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**DEPARTMENT OF CHEMICAL ENGINEERING**

**Optimization of beer production using Maize and different types of barley**

A thesis Submitted to Graduate School of Addis Ababa University, Institute of Technology, Department of Chemical Engineering in partial fulfillment of the requirements for the attainment of the Degree of Masters of Science in Chemical Engineering under Process Engineering stream.

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## Acronyms and Notations

| | |
|---|---|
| A.A | Apparent attenuation |
| AMF | Asella Malt Factory |
| ANOV | AAnalysis of variance |
| BNRP | National Maize Research Project |
| CMC | Carboxymethyl cellulose |
| CSA | Central Statical Agency |
| DCFR | Discounted Cash Flow Return |
| DMS | Dimethylsulfide |
| EDTA | Ethylene di-amine tetra acetic acid |
| F G | Final gravity |
| FAN | Free amino nitrogen |
| GT | Gelatinization temperature |
| MARC | Melkasa Agricultural Research Center |
| OE | Original extract |
| OG | Original gravity |
| OPD | O-phenyl diamine |
| SG | Specific gravity |
| TBN | Thiobarbituric acid number |
| VDK | Vicinal diketones |
| WK | Windisch-kolbacha |

## *Abstract*

*The cereals used in beer production undergo modification during the malting and mashing steps to yield carbohydrates that yeast can convert during the fermentation step into ethyl alcohol and carbon dioxide. In particular highest yield of extract (fermentable sugar) and enough free amino nitrogen as yeast nutrient must be modified. However, in addition to reducing costs, the use of unmalted cereals such as maize contributes palatable flavors to the beer. This study involved the optimization of beer production from maize and different types of barley to reduce the cost and improve the flavor of the beer. Brewing is a critical operation and takes several steps. In connection with various levels of steps the mashing step which is used to produce wort or fermentable sugars solution is the limiting step because the biochemical and physicochemical transformations of starchy biomass occur as a function of operating conditions. At this step full factorial design with three factors, three levels and three replicates ($3*3^3$ or 81 experimental runs) was applied to optimize wort production and to study the interaction of the three factors , namely, maize percentage mashing temperature and mashing time with the response variables which were highest yield of extract and targeted free amino nitrogen. After the optimization of wort had completed at mashing step, beer was produced by boiling wort with hop substance and fermented to give alcohol and $CO_2$. During fermentation the fermentation diagram was prepared and necessary parameters was measured. Once the beer was prepared the final product was characterized and the result of analysis was compared with other brands of brewery product produced in the country and the product obtained was tolerated. Finally the financial analysis and economic evaluation was done and the cost benefit obtained from this project was positive.*

# CHAPTER ONE

# INTRODUCTION

## 1.1. Background

The price of barley to produce beer is now in the world and Ethiopia is exponentially increasing because of the demand of the product. It is very important to find the other starch source grain to solve this problem. Ethiopia has a huge population size (second largest in Africa, 14 largest in the world) and a very young demographic (44 percent of the population is under 15 and 73 percent under 30) which stands out favorably from the consumer goods space perspective.

There are now nine beer companies in Ethiopia, with production capacities of 10.5 million hectoliters, including Zebidar Brewery's expected production capacity of 350,000 hectoliters. According to the Ministry of Industry, Ethiopia's per capita consumption of beer stands at eight liters, and it is expected to reach 10 by the end of 2015/16. According to central statics agency of Ethiopia the exponential increase of beer demand put a pressure on the supply of barley.

Ethiopia is ranked twenty-first in the world in barley production with a share of 1.2 percent of the world's total production. Barley cultivation is widely distributed across the country on over one million hectares of land and by more than four millions small holder farmers. Currently, it is grown exclusively for the domestic market and is neither imported nor exported. Barley is a high-opportunity crop, particularly when connected with the country's commercial brewing and value-added industries. There are two varieties of barley in Ethiopia: food barley for human consumption and malt barley which can be converted into malt.

