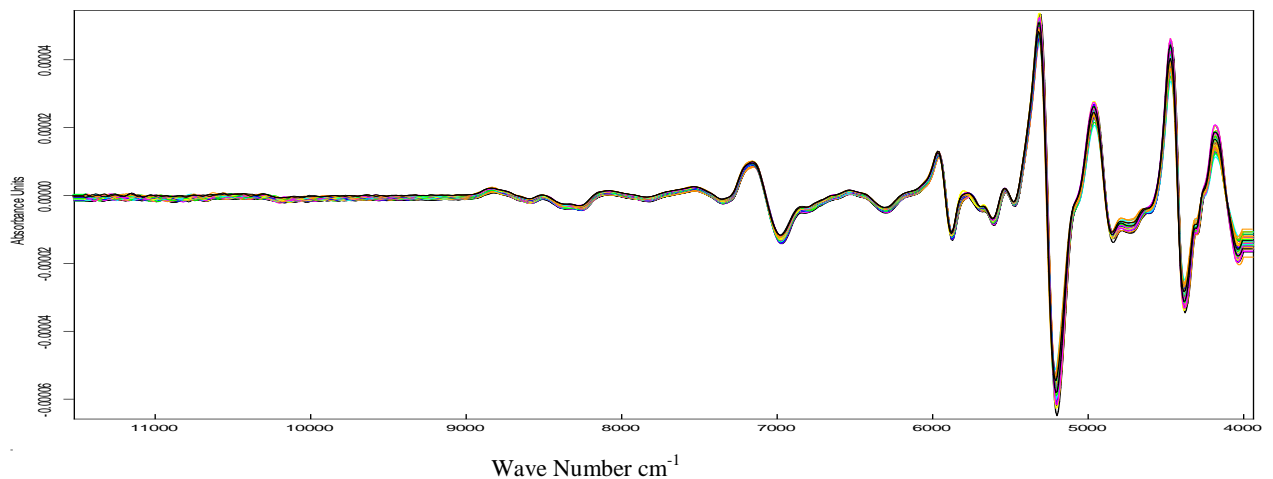
**Figure 14:** Teff samples component matrix raw spectral data collected by NIR

### 3.5. Spectral Data Pre-processing

There were spectral biases and overlaps of wave length bands due to matrix effect, different particle size and spectroscopic condition. For this matter spectral data obtained from the tango direct measurement were pre-processed using OPUS software version 7.5.1. Therefore standard normal variate (SNV) transformations, the 1st derivation with 17 smoothing points were found to be the best method for spectral treatment of barley samples, but second derivative was used for teff samples due to different nature of factors between barley and teff. These methods were selected in this thesis work for spectral data pre-processing after comparing with the other methods through many trials and error.



**Figure 15:** Barley samples component matrix pre-processed spectral data



**Figure 16:** Teff samples component matrix pre-processed spectra data

### **3.6. Calibration and Validation**

The laboratory reference data was correlated with spectral data using the software OPUS version 7.5.1 of Tango (Bruker, Optics GmbH, Germany). In total, 60 spectral data were used for calibration and validation. From 60 spectral data, dataset was splitted into 40 samples data as calibration set and a 20 samples data as validation set with approximately two thirds of the samples in the calibration and one third in the validation set. Validation method used to check the performance of the calibration model was test set validation method. The calibration set was checked to cover the whole variation in terms of spectral data, traits and locations. The following numbers of factors were used for the traits of interest: protein content 8, extract content 8, friability 9,  $\beta$ -Glucan 10 and dry mater 10. The wave number range used for the calibrations was  $11528 - 3944\text{cm}^{-1}$ , after 4 outliers were manually detected and removed. The calibration model result was evaluated by using OPUS software statistical systems (chemometrics) (Krapf et al., 2011). This calibration and validation were conducted in close co-operation with the KWS Chemistry Research & Development Department, Germany.

### **3.7. Calibration Model Statistical Evaluation**

Statistical tools were used to evaluate the efficiency of NIR calibrations and various terms are important in understanding the performance of a calibration model as described by (Williams, 2001). This includes statistics of calibration as well as statistics of validation. For developing calibration model a Partial Least Square (PLS) regression was used with 3 vectors which was performed using OPUS software as referred by (Conzen, 2005). Principal Component Analysis (PCA) was used to analysis the matrix interaction of each component that found the correlation of each component was less than 0.7 except for friability with beta-glucan correlation that could assist to develop individual model for each components.

The Coefficient of Determination ( $R^2$ ), Root Mean Square Error Estimation (RMSEE), Root Mean Square Error of Prediction (RMSEP), Ratio of performance to Deviation (RPD), Standard Deviation (SD) and Standard Error of Prediction) good parameters to evaluate the model performance depending on the reference data and spectra. The calibration was automatically tested by test set validation method (Martens, 2001). The final calibration was determined from an optimization routine of OPUS after the removal of the outliers. During the optimization step,

various frequency regions and also spectral pre-treatments was systemically tested to determine the optimal calibration that was directly stored in the device memory for future quantitative analysis as used by (Krapf et al., 2011).

### **3.8. Wet Chemistry Data Statistical Analysis**

Wet chemistry of barley and teff samples traits like extract, protein, friability,  $\beta$ -glucan and dry matter were analyzed in duplicate. The result was analyzed using SAS statistical software version 9. The means were compared using ANOVA statistical tool of LSD at  $P < 0.05$ .

## 4. Results and Discussion

As previously described in literatures, calibration model development highly depends on selecting a set of good true or calibration samples data obtained by wet chemistry analysis method. The set of calibration samples used in this study also contained the range of chemical and physical variations for which calibration model applied. Accordingly, the calibration experiment was established using a mathematical relationship between the NIR spectrum and physical/chemical properties determined by reference methods. Similarly, comparing the chemical reference data variability and model accuracy is vital point as reported by (Cen, 2007).

### 4.1. Wet Chemistry Data

#### 4.1.1. Barley Sample Traits

##### 4.1.1.1. Malt Extract Content (%)

This Trait is the most important trait either in selecting potential malting varieties and malt commercialization. Therefore developing an easy method for determining extract content is important, so that the trait was chemically analyzed for the purpose of NIR model development.

**Genotypes Variation:** According **table 5**, genotypes NPPT, MBPVT, MBNVT OG and MBNVT N with 79.70%, 80.03%, 80.67% and 80.70% extract content respectively reflected higher value than FBPVT and FBNVT N. But there was statistically significant difference between genotypes at  $P < 0.05$ . Significant difference between the genotypes was also the main objective of the study for independent calibration model development.

In his study (Swanston *et al.*, 2014) also reported that the extract yield varied depending on the extent of enzymatic degradation and the solubility of grain components after malting and mashing. Malting process also permanently affect the amount of extract content as reported by (Nischwitz *et al.* 1999). During malting, enzymes that have an impact on the degradation of substrates, were either synthesised or cleaved from their bound forms. The range of enzymes produced included those that degrade cell wall components, proteins and starch. This is also influenced by the nature of the genotypes performance to produce enough enzymes during such processing. As the objective for most maltsters is to maintain high extract levels and yet

somehow achieve relatively high extract content according to EBC standard from 70-80% based on genotypes.

**Location Variation:** On the other hand as mentioned in **table 6** there was statistically significant difference between the locations Holeta, Debre-Birhan and Bekoji at ( $p < 0.05$ ). Higher extract content was recorded at Holeta and Debre-Birhan having 79.91 and 80.3% mean values respectively at constant genotypes. Therefore location effect was observed in the result as the sample selection depended on creating variability between samples to capture minimum and maximum values later in calibration. As reported by (Fox *et al.*, 2003) extract variability occurred as influenced by several factors such as environmental, growing conditions, temperature, available nitrogen and moisture. The author reflected that these factors were different with location, as the result it created variation in the mean of the traits.

#### **4.1.1.2. Protein (%)**

**Genotypes Variation:** The Protein content of barley throughout genotypes varied from 8-16%. But according to EBC standard range malt barley protein content ranges from 9-11.5%. Similarly, in this study a mean value from 8.50-10.50% was obtained according to **table 5** results. Depending on the result FBPVT and NPPT genotypes with 10.37% and 10.50% protein mean values respectively shown higher than the other genotypes as statistical analysis showed. Other genotypes relatively have lower in protein content as compared to the two. This means that there was significant difference between the genotypes protein content mean values statistically.

In the brewery standard protein content is not needed to be higher as well as lower, but need to be in the range of 9-11.5% as mentioned above, study reported a similar trend in normal malt barley commercial requirement protein content is a maximum of 11.5% protein in the dry matter as (Kunze, 2004). However in this study since some food barley genotypes were included in the samples the values reflected less than 9% protein content. (Emebiri *et al.*, 2007) reported that protein variability occurred due to genotypes variability. Also Emebiri *et al.* (2004) reported negative correlation between protein and extract, a positive correlation between protein and diastatic power, using a low protein breeding population, mean that the quality traits correlation of genotypes could affect the protein variation among genotypes.

**Location Variation:** Also the location wise study reflected in **table 6**, that there was significant difference between Holeta, Bekoji and D/Birhan at  $p < 0.05$  statistically. Accordingly, Bekoji location reflected higher protein content (11.70%) than Holeta and Debre-Birhan locations with 8.93% and 8.31% protein values respectively. Because of purposive sample collection variability was occurred between the genotypes and locations. These variability reason were also reported by Emebiri *et al.* (2003) that barley type (one and two rowed, malt and food type) and a parental irrelative affects protein content. (Emebiri *et al.*,2007) reported that protein variability occurred due to environment and nitrogen fertilizer application. My suggestion was also similar to the literatures soil type, growing season, agricultural practices, amount of rain fall and maturity mainly affected the protein content to be varied among locations and between genotypes.

**Table 5:** Barley Quality Traits Wet Chemistry Data Variation with Genotypes

<b>Genotypes</b>	<b>Extract (%) Mean±SE</b>	<b>Protein (%) Mean±SE</b>	<b>Friability (%) Mean±SE</b>	<b>β-glucan (mg/L) Mean±SE</b>	<b>Moisture (%) Mean±SE</b>
FBPVT	75.10±1.05 <sup>b</sup>	10.37±0.09 <sup>a</sup>	45.33±5.46 <sup>b</sup>	862.7±7.06 <sup>a</sup>	8.75±0.01 <sup>a</sup>
FBNVT N	77.50±1.9 <sup>ab</sup>	9.60±0.95 <sup>ab</sup>	68.33±13.19 <sup>a</sup>	638.0±183.8 <sup>ab</sup>	9.00±0.05 <sup>a</sup>
FBNVT OG	79.38±0.65 <sup>a</sup>	8.50±0.76 <sup>b</sup>	68.67±4.41 <sup>a</sup>	885±109.55 <sup>a</sup>	9.21±0.43 <sup>a</sup>
NPPT	79.70±1.55 <sup>a</sup>	10.50±1.22 <sup>a</sup>	56.33±11.46 <sup>ab</sup>	756±123.78 <sup>ab</sup>	8.85±0.32 <sup>a</sup>
MBPVT	80.03±1.47 <sup>a</sup>	9.80±1.33 <sup>ab</sup>	66.33±16.91 <sup>ab</sup>	528.7±247.45 <sup>b</sup>	10.93±0.30 <sup>a</sup>
MBNVT OG	80.67±2.04 <sup>a</sup>	9.10±1.60 <sup>b</sup>	73.00±18.33 <sup>a</sup>	529.3±239.28 <sup>b</sup>	8.65±0.28 <sup>a</sup>
MBNVT N	80.70±2.10 <sup>a</sup>	9.60±1.82 <sup>ab</sup>	67.33±20.67 <sup>a</sup>	573.3±256.88 <sup>b</sup>	9.77±0.02 <sup>a</sup>
LSD	3.87	1.18	21.15	268.05	0.72

*Results were expressed mean with standard error; means were compared using ANOVA LSD multiple comparison at significance level  $P < 0.05$ . Means indicated with similar superscript lower case letters in the same column were statistically not significant.*

Location was one of the important variations to develop strong and well performed calibration model. Because the genotypes trials were grown at the following different locations using the same package of seed rate, fertilizer rate and genotypes. The only variations occurred might be from soil types, variation of season and other practices. Therefore table 6 described that the locations selected were Holeta, Debre-Birhan and Bekoji for which the comparisons were made.

