Addis Ababa University

College of Natural Science

Center for Food Science and Nutrition

Assessment of Malt Quality Attributes of Barley (*Hordeum Vulgarre L)* Genotypes grown in Bekoji, Holeta and Ankober, Ethiopia

By

Biadge Kefale Antenh

Advisors:

Dr Ashagrie Zewdu

Dr Berhane Lakew

A Thesis submitted to the school of Graduate studies of Addis Ababa University in partial

Fulfillment of the Requirement for the Degree of Master of Science in Food Science and Nutrition

June, 2016

Addis Ababa, Ethiopia

Assessment of Malt Quality Attributes of Barley (*Hordeum Vulgarre L)* Genotypes grown in Bekoji, Holeta and Ankober, Ethiopia

MSc.Thesis

By

Biadge Kefale Antenh

Advisors :Dr Ashagrie Zewdu

Dr Berhane Lakew

A Thesis submitted to the school of Graduate studies of Addis Ababa University in partial Fulfillment of the Requirement for the Degree of Master of Science in Food Science and Nutrition

June, 2016

Addis Ababa, Ethiopia

Acknowledgement

First and above all, I would like to unreservedly thank my advisors Dr.Ashagrie Zewdu, Department of Food Science and Nutrition. His guidance, advice, encouragement, support and research enjoyment had made a high contribution and Dr.Berhane Lakew Holeta Agricultural Research center (HARC), Barley Research Section, for his versatile help and his supervision ,encouragement from the beginning until thesis defense.

I would like to express my sincere gratitude to the experts in Asela Malt Factory quality control laboratory for their valuable assistance in malting process, Diastatic power and malt friability analysis.I never forget their kindness,respect,sincere approach to help and their hard worker character as well. Special thanks should also go to Mr. Eneyew Gebeyhu,Mr.Shemeles Debebe,Mr Biruk Wondemu and Mr.Yadesa Abushu for they informally help in grain and malt analysis.Dr.Berhane Lakew and Mr.Wondemu Fekadu also helped me in identifying the malt barley variety in Ethiopian institute of Agricultural Research,in Barley Research section. I also would like to thank Kulumsa Agricultural Research Center,Holeta Agricaltural Research Center and Debrebrhan Agricultural Research Center Barley Applied Research for providing all the necessary supports starting from sowing (seedling) to harvesting for their unreserved supports.

My parents are always behind my success and with no over statement are the best gifts for me from cheerful God. They helped me morally by encouraging me when I despair and they enable me accomplish my studies. I also thank you to all the Staffs and Graduate Students at the Food science and Nutrition Department particularly Biruk Wendemu for his versatile support in laboratory work procedures, books and journals, and my friend for your support and friendship. Especial thanks to Dr.Ashagrie Zewedu for his SPSS software application. Finally, I would like to thank EIAR for offering me the chance to pursue my second degree , supporting research budget and all my friend who in one or in another way helped or encouraged me.

Table of contents

Acknowledgement ii

Table of contents iii

List of Tables vi

List of Figures vii

List of Abbreviations viii

Abstract ix

1. Introduction 1

1.1.Back ground of the study 1

1.2 Statement of the problem 2

1.3 Significance of the Study 4

1.4. Objective 5

1.4.1. General objective 5

1.4.2. Specific objective 5

2. Literature Review 6

2.1. Malt and malting 6

2.2. Grain quality factors 11

2.2.1. Characteristics of malt Barely Varity 11

2.2.2. Grain size (sieve test) 12

2.2.3. Germination energy 13

2.2.4. Moisture content 13

2.2.5. Kernel plumpness and uniformity 13

2.2.6. Hectoliter weight (HLW) 14

2.2.7. Protein content 14

2.2.8. Amylose and Amylopectine 16

2.3. Malt quality factors 16

2.3.1. Malt extract 16

2.3.2. Friability 18

2.4.wort quality factors 19

2.4.1 Wort color 19

2.4.2. Diastatic power 19

2.4. 3. Free amino nitrogen (FAN) ... 20
2.4.4. Kolbach index (KI) ... 21
2.4.5 Calcium and zinc in wort ... 21
2.4.6. α-Amylase ... 22
2.4.7. β-Amylase ... 22
2.4.8. β-glucan ... 23
2.4.9. Wort viscosity ... 24
2.4.10. Fermentability ... 24
3. Materials and Methods ... 25
3.1. Description of study areas ... 25
3.2. Sample Collection ... 25
3.3 Sample preparation ... 26
3.4. Grain quality analysis of malt barley ... 27
3.4.1. Sieve test (Grain size) ... 27
3.4.2. Germination Energy (GE) ... 27
3.4.3. Moisture content ... 28
3.4.4.Thousand kernel weight ... 28
3.4.5. Hectoliter weight ... 28
3.4.6. Protein content ... 28
3.5. Malt quality analysis of barley ... 29
3.5.1. Micro malting ... 29
3.5.2. Extract determination ... 29
3.5.3. Malt moisture content ... 30
3.5.4. Protein content of the malt ... 31
3.5.5. Friability of malt ... 31
3.6 Wort quality analysis ... 32
3.6.1. Wort color ... 32
3.6.3. Soluble nitrogen in wort ... 32
3.6.4.Diastatic power (DP) of wort ... 33
3.6.5.Free Amino Nitrogen (FAN) of wort ... 33
3.6.6. Kolbach index (ratio S/T) ... 34

3.6.7. Calcium and zinc in wort 34
3.7. Data analysis 34
4. Result and Discussion 35
4. 1.Grain quality Analysis Results 35
4.1.1 Grain size (sieve size) 35
4.1.2 Germination energy (GE) 35
4.1.3 Moisture content 36
4.1.4 Thousand grain weight 38
4.1.5 Hectoliter weight 39
4.1.6 Protein content 39
4.2. Malt quality Analysis Results 41
4.2.1.Fine grind hot water extract (HWE) 41
4.2.2 Coarse grind hot water extract (CWE) 42
4.2.3 Extract difference (ED) 42
4.2.4. Malt moisture content 44
4.2.5. Malt protein content 44
4.2.6. Friability 45
4.3. Wort quality analysis result 48
4.3.1. Color of wort 48
4.3.2. PH of wort 49
4.3.3. Soluble protein content 49
4.3.5. Diastatic power 51
4.3.6 Free amino nitrogen 52
4.3.7. Kolbach index 52
4.3.8 Zinc content 54
4.3.9. Calcium content 55
5. Conclusion and Recommendation 56
5.1. Conclusion 57
5.2 Recommendation 57
6.Reference 58
7. Appendices 70

List of Tables

Table 3.1 varieties used in the study. 26

Tables4.1. Effect of variety on grain size, germination and moisture content of grain. 37

Tables 4.2. Effect of location on grain size, germination and moisture content of grain. 38

Tables 4.3. Effect of variety on Hectoliter weight, Thousand kernel weight and protein content of grain. 40

Tables 4.4. Effect of location on Hectoliter weight, Thousand kernel weight and protein content of grain. 41

Table 4.5 variety effect on malt quality of Fine grind malt extract (%),Caurse grind malt extract (%) and Extract diffrence (%). 43

Table 4.6 Location effect on malt quality of Fine grind malt extract (%),Caurse grind malt extract (%) and Extract diffrence (%). 44

Table 4.7 variety effect on malt quality of Malt Moisture content (%,Malt Protein Content (%) and Friability. 47

Table 4.8. Location effect on malt quality of Malt Moisture content (%,Malt Protein Content (%) and Friability. 48

Table 4.9 effect of variety on Color of Wort, PH of wort, soluble protein content of wort........... 50

Table 4.10. Effect of location on Color of Wort, PH of wort, Solubleprotein content of wort 51

Table 11. effect of location on Diastatic power , Freeamino nitrogen and Kolbach index........... 54

List of Figures

Figure 2.1.structure of barley 7

Figure 2.2. 6-row and 2-row barley.. 9

Figure 2.3. Flow chart for malting and brewing.. 11

Figure 3.1.Leafe, two row plump kernel and sixteen barley (Hordeum Vulgarre L) samples (photo taken during growing period to sample preparation) on 01/03-10/04/2008 E.C 25

figure 3.2 flow chart for sample preparation 27

Figure 3.3.Micro malt apparatus and malt (photo taken in the laboratory on 23/04/2008 E.C) ... 29

Figure3.4 congress mash for extract determination. 30

Figure 3.5 Photo taken in wort filtration, color and PH measurement…………………………...32

Figure 4.1.diastatic power……………………………………………………………………..53

Figure 4.2.kolbach index. 53

Figure 4.3.Free amino nitrogen in wort... 54

Figure 4.4.zinc content in wort... 55

Figure 4.5.calcium content in wort... 56

List of Abbreviations

AACC	American Association of Cereal Chemists
ASBC	American Society of Brewing Chemists
CRD	Complete Randomized Design
CSA	Central Statistical Authority
DP	Diastatic power
EBC	European Brewery Convention
HARC	Holeta Agricultural Research Center
HLW	Hectoliter weight
HWE	Hot water extract
IOB	Institute of Brewing
KARC	Kulumsa Agricultural Research Center
KI	Kolbach index
TKW	Thousand-kernel weight
TKWG	Thousand-kernel weight of grain

Abstract

Barley is a crop of ancient origin in Ethiopia which is an important food source and industrial crop for beer production. This research was conducted to study the malt quality attributes of advanced malt barley (Hordeum vulgare L) genotypes in the central high lands of Ethiopia. Sixteen malt Barely varieties (four released varieties which were in production such as Holker, Traveller, EH1847 and IBON174/03and twelve promising varieties) were analyzed for their grain, malt and wort quality parameters. Except germination energy, the analysis of variance reveled that grain, malt and wort quality parameters were not significantly different among the sixteen varieties. Grain, malt and wort quality parameters were significantly different between the three locations. The Grain quality parameters for sixteen Varieties such as sieve size, germination energy, moisture content, hectoliter weight ,thousand kernel weight and protein content were ranged from 78.83%-92.66%, 13-99.33%, 12-13%, 64.36-67.3 kg/hL, 44.55-51.6 g, 9.0-11.5%, respectively. The malting and wort quality parameters for sixteen Varieties ,fine grind extract, coarse grind extract, extract difference, color of wort, pH of wort, malt moisture content, protein content, soluble protein content, kolbach index, friability, diastatic power, free amino nitrogen, zinc content and calcium content were ranged from 67.18-72.91%,64.91-69.79 ,2.43-4.89%,2.66-4.83%,5.84-6.03%,9.94-11.59%,3.51-5.43,32.92-75.5,50.9-76.7,288.8-428wk,223.48-357.06mg/L,1.72-5.08mg/L,475.75-742.0mg/L, respectively. Varieties MB1, MB3, MB5, MB7, MB9, and MB4 gave better result in grain and malt quality trait compared to the standard check over the three locations Bekoji, Holeta and Ankober. The varieties were differently responded for grain, malt and wort quality parameters. Varieties over locations did not perform consistently for grain, malt and wort quality parameters. However, most of the varieties fulfilled the quality requirements and within the acceptable range of the European Brewery Convention (EBC) and Asela Malt factory standard.

Key words: *malt barley , malt quality, standard check*

1. Introduction

1.1.Back ground of the study

The ancestor of cultivated barely (*Hordeum vulgare L.)* is originated from its wild progenitor (*Hordeum spontaneous).* It is identical in most respects to present day cultivate barley and this species is still found in abundance in many parts of Asia and North Africa (Harlan,1978). Barley was first domesticated in the Fertile Crescent in the Near East, which is the present day Israel, northern Syria, southern Turkey, eastern Iraq and western Iran(Harlan *et al* ,1966). Barley (*Hordeum vulgare,* L.) is a highly adaptable cereal grain that is produced in climates ranging from sub Arctic to sub tropical area.). Historically, barley has been an important food source in many parts of the world, including the Middle East, North Africa, and northern and eastern Europe (mainly Iran, Morocco, Ethiopia, Finland, England, Germany, Denmark, Russia, and Poland), and in Asia (Japan, India, Tibet, and Korea) (Chatterjee and Abrol,1977). At present,only 2%of barley is used for human food (Baik and Ullrich , 2008).

Barley 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 (Berhane, 1991).In Ethiopia barley has a long history of cultivation in the highlands (Firdissa et *al*., 2010). The diversity of barley types found in Ethiopia is probably not exceeded in any other region of comparable size (Bekele., 1983).Barley grain is an excellent source of soluble and insoluble dietaryfiber (DF) and other bioactive constituents, such as vitamin E (including toco-tri-enols), B-complex vitamins, minerals, and phenolic compounds. β-Glucans, the major fiber constituents of barley, have been implicated in lowering plasma cholesterol, improving lipid metabolism, and reducing glycemic index.The effectiveness of barley β-glucans in food products for lowering blood cholesterol has been documented in a number of studies (Newman and others, 1989; Behall and others, 2004).

Barely in Ethiopia is mainly used for making local recipes and drinks in Ethiopia such as Bread, kolo, Genfo,Animal feed,Beso,Tela and Borde. Barley is a rich source of tocols, including tocophenols and toco tri enols,which are known to reduce serum low density lipoprotein cholesterol through their antioxidant action (Qureshi and others,1986).Whole grains are known

for their fiber content, and therefore lower energy density, and as a source of vitamins and mineral components, both of which may increase satiety and reduce energy intake (Slavin ,2003).The process of producing beer from barley involves a series of steps including the production of malt through controlled germination and kilning of the barley. During the brewing process, the malt is mashed and separated to produce wort, which is then boiled prior to fermentation. The brewer demands malt that processes without difficulty and ferments efficiently ensuring maximum beer production (Edney *et al.*, 2014).Barley has been malted, or germinated, prior to consumption for thousands of years. It has been documented that any barley having viable kernel will produce malt, but quality factors would be sacrificed in most cases.In malting operations strict criteria are observed in the selection of barley for malting among the major considerations paramount in the choice of barley for malting include genotypes, kernel size, color, brightness, a germinating capacity of greater than 96%, relatively low protein, less than 12.0 % (Newman *et al*.,2008).

The history of modern malting in Ethiopia was started in 1974 at St.Georege brewery.Asella malt factory was established in 1984 with the aim of supplying malt to local breweries. Malt is the major row material for beer production. Malt barely is a malting crop used to produce malt for breweries. Malt barely grain is mainly produced in the south eastern part of Ethiopia in Aresi and Bale administrative zone (Getachew *et al.*, 2007). Malt is the second largest use of Barely and at the present time it is considered as one of the cash crop in Ethiopia and its demand by malt factory is increased due to expansion of breweries and beer consumption levels in the country (AMF, 2012). As reported by Mohammed and Getachew (2003) and presented by Ethiopian Barely Business (2012) malt barely is among crops demanded in good quantity and quality. Similarly in 2011, Breweries in Ethiopia imported 60% of the malt primarily from international producers (Ethiopian Barely Business case presentation, 2012). The demand from agro industry supply of products in quality and quantity hinder the activity of the brewing industry and their growth.

1.2 Statement of the problem

The development of malting and brewing industry increased interest in high quality malting barley. However, barley grain yield and quality are subject to various factors varying on a large scale. The genotypic peculiarities of a variety and growing climatic conditions are the key factors

influencing grain yield and its quality (Tamm , 2003; Paynter and Young, 2004). The first step to success in the growing systems of malting barley is the choice of appropriate variety. The varietal effect, accounts for 25– 40% of malting performance compared with growing conditions (Tamm , 2003). Thus malt quality traits are influenced by variety and growing location (environmental factors) (Uhlen *et al.*, 1998). But the relative magnitude of variety and growing location effects on malting barley quality are a challenge that makes production of predictable quality difficult (Molina-Cano *et al.*, 1997).

Barley's complex genetics and chemical and physical characteristics and their interactions have contributed to its importance and attempts at improving its grain quality. The mature grain endosperm contains starch, non starch polysaccharides, protein and lipids. Protein and starch are the most important components of the grain and also contribute significantly to its nutritional, processing and commercial value (Peter *et al*., 2014).Protein in barley grain fulfils storage, structural, metabolic and protective functions, and it is often these functions that contribute to its commercial value. Protein generally constitutes 8–27% of the total dry grain weight, while carbohydrates on the other hand attribute approximately 80% of total dry grain weight.

These carbohydrates are divided into starch, sugars and non starch polysaccharides (β-glucan, cellulose and arabinoxylans). Starch, one of the most abundant biopolymers in nature and the main source of energy in human diets and feed, comprises up to 65% of the grain's dry weight. Non starch polysaccharides contribute 2–7% and the remaining 2–4% comprises lipids.Starch serves as a means of energy storage in plants and is produced by means of photosynthesis. It plays an essential role in germination and endosperm composition, which directly relates starch to barley grain quality (Fox *et al*.,2003).

Research on Barley improvement in Ethiopia started in 1955.The research system has produced knowledge, information and technology but due to limited experience and lack of laboratory equipment in small scale malting there was constraints for malt quality data. Therefore this study will assess the current methods and ways of improvement of barely with their quality attributes that are important to characterize barely grain according their physical and chemical characteristics for malting and technology needs.

Thus identifying malt barely advanced variety for verities with respect to quality preference for breeders, malt factory and breweries as well as small scale farmers for appropriate selection, effective quality control and their economic development respectively by growing appropriate and acceptable malt barley variety.

1.3 Significance of the Study

- ✓ As malt barley is used as row material for brewing in Ethiopia, the finding of this research will enable the selection of appropriate malt barley variety.
- ✓ The result for malt quality will help for breeder's final release; increase the economy for small scale farmers and will also used row material for brewing company.
- ✓ Determining and understanding the grain and malt quality of underutilized malt barley is vital to suggest ways and means to improve new malt barley variety.
- ✓ For Import substitution.
- ✓ The results will also be used by Agriculture researchers, university students, and other stakeholders for further studies about malt barley.

1.4. Objective

1.4.1. General objective

To investigate malt quality attributes of Barely genotype grown in Bekoji,Holeta and Ankober , Ethiopia.

1.4.2. Specific objective

- To investigate variability in grain and malt quality parameters among the sixteen malt barely varieties .
- To investigate variability in grain and malt quality parameters among the three locations Bekoji, Holeta and Ankober.
- To identify the appropriate varieties among the sixteen genotypes for malting process.