The market potential for malt barley in Ethiopia is directly related to market demand for beer, which has shown significant growth in terms of consumption. From 2003 to 2011, beer production in Ethiopia increased from 1 million hectoliters to roughly 4 million hectoliters. Malt barley is the major raw material (about 90 percent of the total raw material cost) for beer production. Malt barley grain is mainly produced in the southeastern part of Ethiopia in the Arsi and Bale administrative zones. The total estimated demand for malt barley in 2012/13

was around 72,000 tons, of which 35 percent is supplied from local barley farms. The remaining amount of malt barley is imported from Belgium and France. Asella Malt Factory (AMF), a state-owned facility, was for a long time the only malt processing factory in the country supplying malt to four local breweries. It is very important to find other starch source grain as alternative for beer production. In this context or paper research maize is selected as additional raw material to replace some portion of barley.

In Ethiopia, maize grows under a wide range of environmental conditions between 500 to 2400 meters above sea level. Maize is Ethiopia's leading cereal in terms of production, with 6 million tons produced in 2012 by 9 million farmers across 2 million hectares of land (CSA 2011/12). Currently, maize is the cheapest source of calorie intake in Ethiopia, providing 20.6 % of per capita calorie intake nationally (IFPRI, 2013). Moreover, the crop has potential uses for industrial purposes, serving as a starch, a sweetener for soft drinks, an input for ethanol fuel production and oil extraction, etc.

Due to the lower price of maize compared to other grains, the percapita consumption of maize is estimated to be 45 kg/year in rural areas and 16 kg/year in urban areas. As compared to other cereals, maize can attain the highest potential yield per unit area. World average yield for maize is about 4.5 t/ha and that of developed countries is 6.2 t/ha, with a harvest of 10 t /ha being common. The average yield in developing countries is 2.5 t/ha. However, estimates indicate that the current maize yield could be doubled if farmers adopt higher quality inputs and proven agronomy best practices.

Therefore, in this paper maize will be mixed with malt barley and the extracted sugar and free amio nitrogen to produce beer will be optimized. The main drawback of maize to produce beer is the high percentage of oil compared to other starch source grains. However this can be minimized by processing maize or separating oil part from the germ part before used to mash with malt barley.

## 1.2. Statement of the problem

Demand for beer is growing rapidly due to rising incomes and behavioral changes linked to urbanizations that are increasingly evident in Ethiopia. This is just beginning and will no doubt gather substantial momentum in the next five years. Malt barley is the major raw material for beer production which is mainly produced only in the southeastern part of Ethiopia in the Arsi and Bale administrative zones and this could not cover demand for malt barley. The price of barley malt is expensive in Ethiopia and in the world, while Adjuncts like maize can used to reduce the final cost of the recipe and/or improve beer's color and flavor/aroma**.** In other word maize released higher fermentable sugar in its extracts than barley, but the solubilization of nitrogen and hydrolysis of the soluble nitrogen were higher for high nitrogen barley than for low nitrogen barley and maize. This difference may be making for best fermentation potential.

## 1.3. Objectives

### 1.3.1. Main objective

The main objective of this paper is the production of beer from maize and different type of barley that has the same quality of beer made up of only two row barley

### 1.3.2. Specific objectives

The specific objectives of this paper is summarized as the following

1. To determine the physico-chemical characteristics of malted barley and maize
2. To optimize beer production by using malted barley and maize.
3. Characterization of beer produced from malted barley and maize
4. Cost analysis of beer production using maize and different type of malted barley

### 1.4. Significance of the study

The significance of this study is looked in different dimension. However some of the main point will be explained in the following.