**Table 6:** Barley Samples Wet Chemistry Data Variation with Location

<b>Location</b>	<b>Extract (%)</b> <b>Mean±SE</b>	<b>Protein (%)</b> <b>Mean±SE</b>	<b>Friability (%)</b> <b>Mean±SE</b>	<b>β-glucan (mg/L)</b> <b>Mean±SE</b>	<b>Moisture (%)</b> <b>Mean±SE</b>
Holeta	79.91±1.33 <sup>a</sup>	8.93±0.31 <sup>b</sup>	76.71±7.37 <sup>a</sup>	437.3±100.54 <sup>b</sup>	8.61±0.11 <sup>a</sup>
Debre-Birhan	80.34±0.93 <sup>a</sup>	8.31±0.52 <sup>b</sup>	71.57±5.97 <sup>a</sup>	650.1±107.39 <sup>b</sup>	9.00±0.16 <sup>a</sup>
Bekoji	76.78±0.71 <sup>b</sup>	11.7±0.52 <sup>a</sup>	42.57±6.04 <sup>b</sup>	958.1±24.09 <sup>a</sup>	9.03±0.15 <sup>a</sup>
LSD	2.53	1.43	20.4	240.95	0.47

*Results were expressed mean with standard error; means were compared using ANOVA LSD multiple comparison at significance level  $P < 0.05$ . Means indicated with similar superscript lower case letters in the same column were statistically not significant.*

#### 4.1.1.3. Friability (%)

**Genotypes Variation:** Measuring the friability of commercial malt has increasingly been used as an indicator to malting and brewing quality as well as trouble shooting on samples of poor malt quality. According to the result shown in **table 5**, there was significant difference between friability mean value of genotypes statistically at  $P < 0.05$ . Genotypes FBNVT N(68.33%), FBNVT OG(68.67%) and MBNVT OG(73.00%) were higher in mean values as LSD comparison. But FBPVT (45.33%) was lower in mean value as compared to others. Friability potential of genotypes needs to be higher in breeding lines for the purpose of barley malt commercial as set European Brewery Convection (EBC, 1998). The lower and varied value of friability occurred among genotypes could not be only the genotype variation, also occurred because of relationship with other malt quality parameters and malting process as reported by Chapon *et al.* (1978). These relationships between friability and key malt traits like wort β - glucan, Kolbach Index, wort viscosity along with other malt quality parameters have been studied through detailed experiment examining malt quality (Chapon *et al.* 1980).

**Location Variation:** Location wise variation studied in this study for Holeta, Bekoji and Debre-Birhan locations were showing significant difference among the friability mean for the specified location at  $P < 0.05$  statistically. Holeta (76.71%) and Debre-Birhan (71.57%) locations scored higher friability content than Bekoji(42.57%) which scored significant lower friability content mean as illustrated in **table 6**. However, variations between locations were better than the variation that occurred between genotypes for friability content as the results mean reflected in the table statistically. Even if the friability content scored in both location was varied as need for

model calibration, still it was less than the EBC standard range from 78-81%. This might be because of weather climate and location environment fluctuation to final breeding stage as selection was continued up to VVT (variety verification Trial). Other factors also could affect the result variation due to endosperm modification, such as poor germination, large kernels and high protein which are expected to reduce malt friability as (Edney, 2014) reported in his study rather than locations.

#### **4.1.1.4. $\beta$ -Glucans (mg/L)**

The major constituent of barley endosperm cell walls are -D-(1-3), (1-4)-glucans (75%), with a minor component identified as arabinoxylans (20%) (Fincher 1975; Fincher and Stone 1986; Henry 1987). The range in barley for glucan is 2 to 10% of total grain weight (Henry 1987).  $\beta$ -Glucan content was determined as the method described previously in the materials and method.

**Genotypes Variation:** Depending on the result **table 5**,  $\beta$ -Glucan content mean values were significantly different among genotypes at  $P < 0.05$  statistically. Genotypes like FBPVT with mean 862.7mg/L and FBNVT OG with mean 885.0 mg/L were higher in glucan content as compared to the other genotypes. But even if there was significant difference between the means, other genotypes scored lower glucan content. But the significant variation between genotypes was very important for the objective of this study to develop good calibration model for future easy prediction.

**Location Variation:** The same trend as in genotypes was observed in **table 6** between location variation that the glucan mean values were significantly different at  $P < 0.05$  statistically. Holeta and Debre-Birhan locations with mean value 437.3 mg/L and 650.1mg/L respectively reflected lower value than Bekoji with mean value 958.1mg/L. Higher value of  $\beta$ -Glucan content is not needed for malt commercial for it contributes undesirable effect in other malt quality. The same as in genotypes significant variation between locations were very important to develop good NIR calibration model for future easy prediction.

As the study of (Henry, 1987) reflected both genotypes and location influenced the content of glucan as it has been shown to have a relationship with other malt quality traits. Importantly, high glucan levels may not result in higher or lower extract but relate to other malt quality traits such as Kolbach Index (ratio of soluble to total protein), viscosity or the speed of filtration as

(Evans *et al.* 1999) studied. There was a contradicting idea between that the higher glucan content as lower the amount of extract and indirectly contributing for reducing extract content rather having direct relation with wort speed of filtration and viscosity. But in my opinion I support the idea that the glucan content reduces directly the extract content, because glucans are not easily broken down during mashing to release starch so that the extraction amount will be increased in the mashing time limit given according to the method.

#### **4.1.1.5. Moisture Content (%)**

The moisture content of barley is 8-15% on average. The moisture content can vary between 12% in very dry harvesting conditions and over 20% in wet conditions. More precisely, it is less than 13% in the South region of the European Brewery Convention (EBC) barley and malt committee, and it is more than the 16% in the North region, where consequently the barley should be dried before long term storage.

**Genotypes Variation:** In fact, barley must have moisture content below 15% for long term storage. But according to the study results in **table 5** no variation of moisture content among the genotypes was observed. As compared by using LSD value there was no significant difference between the mean of genotypes at  $P < 0.05$ . But as different literatures mentioned there was moisture content in the accepted range genotypes having 8.65-10.93% range. Normally moisture levels need to be low enough to inactivate the enzymes involved in seed germination as well as to prevent heat damage and the growth of disease microorganisms. Quality and germination capacity may also significantly deteriorate as (Plankinton *et al.*, 2014). Similarly the moisture content result obtained was maximum not higher than 11%. Even if this result supports the literature in having good storage moisture, is not acceptable to build strong NIR calibration model using narrow range mean values according to (Kunze, 2004, Vijaya, 2003).

**Location Variation:** As shown in **table 6** there was also no significant difference between the mean of locations similar to genotypes moisture content at  $P < 0.05$ . But as the literatures mentioned there was moisture content in the accepted range for location having since the mean ranged from 8.61 to 9.03% similar to genotypes variation. But since the results reflected narrow range with almost no significant difference between the values, it was not good for most independent NIR calibration model development to capture wide range moisture content

variability. This was discussed well in the model result. Moreover, the determination of the moisture content is important when the amounts of the other components are related to the dry weight according to (Kunze, 2004, Vijaya, 2003) report.

#### **4.1.2. Teff Sample traits**

##### **4.1.2.1. Protein content (%)**

Teff protein is very important trait which attracted global attention for its gluten free unlike other cereals protein. That is why the study was interested in determining protein content as major component for teff. These protein content of teff was compared across location and genotypes to investigate the variation that occurred by this two factors.

**Genotypes Variation:** As the results in **table 7** show the genotypes protein content ranged from 9.56-12.77%. Genotype VVT scored 12.77% higher protein mean value as compared to the other genotypes. In other genotypes protein content was a little bit lower, even if not very exaggerated variation. According to statistical LSD comparison at  $P < 0.05$ , there was a significant difference between the protein content of genotypes. Faraji *et al.* (2013) reported that the nature of different genotypes from different sources, protein content was varied accordingly. The significant difference between genotypes come from purposive sampling used in the method to expand the nature of calibration model developed for capturing wide range of trait variability.

**Location Variation:** The mean of protein content for location ranged from 9.54% to 11.61%. Similar to genotypes variation there was also significant difference between the protein content of locations at  $P < 0.05$  statistically. Holeta and Debre-Zeit locations with protein mean 9.54% and 10.39% respectively scored lower mean value than Ginchi with mean value 11.61% statistically. This protein content variability was due to fertilizer application and nitrogen in soil. This is in line with the findings for rice reported by Faraji *et al.* (2013), the lowest protein content recorded was (10.4%) and the highest was (12.07%) which almost have similarity with teff protein showing difference in this study among locations. The results in this study also reflected similar teff protein ranges as described in different literatures (Bultosa, 2007).

#### 4.1.2.2. Moisture content (%)

**Genotypes Variation:** Teff grain flours moisture content obtained from selected genotypes in the study ranged 10.13-11.20%, as seen from the result in **table 7**. But the results reflected a very narrow range in moisture content variability in genotypes. Almost there was no significant difference between the moisture content mean of genotypes statistically at  $P<0.05$ . This mean value similarity also affected the NIR calibration model developed. This similarity could occur due to closer grain morphology, almost similar climate, harvesting season and drying condition (Johnson *et al.*, 2009). Also the moisture content of teff grain flour samples obtained in this study was comparable with (Bultosa, 2007) report on teff moisture range.

**Location Variation:** Moisture content results obtained from location treatment ranged 10.47-10.90% as in **table 8**. Like in genotypes, location wise moisture content mean value was also at closer values range. Likewise there was no significant difference between the mean of moisture content of the location statistically at  $P<0.05$ . The same as for genotypes this very closer difference could occur due to closer grain morphology, almost similar climate, harvesting season and drying condition as reported by (Johnson *et al.*, 2009). This literature evidence could be true also for this study because of the barley growing potentials almost obtain closer climate, growing season and drying condition as barley is crop of highland by geographical location.

**Table 7:** Teff Samples Protein and Moisture content Variation with Genotypes

Genotypes	Protein	Moisture Content
	(%)	(%)
	Mean±SE	Mean±SE
PVT	10.07±0.49 <sup>b</sup>	10.13±0.37 <sup>b</sup>
NVT	11.45±0.55 <sup>ab</sup>	11.20±0.33 <sup>a</sup>
NVT-N	10.46±0.75 <sup>b</sup>	11.20±0.41 <sup>a</sup>
PON	9.56±0.61 <sup>cb</sup>	10.53±0.27 <sup>ab</sup>
NPPT	10.76±0.57 <sup>b</sup>	10.3±0.35 <sup>b</sup>
VVT	12.77±0.72 <sup>a</sup>	10.52±0.45 <sup>ab</sup>
LSD	1.75	0.37

*Results were expressed mean with standard error; means were compared using ANOVA LSD multiple comparison at significance level  $P<0.05$ . Means indicated with similar superscript lower case letters in the same column were statistically not significant.*

Teff growing location was one of the important variations to develop well performed calibration model. The genotypes trials were grown at different locations using the same agricultural packages.