2. Literature Review

2.1. Malt and malting

Barley is the fourth largest cereal crop produced in the world (FAO, 2006), ranking behind maize, wheat and rice, respectively. From 1996 to 2005, world barley production averaged 143 million metric tonnes (FAO, 2006), while in Ethiopia, 1.4 million tonnes in the year of 2007/08 was produced (CSA, 2008). The versatile composition of barley makes it suitable for feed, malt and food. Worldwide, barley is predominantly utilized as feed (70%), with 20% use for malt, only 5% for food, and 5% undefined uses (Wang, 2005).

Malting is a controlled germination process consisting of steeping or hydration of grains. The 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).In malt of good quality, cell walls, a part of the small starch granules and the surrounding protein matrix are broken down uniformly throughout the endosperm (Palmer, 1993). This requires rapid distribution of water in the endosperm during hydration as well as fast and homogeneous endosperm modification (Davies 1989; Brennan *et al.,* 1997).

Modification or degradation of endosperm reserves for the needs of the growing seedling involves the coincident action of the enzymes hydrolyzing protein, starch, and cell wall structures. Enzymes are synthesized or activated in the aleurone and scutellar cells by the action of embryonic gibberellins activated signal transduction pathways and are secreted into the starchy endosperm (Cohen and Paleg 1967; Jones and Jacobsen, 1991). Besides enzyme activities, degradation of endosperm reserves, and also hydration, are controlled by the structural pattern at the tissue and sub cellular level (Brennan *et al.* 1996; Chandra *et al.* 1999).

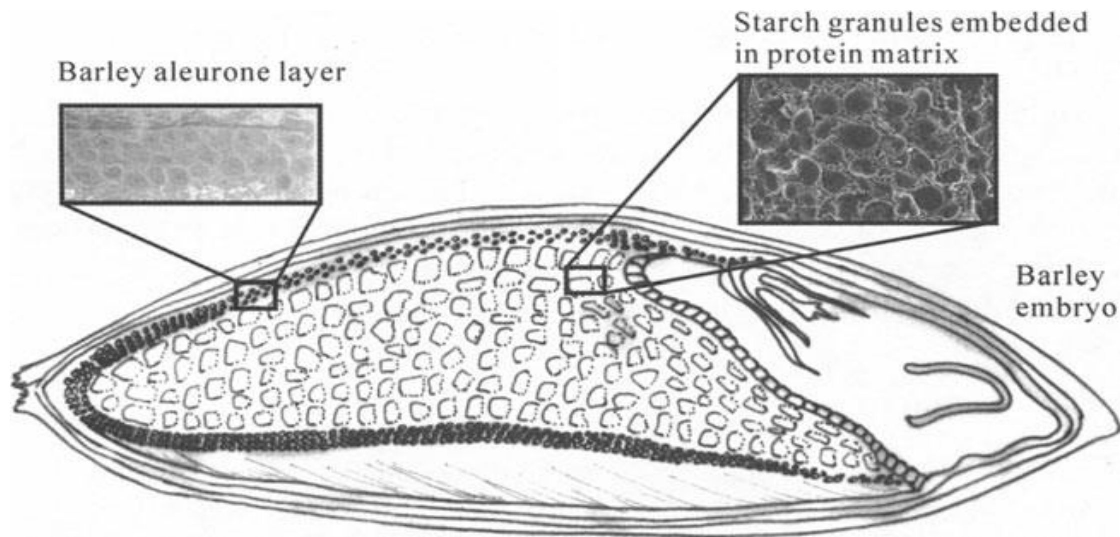


Figure 2.1.structure of barley

Thus both an endosperm structure that is easily hydrated and modified and a good enzyme synthesizing capacity for ensuring fast modification are favorable features of a malting barley cultivar. However, modification should not be too extensive, because this causes too brittle malt which cannot be optimally milled for mashing. Over modification leads also to the breakdown of starch granules and losses in fermentable yield. Overall, the nature of malting quality can be considered complex as it is dependent not only on the grain structure and composition, but also on its germination physiology.

.Malting is a biological process that turns barley into malt. It is a three stage process, including steeping, germination and kilning. During steeping (soaking) the moisture content of the barley is increased to prepare the kernels for germination. Germination (growing) is carefully controlled with temperature, moisture and time to allow the kernels to progress to the point where the enzymes necessary for brewing are generated but the growth of roots and shoots is limited. Kilning (drying) reduces the moisture content of the kernels, stops the biochemical processes within the kernels, carefully preserves the enzymes generated during germination, and generates color and flavor compounds(Plankinton,2014).

Barley grain that is clean, bright yellow white, plump, thin hulled, medium hard, and uniform in size is generally suitable for malt uses and preferred for pearling (Pomeranz,1974). Grain hardness is an important characteristic of barley because it determines subsequent end use

quality of barley. Malting barley varieties are usually soft, whereas non malting varieties are usually hard. Psota and others (2007) also reported significant relationships between hardness of barley grain as assessed using the particle size index and hot water extract of malt as well as the malt quality index of barley malt. Other structural and compositional characteristics of barley endosperm could contribute to grain hardness, including proteins, starch, β-glucan, and their interactions, and packing during grain filling (Henry,1988).

Discolored barley grain often develops undesirable flavors when malted and has poor germination energy and vigor (Li and others, 2003). The grain color of barley can vary from light yellow to purple, violet, blue, and black, which is mainly caused by the level of anthocyanins in the hull, pericarp, and/or aleurone layer. Highly colored types are also receiving attention for applications in functional foods due to their antioxidant properties (Satue-Gracia and others 1997; Nam and others 2006; Philpott and others 2006). However, most of the barley that is produced possesses bright, light yellow grain color, which is generally preferred for malting, brewing, and food purposes. A large number of parameters have been proposed to define malting quality. It is also a fact that the texture of the endosperm influences the malt modification process by affecting water uptake and consequently enzyme synthesis and movement within the endosperm (Chandra and others, 1999). Sulfur deficiency has been shown to affect the composition of proteins in barley grain, with depletion in the S-rich B hordein and the high molecular weight (HMW)Dhordein and an increase in the S poor C hordein (Shewry, 1993).

The malting of hullless barley, however, presents a number of challenges due to differences in chemical and physical characteristics. Malting barley is barley that will produce high quality malt. It is a specialty crop for which a premium price is paid by domestic maltsters and exporters (Haslemore *et al*., 2012). Quality requirements for malting barley are directly related to processing efficiency and product quality in the malting and brewing industries. Others are determined by weather conditions during the growing and harvesting season. High quality malting barley should have the following characteristics Pure lot of an acceptable variety, Germination of 95% or higher (3 day test), Protein content of 11% to 12.5% (dry basis), Moisture content of 13.5%(malt moisture 4-5%) maximum, plump kernels of uniform size. Each

of these factors has an impact on malting and brewing processes and on the quality of the end product beer (Michael, 2014).

Barley is the primary cereal used in the production of malt in the world. Two types of barley are frequently used for the malting process: 6- and 2-row. Two-row barley produces malt with a large extract, lighter color, and less enzyme content than the 6- row type (Broderick, 1977). From the different quality parameters reported in the literature, hotwater extract (HWE), kernel size fractions, kernel weight, β-glucan and protein contents, malting losses, friability, α-amylase activity, viscosity, and soluble nitrogen ratio (SNR) are common assays used to test the quality of barley mutant (Fox and others, 2003). In addition, fast hydration and germination are necessary traits of barley for good malting quality (Ulonska and Baumer, 1976; Briggs, 1998). During malting, barley undergoes an incomplete natural germination process that involves a series of enzyme degradations of barley kernel endosperm.As a result of this enzyme degradation, endosperm cell walls are degraded, and starch granules are released from the matrix of the endosperm in which they are embedded. These structural changes and biochemical degradations of the endosperm components are referred to as endosperm modification (Gunkel and others, 2002). Malting is defined as the controlled germination of cereals, to ensure a given physical and biochemical change within the grain, which is then stabilized by grain drying. Three process steps are necessary to ensure that these changes occur: (1) steeping, to ensure good absorption of water by the grain (from 12% to at least 40% of moisture); (2) germination,to maintain embryo growth, enzyme synthesis and a limited endosperm breakdown; and (3) kilning, to ensure product stability.

Figure 2.2. 6-row and 2-row barley

Different kernel properties have been identified as factors affecting water uptake during steeping of barley, for example, endosperm structure, starch content, protein content, and cell wall properties (Ogushi and others, 2002). Loosely packed endosperm gives soft (mealy) structure and facilitates better moisture and enzyme movements in the endosperm. Thus, a mealy endosperm is more easily degraded by hydrolytic enzymes during malting (Swanston and others, 1995). On the other hand starch granule size and distribution, β-glucan and arabinoxylan content have also been proposed as factors in affecting the hardness of the endosperm (Dombrink and Knutson1997; Tohno Oka and others, 2004). As a result of the malting process, there is an increase in enzyme activity, soluble protein, and breakdown of starch into simple sugars, along with development of the typical color and flavor (Hoseney, 1994).

Mashing is a key step in the beer production process. During mashing, enzymatic degradation of the polysaccharides present in the malt takes place. Fermentable carbohydrates are produced from the degradation of the polysaccharide starch. Such carbohydrates are converted into alcohol in the fermentation step of the beer manufacturing. Nonstarch polysaccharides also degrade during mashing into smaller chain carbohydrates. Different enzymes catalyze all the involved reactions. Because the activity of the different enzymes is highly dependent on temperature, the manipulation of such variable is the main control mechanism for the mashing process (Hardwick, 1995). Malt is one of the key ingredients in brewing another biological process providing starch and the enzymes necessary to produce the fermentable sugars, which yeast then turn into alcohol, as well as other yeast nutrients (Hanning *et al*.,2009). Malt also provides color and flavor compounds which contribute to the final character of a beer. Extract refers to all these compounds that are solubilized from malt during the first stage of brewing, with starch being the main component (Molina *et al*., 1999).

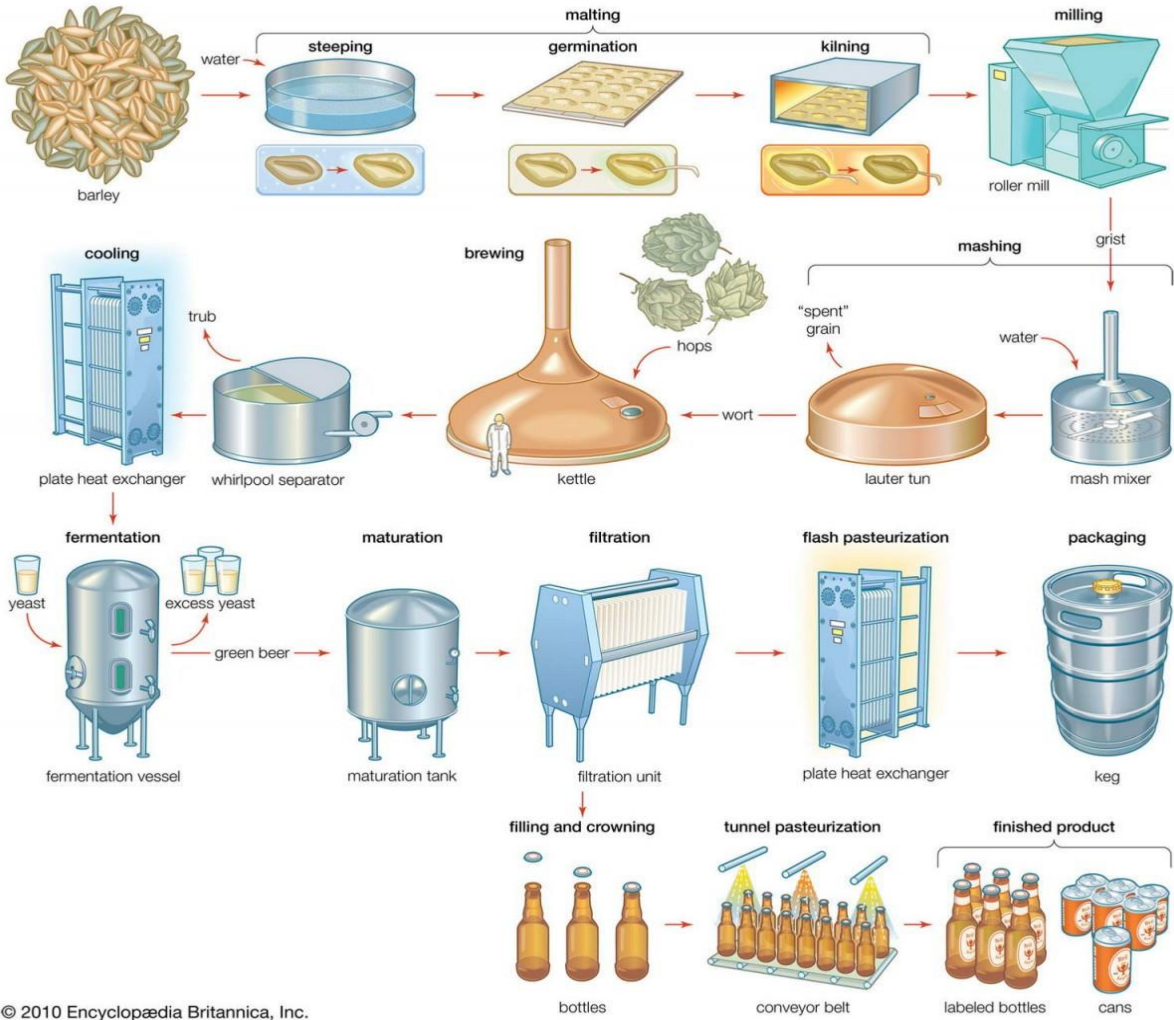


Figure 2.3. Flow chart for malting and brewing

2.2. Grain quality factors

2.2.1. Characteristics of malt Barely Varity

Malting barley varieties possess high levels of several enzyme systems that are necessary to bring about the desired chemical changes within the kernel during malting. They also have other characteristics necessary for the efficient production of quality malt, and high quality beer. Most varieties of feed barley lack one or more of the necessary enzyme systems or have other features that make them unsuitable for malting purposes. Different malting barley varieties behave differently during malting. This is because it is necessary to keep varieties separate as they are grown, harvested, stored, shipped, and processed. Each variety will produce malt with a particular quality profile. Brewers specify which variety or varieties they want in the malt they

use in order to get the quality characteristics they need to make their particular types of beer. The use of certified seed helps ensures varietal purity, as well as overall quality of the seed, and its use is strongly encouraged (Plankinton *et al*., 2014).

2.2.2. Grain size (sieve test)

In Australia, the measurement of grain size is generally based on 4 fractions: <2.2 mm screenings), >2.2 mm, >2.5 mm, and >2.8 mm. (Fox *et al*.,2006) demonstrated the genetic and environmental effects in improving grain size. Industry standards on large grain are based on the total percentage of grain > 2.5mm (IOB, 1997).Increase grain size also showed to provide an increase in the important malt quality trait of diastatic power (Agu *et al*., 2007). Grain size is an important descriptive trait based on the physiology of the grain. The final grain size is determined by environmental effects, which affect the biochemical components within the grain itself (Coventry *et al.,* 2003b).For thousands of years, when grain was used specifically for human consumption the grain was selected based on size. Improvement in grain size and weight has been shown to have occurred through human selection rather than through evolutionary events (Ferrio *et al.,* 2006).

However, there were no corresponding protein determinations to ascertain if the increase in Diastatic Power was related to protein content. (Emebiri *et al.,*2007) reported a strong correlation between increased grain size and yield when using genotypes with low protein content alleles. (Passarella *et al.,*2005) demonstrated the impacts of temperature during grain fill on the level of screenings with high temperature reducing the overall size. (Fox *et al.,* 2006) also showed that from breeding trials, where sites suffered from terminal moisture and/or heat stress, grain size was significantly reduced. The negative impacts of heat and drought stress on grain size and weight have also been shown in Australian barleys under controlled experiments (Savin and Nichols, 1996). The negative effects on heat during grain filling on grain quality and starch synthesis was reported by (Wallwork *et al.* 1998). Industry standards on large grain are based on the amount of grain > 2.5mm. A smaller grain generally has lower starch and higher protein levels, thus reducing the extract or has feed potential. Large grains conversely have increased levels of starch and therefore more potential extract. However, excessively large grain could

impact on malt quality particularly on the rate of water hydration and modification during steeping.

2.2.3. Germination energy

The Germination energy is the total number of grains that germinate over 72 h of incubation under specified conditions (Woonton *et al.,* 2005).Germination is absolutely critical to the malting process. If barley cannot germinate, it cannot be processed into malt. It's that simple. And beer can't be made out of raw barley. A minimum of 95% germination on a 3day germination test is an absolute requirement. Any factor which interferes with the uniformity of germination or reduces the vigour of kernel growth during processing will reduce the quality of malts produced (Michael, 2014).

2.2.4. Moisture content

Grain barley for malting over 13.5% moisture does not store well. 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 germinative capacity may significantly deteriorate (Plankinton *et al.,* 2014).The grower should be sure that the harvested barley is below 13.5% moisture when stored. Because malting barley is a high quality product, storage should be in bins that are dry, clean, and high enough that ground water cannot get in.Walls and floors should be thoroughly swept and measures should be taken to prevent infestation and to make it rodent proof. Ideal storage conditions can be maintained by means of natural aeration or frequent turning of the grain in the bin. If the moisture content of harvested malting barley is above the safe level of 13.5%, it can be dried on farm, but this must be done very carefully. The malt moisture content for long shelf stable storage is recommended 4 to 5% (AOAC, 1990).

2.2.5. Kernel plumpness and uniformity

Maltsters are much more interested in plumpness than in test weight. A plump kernel contains more starch and gives a higher percent of extract which in turn produces a greater amount of beer from a given weight of malt. Extract yield is as important to a brewer as barley yield is to a

producer. Uniformity of kernel size assists in obtaining uniform germination and, therefore, higher quality malt. Data on the homogeneity of barley kernel size, as determined with the Single Kernel Characterization System, have shown that homogeneity is related to malt quality (Edney *et al*., 2012).The hull plays an important role before and after harvest. During the later stages of grain ripening, the hull has been considered to have a role in grain dormancy and therefore pre harvest sprouting resistance (BenechArnold *et al.,* 1999).