- Maize and six row barley are used to reduce the overall material costs, and to utilize indigenous Sources of extract, especially when malted barley is not readily available.
- The problem of malt barley( specially two row barley) scarcity will be minimized
- Farmers will get wide opportunity of maize market and this will encourage the farmers productivity
- The cost to import malted barley will be saved.
- Breweries will get wide alternative malt and adjuncts to produce beer.
- The cost of beer will be decreased. This can increase the consumption of beer in the country.
- Adjuncts can be used to adjust the fermentability of a wort .There are a range of different syrups which can be added to the kettle with different sugar compositions.
- Maize has the effect of diluting malt flavors and allowing subtitle flavors from the fermentation and hops to be fully expressed. Maize tends to impart a fuller flavor to beer.

# CHAPTER TWO

## LITERATURE REVIEW

### 2.1. Introduction

Beer is defined as a fermented alcoholic beverage made of malted cereals, water, hops, and yeast. The cereals used in beer production do not contain sufficient quantities of fermentable sugars in the harvested state and must first undergo modification during the malting and mashing steps to yield carbohydrates that yeast can convert during the fermentation step into ethyl alcohol and carbon dioxide. Many countries allow additional substances to be used. For instance, expensive barley malt is often supplemented with less expensive unmalted cereals such as maize, rice, or wheat. In addition to reducing costs, the use of unmalted cereals contributes palatable flavors to the beer. Hops are the dried flowers from the female hop (Humulus lupulus) plant and contribute flavor and antibacterial compounds to beer. Yeasts are the predominant fermentation organisms used to make beer worldwide.[30]

It is consumed as a refreshing drink or tasting. Moderate consumption of beer, made with intelligence, is recommended. It can bring benefits in terms of health benefits, including by preventing and reducing the risk of cardiovascular disease, gallstones and stomach ulcers and protection of brain against mental decline due to aging. The beer is a tonic, nutritive, appetizer, digestive and sedative beverage. But its abuse is prohibited because at high content the ethyl alcohol contained in the beer may have toxic effects on some physiological processes and organs including the heart, brain, liver and kidney and promoting conditions of anemia-causing disease. This led some countries to encourage the production of low-alcohol beers. [30]

Brewing is a critical operation and takes the following steps: grinding the malt, mashing, wort extraction, cooking and cooling of the wort. In connection with various levels of mashing temperature, biochemical and physicochemical transformations of starchy biomass occur as a function of operating conditions. [13]

## 2.2. The main raw material for beer production

Four raw materials are required for bee production

- Malted grains
- Water
- Hops and
- Yeast

### 2.2.1. Malted grain

In most conventional breweries, the malted grains are primarily barley and wheat, with barley use greatly outdistancing wheat. Rye and sorghum are also malting in some cases. Other grains are primarily used as adjuncts. Adjuncts are selected by the brewer to best fit their brewing process. Wheat and rye either malt or not, are often used as significant contributors to the flavor profile of the final product. Other grains used as adjuncts, however, are typically included to provide a carbohydrate source for alcohol production, and in some cases to add body or foaming characters to the final product. Worldwide, most breweries use alternative starch sources (adjuncts) in addition to malted barley.

#### 2.2.1.1. Barley

Of all the cereal grains, barley is particularly well suited to brewing. It has a short germination period, which makes it good for malting. It has a high starch content that translates into higher yields of extracted sugar in the brewing process. Barley has a low protein content, which reduces haze in beer, and a high level of diastatic enzymes for starch conversion. The majority of barley used in brewing is malted, but it is also used raw or unmalted. Depending on the species of the barley, the plant will expose one or more kernel per node of the ear. Mainly, two species of barley are used in brewing: the two-row barley (Hordeum distichon species) and the six-row barley (Hordeum vulgare species) [9]. Six row barley (Hordeum vulgare ) with 3 spikelets are fertile and each spike produces 25-60 kernels. Two row spikelet arrangements appear to be in two columnsand each spike produces 15-30 kernels. To put it simple, the fewer are the kernels per node, the bigger and richer in starch they are. Conversely, the six-row barley has less starch but higher protein content. Therefore, if the brewer wants to increase the extract content, the two-row barley is the best option, whereas if enzymatic strength is the aim, the six rows will be the best choice (Wunderlich and Back 2009).