**Table 8:** Teff Samples Protein and Moisture content Variation with Locations

Locations	Protein	Moisture Content
	(%) Mean±SE	(%) Mean±SE
Holeta	9.54±0.53 <sup>b</sup>	10.57±0.26 <sup>a</sup>
Debre-Zeit	10.39±0.53 <sup>b</sup>	10.90±0.26 <sup>a</sup>
Ginchi	11.61±0.53 <sup>a</sup>	10.47±0.26 <sup>a</sup>
LSD	0.53	0.26

Results were expressed mean with standard error; means were compared using ANOVA LSD multiple comparison at significance level  $P < 0.05$ . Means indicated with similar superscript lower case letters in the same column were statistically not significant.

## 4.2. Calibration Model

Before the model was developed the NIR software checked the performance of spectral data traits by correlating to each other. As tried to be illustrated in **table 9** below, the component matrix of each trait with one another shown less correlation as it need to be as in the literature. But for traits friability versus beta-glucan, there was high correlation between the two in the component matrix. This could challenge to develop strong independent calibration model, particularly for beta-glucan it resulted in its calibration model to be less trusted as evaluated by model statistics. About each model performances were well discussed in the following result and discussion part. According to (Conzen, 2003) the components correlation should be less than 0.7 to develop most independent calibration model.

**Table 9:** The Traits component Matrix correlation

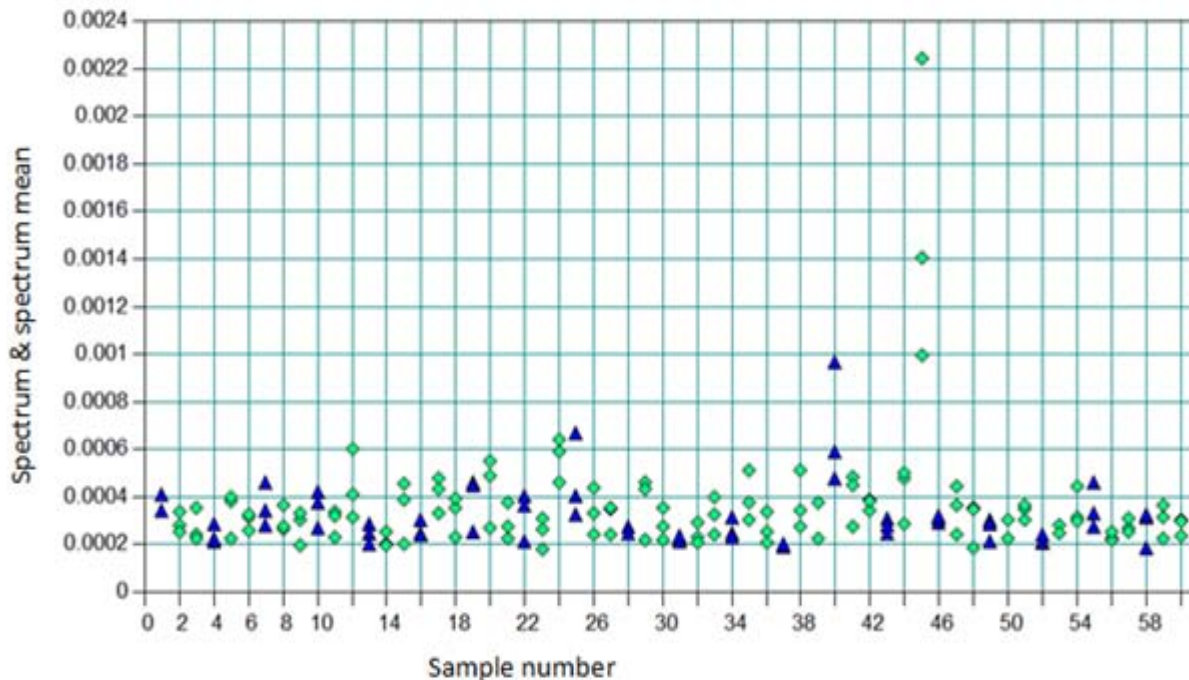
Correlation	$R^2$
Extract v Protein	0.46
Extract v Friability	0.57
Extract v Beta-glucan	0.32
Extract v Moisture Content	0.02
Protein v Friability	0.58
Protein v Beta-glucan	0.18
Protein v Moisture Content	0.005
Friability v Beta-glucan	0.70
Friability v Moisture Content	0.035
Beta-glucan v Moisture Content	0.04
Teff Protein v Teff Moisture Content	0.015

$R^2$  =Coefficient of Determination; V=versus

NIRS calibration models were developed for two teff traits and five barley traits determining quality for malt and food use. The model performance was assessed by the following parameters: coefficient of determination of the calibration ( $R^2_c$ ) and validation set ( $R^2_v$ ), standard errors of prediction (SEP) as well as root mean square error of prediction (RMSEP). The RPD<sub>v</sub> value indicates the suitability of the calibration for the prediction. With a higher RPD<sub>v</sub> value the calibration will more likely be able to predict the right sample values as (Duffus et al, 1992). According to the generally used classification in agriculture (Bamforth et al, 1993) based on RPD<sub>v</sub>-values and  $R^2_c$ -values, the calibration for protein can be regarded as successful calibration.

#### **4.2.1. Barley Traits Calibration Model**

The following barley traits analyzed chemically using wet chemistry methods were assigned for calibration model to fit the true value with predicted spectral value using PLS statistical regression. Spectral data was pre-treated using appropriate statistical tool to remove outliers and to check the status of the chemistry and spectral data for developing calibration model as planned. As we could see from **figure 17** the spectral data were first analyzed using PCA factorial statistics to detect the difference between single spectral data to its mean for the statistically selected samples (sample numbers). Some dots shown extreme far from the mean were later removed as outliers of the spectral data due to instrumental condition reading errors. From the dots shown on the figure triangle forms were spectral mean where as dots with diamond form were single spectral data. But the single spectrum value and its mean was almost very closer except few data outliers observed i.e. the total spectral data for barley was less in variability than observed in teff spectral data variability.



**Figure 17:** Barley Single Spectrum Data Difference from the Mean of the Spectrum Data Figure

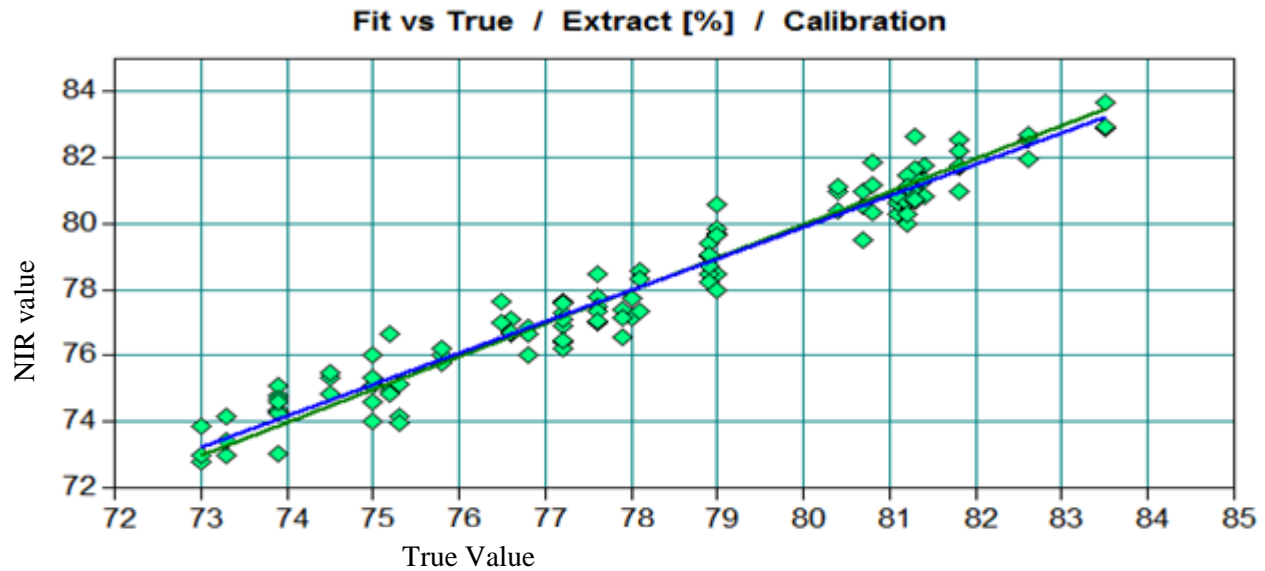
#### 4.2.1.1. Extract Content Model

Extract content prediction delivered good calibrations as in **table 10** for whole grain samples ( $R^2_c = 0.956$ ;  $RPD_C = 4.54$  with variable samples). The model major parameters  $R^2$  and RPD reflect acceptable for most applications and screening purposes. Results from this study was comparable and much better than that of previous researchers who developed promising calibrations for predicting the extract of whole grain ( $R^2_c = 0.78 - 0.85$ ) (Black & Panozzo, 2001) and ground barley ( $R^2_c = 0.77 - 0.96$ ) (Tragoonrung *et al.*, 1990). Because this property is highly influenced by the malting process since enzyme activity during malting influences the malt extract which limits the accuracy of any NIR prediction based on unmalted barley (Henry, 1985). This is why different calibration model performance is reported by different scientists, even if the accuracy of reference sample analysis data is very important. Additionally, the following **figure 18** expressed the calibration curve obtained from chemistry/reference data and NIR predicted spectrum data of extract content.

**Table 10.** Extract content calibration parameters Result

Traits	Mean	SD	Range	Factor	R <sup>2</sup> <sub>C</sub>	SEC	RMSEC	RPD <sub>C</sub>	slope
Extract %	78.1	2.93	73-83.5	8	95.62	0.65	0.67	4.54	0.95

*SD=standard Deviation of reference data; R<sup>2</sup><sub>C</sub>=Coefficient of determination; SEC=Standard Error of Calibration; RMSEC=Root Mean Square Error of Calibration; RPD<sub>C</sub>=Ratio of Performance to Deviation.*



**Figure 18:** Barley extract content calibration curve

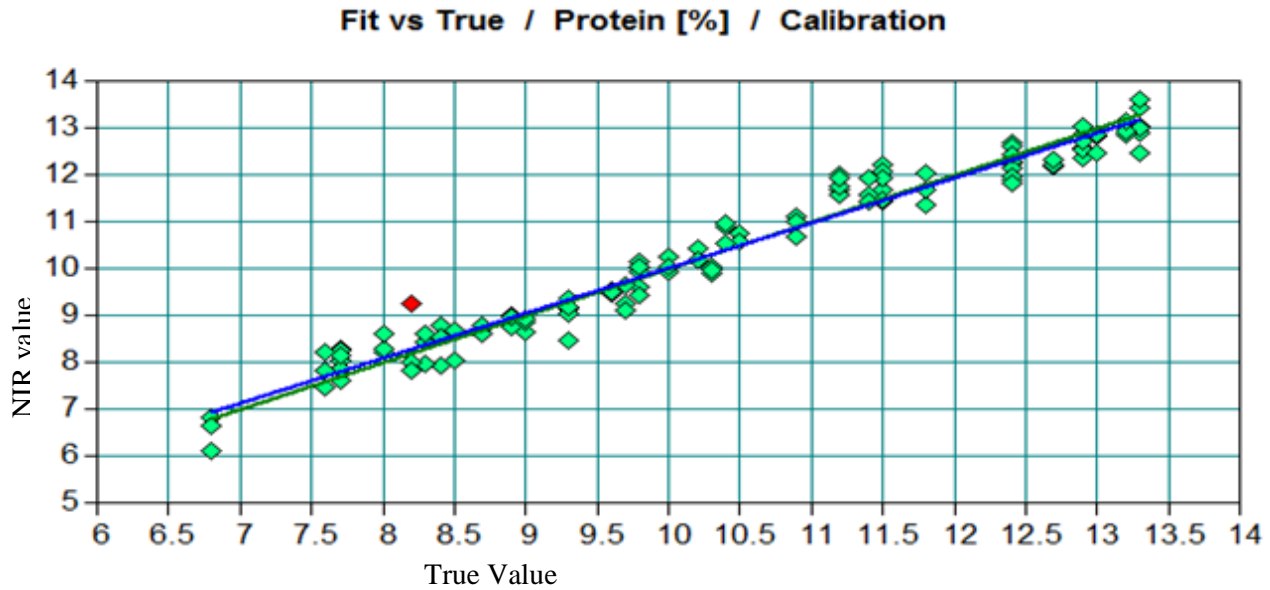
#### 4.2.1.2. Protein Model

Similarly calibration model results were obtained for whole barley grain samples protein content with ( $R^2_c = 0.97$ ;  $RPD = 5.7$  of variable samples) as in **table 11**. The model is more acceptable than the models for other traits which could be usable in most applications, quality assurances and quality control. This prediction of nitrogen content from whole grain barley is well established in the literature and the results from this study compared well with those of previous reports for whole grain barley with  $R^2_c = 0.94$  (Edney *et al.*, 1994) and  $R^2_c = 0.95$  (Sohn *et al.*, 2008). Because NIR prediction is more effective in predicting biochemical properties than physical properties according to my observation from different literatures report. In addition to model parameter information, the following **figure 19** expressed the calibration curve obtained from reference data and NIR predicted spectrum data of protein content.