During harvest, the husk acts to protect the germ during the abrasive threshing process in the harvester. Post harvest, the husk plays a role in processing for the malting, brewing and feed industries. In terms of the malting industry, the husk aids during the malting process by protecting the germ from physical abrasion during handling and preventing the growing acrospires from being damaged during germination and kilning. In the brewing process, the husk aids during filtration of the brewers extract from the lautering process. Huskless barley can filters and reduce filtration rate, thereby adding to the cost of production and potentially impacting on the quality of beer (Edney *et al.,* 1998; Evans *et al.,* 1999b). However, Edney and Langrell (2004) recently demonstrated that good quality malt could be produced from hulless barley if the appropriate malting conditions were used.

2.2.6. Hectoliter weight (HLW)

Test weight (TW) (bulk density or HLW) is an industry standard for classifying malt and feed barley. Barley with plumper grains and a higher test weight should have a greater percentage of starch or energy in the grain and should be lower in fiber (Shewry and Morell, 2001). HLW has been shown to be an influence of the growing environment (Molina-Cano *et al*., 1997), crop management, plant diseases such as *Fusarium* Head Blight (Fox *et al*., 2007), pests such as Russian Wheat Aphid and genetic effects(Bregitzer *et al*., 2003).

2.2.7. Protein content

Proteins are among barley components that are essential for the quality of malt and beer. First, high protein contents decrease available carbohydrates, with a negative influence on the brewing process (Peltonen and others 1994; Fox and others 2002) and second, proteolysis (protease hydrolysis producing amino acids and peptides from hordeins) during malting and mashing is

necessary for yeast metabolism (Moll, 1979). Finally, soluble proteins are important in beer head retention and stability. Malting barley with high protein content results in lower extracts for the brewer. It also slows down water uptake during steeping, potentially affecting final malt quality. A very low protein level, the other hand, results in a lack of enzymes necessary to modify the barley kernel and to break down the starch during brewing. Each brewer defines the malt protein level that's best for them based on their process, their yeast and the type of beer they are making. Protein relevance in grain quality is well known, with composition suggested as a contributing factor to malt quality (Fox, 2010).

Generally, barley protein within the range of 11 – 12.5% can be used by maltsters to meet many brewers' needs. There are limited requirements for malting barleys with protein levels outside of this range. Blending barleys with different protein levels to achieve an acceptable average will also cause real problems during malting because of the effect on water uptake and therefore the uniformity of conversion into malt. Final malt quality will be compromised since the conditions will be set for a protein level that doesn't represent either batch of barley used to achieve an average reading. Protein level is determined primarily by growing conditions. Early planting and high yields usually results in lower percentage protein. Excessive rates of nitrogen fertilizer will increase protein levels, but the application of nitrogen, on the basis of soil tests, to obtain optimum yields will normally have only a minor effect on the protein content of the grain. Good production practices that increase yield will generally tend to reduce protein levels (Wang *et al.*, 2007).Moderate amount of protein is needed for good yeast nutrition, the development of desired enzyme levels, foam stability, and other end product characters. High protein in barley slows water uptake during malting and lowers the ability of the kernel to modify completely. If the protein is too high the amount of extract available to convert to beer will be reduced and beer hazes could form (Plankinton *et al.,* 2014).Several aspects of beer quality are dependent on protein modification such as yeast nutrition, beer foam retention and beer hazes. Soluble protein and Kolbach index had been thought sufficient for monitoring protein modification (Juskiw *et al.*, 2009), with lower grain protein and potential for higher malt extract but with enzyme levels considered low by Canadian standards. Hulless barley cultivars also completed evaluation successfully despite early concerns with their malt quality (Evans *et al.,* 1999).

2.2.8. Amylose and Amylopectine

Both amylose and amylopectin polymers are present in the barley endosperm starch granules (Evers *et al.*, 1999). Starch is a major nutritional component in plant grains consisting primarily of amylose and amylopectin. Amylose is a linear polymer of α-(1→4) linked D-glucose units with few side chains. In contrast, amylopectin has many α-(1→6) linked glucose side chains attached to the main α-(1→4) polymer (Hu *et al.,* 2010). In waxy barley, the amount of amylopectin increases to >90% (Evers *et al.*, 1999). However, waxy barleys generally have lower hot water extract values, and higher β-glucan content and cell wall modification levels (Swanston 1996). Regular grains contain about 20% amylose while waxy grains contain a much lower percentage (Hu *et al.*, 2010). The ratio of amylopectin to amylose is around 3:1 (Palmer, 1983).

Limited starch breakdown occurs during malting, although (Allosio-Ouarnier *et al.,* 2000) reported increased levels of maltose, maltotriose, and maltotetraose during germination. Starch was degraded more in the mashing process by the hydrolytic enzymes a -amylase, ,B-amylase, ,B-glucosidase, and limit dextrinase. High temperature infusion mashes readily solubilise the starch but limit the activity of thermolabile enzymes, in particular a –glucosidase and ,B-amylase (Osman *et al.,* 1996a).

2.3. Malt quality factors

2.3.1. Malt extract

From the brewers point of view, the most essential quality parameter of malt is extract yield. For its measurement, malt is ground and mashed or extracted, usually in a temperature profiled mashing procedure ending at +70°C (Schwartz and Li, 2011). Determined by the specific gravity of the wort produced in mashing, the extract yield reflects the extent of enzymatic degradation and the solubility of grain components after malting and mashing (Swanston *et al.*, 2014). However, because only fermentable sugars are converted to alcohol by yeast in fermentation, the fermentability of the wort is often measured as attenuation limit indicating the proportion of original extract that can be utilized during fermentation (Schwartz and Li, 2011).

Dry basis extract values are a measure of the maximum potential yield of extractable material under ideal mashing conditions. After extraction wort protein had been established and the ratio of soluble protein to total protein, referred to internationally as Kolbach index was used as an indication of protein modification. The method was much more objective than the visual measurement of malt mellowness (mealy versus steely) that had been used to indicate modified starch (Harrison, 1929). The mathematical difference in malt extract values obtained from fine ground versus coarse ground malts (fine/coarse difference) was the first measurement of cell wall breakdown, especially when a 708 ^{0}C isothermal mash was used for the coarse grist (Bendelow ,1977).

Most aspects of grain modification affect final beer quality, including important aspects such as clarity and foam stability. During malting, enzymes which have an impact on the degradation of substrates, are either synthesized or cleaved from their bound forms. The range of enzymes produced includes those that degrade cell wall components, proteins and starch. The process of malt production varies between countries with four day germination schedules in Australia and five to six day germination schedules in most overseas countries. The objective for most maltsters is to maintain high extract levels and yet somehow achieve relatively low protein modification levels 50%.

Mashing is the fourth factor that influences extract. Within the mashing process, there are a number of physical factors that affect the resultant extract. These are pH, mash time, mash temperature, grist/ part icle size and grist to liquor ratio. presented reviews detailing previous and current knowledge and technology available on mashing. While all of the above aspects of mashing would influence the quality of the final extract , most result from the genetic attributes of the starting barley. For example, high DP varieties produce high levels of malt DP under optimal conditions. This in turn impacts on starch hydrolysis and the final fermentable sugar profile. Low DP barley varieties only produce low to moderate levels of starch degrading enzymes which affect the fermentable sugars profile. A number of studies have presented results detailing the relationship between grain and malt physical attributes and extract. Some of these attributes include protein levels and the type of protein fractions , hardness and milling energy (Ellis *et al.,* 1979; Alison, 1986; Swanston and Taylor, 1988),starch properties (Glennie-Holmes,

1995a,b,c,d) , non-starch polysaccharides (Henry, 1985; Molina Cano *et al.,* 1995) and husk thickness (Roumeliotis *et al.,* 2000).

2.3.2. Friability

Refers to the softness of the kernel and is determined by the amount of malt that passes through a rotating screen. Friability is a measure of the breakdown of malt endosperm cell wall components. Malt friability should be >60% (Anonymous, 2012). When barley endosperm is properly modified during malting, the resulting malt is soft and friable. Factors that interfere with endosperm modification, such as poor germination, large kernels and high protein, are expected to reduce malt friability (Edney and Mather, 2004).

The friability or tendency of the endosperm to break into flour in a specified milling process (Chapon *et al.,*1979). This measurement is based on the more brittle structure of enzymatically hydrolysed endosperm compared to native barley endosperm. An increase in friability reflects thus a more extensive modification of the endosperm during malting, mostly with respect to the degradation of the protein matrix and cell walls (Chapon *et al*. 1979; Darlington and Palmer ,1996). The friability measurement enables also the assessment of the homogeneity of modification as the proportion of undermodified endosperm material remaining unmilled.

While extract yield and friability measure the effect of modification in general,several analysis methods exist for measuring either the hydrolysis of cell walls or protein. Cell wall degradation in the starchy endosperm can be assessed by visualizing the main cell wall component, B-glucan, with fluorescent Calcofluor dye (Aastrup *et al*., 1981). The same dye can also be utilized in the analysis of B-glucan content of wort (Jorgensen *et al*., 1985).

2.4.wort quality factors
2.4.1 Wort color

Malt color attribute affect a finished beer color. Malt is normally the largest source of beer color. Wort color is another quality parameter measured from mashed wort. Color variation in wort is due to non-enzymatic browning reactions, the Maillard reaction, that take place during kilning in the malting process, and wort boiling in the brewing process. A non-enzymatic browning reaction is a chemical process that produces a brown color in foods. Browning is caused by the formation of unsaturated, colored polymers with different composition. In this case, the sugars interact with the amino acids, producing a variety of odors and flavors. This reaction is called Maillard reaction and is the basis of the flavoring industry with the type of amino acid involved determining the resulting flavor (Guerrero, 2009).

2.4.2. Diastatic power

Diastatic power is the total activity of starch degrading enzymes in barley malt is considered to be an important quality characteristic for malting and brewing (Hayter and Allison, 1995). The conversion of barley into beer represents mankind's oldest and most complex example of applied enzymology. Indeed, historically some of the most significant advances in enzymology have been linked to the world of brewing, such as Eduard Buchner's extraction of enzymes from brewing yeast (Buchner, 1897) and Adrian Brown's kinetic analysis of invertase (Brown, 1902).In determining the factors that a bearing on the quality of beer, brewers have learned not only how the endogenous enzymes contribute to issues such as ferment ability, filterability, foam, clarity, flavor, so on, but also how to take advantage of exogenous enzymes. There are 3 primary enzyme reactor stages in the conversion of barley to beer (Bamforth, 2006).

Barley kernel, mash tun, and the yeast cell. Only in one of these the mash tun is considered a typical enzyme reactor and has been extensively researched (Boulton and Quain, 2001). More than 40 endopeptidases have been identified in malt, broadly classified into cysteine-, metallo-, aspartic-, and serineproteinases(Jones,2005). There are also exo-peptidases classifiable into carboxypeptidases and amino peptidases (Sopanen and Mikola ,1975). A substantive reason for the limited action of the endo-peptidases in mashing is the presence of inhibitor proteins Principal among such inhibitors are lipid transfer proteins that block the cysteineproteinases

(Jones, 2005). Jones and Budde (2005) suggest that 32% of the soluble protein in malt is already in the ungerminated barley form, 46% is released in malting and the rest solubilized in mashing.

It was shown that over the pH range 5 to 6.6, the proteolytic activity of malt can vary (Jones and Budde 2003). Various factors may come together in causing the release of the enzyme in an active form during malting (Buttimer andBriggs, 2000). It was recently suggested that serine-proteinases have a key role to play here (Schmitt and Marinac, 2008). The least investigated of the endogenous starch degrading enzymes in malted barley is α-glucosidase, although it has been claimed to be 2nd only to α-amylase for its importance in starch degradation during malting (Sun and Henson, 1991). However, the enzyme is thermolabile and likely to be of limited significance during mashing (Muslin and others, 2000). Low calorie beers, so called Light, are the biggest selling style of beers and the exaggerated scares about bad carbs in beer (Bamforth, 2005) led to the advent of low carb beers. For such products, glucoamylase and pullulanase are of great utility in dealing with the dextrins surviving because of the limited action of limit dextrinase (Goode and others, 2005).

Diastatic power, like other quality attributes in barley, has been reported to be determined by a complex interaction of genetic and environmental factors. The desirable range for diastatic power is 90-120°L (Arends *et al*., 1995). Methods for assaying individual starch degrading enzymes, particularly limit dextrinase, are slowly becoming accepted by the malting and brewing industries (Evans *et al*. 2009).

2.4. 3. Free amino nitrogen (FAN)

FAN that can indicate the amount of free amino groups available to yeast nutrition during fermentation. This quality parameter is considered to be a good index for potential yeast growth and fermentation. Protein modification also involves the production of wort amino acids and small peptides (dipeptides and tripeptides), collectively known as free amino nitrogen (FAN.Adequate levels of FAN in wort ensure efficient yeast cell growth and, hence, a desirable fermentation performance.

Enari in 1975, concluded that barley variety, nitrogen content and the malting technique all influence the FAN level of the wort Generally the specifications for a normal fermentation

require FAN levels between 140-160 mg/L. The FAN level of the wort is largely dependent on the malt or the grist used, whilst the mashing program has only a limited influence. The increase in free amino acids in mashing is not more than 50% and is not really significantly influenced by changing the mashing program (Edney and Langrell, 2004; Lekkas *et al*., 2005).

2.4.4. Kolbach index (KI)

The Kolbach Index (KI) are used often in malting as guidelines to determine the extent of modification that has occurred during the malting process. It is a ratio of soluble to total nitrogen. A higher soluble nitrogen or S/T ratio indicates more extensive breakdown of the protein by proteolytic enzymes. The level of KI has also been associated foam properties (Okada *et al*., 2008). The range in KI is around 39-45% although some brewers could request KI levels outside that range. It has been shown to be linked to variety as well as being influenced by growing environment (Wang *et al*., 2007).

2.4.5 Calcium and zinc in wort

Calcium ions play a critical role in the brewing process, as calcium has been shown to increase total soluble nitrogen and free amino nitrogen levels in wort, improve wort runoff, improve wort clarification and protein coagulation, and has also been shown to increase yeast flocculation and growth. Calcium can here provide a cheap, effective and environmental friendly way to remove most of the yeast cells out of the green beer. It has been suggested that Ca^{2+} binds to flocculin proteins and provides them with the correct structural confirmation to form carbohydrate bindings (Vidgren, 2011).

Zinc ions are essential for an effective and vigorous fermentation. The presence of zinc is essential for the structure and function of many enzymes, where it can be involved in the active site (zinc-metalloenzymes). However, zinc can inhibit yeast growth and fermentation at higher concentrations under certain circumstances. Zinc additions during fermentation have also shown to increase the levels of higher alcohols and esters but to reduce acetaldehyde levels. Volatile organic compound levels were higher, this may however also cause an increase of medium fatty acids responsible for undesired soapy, fatty and rancid tastes (Nicola, 2009).Therefore, it is

essential to determine both zinc and calcium concentrations in wort samples to maintain consistency and efficiency within the brewing process (ASBC, 1981).

2.4.6. α-Amylase

After the first day of germination, the aleurone begins to produce α-amylase. The levels of α-amylase formation are highly dependent upon gibberellic acid and in its presence; the enzyme continues to be secreted by the aleurone (MacGregor , 1987). It is important in the metabolism of maltose and maltodextrins. Cereal α-amylases play a very important role in the starch metabolism in developing as well as germinating cereals (Yaldagard, 2008). α-Amylase is an endo-acting enzyme that attacks the starch polysaccharide chain internally to produce various malto oligosaccharides or limit dextrins and some maltose (Burger and LaBerge, 1985).

During mashing the enzyme was shown to be highly active, albeit a number of variables influenced the total activity. The optimal temperature for a-amylase II is around65°C(Briggs *et al.,* 1981) which would allow the enzyme to perform efficiently under most mashing conditions. However, consideration has been given to improving its thermostability in barley by introduction of an alien genetic form of the enzyme from bacteria (Vickers *et al.,* 1996). The preliminary results suggested that mashing could be carried out at temperatures as high as 75°C. In a 65°C infusion mash, the activity of the bacterial enzyme was found to be 1.5 times that of the barley enzyme (Vickers *et al.,* 1996*).*

2.4.7. β-Amylase

B-Amylase is a starch degrading enzyme that hydrolytically cleaves α-1,4-D-glucosidic bonds to liberate β-maltose from the non reducing ends of starch and maltodextrins.Barley β-amylase is synthesized during grain development and stored in mature grains. β-Amylase enzymes occur as a heterogeneous group in both barley grain (Gibson and Solah, 1995) and malts (LaBergc and Marchylo, 1983). β-Amylase activity is highly correlated with DP (Vinje *et al.*, 2010) but is more thermolabile than other diastatic enzymes and therefore more prone to loss of activity during malting (Polakova et al., 2003).

The thermostability of , β -amylase decreases rapidly at temperatures above 55°C (Hoseney, 1986).For mashes (a low initial mash-in temperature followed by rapid heating) , β-amylase remains active until the mash temperature exceeds 55°C. In comparison, for a mashing style where the infusion temperature is greater than 65°C, the activity of , β -amylase is reduced to a few minutes. Fox has shown that in high temperature mashes, with a low grist liquor ratio, the maltose level in the final wort is higher. This suggests substrate protection of the enzyme within a thick mash. An increase in the thermostability of , β -amylase would provide brewers with opportunities to increase efficiencies in wort production. This enzyme has been shown to remain active at temperatures greater than 60°C under simulated commercial mashing conditions.

2.4.8. β-glucan

In the brewing industry,a high content of β-glucan in barley may lead to insufficient degradation of cell walls, which in turn hinders the diffusion of enzymes, germination, and the mobilization of kernel reserves,and hence reduces malt extract. Residual β-glucan may also lead to highly viscous wort, giving rise to a filtration problem in the brewery, and it may participate in maturing of beer, causing chill haze (Bamforth, 1982). The degradation of endosperm cell walls and subsequent changes in β-glucan levels during malting are, to a great extent, related to β-glucanase activity, which depolymerizes β-glucan (Etokakpan ,1993). Therefore, better malting performance is expected to be associated with lower levels of β-glucan in grains and higher levels of β-glucanase in malt. Historically,reduced beer filtration efficiency has been mainly attributed to β-glucan in the brewing process. β-Glucan may increase the viscosity of beer by forming gels (Home and others, 1999).