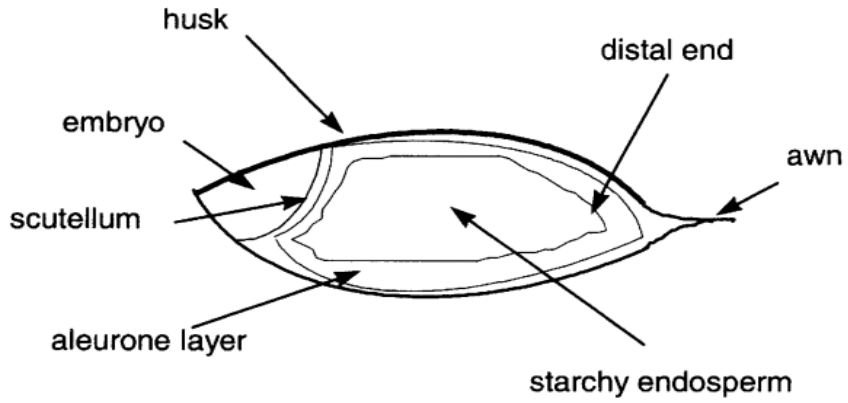



Figure 2.1 Diagrammatic views (longitudinal section) of a barley grain

Malt barley production in Ethiopia actually has dual use: it can be used for food (bread and several traditional dishes) and also for malting. Asella Malt Factory (AMF), a state-owned facility, was for a long time the only malt processing factory in the country supplying malt to local breweries.

**2.2.1.2. Adjuncts**

A great deal of effort has been expended to improve the performance of various adjuncts and to examine their contribution to the characteristics of the finished beer. In general, maize tends to give a fuller flavor to beers than wheat, which imparts certain dryness. Barley will give a stronger harsher flavor. Both wheat and barley adjuncts can considerably improve head retention (foam). Rice will also give a very characteristic flavor to beer. The overall brewing value of an adjunct may be expressed by the following equation.

*Brewing value = Extract + Contribution to beer quality - Brewing cost*

**2.2.1.2.1. Maize**

Whole grain maize or corn consists of 76-80% carbohydrate, 9-11% protein and 4-5% oil. The oil fraction is located in the germ of the maize. Therefore maize is de-germed to limit beer foam damaging effects that would otherwise occur. During processing to grits or flakes the protein content is decreased to 7-9%. However, this protein remains largely undissolved during mashing and so free amino nitrogen (FAN) can be a limiting factor when brewing with high maize levels. Maize is processed to make corn grits, maize flakes, refined grits and corn syrup. Maize flakes are pre-gelatinised and so can be mashed directly with malted barley.

Maize grits are the most widely used adjunct in the United States and Canada. They are produced by dry milling corn. The milling process removes the hull and outer layers of the endosperm along with the oil-rich germ, leaving behind almost pure endosperm fragments. These fragments are further milled and classified according to brewers' specifications. [24]

The germ or embryo of the maize kernel is high in fat (33.3%) in addition to enzymes and nutrients for new maize plant growth and development. The germ also contains vitamins B complex and antioxidants such as vitamin E. Maize germ oil is particularly high in polyunsaturated fatty acids (54.7%), which are subject to oxidative and other forms of rancidity resulting in off or objectionable flavors from full-fat maize products. Pericarp is a high-fiber (8.8% crude) semipermeable barrier surrounding the endosperm and germ, covering all but the tip cap. It is the outer skin or hull of the kernel which serves to protect the seed. The tip cap is the structure through which all moisture and nutrients pass through during development and kernel dry down. [16]. The next picture is the component of the maize kernel.