**Table 11:** Protein calibration model parameters Result

Traits	Mean	SD	Range	Factor	$R^2_c$	SEC	RMSEC	RPD <sub>c</sub>	slope
Protein %	10.4	1.88	7-14.1	8	96.93	0.33	0.34	5.7	0.97

*SD=Standard deviation of reference,  $R^2_c$  = Coefficient Determination of Calibration, SEC=Standard Error of Calibration, RMSEC=Root Mean Square Error of Calibration, RPD=Ratio of Performance to Deviation*



**Figure 19:** Barley protein content calibration curve

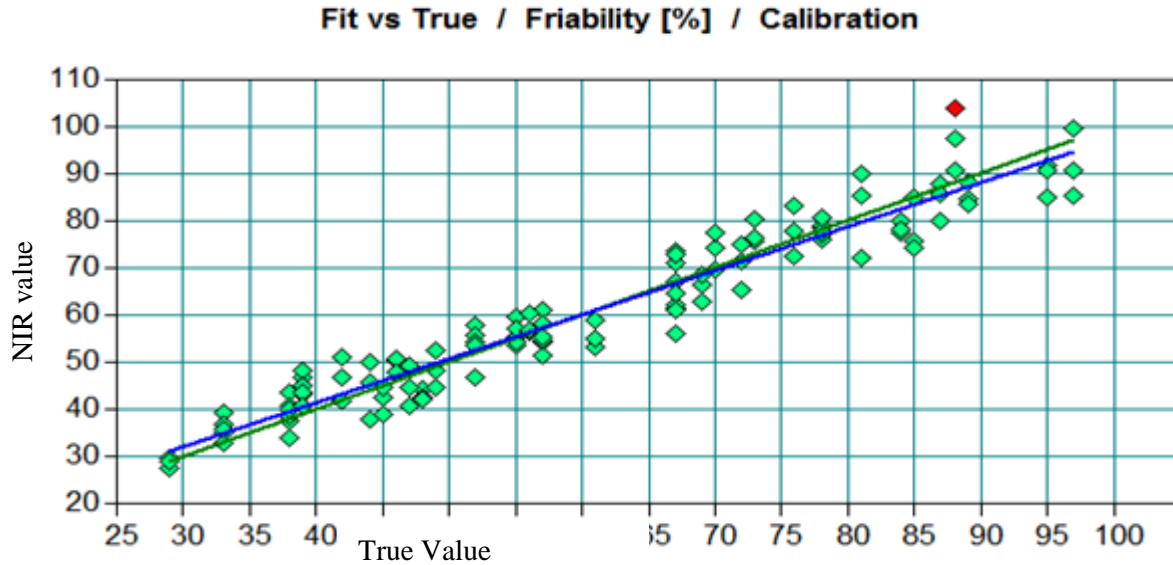
#### 4.2.1.3. Friability Model

Friability prediction performance model shown in **table 12** of this study was ( $R^2_c=0.95$ ; RPD=4.36), which was excellent like the protein model which could be used in most applications including in quality assurances, but in our case it was trustful model to identify excellent barley friability for malt factories at breeding final stage. Almost similar performed model was also reported in the literature with parameters value ( $R^2_c=0.91$ ; RPD=3.33) by (selioni, 2011). Therefore the friability calibration model was not as such challenging like in dry matter and beta-glucan as observed from similar model reported by different authors. Additionally, **figure 20** expressed the calibration curve obtained from chemistry/reference data and NIR predicted spectrum data of friability content. This curve was built after exaggerated outliers were removed from the spectrum data manually as well as by the OPUS system.

**Table 12:** Friability calibration Model Parameters Result

Traits	Mean	SD	Range	Factor	$R^2_c$	SEC	RMSEC	RPD <sub>c</sub>	slope
Friability %	61.0	18.96	29-97	9	94.75	4.35	4.5	4.36	0.95

*SD=Standard deviation of reference,  $R^2_c$  = Coefficient Determination of Calibration, SEC=Standard Error of Calibration, RMSEC=Root Mean Square Error of Calibration, RPD=Ratio of Performance to Deviation*

**Figure 20:** Barley Friability Calibration Curve

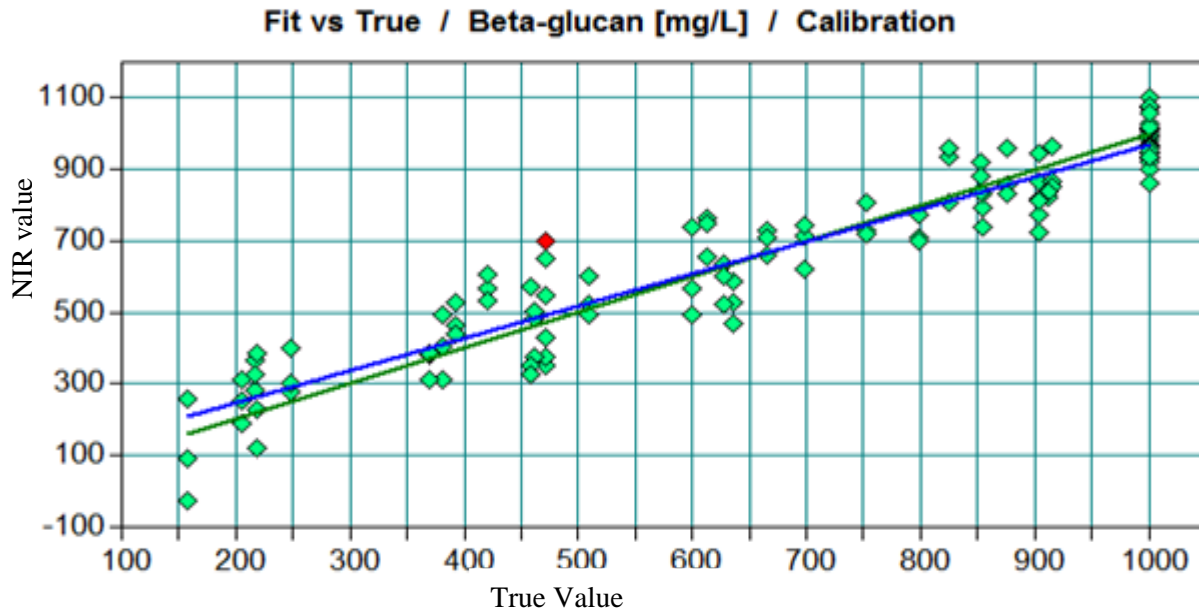
#### 4.2.1.4. $\beta$ -Glucan Model

Beta-glucan prediction model was successful in this study for whole grain barley samples having ( $R^2_c=0.90$ ; RPD=3.18) as in result **table13**. According to (Williams, 2001) this type of model performance is usable with caution specially for screening purposes such like in early stage breeding lines. But a similar study have been reported in the literature with much lower ( $R^2_c=0.25$ ) as compared to this study by (Black & Panozzo, 2001). On the other hand (Roux, 2011) reported ( $R^2_c=0.61$ ) and he referred that the poor distribution of reference values in the sample range may be the reason for the poor results in Beta-glucan model of barley grain that were obtained in his study. But in this study the good modifying malting process, excellent sample variability selection and moderate accurate chemistry analysis made the model better as compared to the reported literature. Additionally, the **figure 21** expressed the calibration curve obtained from chemistry/reference data and NIR predicted spectrum data of  $\beta$ -glucan content.

**Table 13:** Beta-glucan Calibration Model Parameters Result

Traits	Mean	SD	Range	Factor	$R^2_c$	SEC	RMSEC	$RPD_c$	slope
<b>Beta-glucan (mg/L)</b>	685.0	279.5	158-1000	10	90.11	87.90	91	3.18	0.90

*SD=standard Deviation of reference data;  $R^2_c$ =Coefficient of determination; SEC=Standard Error of Calibration; RMSEC=Root Mean Square Error of Calibration;  $RPD_c$ =Ratio of Performance to Deviation.*



**Figure 21:** Barley Beta-glucan Calibration Curve

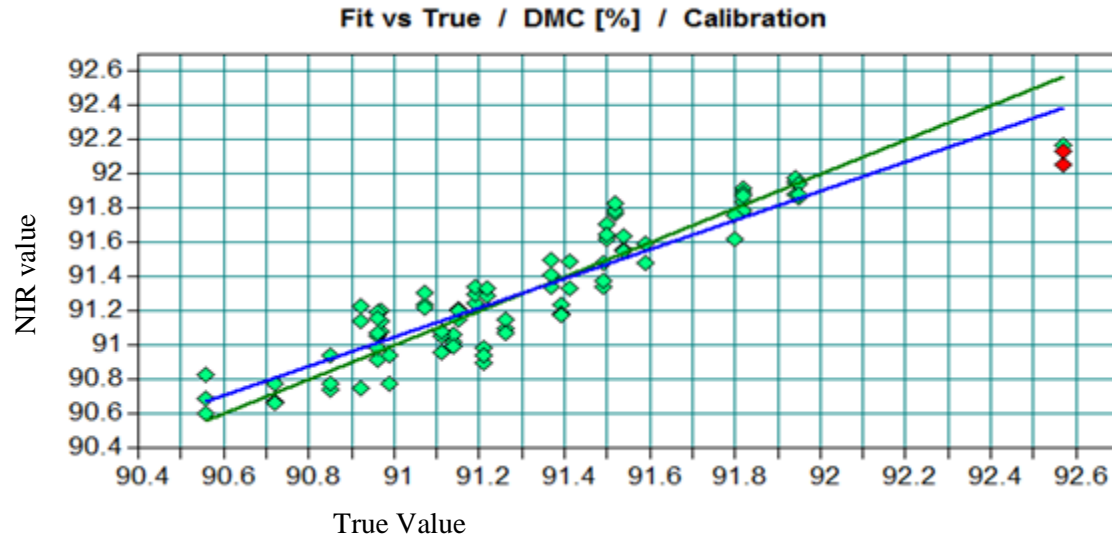
#### 4.2.1.5. Dry Matter Model

Moisture predictions model from the same whole barley grain samples were good as in **table 14**, but was only acceptable for some screening purposes, because ( $R^2_c = 0.86$ ;  $RPD_c=2.69$ ) was less as compared to the recommended range by(Williams, 2001). Results of moisture content from this study are not comparable well to literature reports, this is due to the smaller sample moisture content ranges (90.6-92.3 % DM) utilized compared to those used by previous researchers. Similar problem was also observed in literatures with the small sample range (78.4 - 83.4% DM) with in reference values obtained as studied by (Roux, 2011). The range of samples needs to be expanded in order to obtain acceptable calibrations model. Additionally, the **figure 22** expressed the calibration curve obtained from chemistry/reference data and NIR predicted spectrum data of dry matter content.