During germination or malting, the modification of the endosperm relates to the breakdown of the cell wall and endosperm components. Although endosperm modification is measured through the solubilisation of endosperm protein reserves, access to those reserves is only possible after cell wall breakdown.The results indicated that by day 4 of germination, barley ,B-glucan had decreased from 5% to around 1%. Similarly, Allosio -Ouarnier reported an increased level of sugars derived from ,B-glucan and arabinoxylan components during malting. Although these studies analysed the complete malting process, (Walker *et at.,* 2001) suggested that by day 2 of germination, the ,B-glucan level could be used to indicate if a cultivar had desirable extract

potential. (Stewart *et al.*, 2000) demonstrated that both ,B-glucan and pentosan impact on wort viscosity and beer filtration rates . Thus, the enzymic breakdown of ,B-glucan and pentosan during malting is critical for efficient brewing. Mashing temperature has an influence on the solubility of ,B-glucan. Palmer and (Agu,1999) demonstrated the difference in solubility of ,B-glucan when malt was mashed at 45°C or 65°C, with an increased level of solubilisation at the latter temperature.

The level of B-glucan has been shown to have a relationship with other malt quality traits. Importantly, high ,B-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 (Evans *et al.,* 1999). Views differ on the relationship between, B-glucan and foam stability (Lusk *et al., 2001).*

2.4.9. Wort viscosity

It is a measure of a liquid's ability to resist flow through a capillary column. The measurement is the amount of time required for a predetermined volume to flow through the thin tube in comparison with water. The main offenders that cause viscosity are β-glucan and arabinoxylan. These have been shown to be influenced by variety and growing environment (Henry, 1986; Fox, 2008). It is linked to wort β-glucan but also another important non-starch polysaccharide, arabinoxylan. Viscosity, like hot water extract, cannot be related to a single trait within barley. The breakdown of β-glucan during malting has been shown to have a direct impact on viscosity in high temperature infusion mashing (Fox *et al*., 2003).

2.4.10. Fermentability

Ferment ability is dependent on a range of variable including variety, variety growing environment, malting process, mashing style and fermentation conditions (Fox, 2008). over-modification could also limit fermentability due to increased levels of soluble protein and reduced levels of fermentable sugars due to elevated malting losses. Viscosity and β-glucan also affected fermentability wort ferment ability can differ when different yeasts are applied (Evans *et al.*, 2005).

3. Materials and Methods

3.1. Description of study areas

The experiment was conducted during the year 2014-2015 at three locations. These were in Arsi zone of Oromia region (Kulumsa agricultural research center), Amahara region north shewa (Deberebrhan regional research center) and west shewa at Holeta agricultural research center located in the central high land of Ethiopia.The experiment on the malting process was conducted at the department of malt quality analysis laboratory Asela malt Factory. The Laboratory analysis of barley grain and malt quality was conducted at Holeta grain laboratory, Addis Ababa University (food science and nutrition research laboratory) and Asela malt Factory.

3.2. Sample Collection

Trial planting and field management was implemented as per the standard procedure (figure 3.1). Sixteen barley varieties were collected from the National Variety Trial (NVT) which was conducted at Holeta,Debrebrhan and Kulumsa Agricultural Research Center. Four variety (Holker,traveler,EH 1847,IBON174/03) were used as standard check (they were released varieties)and selected based on their yield. To prevent absorption of moisture the malting barley varieties was stored in a dry place until malting .A composite sample was prepared from three locations for analysis at Ethiopian institute of agricultural research.

Leaf 2-row barley

Sixteen barley samples

Figure 3.1.Leafe, two row plump kernel and sixteen barley (Hordeum Vulgarre L) samples (photo taken during growing period to sample preparation) on 01/03-10/04/2008 E.C

Table 3.1 varieties used in the study

Sample in breeding line	Sample representation
1.MBF-P#26	MB1
2.MBPON2013/P#41	MB2
3.MBF5-P#31	MB3
4.MBPON2013/P#15	MB4
5.MBF5-P#11	MB5
6.MBPON2013/P#74	MB6
7.MBF5-P#19	MB7
8.MBF5-P#3	MB8
9.MBF5-P#10	MB9
10.MBPON2013/P#7	MB10
11.MBF5-P#1	MB11
12.MBPON2013/P#32	MB12
13.Holker	MB13
14.Trveller	MB14
15.EH 1847	MB15
16.IBON174/03	MB16

3.3 Sample preparation

Upon arrival, the grain brought to laboratory were separated cleaned to remove surface foreign matters using sieve and hand carefully .Then ,about 500 gram of grain sample was weighted. For this purpose the weighted samples were go to in to micro malt apparatus and the malting process were run. After malting the dry malt were milled using a mill fine and course grind miller. The milled sample were labeled and stored in dry place for later analysis.50 gram was milled through 0.425 mm mesh size. Finally the flours were bagged in polythene bags which were properly labeled and stored in dry place.

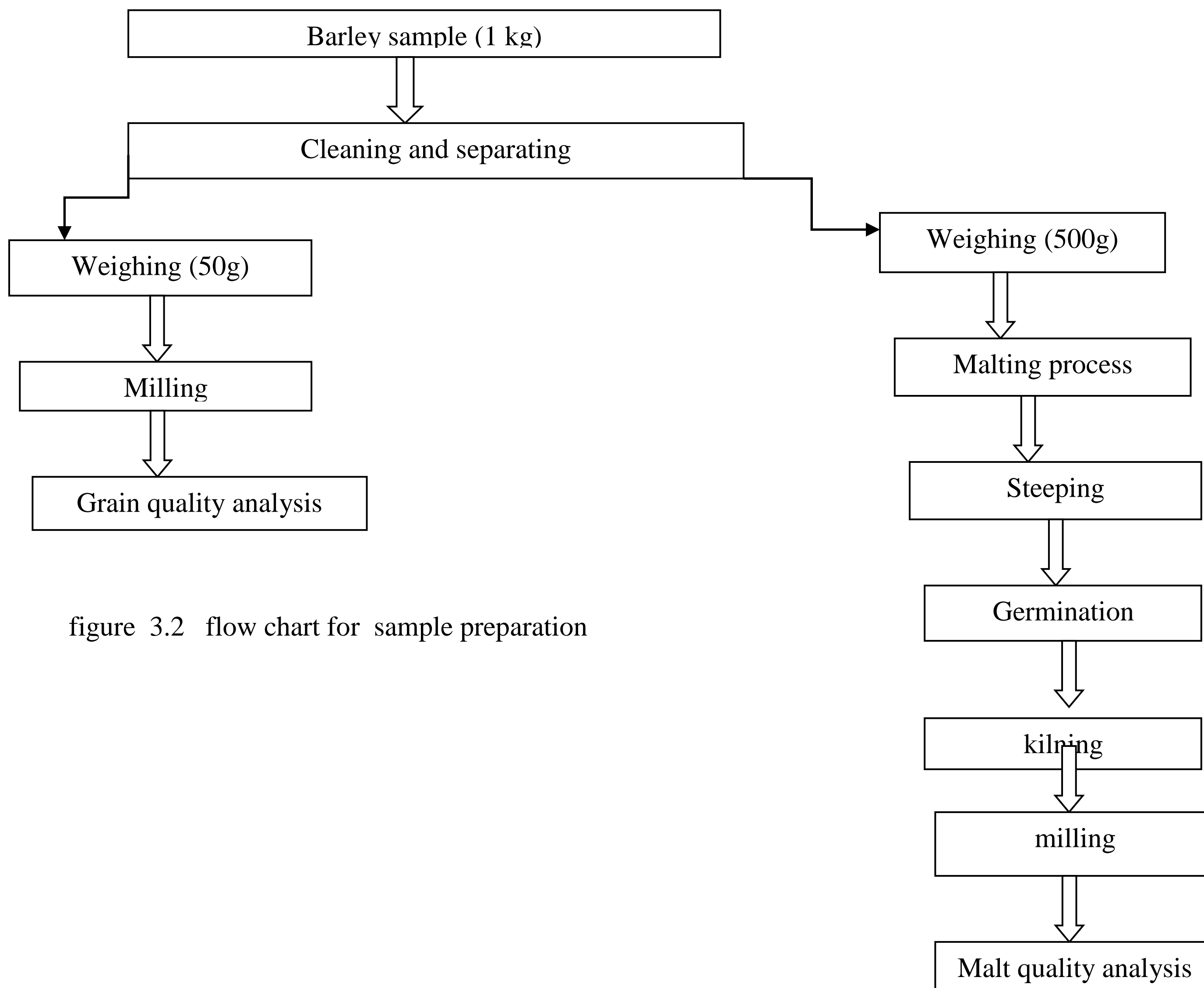


figure 3.2 flow chart for sample preparation

3.4. Grain quality analysis of malt barley

The standard operating procedures were according to European breweries convention (EBC) methods (Analytical EBC and AACC Method).

3.4.1. Sieve test (Grain size)

Grain size analyzer was used to determine the size of kernals. Grain size analysis was performed by 2.8mm, 2.5mm and 2.20mm vibrating sieves in to four components. Taking 100gm of grain barley sample and put on the sieve then allow the sieve to run for 5 minute and weigh out the sample as percentage.

3.4.2. Germination Energy (GE)

Germination energy of barley was determined as described by Doran and Briggs (1992). Barley kernels (100) were spread on wetted (4ml distilled water) filter paper lined on Petri dishes (90mm) and allowed to germinate at nearly 100% relative humidity set at a temperature of 16°C

germination cabinet for 3 days as described in EBC (1998) method 3.6. The germinated (or chitted) kernels of each variety were counted and removed after 24, 48, 72 hr. The total percentage of kernels germinated by the end of 72 hr was counted and the result was expressed in percent.

3.4.3. Moisture content

Malt moisture content- The dried and polished malt at each variety was grounded and passed through a 1-mm sieve. A sample of about 3-g flour was weighed on analytical balance and oven-dried at 105°C for 3 hours. The moisture lose on drying was calculated and expressed in % of the pre-drying sample mass as described in the EBC(1998) Method 4.2.1.

$$\%\text{Moisture} = \frac{\text{intial weight} - \text{final weight}}{\text{intial weight}} \text{x}100$$

3.4.4. Thousand Kernel weight

Thousand kernel weights was determined with a counting device and balance. Broken and foreign corns must were removed previously and their weight is subtracted. Take 40 gram barley sample were counted and calculated as percentage corn weight**.**

3.4.5. Hectoliter weight

The grain bulk density (hectoliter weight) was determined on dockage- free samples using a standard laboratory hectoliter weight apparatus (grain analysis computer (GAC) 2100). as described in the AACC (2000) method no 55-10.

3.4.6. Protein content

The nitrogen content of each barley variety was determined by Kjeldahl method as stated in the AACC (2000) Method 46-11

3.5. Malt quality analysis of barley

3.5.1. Micro malting

Malts was prepared using a Phoenix Automated Micro malting system (Phoenix Bios stems, Adelaide, Australia) designed to process twenty four 500g (1kg 12 barley samples) barley samples per batch. Sixteen Samples were steeped with alternating periods of water immersion and air rest for thirty two hour (32h) with temperature of sixty degree centigrade(16 ^{0}C),Germination for ninety six hour (96h)with temperature sixty degree centigrade(16 ^{0}C) and kilning fifty hour(15h) with temperature ninety degree centigrade(90 ^{0}C).

Figure 3.3.Micro malt apparatus and malt (photo taken in the laboratory on 23/04/2008 E.C)

3.5.2. Extract determination

Mashing procedure

The mashing process was according to the EBC congress mashing method. 55g of malt sample from each varieties were weighed (at room temperature) in to mash beaker and grinded through mill set for standardized fineness of grind. Then, ground malt was collected in same mash beaker, carefully brushing malt particles remaining in mill in to mash beaker. Mix, and without delay, the mash beaker was placed with content on balance accurate to within ±0.05g under 750g load and adjust weight of malt to 50 ± 0.05 g by removing excess in to tared dish for moisture determination. The mashing procedure was done by adding 200 mL of distilled water at 45 ℃ to 50 g of ground malt, and then the vessel was placed in a mashing apparatus. The sample was held at 45 ℃ for 30 min, then the temperature was raised to 70 ℃ by 1 ℃ for every 1-min

increase for 25 min, and then 100 mL 70 ℃ distilled water was added to each sample and held at 70 ℃ for 1 h. After 10 min and 15 min (for late saccharified samples), saccharification test EBC (1998) was done with 0.02N iodine solution. At the completion of mashing, the sample was cooled to room temperature and then distilled water was added to adjust weight of the content in mash vessel to 450 g. The extract was filtered through 32 cm fluted filter paper in 20 cm funnel. The time elapsed by each sample to filter fully into a flask was recorded to determine filtration time. The density of the clear wort was determined using an wort hydrometer and expressed in degrees Plato (^{o}P). The extract obtained was converted and expressed in percentage on wet basis (% wb) using the following equation.

$$Extract\ wet\ basis = P\frac{(800 + M)}{(100 - P)}$$

$$\textbf{Extract dry basis} = \frac{(\mathbf{E \times 100})}{(\mathbf{100 - M})}$$

Where: P is g extract in 100 g wort (° Plato), M is % moisture in the malt and E is extract as wet basis.

Figure3.4 congress mash for extract determination.

3.5.3. Malt moisture content

The dried and polished malt at each variety was grounded and passed through a 1-mm sieve. A sample of about 3gram flour was weighed on analytical balance and oven dried at 105^{0}C for 3 hours. The moisture lose on drying was calculated and expressed in % of the pre drying sample mass as described in the EBC(1998) Method 4.2.1.

$$\%\textbf{Moisture} = \frac{\textbf{intial weight} - \textbf{final weight}}{\textbf{intial weight}}\ \mathbf{x100}$$

3.5.4. Protein content of the malt

The nitrogen content of the malt from each variety was determined by Kjeldahl method as stated in the AACC (2000) Method 46-11. About 1 g grind sample was weighed into Kjeldahl digestion flasks and catalyst mixture (K_2SO_4 mixed with $CuSO_4.5H_2O$ and selenium in the ratio of (10:6:1) was added in to each flask. Then, 20 mL of concentrated H_2SO_4 (98%) was added and the sample was digested for 2 hours at a temperature of 400 ℃ until the solution was clear white. With the completion of the digestion (when the digested sample becomes colorless or light blue) the samples were allowed to cool. After the samples were cooled, 50 mL of distilled water was added into each digestion flask followed by 40 ml of 40% NaOH. Immediately the contents were distilled by inserting the digestion tube line into the receiver flasks that contain 25 mL of 2% boric acid solution. The collected ammonia distillate was then titrated against a standardized 0.1N HCl until the end of the titration is attained (where the titration color changes from blue to pink). Then the volume of HCl consumed to reach the titration end point was read from the burette and the %nitrogen content was calculated as follows:

$$\mathbf{Nitrogen(\%)} = \frac{\mathbf{V_{HCl} \times N_{HCl} \times 14.00}}{\mathbf{sample\ weight\ in\ dry\ matter\ base}}$$

Where V_{HCl} is volume of HCl in litter consumed to the end point of titration N_{HCl} is normality of HCl used and 14.00 is the molecular weight of nitrogen. Percent nitrogen was expressed on wet matter basis and the resulting value multiplied by a factor of 6.25 to obtain protein content of each variety at each location.

3.5.5. Friability of malt

Friability- Samples were analyzed using a Pfeuffer Friabilimeter, which uses 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). Malt sample, 50g, was run in the friability meter for 8 min, and the non-friable fraction was weighed.

$$\mathbf{Friability(\%) = 100 - R * 2}$$

Where: R is mass of non friable one retained over the Friabilimeter sieve from 50g sample used for the test.

3.6 Wort quality analysis

Sampling of wort

Before being used in the mashing process, dry cylinders were weighted and the milled and weighted malt was added. In the mashing process 300ml of demineralised water was added, after which the mashing cylinders were cooled down to room temperature (20°C) and the content adjusted to 450g by adding more demineralised water After mashing cylinders were standardized to 450g and thoroughly mixed. Filtration was done by using glass and plastic funnels and filter paper seen in figure 3.5.Wort was collected into 500ml vials.

3.6.1. Wort color

The wort obtained from malt extract was used for wort color measurement by EBC standards using a portable comparison of the wort color with an appropriate color disc in the Hellige Neo compactor apparatus.

Color measurement Wort filtration

Figure 3.5 Photo taken in wort filtration, color and PH measurement.

3.6.2. PH of Wort

PH of wort was measured 30 minute after the start of filtration with a glass electrode PH meter.

3.6.3. Soluble nitrogen in wort

. 20 ml wort was pipated in to 500ml kjeldahl flask and 3ml of sulfuric acid was added. Antifoam was added to prevent excess foaming. This stage was drying stage. After drying 20ml sulfuric acid and 10g of catalyst was added. The digestion, distillation and titration were completed as described in EBC method 3.3.1.

$$\text{Total nitrogen} = \frac{T.14}{V_{wort}} .100$$

3.6.4.Diastatic power (DP) of wort

Finely milled (20g) pale malt was prepared and Dissolved in 480ml distilled cold water and put into a bath at 40c^0 for an hour. the content was cooled and made to a weight of 520 and 540 for pale malt. The content was filtered and removed 200ml of the first filtrate was used the next 50ml filtrate was used for analysis. four graduated flasks (label1 & 2 for main and 3&4 for blank analysis (test) was prepared. starch solution (10g starch was dissolved in a small amount of water, boil 400ml distilled water utill evaporation, add the dissolved starch in 400ml boiled water and stirred for 5min, then cool and made the volume to 500ml.100ml of starch aliquot was Transferred to each of the four flasks. 5ml acetate buffer was Add to flask 1&2 and put the four flasks at 20c^0 for 20min. At the end of 20 min 5ml of malt extract was added to the main test (1&2) shake gently and kept at 20c^0 for 30 min. At the end of 30min 4ml of NaOH was added to the main test to deactivate diastase enzymes. 2.35ml NaOH was Added to 3&4 and 5ml of extract after shaking. All the four flasks were made to 200ml with distilled water.50ml from each flask was Transferred to four corresponding 150ml flasks. 25ml of iodine solution and 3ml of NaOH was added for each. Shake and allow to stand for 15min (cover the flasks to prevent loss of iodine solution, since iodine is light sensitive). At the end of 15min the solution was acidified by addition of 4.5ml H_2SO_4. the four solutions was titrated with sodium thiosulfate solution to the disappearance of the iodine to blue color. The reacted iodine should be between 6-12ml unless repeat the test.The result was calculated from the titration.