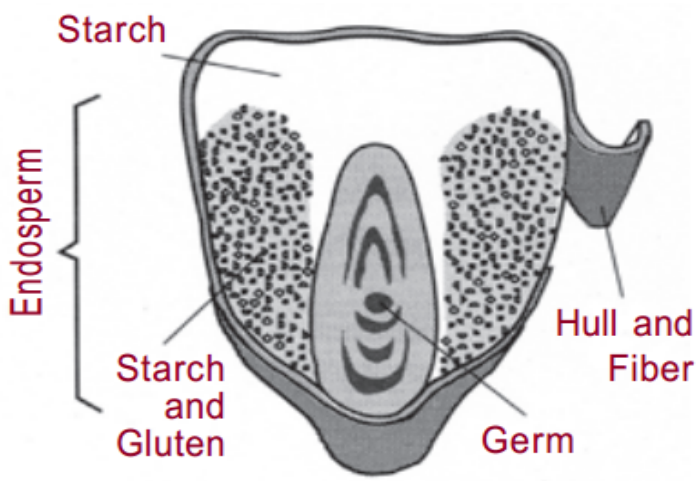



Figure 2.2**.** Components of the maize kernel

Maize is mainly grown in the four big regions of the country: Oromia, Amhara, SNNP, and Tigray. Oromia and Amhara contribute to almost eighty percent of the maize. Three federal research centers/projects are particularly relevant for the maize sector: Bako National Maize Research Project (BARP), which focuses exclusively on maize research; and Melkasa Agricultural Research Center (MARC), which focuses on drought tolerant maize varieties (DTM). Ambo ARC works on maize research for highland sub-humid maize producing

agroecologies. Some maize hybrid varieties such as BH660, BH540, and are relatively well known by farmers.

#### 2.2.1.2.2. Industrial maize processes

There are two basic categories of industrial processing employed for transforming maize into products for human consumption. They are known as dry and wet milling. In the wet milling process, maize is separated into relatively pure chemical compound classes of starch, protein, oil, and fiber. The products and coproducts obtained from wet maize milling are not typically directly used by the consumer and often require further industrial processing before consumption. The products of wet maize milling are not typically produced on a small scale commercially or in the home. The primary product, starch, can be processed into a variety of starch products or further refined into a variety of sweeteners sold in liquid and dry forms

### 2.2.2. Water

Water is the primary raw material used not only as a component of beer, but also in the brewing process for cleaning, rinsing, and other purposes. In addition, different beer styles require different compositions of brewing liquor. Calcium is perhaps the most important ion in the brewing liquor. It protects α-amylase from the early inactivation by lowering the pH toward the optimum for enzymatic activity. Throughout boiling, it not only supports the precipitation of the excess of nitrogen compounds, but also acts in the prevention in over-extraction of hops components. In respect, different functions can be categorized as the following. [10]

**1. Brewing water**: - Water actually contributing directly as an ingredient to beer. This is liquor used in wort production and for standardization to target alcohol content (as in high-gravity brewing). Brewing water requires treatment/adjustment to achieve the correct composition relevant to the beers being brewed.

**2. Process water**: - Comprises water used for washing and sterilizing of vessels and pipe work and for container cleaning and rinsing (i.e. all beer process contact surfaces). It should be of potable standard and ideally softened; it may also be used for pasteurization and refrigeration. [3]

**3. General-Purpose water**: Covers the water for general washing down, site hygiene, and office use, and will generally require no further on-site treatment.

**4. Service water**: Includes water for boiler feed (i.e. steam raising). It should not produce scaling and so must be softened or, ideally, be fully de-mineralized. [14]

### 2.2.3. Hop

Compared to water and malts, hops are lesser of the ingredients used in brewing, but no lesser is the contribution it makes to the final beer. Hops influence to a large extent the final character of beer. Brewers use the flowers (cones) from the female plants of *Humulus lupulus*. As there are numerous varieties of this plant spread worldwide, it is predictable that the quality and characteristics of the flowers also vary. Thus, some hops are known as "aroma/flavor hops" while others as "bitter hops." The α-acids are responsible for the bitterness of a given hop, whereas aroma is tied to essential oils from hop cones. Thus, aroma hops are usually weaker in α-acids but rich in essential oils. Conversely, bitter hops have higher contents of α-acid but may lack on essential oils. Because of the presence of the oil- and resin-rich lupulin glands, the overall composition of fresh, dried hop cones shows them to be unlike that of other plant material, though the leafy nature of the hop petals ensures the presence of such ubiquitous substances as proteins and carbohydrates. [27]

### 2.2.4. Yeast

Genus of *Saccharomyces* has always been involved in brewing since ancient times, but through the vast majority of the brewing history our ancestors had no idea that living cells were the responsible entities for fermentation.