**Table 14:** Dry Matter calibration parameters Result

Traits	Mean	SD	Range	Factor	$R^2_C$	SEC	RMSEC	$RPD_C$	slope
Dry matter %	91.34	0.4	90.6-92.3	10	86.23	0.15	0.153	2.69	0.86

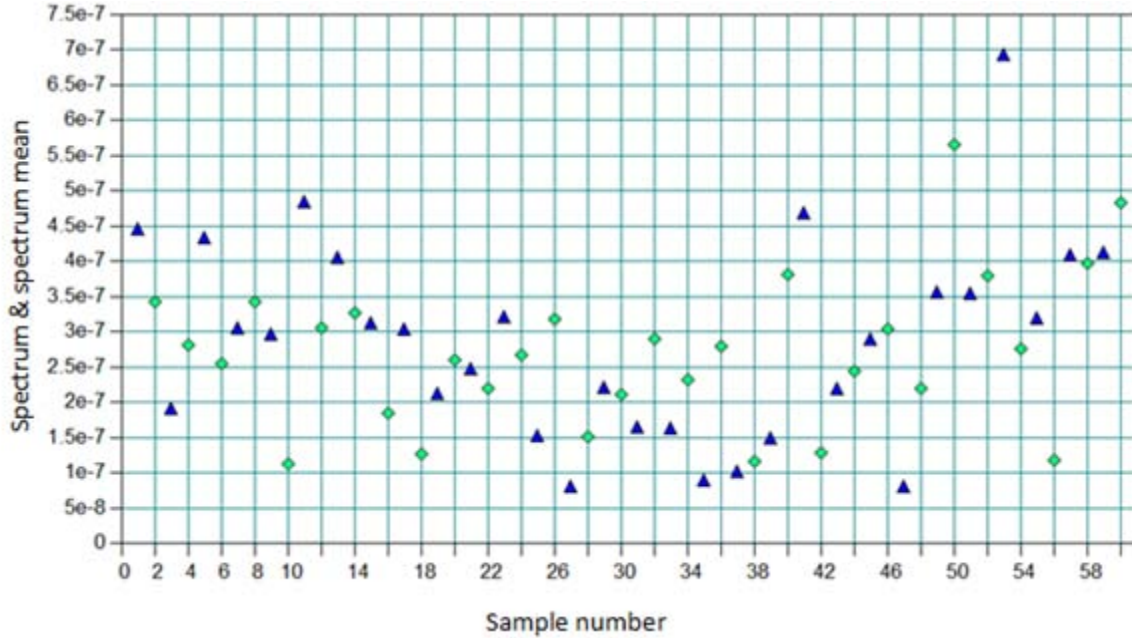
*SD=standard Deviation of reference data;  $R^2_C$ =Coefficient of determination; SEC=Standard Error of Calibration; RMSEC=Root Mean Square Error of Calibration;  $RPD_C$ =Ratio of Performance to Deviation.*



**Figure 22:** Moisture content calibration curve

#### 4.2.2. Teff Traits Calibration Model

The Teff protein and moisture content traits analyzed chemically using wet chemistry methods were assigned for calibration model to fit the true value with predicted spectral value using PLS statistical regression. Spectral data was treated using appropriate statistical tool to remove outliers and to check the status of the chemistry and spectral data for developing calibration model. As we could see from **figure 23** the spectral data were first analyzed using PCA factorial statistics to detect the difference between single spectral data to its mean for the statistically selected sample numbers. Some dots shown extreme far from the mean were considered as outliers of the spectral data occurred due to instrumental condition and reading errors. Which later were removed from the data before a calibration model was developed. The signs shown on the figure in triangle form were spectral mean where as signs with diamond form was single spectral data.



**Figure 23:** Teff Single Spectrum Data Difference from the Mean of the Spectrum Data Figure

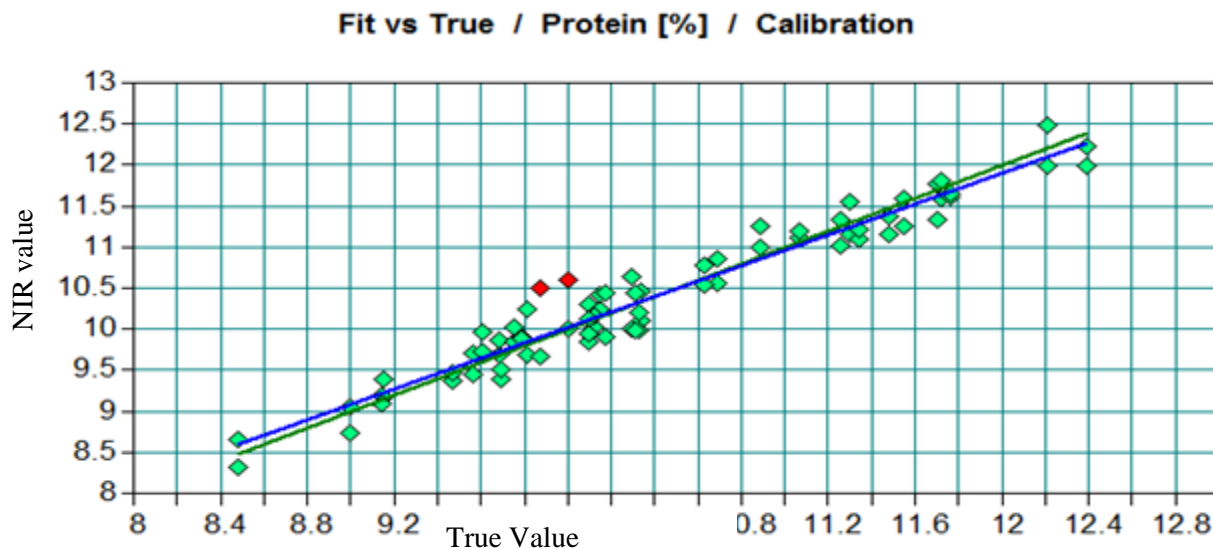
#### 4.2.2.1. Protein Model

Whole teff grain protein content model result **table 15** was ( $R^2_c=0.94$ ;  $RPD=4.16$ ) which was also well performed model similar to barley protein model and could be applied for most prediction purposes. But for development of more effective and excellent prediction models for this property, chemical analysis data should be precise as well as sample variability ranges should be expanded. Additionally, the **figure 24** expressed the calibration curve obtained from reference data and NIR predicted spectrum data of teff protein content.

**Table 15:** Protein Calibration Model Parameters Result

Traits	Mean	SD	Range	Factor	$R^2_c$	SEC	RMSEE	$RPD_c$	Slope
<b>Protein %</b>	10.47	1.09	8.5-12.4	9	94.20	0.26	0.24	4.16	0.94

*SD=standard Deviation of reference data;  $R^2_c$ =Coefficient of determination; SEC=Standard Error of Calibration; RMSEC=Root Mean Square Error of Calibration;  $RPD_c$ =Ratio of Performance to Deviation.*



**Figure 24:** Calibration Curve of Teff Protein Content

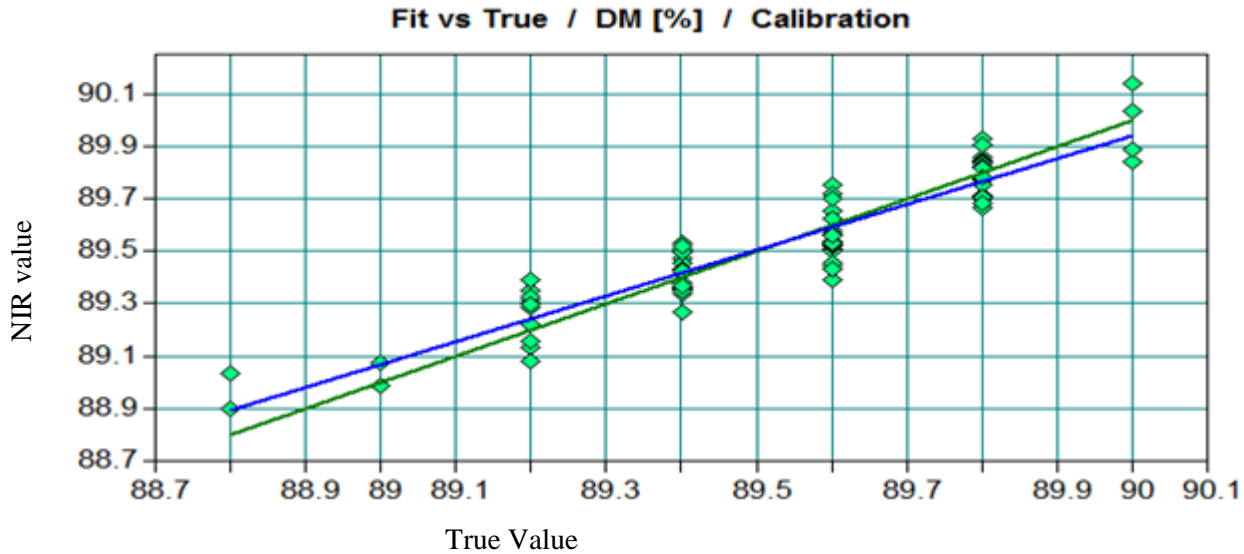
#### 4.2.2.2. Dry Matter Model

As **table 16** teff moisture content model result with ( $R^2_c=0.87$ ;  $RPD=2.77$ ) was similar performance as in barley moisture. But these models were less trusted similar to barley moisture content than moisture content determinations model well established in literatures ( $R^2_c = 0.94 - 0.96$ ) (Downey, 1985; Halsey, 1987; Sohn *et al.*, 2008) with 84.1-90.6% DM sample ranges. Results of moisture content from this study did not compare well to literature reports, this is due to the smaller sample moisture content ranges (90.6-92.3 % DM) utilized compared to those used by previous researchers. A similar problem was also observed in literatures with small sample range (78.4 - 83.4% DM) with in reference values obtained as studied by (Roux, 2011). The range of samples needs to be expanded in order to obtain acceptable calibrations model. Additionally, the following **figure 25** expressed the calibration curve obtained from chemistry/reference data and NIR predicted spectrum data of teff dry matter content.

**Table 16:** Moisture Content Calibration Model Result

Traits	Mean	SD	Range	Factor	$R^2_c$	SEC	RMSEE	$RPD_c$	Slope
DM %	87.6	0.27	88.8-90	9	86.95	0.10	0.11	2.77	0.87

*SD=standard Deviation of reference data;  $R^2_c$  =Coefficient of determination; SEC=Standard Error of Calibration; RMSEC=Root Mean Square Error of Calibration;  $RPD_c$ =Ratio of Performance to Deviation.*



**Figure 25:** Calibration Curve of Teff Moisture Content

### 4.3. Model Validation

Model evaluation was performed by test set validation; because test set validation is a more independent validation method than cross-sectional validation as (Williams, 2001). In this method, the set of calibration samples is divided into a calibration set and validation set. According to this study from 60 samples, 20 samples spectral data and chemistry data was set for validation. Using this process, the models were validated and checked for their prediction capacities. The principle was predicting quantitative value using the model from the validation set spectral data and comparing the predicted value against the chemistry data set for validation. Validation parameters for each barley and teff traits are well discussed below.