The volume of thiosulfate consumed to reach the titration end point was read from the burette and calls it “B”. Finally, the diastatic power was calculated by the following equation.

$$\text{Diastatic power, }^\circ(\text{wet basis}) = (B - A) \times 23$$

$$\text{Diastatic power, }^\circ(\text{dry basis}) = \frac{^\circ(\text{as is}) \times 100}{100 - M}$$

Where: A is the volume of sodium thiosulfate in milliliter used for direct titration, B is the volume of sodium thiosulfate in milliliter used for blank correction, M is % moisture in malt and 23 is conversion factor specific for the procedure outlined.

3.6.5.Free Amino Nitrogen (FAN) of wort

The free amino nitrogen value was determined from the wort sample and was based on a small scale version of the IoB Ninhydrin method. Wort (1.0 mL) was diluted to 100mL with distilled

water in a volumetric flask. A 2mL aliquot (in triplicate) was transferred to test tubes and 1mL of ninhydrin solution was added. The tubes were placed in a boiling water bath for 16 min and then in a cool water bath for 20 min. A dilution solution containing potassium iodate (5 mL) was added to each tube to ensure that the ninhydrin remained oxidized. The absorbance was measured at 570 nm. Average absorbance readings were obtained for the triplicate tubes. Average blank absorbance was subtracted from absorbance of the samples and from the glycine standard. The FAN values were expressed as mg/L.

$$\text{Free amino nitrogen (FAN)}, {}^{mg}/_{L} = \frac{\text{Net absorbace of test solution}}{\text{Net absorbance of glycine standard}} \times 2 \times \text{dilution}$$

3.6.6. Kolbach index (ratio S/T)

Kolbach index was calculated according to ASBC (2008) by using the following formula.

$$\text{Kolbach index (KI)} = \frac{\%\text{soluble protein}}{\%\text{ malt protein}} \text{ x100}$$

3.6.7. Calcium and zinc in wort

The content of metal ions in solutions was determined by atomic absorption spectrometry with a flame atomization technique using an automatic dispensing sample system, Gas flow was an air acetylene mixture of acetylene and air. Calcium and Zinc concentrations were determined by reference to an appropriate metal solution made of 1000ppm standards. The wort samples were homogenized by shaking before used. It was filtered through dry zinc free filter paper and the first 4 ml filtrate was discarded before collecting. Successively pipette in a test tube 2.0 ml wort sample, 8.0ml 0.01 mol/l HCl was mixed well. For calcium 2ml $LaCl_3$ was added. The atomic absorption spectrophotometer was used to determine for zinc and Calcium.

3.7. Data analysis

Analysis of variance was computed using appropriate software (SPSS version 20) statistical software package. Data were expressed as mean±standered deviation. One way analysis of variance (ANOVA) was used with respect to studied parameters. The result was summarized as table of mean and LSD at $P<0.05$ was used to determine which mean were significant different.

4. Result and Discussion

The finding of this study are presented and discussed in detail to address the objectives of the study. The data used for the statistical analysis are stated in wet basis.

4. 1.Grain quality Analysis Results

4.1.1 Grain size (sieve size)

Grain size is an important descriptive trait based on the physiology of the grain. The final grain size is determined by several environmental factors as well as biochemical components within the grain itself (Coventry *et al*., 2003). Growing location showed significance difference (P<0.05 Table4.2) in grain size. Varieties grown at Bekoji had higher mean grain size than varieties grown at Ankober and Varieties grown at Ankober had higher mean grain size than Holeta (94.07, 92.87and 72.62 %respectively) but the requirement in the brewing industry is >80 % must be above 2.8 and 2.5 mm sieve size according to Ethiopian standard requirement for malt purpose. Mean grain size in Holeta is very small compared to Bekoji and Ankober. (Fox *et al*.,2006) demonstrated the genetic and environmental effects in improving grain size. Industry standards on large grain are based on the total percentage of grain > 2.5mm (IOB, 1997). Increase grain size also showed to provide an increase in the important malt quality trait of diastatic power (Agu *et al*., 2007).

The analysis of variance for grain size not significantly different (P<0.05, Table 4.1) among varieties. Highest mean grain size percentage were obtained in MB15 (92.56%) and MB1 (92.66%) while varieties MB12(78.83%)and MB11(80.1%)had lower values (Table 1).The grain size percentage should be >90% for 2-rowed barley and >80% for 6-rowed barley (Anonymous, 2012).in this study the grain size fulfill the standard requirement of the Industry according to EBC and Ethiopia malt factory except variety MB12(78.83%).

4.1.2 Germination energy (GE)

The Germination energy is the total number of grains that germinate over 72 h of incubation under specified conditions (Woonton *et al.,* 2005).The analysis result of germination energy was significantly different (P<0.05, Table4.2) between locations. As it was indicated on (Table 1) GE was higher at Debrebrhan (Ankober) (85.93%) than Holeta and Bekoji (76.87% versus,76.37%

respectively). A minimum of 95% germination on a 3day germination test is an absolute requirement. Any factor which interferes with the uniformity of germination or reduces the vigour of kernel growth during processing will reduce the quality of malts produced (Michael, 2014).The analysis of variance of germination energy was significantly diffrent(P<0.05, Table 2) among varieties. The germination energy of varieties ranged from 13-99.33% (Table4.1). The highest value were for MB10(99.33%)followed by MB13(98.33) and MB3 (96.33%) while the lowest was observed in MB14, followed with MB15 .it has been indicated that varietal different showed the difference in germination energy (Bhatty, 1996), which support this study. Thomas (cited by Swanston *et al*., 2002) also noted differences in the genetic factors determining germination after three days and also suggested that there were environmental effects on their expression. In this study, the number of grains germinating within 24, 48 and72 hr was significant different between locations and among varieties.

4.1.3 Moisture content

The analysis of variance revealed significant differences between locations for grain moisture content (P<0.05, Table 4.2). As the mean indicated in Table 2 the moisture content of grain was higher at Bekoji than Holeta and the moisture content of grain was higher at Holeta than Ankober (13.83, 11.44% and 8.36%, respectively). 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 germinative capacity may also significantly deteriorate (Plankinton *et al.,* 2014

The result (Table 4.1) showed that the moisture content were not significantly different (P<0.05, Table 4.1) among the varieties. The moisture content of varieties varied between 10.00-11.9%. The highest moisture content was for variety MB9 (11.83%) and the least was observed in MB10 (10.7%).According to Fox *et al.* (2003) the maximum reasonable industrial specification of malt barley moisture content for safe storage is 12.5%, whereas, the EBC standard, a moisture content of 12 -13% is accepted. In this study the moisture content were in the acceptable range.

Tables4.1. Effect of variety on grain size, germination and moisture content of grain.

Genotype/Varity	Grain size (%)	Germination energy (%)	Moisture content (%)
1.MB1	92.66±3.46[c]	89.33±5.13[e]	11.46±2.84
2.MB2	86.00±15.82[b]	64.33±23.54[c]	11.10±2.65
3.MB3	90.8±7.70[b]	96.33±1.15[f]	11.30±2.72
4.MB4	80.50±25.89[a]	87.66±9.29[d]	10.83±2.85
5.MB5	91.93±5.74[c]	93.66±7.09[f]	11.00±3.00
6.MB6	83.40±13.13[a]	68.66±27.30[c]	11.26±2.96
7.MB7	89.90±7.18[b]	65.00±34.04[c]	10.96±2.70
8.MB8	86.06±9.30[b]	76.33±10.69[d]	11.33±2.85
9.MB9	87.96±10.48[b]	96.00±6.08[f]	11.83±2.70
10.MB10	85.80±20.36[b]	99.33±1.15[f]	10.73±2.55
11.MB11	80.10±17.95[a]	94.33±8.14[f]	11.10±3.05
12.MB12	78.83±17.87[a]	87.00±13.52[e]	11.33±2.80
13.MB13	83.63±10.53[a]	98.33±2.08[f]	11.33±2.99
14.MB14	86.60±18.62[b]	13.00±4.58[a]	11.73±2.22
15.MB15	92.56±3.63[c]	55.00±6.55[b]	11.10±2.88
16.MB16	87.63±9.036[b]	91.33±9.01[e]	11.00±2.68

- Means ± standard deviation with different letters after data within a column represents significant differences at 95% probability level significantly different at P=0.05. GS =Grain size MC=moisture content of grain; GE=germination energy.
- A composite sample was prepared from three locations (Holeta,Kulumsa and Ankober) for analysis at Ethiopian institute of agricultural research.

Tables 4.2. Effect of location on grain size, germination and moisture content of grain.

Location	Grain quality parameter		
	Grain size	Germination energy	Moisture content
Holeta	72.62±11.38^{a}	76.87±24.58^{a}	11.44±.51^{b}
Debrebrhan(Ankober)	92.87±4.19^{b}	85.93±26.34^{a}	8.36±.62^{a}
Kulumsa(Bekoji)	94.07±2.96^{b}	76.37±24.45^{a}	13.83±.34^{c}

Means ± standard deviation with different letters after data within a column represents significant differences at 95% probability level indicated value is significantly different at P=0.05. GS =Grain size MC=moisture content of grain; GE=germination energy.

4.1.4 Thousand grain weight

The analysis of variance of thousand kernel weight was significant difference (P<0.05 Table 4.4) between locations.Bekoji revealed a greater TKW than Ankober and Ankober revealed agreater TKW than Holeta (51.6 g 48.0 And 44.17 g, respectively). Between the locations, lowest values were obtained at Holeta (44.17g). However, highest values (> 46 g) were obtained at Ankober and Bekoji Thousand grain weight (g) should be >45 g for 2-rowed barley and > 42 g for 6-rowed barley (Anonymous, 2012).

The thousand kernel weight result showed that there was no significant difference (P<0.05, Table 4.3) among the varieties. In this study, thousand kernel weight varied between 44.55-51.6 g (Table 4.3). The highest Thousand kernel weight was for MB10 (51.6 g) and the least was observed in MB12(44.5 g). Lu et al. (2001) also reported the minimum and maximum thousand kernel weight of malt barley which is ranged from 38.6-53.0 g and this result is above to our finding and it fulfill EBC (23-35%) standard requirement for industry.

4.1.5 Hectoliter weight

The hectoliter weight was significantly affected by location ($P<0.05$) and there was effect of growing location on all the sixteen varieties (Table 4.3).The hectoliter weight was relatively highest obtained at Holeta and Ankober (67.88 kg/hL,67.16 kg/hL) than Bekoji (62.33 kg/hL) (Table 4.4). Hectoliter weight has been shown to be influenced by growing environment (Molina-Cano *et al*. 1997). Test weight (TW) (bulk density or HLW) is an industry standard for classifying malt and feed barley. Barley with plumper grains and a higher test weight should have a greater percentage of starch or energy in the grain and should be lower in fiber (Shewry and Morell, 2001).

MB9(67.9 kg/hl) followed by MB2 (67. kg/hl), MB3 (66.6 kg/hl) were highest hectoliter weight value. The varieties MB13 and MB14 was have lower Hectoliter weight (64.56 and 64.53 respectively).Verma *et al.*(2008) had shown that hectoliter weight is one of the best correlated parameter for malt quality and the effect of location had significant effect on hectoliter weight.

4.1.6 Protein content

The analysis of variance revealed significant differences between locations for grain protein content ($P<0.05$,Table 4). The protein content was relatively higher at Kulumsa (Bekoji)(11.87) than Holeta and Ankober(10.6 ,9.0 respectively) in (Table 4.4). The Grain Protein Content is influenced to a large extent by both genotype and environment (Bathgate, 1987).

The protein content of the grain result showed that there was no significant difference ($P<0.05$, Table 3) among the varieties. In this studythe protein content of the grain of barley variety varied between 9.66-11.50(Table 4.3). Lowest mean protein content was obtained in MB14 (9.66%), followed by MB9 (9.86). The protein content was on higher side in MB7 (11.50) and MB15 (11.30%) (Table 3). Desirable protein content range for 2-rowed barley is 9.0-11.0% and for 6-rowed barleys 9.0-11.5% (Anonymous, 2012). Barley used for malt should have a grain protein concentration (GPC) below11.5%, as higher protein content will deteriorate malting produce and final beer quality.

Tables 4.3. Effect of variety on Hectoliter weight, Thousand kernel weight and protein content of grain.

Genotype/Varity	Grain quality parameter		
	Hectoliter weight	Thousand kernel weight	Protein content
1.MB1	64.36±4.350[a]	48.96±3.53[c]	10.03±1.501[a]
2.MB2	67.00±4.026[b]	46.80±5.23[b]	10.43±2.064[b]
3.MB3	66.66±3.82[b]	47.80±3.56[b]	10.80±1.41[b]
4.MB4	65.73±2.36[a]	50.13±6.21[c]	10.23±2.48[a]
5.MB5	65.86±3.34[b]	47.53±3.66[b]	10.50±1.91[b]
6.MB6	66.60±2.94[b]	50.63±4.25[c]	11.10±1.45[b]
7.MB7	66.20±3.85[b]	46.50±3.051[a]	11.50±1.45[c]
8.MB8	65.93±3.11[b]	45.40±4.51[a]	10.23±.92[a]
9.MB9	67.90±3.06[c]	47.43±4.90[b]	9.86±1.30[a]
10.MB10	65.33±3.80[a]	51.60±5.55[d]	10.46±1.27[b]
11.MB11	65.30±4.38[a]	47.83±2.61[b]	10.33±1.87[a]
12.MB12	66.56±1.98[b]	44.56±7.83[a]	10.96±2.00[b]
13.MB13	64.566±2.87[a]	50.00±5.56[c]	9.96±1.46[a]
14.MB14	64.53±3.35[a]	48.13±8.46[b]	9.66±.66[a]
15.MB15	65.43±3.16[a]	46.66±3.00[b]	11.30±1.55[c]
16.MB16	64.66±2.84[a]	51.10±1.94[d]	10.46±1.78[b]

- Means ± standard deviation with different letters after data within a column represents significant differences at 95% probability level indicated value is significantly different at P=0.05.
- A composite sample was prepared from three locations (Holeta,Kulumsa and Ankober) for analysis at Ethiopian institute of agricultural research.

Tables 4.4. Effect of location on Hectoliter weight, Thousand kernel weight and protein content of grain.

Location	Grain quality parameter		
	Hectoliter weight	Thousand kernel weight	Protein content
Holeta	67.88 ± 1.61^{c}	44.17 ± 3.62^{a}	$10.60\pm.95^{b}$
Debrebrhan(Ankober)	67.16 ± 1.63^{b}	48.01 ± 2.62^{b}	$9.00\pm.60^{a}$
Kulumsa(Bekoji)	62.33 ± 1.71^{a}	52.39 ± 2.93^{c}	$11.87\pm.88^{c}$

Means ± standard deviation with different letters after data within a column represents significant differences at 95% probability level indicated value is significantly different at P=0.05.

4.2. Malt quality Analysis Results

4.2.1.Fine grind hot water extract (HWE)

Fine grind hot water extract was significant difference between locations (P<0.05, Table 4.6). The extract amount was higher at Holeta (72.74%) than Bekoji and Ankober (70.04%, 68.66%, respectively) and the lowest value is found at Ankober. Howard *et al*., (1996) also found that variation in growing conditions resulted in a wide range of malt extract values. According to Fox et al. (2003) the quality of the extract is influenced by several factors such as environmental, growing conditions, temperature, fertilizer, available nitrogen, or moisture.

The result showed no significantly different (P<0.05, Table 4.5) among varieties for fine grind hot water extract. The mean hot water extract among varieties ranged from (67.18-72.91%) (Table 4.5). The highest (72.91) fine grind hot water extract was recorded for MB1 whereas, the lowest (67.18) value was detected in MB4. The extract content of promising varieties were having the same potential with released varieties. The best extract content was obtained from variety MB1 which is a promising one which is compared to the released variety MB14and MB13.The extract yield reflects the extent of enzymatic degradation and the solubility of grain

components after malting and mashing (Swanston *et al*., 2014). Low malt extract in barley seeds is ungerminated and incompletely modified seeds because High glucan content, slow filtration rate and high molecular nitrogen in extract, which result in low quality of beer (Bathgate et al., 1974, Lersrutaiyotin *et al*., 1991). Mean EBC hot water extract value ranged from 75.0-80.7% but this result were below the EBC standard. This study result indicate low malt extract compared to EBC range for the Varites.

4.2.2 Coarse grind hot water extract (CWE)

Coarse grind hot water extract was significantly different between locations ($P<0.05$, Table 4.6). The extract amount was higher at Holeta (69.47%) than Bekoji and Ankober (66.96%, 64.67%, respectively) and the lowest value is found at Ankober.the extract yield reflects the extent of enzymatic degradation and the solubility of grain components after malting and mashing (Swanston *et al*., 2014).in this study the highest extract yield is found in Holeta and the lowest value were found in Debrebrhan (Ankober).The result (table 5) showed no significant difference ($P<0.05$,Table 5) among varieties for course grind hot water extract. The mean hot water extract among varieties ranged from (64.91-69.79%) (Table 4.5). The highest (69.79) course grind hot water extract was recorded for MB1 whereas, the lowest (64.91) value was in MB4.The best extract content was obtained from variety MB1.

4.2.3 Extract difference (ED)

Extract difference was significantly different between locations ($P<0.05$, Table 4.5). Extract difference was higher at Bekoji (3.71%) than Holeta and Ankober (3.59%, 3.25%, respectively) and the lowest value is found at Ankober. The mathematical difference in malt extract values obtained from fine grind versus coarse grind malts (fine/coarse difference) was the first measurement of cell wall breakdown, especially when a 708 ^{0}C isothermal mash was used for the coarse grist (Bendelow ,1977). The result (table 4.6) showed no significant difference ($P<0.05$, Table 6) among varieties for extract difference. The mean extract difference among varieties ranged from (2.43-4.89) (Table4.6). The highest (4.89) extract difference was recorded for MB13 whereas, the lowest (2.43) value was detected in MB16 the new genotype were found in the range between the standard check MB13 and MB16.