#### 4.3.1. Barley Traits Model Validation

##### 4.3.1.1. Extract Content

The extract content calibration model was validated using test set validation method according to pre-specified sample set. For this purpose the validation parameters mentioned in **table 17** were the major important parameters to evaluate the model, similar to calibration model parameters RPD and  $R^2_v$  was the main parameter to discuss about the validation. For extract model  $R^2_v$  with 0.81 and RPD 2.28 respectively described the model was in good performance for prediction. Also similar study reported correspondent result with this study (Maertin et al, 2015). Again

other parameters like SEP\RMSEP and Bias with lower value reflected that the model was well performed.

#### **4.3.1.2. Protein**

As we saw in the calibration model,  $R^2_C$  and  $RPD_C$  were shown good parameters value which was promising for good prediction. The same trend was also observed in **table 17** validation parameters in which  $R^2_V$  and  $RPD_V$  with 0.93 and 3.78 respectively reflected that the developed calibration model for protein was strong enough to predict most nearest value to the reference value. Other validation parameters also showed very convincing value that the user could believe the model to use for prediction of protein. Similarly a study by (Edney *et al.*,1994) delivered excellent nitrogen content prediction models that could be used in most applications, as indicated by the high  $R^2$  (0.94) and the low SEP (0.31%) values.

#### **4.3.1.3. Friability**

The model prediction for friability was better as compared beta-glucan and dry matter , but it was usable for screening as the model parameter in **table 17** reflected  $R^2_V$  and  $RPD_V$  with values 0.59 and 1.57 respectively . Other parameters also reflected less trusted values like SEP and RMSEP were higher unlike reflected in literatures. This should be due to the nature of the sample and less modifying malting process occurred (Selioni, 2011).

#### **4.3.1.4. $\beta$ -glucan**

The results for the prediction of malt beta-glucan properties from whole grain barley study are shown in **Table 17**. An attempt to predict  $\beta$ -glucan levels from NIR spectra of whole grain barley delivered poor results ( $R^2_V = 0.49$ ) and ( $RPD_V = 1.41$ ) the model was not widely usable for prediction as (Black & Panozzo, 2001) reported. But this type of model could be used for simple rough screening. Prediction results for  $\beta$ -glucan content of raw barley were suitable for screening and could be used for classification of barley into low and high groups. This was possibly due to the complex nature of the constituent and the proteins and starches in unmalted barley that were not yet modified by the action of enzymes during malting.

#### **4.3.1.5. Dry Matter Content**

Prediction models based on test set validation for moisture delivered less trusted model with  $R^2 = 0.51$  and  $RPD=1.43$  as result mentioned in **table 17**, it allows only for rough screening in some

applications. But SEP=0.18 and RMSEP=0.29 reflected that confirm the usable of the model for some screening applications only. A study for prediction of barley moisture content delivered model that were considered to be satisfactory for predicting these values when considering the low RMSEP and SEP values of 0.10% and the excellent correlations obtained ( $R^2 = 0.98$ ) between predicted and reference values (Marte *et al.*,2009). The study delivered lower trusted model as compared to the literatures, this may be because of using small range of sample value and chemistry data inaccuracy result.

**Table 17. Barley Traits Model Validation Parameters Result**

Traits	Mean	SD	Range	Factor	$R^2_v$	SEP	RMSEP	RPD <sub>v</sub>	Bias
<b>Extract %</b>	78.1	3.12	69.9-84	6	0.81	1.37	1.34	2.28	0.062
<b>Protein %</b>	10.5	2.08	6.8-13.3	2	0.93	0.55	0.54	3.78	-0.03
<b>Friability %</b>	59	22.13	20-98	5	0.59	14.10	13.8	1.57	0.32
<b>Beta-glucan mg/L</b>	699	358.6	50-1000	3	0.49	254.33	249	1.41	-22.6
<b>DM %</b>	91.28	0.26	90.8-91.9	6	0.51	0.18	0.29	1.43	-0.001

*SD=standard Deviation of reference data in validation;  $R^2_v$ =Validation Coefficient of determination; SEP=Standard Error of Prediction; RMSEP=Root Mean Square Error of Prediction; RPD<sub>v</sub>=Ratio of Performance to Deviation for Validation.*

### 4.3.2. Teff Traits Model Validation

#### 4.3.2.1. Protein

The prediction performance model for teff grain protein content result **table 18** was ( $R^2_v=0.75$ ; RPD<sub>v</sub>=1.08) which was also could be usable for screening purpose unlike to barley protein model which could be not applied most prediction purposes. Parameters like SEP=0.62 and RMSEP=0.9 reflected trustful result to use the model for prediction purposes of some applications. But for development of more effective and excellent prediction models for this property, chemical analysis data should be precise as well as sample variability ranges should be expanded.

#### 4.3.2.2. Dry Matter Content

As shown in **table 18** teff moisture content prediction performance model result with ( $R^2_v=0.27$ ; RPD<sub>v</sub>=0.88) was less performed as compared to the other models. But these models were less trusted similar to barley moisture content than moisture content determinations model well established in literatures. But in some cases the model can be used for simple screening, even if

the performance is less than the expected depending on the good promising result from SEP=0.66 and RMSEP=0.61 as good as expected. A similar problem was also observed in literatures with the small sample range (78.4 - 83.4% DM) with in reference values obtained as studied by (Roux, 2011). The range of samples needs to be expanded in order to obtain acceptable calibrations model. Additionally, the following figure 14 expressed the calibration curve obtained from chemistry/reference data and NIR predicted spectrum data of teff moisture content.

**Table 18. Teff Protein and Moisture Content Model Validation Parameters Result**

Traits	Mean	SD	Range	Factor	$R^2_v$	SEP	RMSEP	RPD <sub>v</sub>	Bias
<b>Protein %</b>	10.46	0.67	9.11-12.7	3	0.75	0.62	0.90	1.08	-0.05
<b>DM %</b>	89.46	0.58	87.6-90	5	0.27	0.66	0.61	0.88	0.027

*SD=standard Deviation of reference data for Validation;  $R^2_v$ =Coefficient of determination for Validation; SEP=Standard Error of Prediction; RMSEP=Root Mean Square Error of Prediction; RPD<sub>v</sub>=Ratio of Performance to Deviation for Validation*

## 5. Conclusion

Barley is the fourth most important cereal crop worldwide. Also it is a crop of ancient origin in Ethiopia and is considered as a center of diversity for barley, because of the presence of great diversity in ecology. Most of the Ethiopian barley and teff production is consumed as food at home also indicating the status of barley as “poor man’s bread”. At the same time using barley for malt production establishes new value-added chains from which Ethiopian small holders can benefit substantially. So the main objective of this study was enabling the breeding programs to select the appropriate genotypes easily by developing calibration model using near infrared spectroscopy. Depending on the objective Samples wet chemistry for barley (Extract, protein, friability, beta-glucan and moisture content) and for teff (protein and moisture content) were analyzed using the international official methods described in materials and method part. Similarly calibration models were developed for all above mentioned traits NIR OPUS software statistical parameters. These wet chemistry and calibration model results demonstrated a realistic approach to predict quality traits in Ethiopian barley and teff such as barley protein content, barley extract content, barley friability, barley  $\beta$ -glucan, barley dry matter, teff protein content and teff dry matter by applying NIRS. This could not only be shown for one set of barley samples, but rather for sets with different genetics from diverse genotypes and locations. Almost all of the traits models were usable for different purposes depending on each predicting performances accordingly, except for beta-glucan and moisture content which need further improvements.

Therefore, this prediction model will enable the selection of appropriate food barley, malt barley and teff genotypes for future end goals. Since NIRS is fast and cost-efficient the barley and teff breeding programs can increase the intensity of selecting superior candidate lines for variety verification. With regard to broadening calibration performance Ethiopian landraces were included in the samples to capture the whole Ethiopian barley variability.

## 6. Recommendation

- ✓ All parameters that could have direct or indirect correlation with these identified traits should be included for model development to reduce matrix effect
- ✓ To more improve and develop most independent model for each quality traits for teff should be studied further as country endogenous crop.
- ✓ For some of the traits like dry matter and beta-glucan further study is very important to distinguish between sample variability range and wet chemistry analysis inaccuracy which could contribute to be less trusted model observed in most studies.
- ✓ To develop most excellent prediction method for barley and teff, excellent sample variability, accurate wet chemistry data and having good instrument condition is very important.

## 7. References

- Angelino S.A.G.F., 1996. Determination of the moisture and nitrogen contents of barley and malt by near infrared spectroscopy (NIRS). *Journal of the Institute of Brewing*, 102, 73-74.
- Anger H., Schildbach S., Harms D. and Pankoke K., 2009. Analysis and quality control. In: *Handbook of Brewing: Processes, Technology, Markets* (edited by H.M. Eßlinger). Pp. 437- 475.
- AOAC 2005. Official methods of Analysis. 17th edn. Association of Official Analytical Chemists, Washington DC.
- Arends A.M., Fox G.P., Henry R.J., Marschke R.J. and Symons M.H., 1995. Genetic and environmental variation in the diastatic power of Australian barley. *Journal of Cereal Science*, 21, 63-70.
- Arogundade LA, 2006. Functional characterization of teff (*Eragostis tef*) protein concentrate: Influence of altered chemical environment on its gelation, foaming, and water hydration properties. *Food Hydrocolloid*. 20:831-838.
- B.G. Osborne T. and Fearn P.H., 1993. Hindle, *Practical NIR Spectroscopy*, Longman, Harlow, 49-78
- Bamforth C.E. and Barclay A.H.P., 1993. Malting technology and the uses of malt. In: *Barley: Chemistry and Technology* (edited by A.W. MacGregor & R.S. Bhatt). Pp. 297-354. St. Paul, Minnesota, USA: American Association of Cereal Chemists, Inc.
- Barnes R.J., Dhanoa M.S. and Lister S.J., 1989. Standard normal variate transformation and detrending of near-infrared diffuse reflectance spectra. *Applied Spectroscopy*, 43, 772-777.
- Baye, K., 2015. Teff: nutrient composition and health benefits. ESSP Working Paper 67. Washington, D.C. and Addis Ababa, Ethiopia: International Food Policy Research Institute (IFPRI) and Ethiopian Development Research Institute (EDRI)
- Beebe K.R., Pell R.J. and Seasholtz M.B., 1998. In: *Chemometrics: a Practical Guide*. Pp. 1-8, 26-55.
- Bekele E., 1983. A differential rate of regional distribution of barley flavonoid patterns in Ethiopia and a view on the center of origin of barley. *Hereditas*, 98:269-280.
- Berhane L., 1991. Barley Dependence Cereal in Ethiopia, IAR, News Letter Agricultural Research (2):3.
- Black, C. & Panozzo, J.F., 2001. Utilising Near infrared spectroscopy for predicting malting quality in whole grain barley and whole grain malt. *Proceedings of the 10th Australian Barley Technical Symposium, Canberra, ACT, Australia, 2001*.
- Bokobza L., 1998. Near infrared spectroscopy. *Journal of Near Infrared Spectroscopy*, 6, 3-17.
- BOSTID (Board on Science and Technology for International Development, National Research Council), 1996. Lost Crops of Africa. National Academy Press, Washington, DC.
- Brennan CS, Harris N, Smith D and Shewry PR., 1996. Structural differences in the mature endosperms of good and poor malting barley cultivars. *Journal of Cereal Science* 24, 171-177.
- Briggs D.E., Hough J.S., Stevens R. and Young T.W., 1981. Barley. In: *Malting and Brewing Science*. Pp. 15-38.

- Bultosa, G., 2007. Physicochemical characteristics of grain and flour in 13 tef [*Eragrostis tef* (Zucc.) Trotter] grain varieties. *J. Appl. Sci. Res*, 3(12), 2042-2051.
- Cen H.Y. and He Y., 2007. Theory and application of near infrared reflectance spectroscopy in determination of food quality. *Trends in Food Science & Technology*, 18, 72-83.
- Chapon L, Gromus J, Erber HL and Kretschmur H., 1980. Physical method of the determination of malt modification and their relationship to wort properties. European Brewery Convention, Monograph VI - Symposium on the relationship between malt and beer, Helsinki, 45-70.
- Chapon, L., Maucourt ,JM. and Gobert, J., 1979. The friabilimeter an apparatus for objective measurement of malt friability. 32: 160–167. Chemists. Washington DC.
- Conzen J.-P., 2005. Multivariate Kalibration. Ein praktischer Leitfadens zur Methodentwicklung in der quantitativen Analytik. 4., neu bearb. Aufl. [Ettlingen]: Bruker
- Conzen, J. P., 2003. "Multivariate Calibration", Bruker Optik GmbH.
- Cowe I.A. and McNicol J.W., 1985. The use of principal components in the analysis of nearinfrared spectra. *Applied Spectroscopy*, 39, 257-266.
- Cruciani, G., Baroni, T., Clementi, S., Costantino, F., Riganelli D. and Skagerberg, B., 1989. "Predictive ability of regressions models. Part I: standard deviation of prediction errors (SDEP)", *Journal of Chemometrics*, Vol. 3, pp. 499–509.
- CSA (Central Statistic Authority), 2012. Agricultural Sample Survey: report on Area and production of major Crops (Private peasant holdings "meher" season), Volume I, Addis Abeba, Ethiopia.
- Daszykowski M., Walczak B. and Massart, D.L., 2002. Representative subset selection. *Analytica Chimica Acta*, 468, 91-103.
- Davies A.M.C., 2001. Uncertainty testing in PLS regression. *Spectroscopy Europe*, 13, 16-19. 55
- Davies T., 1998. The history of near infrared spectroscopic analysis: Past, present and future "From sleeping technique to the morning star of spectroscopy". *Analisis*, 26, 17-19.
- Delwiche S.R., 2004. Analysis of Small Grain Crops. In: *Near-Infrared Spectroscopy in Agriculture* (edited by C.A. Roberts, J. Workman & J.B. Reeves). Pp. 269.
- Downey, G., 1985. Estimation of moisture in undried wheat and barley by near infrared reflectance. *Journal of the Science of Food and Agriculture*, 36, 951-958.
- Duffus C.M. and Cochrane, M.P., 1992. Grain structure and composition. In: Shewry, P.R. (Ed.), *Barley: Genetics, Biochemistry, Molecular Biology and Biotechnology*. CAB International, pp. 291e317.
- Duffus C.M. and Cochrane, M.P., 1993. Formation of the barley grain - Morphology, physiology and biochemistry. In: *Barley: Chemistry and Technology* (edited by A.W. MacGregor & R.S. Bhatt). Pp. 31-67. St. Paul, Minnesota, USA: American Association of Cereal Chemists, Inc.
- Edney M.J., Morgan J.E., Williams P.C. and Campbell L.D., 1994. Analysis of feed barley by near infrared reflectance technology. *Journal of Near Infrared Spectroscopy*, 2, 33-41.
- Edney, M., MacLeod, A. and LaBerge, D., 2014. Evolution of a quality testing program for improving malting barley in Canada. *Canadian journal of plant science*. (2014) 94.
- Emebiri LC, Moody DB, Black C, van Ginkel M and Hernandez E 2007. Improvement in malting quality barley grain yield by manipulation of genes influencing grain protein content. *Euphytica* 156, 185-194.

- Esbensen K., 2002. Uncertainty estimates, significance and stability (Marten's uncertainty test). In: *Multivariate data analysis - In practice*. Pp. 327-329. Oslo, Norway: CAMO Process AS.
- European Brewery Convention, 1998. *Analytica EBC*. 5th ed. Verlag Hans Carl, Nurnberg, Germany.
- Evans DE & Hejgaard J., 1999. The impact of malt derived proteins on beer foam quality Part I: The effect of germination and kilning on the level of protein Z4, Protein Z7 and LTP1. *Journal of the Institute of Brewing* 105, 159-169.
- Evette Roux, 2011. near infrared (nir) spectroscopy for selection of malting barley in south african breeding programmes, Book.
- Faraji,F., Esfahani,M., Kavousi, M.,Nahvi,M & Forghani, A., 2013. Effect of nitrogen fertilizer levels on Fe and protein content, grain breakage and grain yield of rice (*Oryza sativa* L. cv. Khazar). *Biharean Biologist*, 7(1), 25-28.
- Ferrio JP, Alonso N, Voltas J and Araus J., 2006. Grain weight and changes over time in ancient cereal crops: Potential roles of climate and genetic improvement. *Journal of Cereal Science* 44, 323-332.
- Fincher G.B. and Stone B.A., 1993. Physiology and biochemistry of germination in barley. In: *Barley: Chemistry and Technology* (edited by A.W. MacGregor & R.S. Bhatti). Pp. 247-296. St. Paul, Minnesota, USA: American Association of Cereal Chemists, Inc.
- Fox G.P., Kelly A., Bowman J., Inkerman A., Poulsen D. and Henry R., 2009. Is malting barley better feed for cattle than feed barley? *Journal of the Institute of Brewing*, 115(2), 95-104. 56
- Fox, G., Panozzo, J., Li, C., Lance, R. C, Inkerman, P., and Henry, R. J., 2003. Molecular basis of barley quality, *Australia journal of agriculture research*. 54, 1081–1101.
- Fridissa E., Woldeyeseus S., and Heinrich G., 2010. On farm Diversity and Characterization of Barley (*Hordeum vulgare* L.) Landraces in the Highlands of West Shewa, Ethiopia vol.8:025 034.
- Geladi P., Macdougall D. and Martens H., 1985. Linearization and scatter-correction for NearInfrared Reflectance Spectra of meat. *Applied Spectroscopy*, 39, 491-500.
- Gothard P.G., Morgan A.G. and Smith D.B., 1980. Evaluation of a micro-malting procedure used to aid a plant breeding programme. *Journal of the Institute of Brewing*, 86, 69-73.
- H. Mark, 1992. 'Qualitative Discriminant Analysis', in *Handbook of Near-Infrared Analysis*, eds. D.A. Burns, E.W. Ciurczak, Marcel Dekker, New York, 329–363
- Hailu Tefera, Kebebew Assefa, Fufa Hundera, Tiruneh Kefyalew and Tesfaye Teferra, 2003. Heritability and genetic advance in recombinant inbred lines of tef (*Eragrostis tef*). *Euphatica*, 131: 91-96.
- Halsey S.A., 1986. The application of transmission near infrared spectroscopy to the analysis of worts. *Journal of the Institute of Brewing*, 92, 387-393.
- Halsey, S.A., 1987. Analysis of whole barley kernels using near infrared reflectance spectroscopy. *Journal of the Institute of Brewing*, **93**, 461-464.
- Henry R.J., 1985. Use of a scanning near-infrared reflectance spectrophotometer for assessment of the malting potential of barley. *Journal of the Science of Food and Agriculture*, 36, 249-254
- Hough J.S., 1991. Malt - a package of enzymes and food substances. In: *The Biotechnology of Malting and Brewing* (edited by J. Baddiley, N.H. Carey, J.F. Davidson, I.J. Higgins & W.G. Potter). Pp. 19-39. Cambridge: Cambridge University Press.

- Hruschka W.R., 2001. Data analysis: Wavelength selection methods. In: *Near-Infrared Technology in the Agricultural and Food Industries* (edited by P.C. Williams & K.H. Norris). Pp. 39-58. St. Paul, Minnesota, USA: St. Paul, Minnesota, USA.
- Huang H., Yu H., Xu H. and Ying Y., 2008. Near infrared spectroscopy for on/in-line monitoring of quality in foods and beverages: a review, *J. Food Eng.*, **87**, 303–13.
- Hunter H., 1962. The science of malting barley oroduction. In: *Barley and Malt: Biology, Biochemistry, Technology* (edited by A.H. Cook). Pp. 25-44. London: Academic Press, Inc.
- Ilari, J.L.; Martens, H.; Isaksson, T., 1988. Determination of particle-size in powders by scatter correction in diffuse near-infrared reflectance. *Appl. Spectrosc.* **1988**, *42*, 722–728.
- Institute of Brewing, 1982. Recommended Methods of Analysis. IOB, London
- Johnson, G. A., Qian, Y. L., & Davis, J. G., 2009. Topdressing Kentucky bluegrass with compost increases soil water content and improves turf quality during drought. *Compost Science & Utilization*, *17*(2), 95-102.
- Kaczmarek Z., Adamski T., Surma M., Jezowski S. and Lesniewska-Fratczak M., 1999. Genotype-environment interaction of barley doubled haploids with regard to malting quality. *Plant Breeding*, *118*, 243-247.
- Kawano S., 2002. Sampling and sample presentation. In: *Near Infrared Spectroscopy: Principles, Instruments, Applications* (edited by H.W. Siesler, Y. Ozaki, S. Kawata & H.M. Heise). Pp. 115-124, Germany
- Kessler W., 2007. Multivariate Datenanalyse. Für die Pharma-, Bio- und Prozessanalytik; ein Lehrbuch. Weinheim: Wiley-VCH.
- Kotze G.J., 2009. Guidelines for the production of malting barley: winter dryland 2009. SAB Maltings, Kimberley, South Africa.
- Krapf L. C., Gronauer A., Schmidhalter U. and Heuwinkel H., 2011. Near infrared spectroscopy calibrations for the estimation of process parameters of anaerobic digestion of energy crops and livestock residues (19), S. 479–493.
- Kreisz S., 2009. Malting. In: *Handbook of brewing: Processes, Technology, Markets* (edited by H.M. Eßlinger). Pp. 147-164. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.
- Kunze, W., “Technology Brewing and Malting” VLB, Berlin, 2004.
- Macleod L.C., Dowling M.A., Sparrow D.H.B. and Lance R.C.M., 1991. In: The Proc. 5th Barley Tech. Sym., Australia, p. 125-128.
- Marte L., Belloni P., Genorini E., Sileoni V., Perretti G., Montanari L. and Marconi O., 2009. Near-infrared reflectance models for the rapid prediction of quality of brewing raw materials. *Journal of Agricultural and Food Chemistry*, *57*, 326-333.
- Martens H. and Martens M., 2001. Multivariate analysis of quality. An introduction. Chichester [u.a.]: Wiley. Martens, H., Naes, T. (2001): Multivariate calibration. Chichester [u.a.]: Wiley.
- Martens, H. and Naes, T., 1989. “Multivariate Calibration“, J. Wiley and Sons, New York.
- Marwat S. K., Hashimi M., Khan K. U., Khan M. A., Shoaib M. and Fazal-ur-Rehman, 2012. Barley (*Hordeum vulgare L.*) A Prophetic Food Mentioned in Ahadith and its Ethnobotanical Importance. *American-Eurasian Journal of Agricultural and Environmental Sciences*. *12*(7). P. 835–841.
- Massart D.L., Vandeginste B.G.M. and Buydens L.M.C., 1988. *Chemometrics: A Textbook*. Amsterdam: Elsevier. McClure, W.F. (2003). 204 years of near infrared technology: 1800-2003. *Journal of Near Infrared Spectroscopy*, *11*, 487-518.