Table 4.5 variety effect on malt quality of Fine grind malt extract (%),Caurse grind malt extract (%) and Extract diffrence (%).

Genotype/Varity	Malt quality parameter		
	Fine grind malt extract	Caurse grind malt extract	Extract diffrence
1.MB1	72.91±3.475[c]	69.79±3.41[c]	3.12±1.59[b]
2.MB2	71.70±4.978[c]	67.64±4.27[b]	4.03±1.26[c]
3.MB3	70.91±1.940[b]	67.60±3.06[b]	3.31±1.55[b]
4.MB4	67.18±4.69[a]	64.91±4.21[a]	2.75±1.01[a]
5.MB5	71.21±2.23[b]	66.61±2.14[b]	4.59±.30[d]
6.MB6	71.90±1.20[c]	67.70±1.10[b]	3.80±1.09[c]
7.MB7	71.86±2.71[c]	68.70±3.05[c]	3.30±.44[b]
8.MB8	68.67±4.614[a]	65.58±4.80[a]	3.25±1.29[b]
9.MB9	71.98±1.88[c]	68.69±2.23[c]	3.45±.96[b]
10.MB10	72.19±4.96[c]	68.81±4.02[c]	3.52±1.20[b]
11.MB11	69.75±3.28[b]	66.29±4.19[a]	3.41±.92[b]
12.MB12	68.59±4.37[a]	65.12±3.96[a]	3.46±1.19[b]
13.MB13	70.77±3.16[c]	65.70±3.63[a]	4.82±1.50[d]
14.MB14	71.70±1.52[c]	67.99±3.51[b]	4.02±2.02[c]
15.MB15	68.74±6.11[a]	65.72±5.89[a]	3.02±1.30[b]
16.MB16	67.65±3.40[a]	65.75±3.5[a]	2.43±.14[a]

- Means ± standard deviation with different letters after data within a column represents significant differences at 95% probability levelindicated value is significantly different at P=0.05, FGME = fine grind malt extract; CGME=coarse grind malt extract; and ED=extract difference.
- A composite sample was prepared from three locations (Holeta,Kulumsa and Ankober) for analysis at Ethiopian institute of agricultural research.

Table 4.6 Location effect on malt quality of Fine grind malt extract (%),Caurse grind malt extract (%) and Extract diffrence (%).

Location	Malt quality parameter		
	Fine grind malt extract (%)	Caurse grind malt extract (%)	Extract diffrence (%)
Holeta	72.74±1.74[b]	69.47±1.97[c]	3.55±1.24[a]
Debrebrhan(Ankober)	68.04±2.77[a]	64.67±2.35[a]	3.29±.98[a]
Kulumsa(Bekoji)	70.66±3.99[a]	66.96±3.85[b]	3.71±1.29[a]

Means ± standard deviation with different letters after data within a column represents significant differences at 95% probability level indicated value is significantly different at P=0.05, FGME = fine grind malt extract; CGME=coarse grind malt extract; and ED=extract difference.

4.2.4. Malt moisture content

Malt moisture content of malt was significantly different ($P<0.05$, Table 4.8) between growing locations. As it was presented in Table 8 the higher Malt moisture content was recorded at Debrebrhan(Ankober) (6.02) than Kulumsa(Bekoji) and Holeta (5.35 versus, 4.87 respectively).

The analysis results showed that, there was not significantly different ($P<0.05$, Table 4.7) among varieties for Malt moisture content. The mean Malt moisture content of the varieties ranged from (4.26-6.03)(Table 4.7). The highest Malt moisture content was for MB15 (6.03), while the least was observed in MB11(4.26). The malt moisture content for long shelf stable storage is recommended 4 to 5% (AOAC, 1990).but in this study the malt moisture content was above the EBC and the specified range.

4.2.5. Malt protein content

Protein of malt was significantly different ($P<0.05$, Table 4.8) between growing locations. As it was presented in Table 8 the higher protein content was recorded at Ankober (12.65) than Holeta and Bekoji (10.87% versus 8.94%, respectively).

The analysis results showed that, there was not significantly different (P<0.05, Table 4.7) among varieties for protein content. The mean protein content of the varieties ranged from 9.94-11.59% (Table 4.7). The highest malt protein content was for MB4 (11.59%), while the least was observed in MB3(9.94%). Moderate amount of protein is needed for good yeast nutrition, the development of desired enzyme levels, foam stability, and other end product characters. High protein in barley slows water uptake during malting and lowers the ability of the kernel to modify completely. If the protein is too high the amount of extract available to convert to beer will be reduced and beer hazes could form (Plankinton *et al.,* 2014).Barley used for malt should have a grain protein concentration (GPC) below 11.5%, as higher protein content will deteriorate malting produce and final beer quality. However, it is often difficult to keep it below this upper limit, since the GPC is influenced, to a large extent, by both genotype and environment (Bathgate, 1987). In this study the result were satisfied a malt protein concentration below 11.5%.

4.2.6. Friability

Friability is a measure of the breakdown of malt endosperm cell wall components. Malt friability should be >60% (Anonymous, 2012).The variation for friability was not significant (P<0.05, Table 4.8) between locations. The mean value for locations was 56.95%, 57.65% and 63.46which were at Holeta, Ankober and Bekoji respectively. The friability at Bekoji was highest. The lowest friability were observed at Holeta. An increase in friability reflects thus a more extensive modification of the endosperm during malting, mostly with respect to the degradation of the protein matrix and cell walls (Chapon *et al*. 1979; Darlington and Palmer ,1996).

The analysis results showed that, there was no significantly different (P<0.05, Table 4.7) among varieties for friability content. The mean friability content of the varieties ranged from (50.9-71.7) (Table 4.7). The highest malt friability content was for MB16 (71.7), while the least was observed in MB15 (50.9) the range is in between the standard check. But most of the varieties also had friability percentage of <60%, When barley endosperm is properly modified during malting, the resulting malt is soft and friable. Factors that interfere with endosperm modification,

such as poor germination, large kernels and high protein, are expected to reduce malt friability (Edney and Mather, 2004).in this study the friability contradict the required standard. Since the friability of the variety need modification.

Table 4.7 variety effect on malt quality of Malt Moisture content (%,Malt Protein Content (%) and Friability.

Genotype/Varity	Malt quality parameter		
	Malt Moisture content (%)	Malt Protein Content (%)	Friability (%)
1.MB1	5.46±.49[a]	10.30±2.00[a]	54.73±7.5[b]
2.MB2	5.33±.61[a]	10.69±2.34[a]	62.56±7.88[c]
3.MB3	4.93±.87[a]	9.94±1.68[a]	69.60±10.71[d]
4.MB4	5.03±.72[a]	11.59±2.46[b]	61.00±3.29[c]
5.MB5	5.60±.34[a]	11.19±1.67[b]	52.96±15.02[a]
6.MB6	5.73±.95[a]	10.98±1.31[b]	51.80±0.91[a]
7.MB7	5.83±1.75[a]	11.23±2.09[b]	53.23±11.47[a]
8.MB8	5.10±.91[a]	10.72±2.3[a]	54.00±9.6[a]
9.MB9	4.49±.49[a]	10.60±1.23[a]	60.50±0.7[c]
10.MB10	5.40±1.45[a]	10.99±2.05[b]	60.60±0.72[c]
11.MB11	4.26±.40[a]	11.02±1.89[b]	55.92±4.53[b]
12.MB12	5.23±.15[a]	11.28±3.2[b]	61.40±4.97[c]
13.MB13	5.46±.75[a]	10.29±2.04[a]	61.65±1.81[c]
14.MB14	5.86±1.72[a]	10.81±2.06[b]	67.16±10.42[d]
15.MB15	6.03±.80[b]	11.43±2.57[b]	50.90±14.16[a]
16.MB16	5.93±.85[a]	10.07±0.77[a]	71.70±5.99[e]

- Means ± standard deviation with different letters after data within a column represents significant differences at 95% probability levelindicated value is significantly different at P=0.05, MC = Malt moisture content; MPC=malt protein content; and Friability=friablity.
- A composite sample was prepared from three locations (Holeta,Kulumsa and Ankober) for analysis at Ethiopian institute of agricultural research.

Table 4.8. Location effect on malt quality of Malt Moisture content (%,Malt Protein Content (%) and Friability.

Location	Malt quality parameter		
	Malt Moisture content (%)	Malt Protein Content (%)	Friability (%)
Holeta	4.87±.87[a]	10.87±0.71[c]	56.95±9.95[a]
Debrebrhan(Ankober)	6.02±.96[c]	12.65±1.25[b]	57.65±7.31[a]
Kulumsa(Bekoji)	5.35±.44[b]	8.94±0.14[a]	63.46±9.37[b]

Means ± standard deviation with different letters after data within a column represents significant differences at 95% probability level indicated value is significantly different at P=0.05, MC = Malt moisture content; MPC=malt protein content; and Friability=friablity.

4.3. Wort quality analysis result

4.3.1. Color of wort

Color of wort was not significantly diffrent between locations ($P<0.05$,Table 4.10). Color of wort was higher at ankober (3.82) than Holeta and Bekoji (3.42%, 3.18%, respectively) and the lowest value is found at Bekoji. The analysis of variance of color of wort was not significant ($P<0.05$, Table 4.9) among varieties for color of wort. The mean color of wort among varieties ranged from (2.66-4.83) (Table 4.9. The highest (4.83) color of wort was recorded for MB5 whereas, the lowest (2.66) value was detected in MB8. Wort color is another quality parameter measured from mashed wort. Color variation in wort is due to non-enzymatic browning reactions, the Maillard reaction, that take place during kilning in the malting process, and wort boiling in the brewing process.In this case, the sugars interact with the amino acids, producing a variety of odors and flavors. This reaction is the basis of the flavoring industry with the type of amino acid involved determining the resulting flavor and color (Guerrero, 2009).in this study color of wort were in the EBC standred range except MB5and MB10.

4.3.2. PH of wort

PH of wort was significantly different (P<0.05, Table 4.10) between growing locations. As it was presented in Table 8 the higher PH of wort content was recorded at Debrebrhan(Ankober) (6.32) than Holeta and Bekoji (6.12 versus, 5.61 respectively).

The analysis results showed that, there was not significantly different (P<0.05, Table 4.9) among varieties for PH of wort content. The mean PH of wort content of the varieties ranged from (5.84-6.30)(Table 4.9). The highest malt PH of wort content was for MB16 (6.30), while the least was observed in MB1 (5.84). It was shown that over the pH range 5 to 6.6, the proteolytic activity of malt can vary (Jones and Budde, 2003).PH variation limit the growth of microorganism in this case the growth of fermenting yeast is influenced with in the varation of PH .but in this study the PH of wort is in the specified range.

4.3.3. Soluble protein content

Soluble Protein of malt was not significantly different (P<0.05, Table 4.10) between growing locations. As it was presented in (Table 4.10) the higher soluble protein content was recorded at Bekoji (4.85%) than Holeta and Ankober (4.40 versus, 4.41% respectively).

The analysis results showed that, there was not significance difference (P<0.05,Table 4.9) among varieties for soluble protein content. The mean soluble protein content of the varieties ranged from (3.51-5.43%) (Table 4.9). The highest malt soluble protein content was for MB7 (5.43), while the least was observed in MB11(3.51%).In protein protein linkages, the stabilize foams and are responsible for mouth feel and flavor stability, and in combination with polyphenols, they are thought to form haze. As amino acids and peptides they are important nitrogen sources for yeast (Steiner *et al.,* 2009).

Table 4.9 effect of variety on Color of Wort, PH of wort, soluble protein content of wort.

Genotype/Varity	malt quality parameter		
	Color of Wort	PH of wort	Solubleprotein content of wort
1.MB1	3.41±.28[b]	5.84±.21[a]	4.66±1.18[c]
2.MB2	3.16±.14[b]	5.99±.42[b]	3.94±0.60[b]
3.MB3	3.25±.25[b]	5.96±.44[a]	4.21±0.62[b]
4.MB4	3.00±.25[a]	5.97±.19[a]	4.80±0.39[d]
5.MB5	4.83±.80[e]	6.06±.36[b]	4.25±0.69[b]
6.MB6	2.83±.52[a]	6.17±.30[c]	4.79±0.68[d]
7.MB7	4.25±1.32[d]	6.10±.28[b]	5.43±0.86[e]
8.MB8	2.66±.94[a]	5.81±.57[a]	4.61±0.38[c]
9.MB9	4.16±1.66[c]	6.02±.41[b]	5.06±1.28[d]
10.MB10	4.41±.76[d]	5.94±.15[a]	4.46±1.22[c]
11.MB11	3.16±.80[b]	5.97±.49[a]	3.51±0.83[a]
12.MB12	2.83±.80[a]	5.89±.68[a]	5.29±1.45[e]
13.MB13	2.91±.57[a]	6.25±.48[c]	4.82±0.42[d]
14.MB14	3.00±.43[a]	5.99±.57[b]	4.09±0.22[b]
15.MB15	3.66±.52[c]	6.00±.14[b]	4.38±0.82[c]
16.MB16	4.08±1.52[d]	6.30±.52[d]	4.56±1.29[c]

- Means ± standard deviation with different letters after data within a column represents significant differences at 95% probability level indicated value is significantly different at P=0.05, CW= color of wort; PHW =PH of wort; and SPC=soluble protein content.
- A composite sample was prepared from three locations (Holeta,Kulumsa and Ankober) for analysis at Ethiopian institute of agricultural research.

Table 4.10. Effect of location on Color of Wort, PH of wort, Solubleprotein content of wort

Location	Malt quality parameter		
	Color of Wort	PH of wort	Solubleprotein content of wort
Holeta	3.42±.53[b]	6.12±.23[c]	4.40±0.69[a]
Debrebrhan(Ankober)	3.82±1.42[c]	6.32±.22[b]	4.41±1.19[a]
Kulumsa(Bekoji)	3.18±.57[a]	5.61±.22[a]	4.85±0.61[b]

Means ± standard deviation with different letters after data within a column represents significant differences at 95% probability level indicated value is significantly different at P=0.05, CW= color of wort; PHW =PH of wort; and SPC=soluble protein content.

4.3.5. Diastatic power

The variation for Diastatic power was not significant ($P<0.05$, Table 4.11) between locations. The mean value for locations was higher at Ankober (372.01WK) than Holeta and Bekoji (370.69 and 352.97WK) respectively. The Diastatic power at Ankober (372.01WK)was highest. The lowest Diastatic power was observed at Bekoji (352.97WK).Diastatic power, the total activity of starch degrading enzymes in barley malt, is considered to be an important quality characteristic for malting and brewing (Hayter and Allison, 1995). The conversion of barley into beer represents mankind's oldest and most complex example of applied enzymology. Indeed, historically some of the most significant advances in enzymology have been linked to the world of brewing, such as Eduard Buchner's extraction of enzymes from brewing yeast (Buchner, 1897) and Adrian Brown's kinetic analysis of invertase (Brown, 1902).

The analysis results showed that, there was no significant difference ($P<0.05$) among varieties for Diastatic power content. The mean Diastatic power content of the varieties ranged from (288.80-428.60WK) (figure 4.1). The highest malt Diastatic power content was for MB16 (428.60WK), while the least was observed in MB11 (288.80WK).The desirable range for diastatic power is 90-110°L or 200-300WK for 2-rowed cultivars and 90-120°L for 6-rowed

ones. The mean values of DP were not in optimum range. Mean DP value was however higher compared to the standard. The development of improved varieties for quality purposes always requires identification of important traits affecting quality. In this study the result were showed higher which indicate active enzymatic activity for fermentation to be fast.

4.3.6 Free amino nitrogen

The analysis results showed that, there was no significant difference (P<0.05, Table 4.11) between location. The free amino nitrogen value in location Holeta were highest (275.29ppm) and the lowest value were kulumsa (bekoji) (235.71ppm). The analysis results showed that, there was no significant difference (P<0.05) among varieties for FAN.the vale was ranged (223.48 - 357.06 ppm). The variety MB7,MB14,MB9(357.06,294.59, 280.86 ppm) were highest in free amino nitrogen content. High FAN Value is considered to be a good index for potential yeast growth and fermentation. Protein modification also involves the production of wort amino acids and small peptides (dipeptides and tripeptides), collectively known as free amino nitrogen (FAN). Adequate levels of FAN in wort ensure efficient yeast cell growth and, hence, a desirable fermentation performance.MB1,MB6,MB8 were lowest in FAN Value(229.27,223.48,230.5ppm) Enari in 1975 concluded that barley variety, nitrogen content and the malting technique all influence the FAN level of the wort generally the specifications for a normal fermentation require FAN levels between 140-160 mg/L(250-400ppm).in this study the FAN value have comparable value in the standard requirement indicating rich in free amino nitrogen content for yeast nutrition.

4.3.7. Kolbach index

The variation for kolbach index was not significantly different (P<0.05, Table 4.11) between locations. The mean value for locations was 54.28%, 40.6% and 36.06% which were at Bekoji ,Holeta and Ankober respectively.Kolbach index had been thought sufficient for monitoring protein modification (Juskiw *et al*., 2009), with lower grain protein and potential for higher malt extract considered by Canadian standards.

The Kolbach Index (KI) is an important parameter that provides information on the level of protein modification (breakdown) that has occurred during the malting and mashing process. The analysis results of KI was not significantly different (P<0.05, figure 4.3) among varieties. The means of Kolbach was varied between 32.92-47.55%. The highest was for variety MB13 and MB14 which were the standard check. lowest value were for MB1(32.92)respectively.According to Altunkaya et al. (2001) Kolbech index that gives the degree of solubility of barley protein during malt production should be between 39-44%.but in this study the Kolbach index was above the range this is the result of high modification in malt protein.

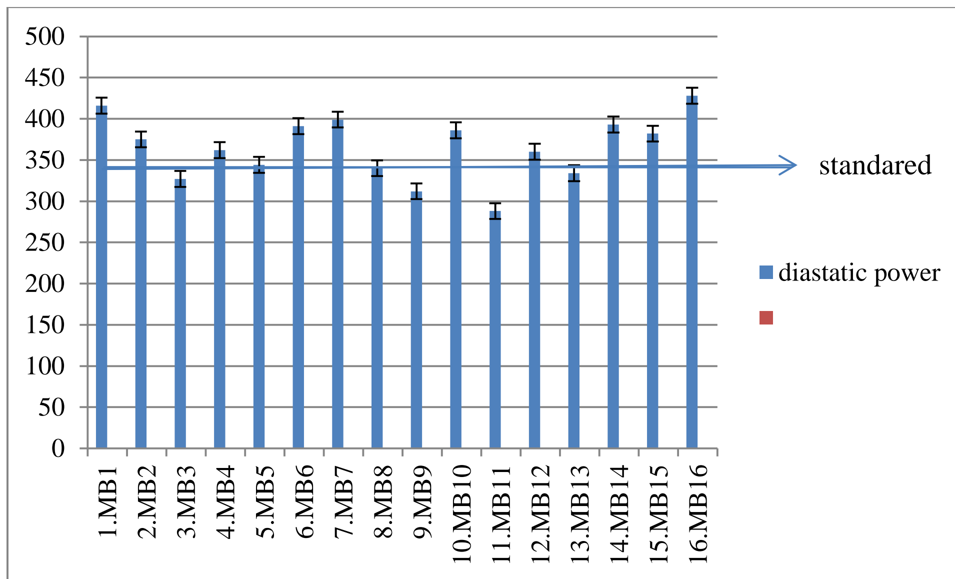


Figure 4.1.diastatic power in the barley malt (wort)

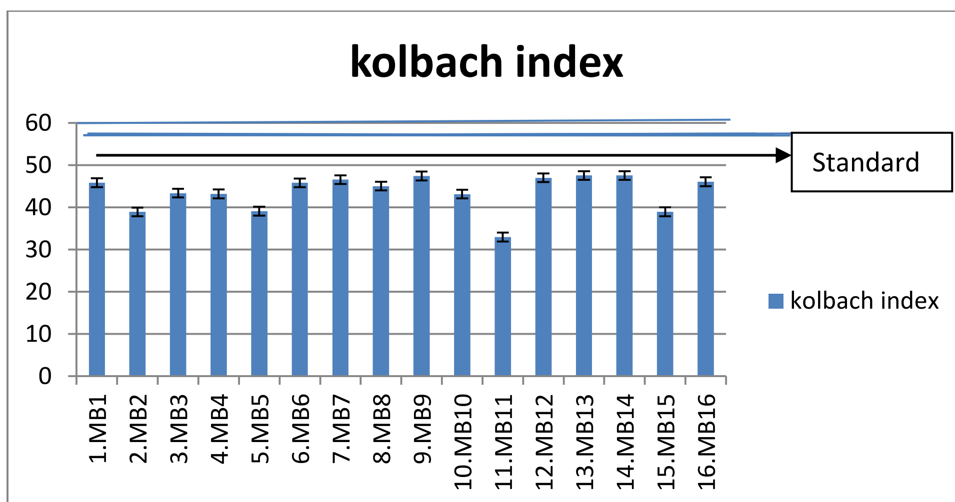


Figure 4.2.kolbach index.

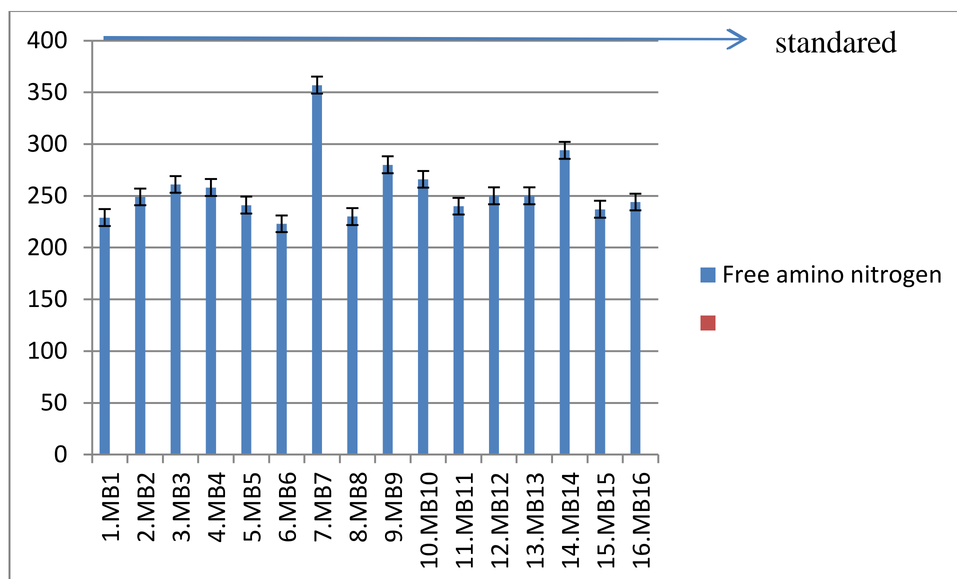


Figure 4.3.Free amino nitrogen in wort.

Table 4.11. effect of location on Diastatic power , Freeamino nitrogen and Kolbach index.

Location	Malt quality parameter		
	Diastatic power	Freeamino nitrogen	Kolbach index
Holeta	370.69±57.68[a]	275.29±54.06[b]	40.60±5.46[b]
Debrebrhan(Ankober)	372.01±69.77[a]	260.90±40.57[b]	36.07±8.7[a]
Kulumsa(Bekoji)	352.97±49.87[a]	235.71±49.74[a]	54.28±6.76[c]

Means ± standard deviation with different letters after data within a column represents significant differences at 95% probability level indicated value is significantly different at P=0.05, DP= Diastatic power ; FAN =free aminonitrogen; and KI=kolbach index.

4.3.8 Zinc content

The analysis results showed that, there was no significance difference ($P<0.05$) among the variety for zinc content in the wort. The mean value for variety MB1 and MB4 was higher in zinc content 5.07mg/L, 5.08mg/L respectively. The lower value in zinc content was for variety MB9 and MB3 1.72mg/L,1.75mg/L respectively. The zinc content was ranged 1.72-5.08 mg/L. Zinc ions are essential for an effective and vigorous fermentation. The presence of zinc is essential for the structure and function of many enzymes, where it can be involved in the active site (zinc-metalloenzymes). However, zinc can inhibit yeast growth and fermentation at higher concentrations under certain circumstances. Zinc additions during fermentation have also shown to increase the levels of higher alcohols and esters but to reduce acetaldehyde levels. Volatile organic compound levels were higher, this may however also cause an increase of medium fatty acids responsible for undesired soapy, fatty and rancid tastes (Nicola, 2009).

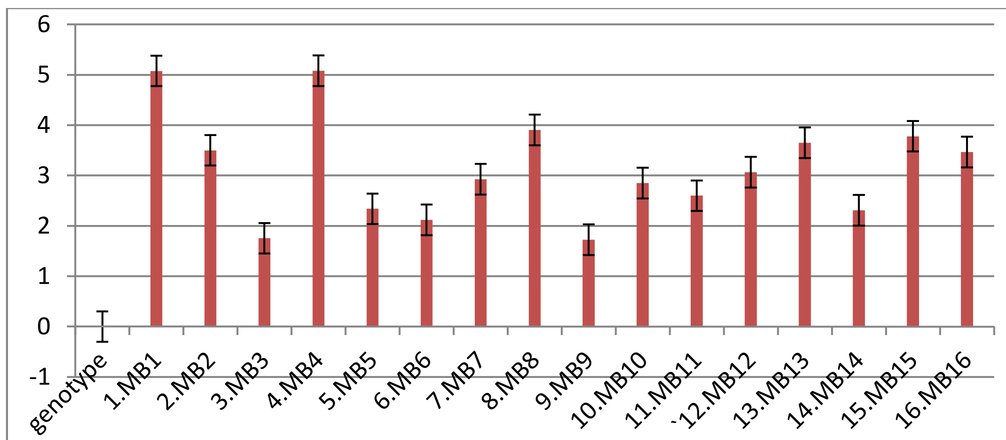


Figure 4.4.zinc content in wort.

4.3.9. Calcium content

The analysis results showed that, there was no significance difference ($P<0.05$) among the variety for calcium content in the wort.MB12(742.0 mg/L) was higher in calcium content among the variety and MB11(475.75 mg/L) was lower in calcium content. The calcium content was ranged 475.75-742.0 mg/L. Calcium ions play a critical role in the brewing process, as calcium has been shown to increase total soluble nitrogen and free amino nitrogen levels in wort, improve wort runoff, improve wort clarification and protein coagulation, and has also been shown to increase yeast flocculation and growth. Calcium can here provide a cheap, effective and environmental friendly way to remove most of the yeast cells out of the green beer. It has been suggested that Ca^{2+} binds to flocculin proteins and provides them with the correct structural confirmation to form carbohydrate bindings (Vidgren, 2011). In this study the calcium content was higher value compared to metal ion concentrations found in other studies.

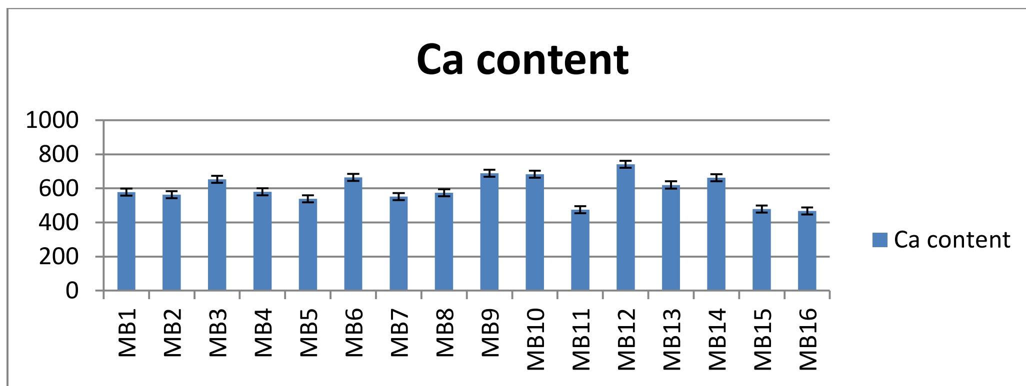


Figure 4.5.calcium content in wort.

5. Conclusion and Recommendation

5.1. Conclusion

In general, The study showed that, growing locations had a significant effect for grain quality (grain size, germination energy, moisture content, thousand grain weight, hectoliter weight, protein content). Except germination energy there was no significance difference among varieties. The malt quality parameters, fine grind hot water extract, coarse grind hot water extract, extract difference, color of wort,PH of wort,malt moisture content, malt protein content, soluble protein content, kolbach index, friability, diastatic power, zinc and calcium content were not significantly different among the varieties. malt quality traits such as diastatic power ,friability, soluble protein content, kolbach index and Free amino nitrogen were not significant difference across the location .

The result of this study showed that the varieties MB1,MB3,MB5,MB7,MB9,MB10 and MB4 were acceptable grain quality (grain size, germination energy, moisture content, hectoliter weight, thousand kernel weight, protein content) and malt quality (extract amount, malt protein content, PHof wort, soluble protein, kolbach index ,free amino nitrogen, diastatic power ,Zinc and calcium content) results compared to the standard checks (MB13,MB15,MB14 and MB16).These varieties will be usefull for the breeding program in the future development of malt barley Varities. The result of this study also showed that among the three locations Kulumsa(Bekoji) was very suitable for quality malt barley production as the grain and malt quality traits are in the acceptable range followed by Dbrebrhan (Ankober).

5.2 Recommendation

Generally, the findings of this study will contribute by providing malt quality data for the sixteen advanced malt barley varieties for further evaluation and use by the breeding program.Similar studies are required in the potential regions of the country to identify suitable malt barley growing environments for increased malt barley production and to solve the demand and supply gap of malt barley as raw material for the beer industry. Continuous research will be needed for improved malt barley generation and development in the country.

6.Reference

Aaron, L., MacLeod, D., Langrell, E. and Michael, J. Edney,. (2014). Comparison of harvest and export surveys of Canadian malting barley quality. *Canadian Journal of plant science.*94: 587_592.

Aastrup, S., Gibbons, GC., Munck, L. (1981). A rapid method for estimating the degree of modification in barley malt by measurement of cell wall breakdown. *Carlsberg Research Communications.* 46: 77–86.

Agu, R.C., Brosnan, T.,Bringhurst, A.,Palmer, G.H. and Jack, F.R.. (2007). Influence of corn size distribution on diastatic power of malted barley and its impact on other malt quality parameters. *Journal of Agricultural and Food Chemistry*, 55:3702-3707.

Andersson, AAM., Elfverson, C., Andersson, R., Regner, S., Aman ,P. (1999). Chemical and physical characteristics of different barley samples.79:979–86.

AOAC, Association of Official Agricultural Chemists. (1990). Official Methods of Analysis. Arlington, Virginia, USA.

Arends, AM., Fox, GP., Henry, RJ., Marschke RJ., and MH Symons. (1995). Genetic and environmental variation in the diastatic power of Australian barley. *Journal of Cereal Science* .21:63-70.

American Society of Brewing Chemists. Report of Subcommittee on Atomic Absorption Spectrophotometer. *Journal* 39"89, 1981.

Anonymous,. (2012). Progress report of All India coordinated wheat and barley improvement project. 2011-12. Vol. VI.Barley Network. Directorate of Wheat Research,Karnal, India..

Allosio-Ouarnier, N., Quemener, B., Bertrand.D, Boivin,P. (2000). Application of High Performance Anion Exchange Chromatography to the Study of Carbohydrate Changes in Barely during Malting. *Journal of Institute of brewing*, 106: 45-52.

Bamforth, CW. (2006). Scientific principles of malting and brewing. *American Society of Brewing Chemists*.

Baik, BK., Ullrich, SE. (2008). Barley for food characteristics improvement and renewed Interest Critical review. *Journal of Cereal Science.* 30:1–10.

Bamforth, CW. (1982). Barley *β*-glucans: their role in malting and brewing. Brew 3:22–35.

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.

Bendelow, V. (1977). Malting quality selection methods in Canadian barley breeding programs. *Journal of American society of brewing chemist* 35: 81-85.

Berhane,L. (1991). Barley A Dependence Cereal in Ethiopia, IAR, News Letter Agricultural Research (2):3.

Berhane, L., Hailu, G. and Fekadu, A., (1996). Barley Production and Research in Ethiopia. pp.1-8. In: Hailu Gebre and A.G. Van lure Joop (ed.) Barley research in Ethiopia Past Work and Future Prospect. IAR, Addis Ababa, Ethiopia.

Benech-Arnold, R L., Giallorenzi ,M C., Frank ,J. , Rodriguez, V. (1999). Termination of Hull-imposed Dormancy in Developing Barley Grain is Correlated in Change With Embryonic ABA Levels and Changes. Seed Science Research., 9: 39-47.

Brennan ,CS., Harris ,N., Smith, D., Shewry, PR..(1996). Structural differences in the mature endosperms of good and poor malting barley cultivars. *Journal of Cereal Science* 24: 171–177.

Boulton, C., Quain, D.(2001). Brewing yeast and fermentation. Oxford UK Blackwell Science.

Brown, A. (1902). Enzyme action. *Jornal of Chemical Society* 81:373–88.

Buttimer ,ET., Briggs, DE. (2000). Mechanisms of the release of bound beta-amylase. *Journal of institute of brewing* 106:83–94.

Bregitzer, P., Mornhinwig D., and Jones, B. (2003). Resistance to Russian wheat aphid damage derived from STARS 9301B Protects agronomic performance and malting quality when transferred to adapted barley germplasm. *Crop Science*, 43: 2050-2057.

CSA (Central Statistical Authority), 2008. Agricultural Sample Survey for 2007/08: Report on Area and Production of Crops for Private Peasant Holdings, Addis Ababa, Ethiopia. 13pp.

Chapon, L., Maucourt ,JM., Gobert, J. (1979). The friabilimeter an apparatus for objective measurement of malt friability. 32: 160–167.

Chandra, GS., Proud love, MO., Baxter, ED. (1999). The structure of barley endosperm an important determinant of malt modification. *Journal of Food science and Agricalture* 79:37–46.

Cohen, D., Paleg, LG. (1967). Physiological effects of gibberellic acid. X. The release of gibberellins like substances by germinating barley embryos. *Plant Physiology* 42:1288–1296.

Eglinton ,J K., Langridge, P., Evans, D E. (1998). Thermo stability Variation in Alleles of Barley Beta-amylase. *Journal of Cereal Science,* 28: 301-309.

Edney, M.., MacLeod,A. , LaBerge, D. (2014). Evolution of a quality testing program for improving malting barley in Canada. *Canadian journal of plant science.* (2014) 94: 535_544

Davies, NL. (1989). Patterns of hydration in barley endosperm during steeping and their relation to malting performance. Proceedings of the 22nd European Brewery Convention Congress. Oxford University Press, 221–228.

Dombrink, MA., Knutson, CA. (1997). A study of maize endosperm hardness in relation to amylose content and susceptibility to damage. *Cereal Chemistry* 74:776–80.

Edney, M J. (1999). Canadian malting barley varieties for the future. MBAA Tech. Q. 36: 359-363.

Edney, M. J., O'Donovan, J. T., Turkington, T. K., Clayton, G. W., McKenzie, R., Juskiw, P., Lafond, G. P., Brandt, S.,Grant, C. A., Harker, K. N., Johnson, E. and May, W. (2012). Effects of seeding rate, nitrogen rate and cultivar on barley malt quality. J. Sci. Food Agric. 92: 2672-2678.

Edney, MJ. and Mather, DE. (2004). Quantitative trait loci affecting germination traits and malt friability in a two-rowed by six-rowed barley cross. *Journal of Cereal Science* 39:283-290.

Edney, M.J. and D.E. Langrell, (2004). Evaluting the malting quality of hulless CDC down, acid-dehusked harington and harington barley. *Journal of American Society of Brewing Chemist*, 62(1): 18-22.

Emebiri, L C., Moody, D B., Black, C., Van Ginkel, M., Hernandez, E. (2007). Improvement in Malting Quality Barley Grain Yield By Manipulation of Genes Influencing Grain Protein Content. 156: 185-194

Etokakpan, OU. (1993). Enzymatic degradation and nature of the endosperm cell-walls ofgerminating sorghums and barley. *Journal of Science and Food Agriculture* 61:389–93.