- McClure W.F. and Tsuchikawa S., 2007. Instruments. In: *Near-infrared Spectroscopy in Food Science and Technology* (edited by Y. Ozaki). John Wiley & Sons, Inc. McGuire, C.F. (1982). Near-infrared reflectance estimates of malt extract. *Cereal Chemistry*, 510-511.
- Mengesha, M.H., 1966. 'Chemical composition of teff (*Eragrostis tef*) compared with that of wheat, barley and grain sorghum'. *Economic Botany*, 20:268-273.
- Meredith W.O.S., Anderson J.A. and Hudson L.E., 1962. Evaluation of malting barley. In: *Barley and Malt: Biology, Biochemistry, Technology* (edited by A.H. Cook). Pp. 207-269. London: Academic Press, Inc.
- Morgan, A. G. and Gothard, P. G. 1979. Rapid prediction of malt hot water extract by near infrared reflectance spectroscopy studies on barley. *J' Inst. Brew.* 85: 339-341.
- Næs T., Isaksson T., Fearn T. and Davies T., 2002. Scatter correction of spectroscopic data. In: *A user-friendly guide to multivariate calibration and classification*. Pp. 105-125. Chichester, UK: NIR Publications.
- Nilan R.A. and Ullrich S.E., 1993. Barley: Taxonomy, origin, distribution, production, genetics and breeding. In: *Barley: Chemistry and Technology* (edited by A.W. MacGregor & R.S. Bhatti). Pp. 3-25. American Association of Cereal Chemists, Inc. St. Paul, Minnesota, USA.58
- Nilsen M. and Panozzo J., 1995. In: *Leaping ahead with Near Infrared Spectroscopy*, Ed by Batten, G.D., Flinn, P.C., Welsh, L.A. and Blakeney, A.B. NIR Spectroscopy Group, Australia, p. 174-177.
- Osborne B.G., 1981. Principles and practice of near infra-red (NIR) reflectance analysis. *Journal of Food Technology*, 16, 13-19.
- Osborne B.G., 2000. Near-Infrared spectroscopy in food analysis. In: *Encyclopedia of Analytical Chemistry* (edited by R.A. Meyers). Pp. 1-13. Chichester: John Wiley & Sons.
- Osborne B.G., 2006. Review: Applications of near infrared spectroscopy in quality screening of early-generation material in cereal breeding programmes. *Journal of Near Infrared Spectroscopy*, 93-101.
- Osborne B.G., Fearn T. and Hindle P.H., 1993. Fundamentals of near infrared instrumentation. In: *Practical NIR Spectroscopy with applications in food and beverage analysis* (edited by D. Browning). Pp. 49-77. Essex, UK: Longman Scientific and technical.
- Pasquini C., 2003. Near Infrared Spectroscopy: fundamentals, practical aspects and analytical applications. *Journal of the Brazilian Chemical Society*, 198-219.
- Plankinton, Ave. and Milwaukee, 2014. Malting barley quality requirements, American Malting Barley Association, Inc. vol. (414) 272-4640.
- Poehlman J.M., 1985. Adaptation and distribution. In: *Barley* (edited by D.C. Rasmusson). Pp. 1-17. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, Wisconsin.
- Pollock J.R.A., 1962. The analytical examination of barley and malt. In: *Barley and Malt: Biology, Biochemistry, Technology* (edited by A.H. Cook). Pp. 400-427. London: Academic Press, Inc.
- Pomeranz Y., 1988. Wheat: chemistry and technology American Association of Cereal Chemists, St. Paul, Minn., USA.
- R. M. Haslemore , C. R. Slack & K. N. Brodrick (1982) Assessment of malting quality of lines from a barley breeding program, *New Zealand Journal of Agricultural Research*, 25:4, 497-502.

- Ratcliffe M. and Panozzo, J.F., 1999. The application of near infrared spectroscopy to evaluate malting quality. *Journal of the Institute of Brewing*, 105, 85-88.
- Rinnan , A., van den Berg , F., and Balling Engelsen S., 2009. “Review of the most common preprocessing techniques for near-infrared spectra”, *Trends in Analytical Chemistry*, Vol. 28 (10), 1201-1222.
- Roumeliotis S., Logue S.J., Jefferies S.P. and Barr A.R., 2000. In: *Near Infrared Spectroscopy: Proceedings of the 9th International Conference*, NIR Publications, UK, p. 673-678.
- Rutger J.N., Schaller C.W. and Dickson A.D., 1967. Variation and covariation in agronomic and malting quality characters in barley. II. Interrelationships of characters. *Crop Science*, 7, 325-326.
- Savin R. and Molina-Cano J.L., 2002. Changes in malting quality and its determinants in response to abiotic stresses. In: *Barley Science: Recent advances from molecular biology to agronomy of yield and quality* (edited by G.A. Slafer, J.L. Molina-Cano, R. Savin, J.L. Araus & I. Romagosa). Pp. 523-549. Binghamton, New York: The Haworth Press.
- Shao, Y.; He, Y.; Bao, Y.; Mao, J., 2009. Near-infrared spectroscopy for classification of oranges and prediction of the sugar content. *Int. J. Food Prop.* **2009**, 12, 644–658.
- Siesler H.W., Ozaki Y., Kawata S. and Heise H.M., 2002. *Near-infrared spectroscopy*. Weinheim, Germany, WILEY-VCH Verlag GmbH.
- Sileoni V., van den Berg F., Marconi O., Perretti G., Fantozzi P., 2011. “Validation strategies for long term effects in NIR calibration models”, *Journal of Agricultural and Food Science*”, Vol. 59, pp. 1541–1547.
- Sissons M., Osborne B.G. and Sissons S. 2006. Application of near infrared reflectance spectroscopy to a durum wheat breeding programme. *Journal of Near Infrared Spectroscopy*, 17-25.
- Sohn, M., Himmelsbach, D.S., Barton, F.E., Griffey, C.A., Brooks, W. & Hicks, K.B., 2008. Nearinfrared analysis of whole kernel barley: Comparison of three spectrometers. *Applied Spectroscopy*, **62**, 427-432.
- Stewart, R.B. and Getachew, A., 1962. Investigations of the Nature of Injera. *Economic Botany*, 16:127-130.
- Swanston JS, Wilhelmson A, Ritala A, Gibson BR. 2014. Malting, brewing and distilling. In: Shewry PR, Ullrich SE, eds. *Barley: Chemistry and Technology*. St. Paul: AACC International, 193–222.
- The American Society for Testing and Materials (ASTM) Practice E1655-00. ASTM Annual Book of Standards, West Conshohocken, PA 19428-2959 USA, 2001, Vol. 03.06, pp. 573-600.
- The American Society for Testing and Materials (ASTM) Practice E1655-00. ASTM Annual Book of Standards, West Conshohocken, PA 19428-2959 USA, 2001, Vol. 03.06, pp. 573-600.
- Tragoonrung, T., Hayes, P.M. & Broich, L., 1990. Near-infrared reflectance estimates of grain protein and malt extract in hill and row plot evaluations of spring malting barley. *Canadian Journal of Plant Science*, **70**, 71-78.
- Tsuchikawa S., 2007. Sampling techniques. In: *Near-Infrared Spectroscopy in Food Science and Technology* (edited by Y. Ozaki, W.F. McClure & A.A. Christy). Pp. 133-162. Hoboken, New Jersey: John Wiley & Sons, Inc.
- Twidwell EK, Boe A and Casper DP., 2002. Tef: A New Annual Forage Grass for South Dakota, South Dakota State University. Brookings, SD. Coop. Ext. Serv. Bul. Ex 8071.

- Ullrich S.E., 2002. Genetics and breeding of barley quality attributes. In: *Barley Science: Recent advances from molecular biology to agronomy of yield and quality* (edited by G.A. Slafer, J.L. Molina-Cano, R. Savin, J.L. Araus & I. Romagosa). Pp. 115-142. Binghamton, New York: The Haworth Press.
- Vijaya Raghavan, G., S., 2003. "Commercial Grain Dryers" in Handbook of Postharvest Technology Cereals - Fruits, Vegetables, Tea, and Spices, Amalendu Chakraverty, Indian Institute of Technology Kharagpur, India, pp. 167-183.
- Visoottiviset, P., Francesconi, K. and Sridokchan, W., **2002**. The potential of Thai indigenous plant species for the phytoremediation of arsenic contaminated land. *Environ. Pollut.*, *118*, 453- 461.
- Wang W. and Paliwal J., 2007. Near-infrared spectroscopy and imaging in food quality and safety, *Sens. and Instrumen. Food Qual.*, *1*, 193–207.
- Westad F. & Martens H., 2000. Variable selection in near infrared spectroscopy based on significance testing in partial least squares regression. *Journal of Near Infrared Spectroscopy*, 117-124.
- Wetzel D.L., 1983. Near-infrared reflectance analysis: Sleeper among spectroscopic techniques. *Analytical Chemistry*, 1165A-1176A.
- Wetzel D.L., 2001. Contemporary near-infrared instrumentation. In: *Near-Infrared Technology in the Agricultural and Food Industries* (edited by P.C. Williams & K.H. Norris). St. Paul, Minnesota, USA: The American Association of Cereal Chemists, Inc.
- Williams P.C., 2001. Implementation of near-infrared technology. In: *Near-Infrared Technology in the Agricultural and Food Industries* (edited by P.C. Williams & K.H. Norris). Pp. 145-169. St. Paul, Minnesota, USA: St. Paul, Minnesota, USA.
- Williams P.C., 2007. Grains and seeds. In: *Near-infrared Spectroscopy in Food Science and Technology* (edited by Y. Ozaki, W.F. McClure & A.A. Christy). Pp. 165-202. John Wiley & Sons.
- Wold S., 1995. Chemometrics; what do we mean with it and what do we want from it. *Chemometrics and Intelligent Laboratory Systems*, *30*, 109-115.
- Wold S., Sjöström M. and Eriksson L., 2001. PLS-regression: a basic tool of chemometrics. *Chemometrics and Intelligent Laboratory Systems*, *58*, 109-130.
- Woodcock T., Downey G. and O'Donnell C.P., 2008. Better quality food and beverages: the role of near infrared spectroscopy. *Journal of Near Infrared Spectroscopy*, **16**, 1-29.

## 8. ANNEXES

### Annex I: Holeta and Berlin (VLB) Laboratory performance Comparison based on Results

Lab	N	Variable	mean	SD	SE	CV	F	P
Holeta Lab	60	Extract	78.12	2.97	0.38	3.8	0.03	0.87
	60	Protein	10.42	1.93	0.24	10.54	0.06	0.81
	60	Friability	60.17	19.91	1.57	13.09	0.00	0.95
	60	Moisture	8.67	0.41	0.05	4.75	0.07	0.79
Berlin (VLB)	60	Extract	78.03	2.93	0.38	3.76	0.03	0.87
	60	Protein	10.34	1.95	0.25	10.82	0.06	0.81
	60	Friability	60.36	19.93	1.57	13.00	0.01	0.96
	60	Moisture	8.69	0.36	0.04	4.17	0.07	0.79

*N=number of sample; SD=standard Deviation; SE=Standard Error; CV=Coefficient of Variation; F=F-Value; P=Probability*

### Annex II: Control Reference (MB3012) Used as Analysis Control in the Wet Chemistry

Trait	Actual Value	Recovery Value	SD	CV
Extract	81.67	82.01	0.24042	0.29
Protein	10.01	9.87	0.09899	0.99
Friability	73.4	72.98	0.29698	0.41

*SD=standard Deviation; CV=Coefficient of Variation;*