Evans, D.E., Lance, R.C., Elington, S.J., Logue and Barr, A.R (1995). The Influence of β-amylase isoform pattern on β-amylase activity in barley and malt. Proceeding of 45th Australian Cereal Chemist Conference, Adelaide, 357-364pp.

Evans,D. E., Harasymow, S., Roumeliotis, S. and Eglinton, J. K. (2009). Improved prediction of malt ferment ability by measurement of the diastatic power enzymes betaamylase, alpha-amylase, and limit dextrinase.

Evans, D.E., H.Collins, J.Eglinton, and Wilhelmson, A.,(2005). Assessing the impact of the level of diastatic power enzymes and their thermostability on the hydrolysis of starch during wort production to predict malt fermentability. *Journal American Society of Brewing Chemists,* 63(4):185-189.

FAO, 2006. Food and Agriculture Organization of the United Nations online database. http://faostat.fao.org. Retrieved on 2009-09-10.

Ferrio, J P., Alonso, N., Voltas, J., 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

Fox, G P., Kelly, A M., Poulsen, D M E., Inkerman, P A., Henry, R J. (2006a) Genetic and Environmental Effects on Selecting Improved Barley Grain Size in Dry Environments. *Journal of Cereal Science*, 43: 198-208

Fox,G. (2010). Chemical composition in barley grains and malt quality, in Genetics and Improvement of Barley Malt Quality, (Zhang, G. and Li, C., Eds) pp. 63–98, Springer, Berlin.

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.

Fox, GP.,Kelly,J.G., Bowman, P.A., Inkerman, D.M.E., Poulsen and Henry, R.J (2007). Relationship between malt and feed quality in barley (*Hordeum vulgare). Journal of the Science of Food and Agriculture*, 46: 1318-1322

Fox, GP., (2008). Biochemical and molecular evaluation of quality for malt and feed barley. PhD thesis, Southern Cross University, Lismore, NSW.

Fridissa, E.,Woldeyeseus,S., 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.

Getachew, L., Sentayehu,D., Tolosa, A., (2007). assessing the uncompanative advantage of malt barley production in Ethiopia.vol.8.pp.1227-1230.

Gunkel ,J., Votez, M., Rath, F.(2002). Effect of the malting barley variety (*Hordeum vulgare L.*) on fermentability. *Journal of institute of brewing*. 108:355–61.

Guerrero, B.G., (2009). Effects of amino acid profile, endoprotease activities and wort quality on fermentability under different malting and brewing conditions. A Thesis presented to the Faculty of Graduate Studies of the University of Manitoba. 227p.

Gibson, T.S. and Solah, V. (1995). Diastatic power in malted barley: contributions of malt parameters to its development and the potential of barley grain β-amylase to predict malt diastatic power. *Journal of the Institute of Brewing*, 101: 277-280.

Glennie Holmes, M .(1995). Studies on Barley and Malt with the Rapid Viscoanalyser I: the Effect of Variations in Physical and Chemical Parameters. *Journal of institute of brewing* . 101: 11-18.

Goode, DL., Ulmer ,HM., Arendt, EK. (2005). Model studies to understand the effect of amylase additions and pH adjustment on the rheological behavior of simulated brewery mashes. *Journal of institute of brewing* .111:153–64.

Hardwick, WA. (1995). Handbook of brewing. New York, Marcel Dekker Inc.

Hassona, HZ. (1993). High fibre bread containing brewers spent grains and its effect on lipid metabolism in rats. Die Nahr 37:576–82.

Hanning, Chuck.(2009). Yeast and Fermentation. *BJCP Exam Study Guide.* April 15, 2009

Harlan, J.R., (1978). On the Origin of Barley. U.S. Department of Agriculture, Washington, DC. Pages, pp: 10-36.

Harrison,T J. (1929). A basis for the valuation of malting barley. Science and Agricalture. 9: 599_609.

Harlan,J.R. and Zohar, D. (1966). Distribution of wild wheats and barleys. Science, 153: 1074-1080.

Henry, RJ. (1988). Changes in β-glucan and other carbohydrate components of barley during malting. *Journal of Science and Food Agriculture*. 42:333–41.

Henry, R.J., (1986). Genetic and environmental variation in the pentosan and β-glucan contents of barley and their relation to malting quality. *Journal of Cereal Science* .4, 269-277.

Hoseney, RC. (1994). Principles of cereal science and technology. 2nd ed. U.S.A.*: American Assn. of Cereal Chemists*, Inc. p 179–82.

Home,S., Stenholm, K., Wilhelmson, A., Autio, K. (1999). Properties of starch and cell wall components and their effects on processing. In: Proceedings of 9th Australian Barley TechnicalSymposium, (2009), September 12–16; Melbourne, Australia. p 2.37.1–2.37.8.

Jones, BL.(2005b). The endogenous endoproteinase inhibitors of barley and malt and their roles in malting and brewing. *Journal of Cereal Science*. 42:271–80

.

Jones ,BL., Budde, AD. (2005). Various malt endoproteinase classes affect wort soluble protein levels. *Journal of Cereal Science.* 41:95–106.

Jorgensen, KG., Jensen, SA., Hartley, P., Munck, L. (1985). The analysis of *B*-glucan in wort and beer using Calcofluor. *Proceedings of the 20th European Brewery Convention Congress. Helsinki .1985*.403–410.

Juskiw, P. E., Helm, J. H., Oro, M., Nyachiro, J. M. and Salmon, D. F. (2009). registration of 'Bentley' barley. *Journal of Plant Registrations* 3: 119-123.

LaBergc, D. E. and B.A. Marchylo, (1983). Hetrogenity of the β-amylase enzymes of barley. *American Society of Brewing Chemists Journal*, 41:120-124.

Li, J., Kaneko ,T., Qin, LQ., Wang, J., Wang, Y. (2003). Effects of barley intake on glucose tolerance, lipid metabolism, and bowel function in women. Nutrition. 19:926–9.

MacGregor,A.W., (1987). α-Amylase, limit dextrinase and α-glucosidase enzymes in barley and malt. *CRC Crtitical Reviews in Biotechnology*, 5:117-128.

MacNicol, P K., Jacobsen, J V., Keys, M M., Stuart, I M. (1993) .Effects of Heat and Water Stress on Malting Quality and Grain Parameters of Schooner Barley Grown in Cabinets. *Journal of Cereal Science*, 18: 61-68

Michael Brophy, (2014). quality factors in malting barley , Canada brewing and malting barley research institute ,vol. 204-927-14014640.

Molina-Cano, J.L., M. Francesch , A.M.Perez-VendrelL , T.Ramo, J. Voltas and J.Brufau, (1997). Genetic and environmental variation in malting and feed quality of barley. *Journal of Cereal Science,* 25: 37-47.

Moll, M. (1979). Water in malting and brewing. In: Pollock JRA, editor. *Brewing science*. Vol.1. London: Academic Press. p 1–327.

Newman, R.K. and C.W. Newman, (2008). Barley for Food and Health: Science, Technology and Products. John Wiley & Sons.

Newman, RK., Lewis, SE., Newman, CW., Boik, RJ., Ramag, RT.(1989). Hypocholesterolemic effectof barley foods on healthy men. 39:749–60.

Newman, CW., Newman, RK. (2006). A brief history of barley foods. *Cereal Foods World* 51(1):4–7.

Nicola, R.D.,(2009). Influence of zinc on distillers's yeast. cellular accumulation of zinc and impact on spirit congeners. *Journal of institute of brewing*.115(3): p. 265-271.

Ogushi, K., Lim ,P., Barr, AR., Takahashi ,S., Asakura ,T., Ito K. (2002). Japanese barley meets Australia quality performance of malting barley grown in different countries. *Journal of institute of brewing*.108:303–9.

Okada, Y., Iimure, K., Takop, T., Kaneko, M., Kihara, K., Hayashi, K., Ito, K., Sato and K.Takeda, (2008). The influence of barley malt protein modification on beer foam stability and their relationship to the barley dimeric α-amylase inhibitor-I (BDAI-I) as a possible foam promoting protein. *Journal of Agricultural and Food Chemistry*, 56:1458-1464.

Osman, A M., Jersey, J. , Inkerman, P A. (1996b). the Specific Measurement of Starch Degrading Enzymes in a Common Extract from Barley Malt. In: Wrigley C W (eds.) Proc of the 46th Australian Cereal Chemistry Conference. Royal Australian Chemical Institute, Melbournea, 208-212.

Passarella, V S., Savin, R., Slafer, G A. (2005). Breeding Effects on Sensitivity of Barley Grain Weight and Quality to Events of High Temperature during Grain Filling.Euphytica, 141: 41-48

Paynter, B.H., and. Young, K.J. (2004). Grain and malting quality in two row spring barley. *Australian Journal of Agricultural Research.* 55(5): 539–50.

Peter,W., Gous, Robert, G., Gilbert and Glen, P. (2014.)Drought-proofing barley (*Hordeum vulgare*)and its impact on grain quality. *Institute of brewing*.2015; 121: 19–27.

Peltonen, J., Rita, H., Aikasalo, R., Home, S. (1994). Hordein and malting quality in northern barleys. *Hereditas* 120:231–9.

Plankinton,Ave., Milwaukee (2014). malting barely quality requirements, American Malting Barley Association, Inc.vol. (414) 272-4640.

Pomeranz, Y. (1974). Food uses of barley. *Critical Review Food Technology*. 4:377–94.

Polakova, K., D. Laurie, K. Vaculova and J. Ovesna, (2003). Characterization of β-amylase alleles in 79 barley varieties with Pyrosequencing. *Plant Molecular Biology Reporter,* 21: 439–447.

Psota, V., Vejrazka, K., Famera O., Hrcka, M. (2007). Relationship between grain hardness and malting quality of barley (Hordeum vulgare L.). Journal of institute of brewing. 113:80–86.

Roumeliotis, S., Collins, H M., Logue, S J., Willsmore, K L., Jefferies, S P., Barr A R .(1999) Implication of Thin Husk. In Proceedings of the 9th Australian Barley Technical Symposium.

Satue Gracia, M., Heinonen, IM., Frankel, EN. (1997). Anthocyanins as antioxidants on human Low density lipoprotein and lecithin-liposome systems. *Journal of Agricalture and Food Chemistry*. 45:3362–7.

Schwartz, P, Li, Y. (2011). Malting and brewing uses of barley. In Ullrich SE, ed. Barley: Production, Improvement and Uses. Chichester, UK: Wiley-Blackwell.478–521.

Shewry, PR. (1993). Barley seed proteins. In: MacGregor AW, Bhatty RS, editors. Barley: chemistry and technology. St. Paul, Minn., U.S.A.: *American Assocation of Cereal Chemists* .131–97.

Schmitt, MR., Marinac, L. (2008). Beta amylase degradation by serine endoproteinases from green barley malt. *Journal of Cereal Science*. 47:480–8.

Shewry, P.R. and Morell, M. (2001). Manipulating cereal endosperm structure, development and composition to improve end use properties. *Advanced Botany Research*, 34:165-234.

Swanston, JS., Ellis, RP., Stark ,JR. (1995). Effects on grain and malting quality of genes altering barley starch composition. *Journal of Cereal Science*. 22:265–73.

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.

Tamm, U. (2003).The variation of agronomic characteristics of European malting barley varieties. *Agronomy Research.* 1: 99–103.

Uhlen, K. A., Hafskjold, A.,Kalhovd, M., Sahlstro, A. ,Langva and Magnus, (1998). Effect of cultivars and temperature during grain fills on wheat protein content, composition, and dough mixing properties. *Cereal Chemistry* 75: 460-465.

Vickers, J E., Hamilton, S E., De Jersey, J. , Henry, R J. , Marschke, R J. , Inkerman ,P A. (1996). Assessment of Bacillus Licheniformis ,8-amylase as a Candidate Enzyme for Genetic Engin eering of Malting Barley. *Journal of institute of brewing*.102: 75-78.

Vidgren, V. and Londesborough,J.(2011)125th Anniversary Review: Yeast Flocculation and Sedimentation in Brewing. *Journal of institute of brewing* .117(4). 475-487.

Walker, C., Dickie, K., Biawa, J P., Ueda ,T. , Muller, R E. (2001). Prediction of Extract Potential in New Barley Cultivars By Measuring Cellwall Breakdown. *Journal of institute of brewing* . 107: 167-174.

Wang, J. M., Chen, J. X., Dai, F., Wu, F. B., Yang, J. M., and Zhang, G. P.(2007). Protein fractions in barley grains as affected by some agronomic factors and their relationships to malt quality. 53, 129–140.

Wang, J.M., J.X. Chen, F. Dai, F.B. Wu, J.M. Yang and G.P. Zhang, (2007). Protein fractions in barley grains as affected by some agronomic factors and their relationships to malt quality. *Cereal Research Communications*, 35:129-140.

Woonton, B.W., Jacobsen, F., Sherkat and. Stuart,I.M , (2005). Changes in germination and malting quality during storage of barley. *Journal of Institute of Brewing,* 111(1): 33–41.

Yaldagard, M., S. A., Mortazavi and F.Tabatabaie, (2008). Influence of ultrasonic stimulation on the germination of barley seed and its alpha amylase activity. *African Journal of Biotechnology*, 7 (14): 2465-2471.

7. Appendices

Appendices 1.photo depicting sample preparation and malting in Asela malt factory**.**

a) Barley plantation in Holeta **b)Malting in AMF**

c) Malt

Appendix 2 Photo taken in the laboratory.

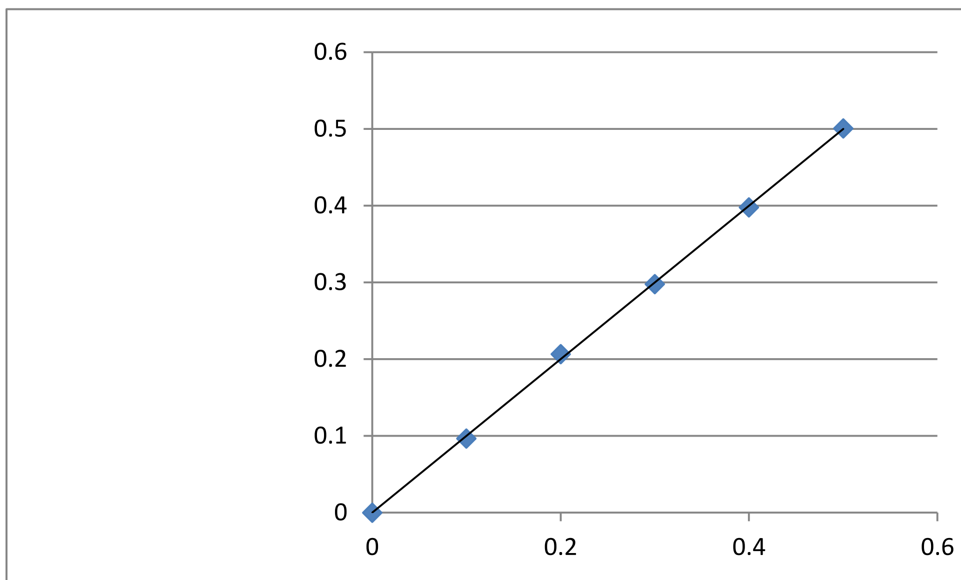
a)digestion for protein determination. B) distillation for protein determination

Appendix 3. Photo taken in wort extraction.

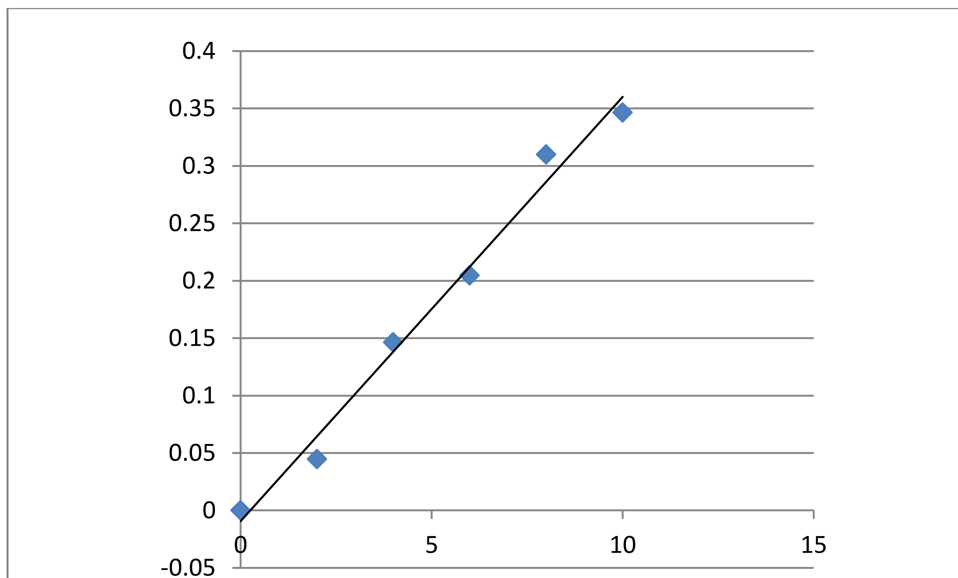
c) Wort formation d) extract determination

e) Wort for soluble protein content determination. d) wort for free amino nitrogen

Equation for zinc determination $Y=0.40x-0.0021, R^2=0.997$



Appendix 4.Calibration curve for zinc determination in wort sample, absorbance vs concentration.



Equation for calcium determination $y=0.038655x+0.02269, R^2=0.9914$

Appendix 5.Calibration curve for calcium determination in wort sample absorbance vs concentration.

DECLARATION

I, the undersigned, declare that this thesis is my original work and has not been presented for any degree in this or any other institution and that all sources of materials used in this thesis have been duly acknowledged.

Candidate:____________________________ signature____________________-

This thesis has been submitted for examination with my approval as a university advisor. in addition, I declare that this thesis is the original work of my student and has been done under my supervision.

Advisors Name: signature

1.______________________________ __________________

2.______________________________ __________________

Approved by the examining Board

Name signature

1._________________________________ ___________________

2._________________________________ ____________________

3._________________________________ _____________________

4._________________________________ _____________________

Place and Date of Submission: Addis Ababa University School of Graduate studies

Center for food science and Nutrition

Addis Ababa, Ethiopia

June, 2016