Wort represents a rich source of nutrients for yeast. It contains fermentable sugars, assimilable nitrogen, minerals and vitamins, as well as minor growth factors. The important oxygen must be supplied by aeration. By careful brewhouse work all malt worts can be created containing all essential nutrients in ample amounts. Only zinc and, rarely, biotin can be critical. Different conditions may occur by applying high- gravity brewing under utilization of large amounts of unmalted cereals or sugar, where a deficiency in nutrients can be observed. Commercially available, so called yeast nutrients are employed to cover the requirements. During yeast propagation and fermentation, the concentration of various nutrients changes. The yeast has to respond immediately; therefore, the nutrient requirements and intake are of tremendous importance. [25]

For all malt wort (12° P) an amount of 900 – 1200 mg/l total soluble nitrogen and 200 – 240 mg/l free amino nitrogen are considered adequate. It should be taken into account that the required amount is also strain dependent. Some strains show perfect fermentation results

despite a much lower nitrogen supply Flocculation, a property of the yeast cell wall, is also the characteristic of brewing yeast that allows the separation of yeast from beer, and is strongly correlated to the physical surface properties of the cell. Flocculation can be defined as the phenomenon where in yeast cells adhere in clumps and either sediment rapidly from the medium in which they are suspended or rise to the surface. [26]

## 2.3. Beer production process

### 2.3.1. Malting

Quality specifications for brewing barley are the most challenging specifications in comparison to other cereals in the food industry. The high quality demanded is specified by several quality parameters like germinative capacity, protein content, sorting (size of the kernels), water content, kernel abnormities and infestation. Therefore, an effective quality check before barley intake is essential. Malting develops the *diastatic enzymes* that accomplish the conversion of starch to sugar during brewing and begins a limited process of conversion that makes the starches more accessible the brewer. Only the highest quality grain, called *brewing grade,* is selected for malting. Diastatic power is the ability of grains to break down complex starch molecules into simpler sugars for brewing. It is determined by the amount of diastatic enzymes in the grain. [25]

#### 2.3.2.1. Malting process

Malting is a three-step process consisting of steeping and germination, drying, and finally kilning.

**1. Steeping and Germination**

The malting process begins when the grains are steeped for thirty-eight to forty-six hours until they have absorbed almost 50% of their initial weight in water. Steeping-in has two major functions: It raise the moisture content to initiate germination and wash the grain and remove germination inhibitors as well as all floating material by skimming. They are then drained and moved to a germination room where they are kept at a constant humidity and temperature for almost four days. They are turned periodically to maintain an even grain bed temperature of 60°F to 75°F Fahrenheit (all temperatures are given in Fahrenheit), which promotes germination. The germination step takes advantage of the plant's natural growth cycle, activating enzymes already present in the grain that begin the process of unpacking and breaking down the proteins and starches at the kernel's center.

A] Steeping B] Germination

Figure 2.3. Steeping and Germination step

The objectives of germination are:

- ✓ To Control breakdown of cell walls and matrix proteins.
- ✓ Produce optimal level of hydrolytic enzymes.
- ✓ Hydrolyze certain barley reserves [e.g. protein to form free amino nitrogen ( FAN )].
- ✓ Minimize loss of potential extract from growth and respiration while achieving optimal modification.
- ✓ Produce balanced, well - modified green malt for kilning.

The degree to which this breakdown occurs is referred to as *modification.* Most brewing malt produced today is highly modified, meaning a significant amount of enzyme development and starch conversion has taken place, making these essential elements easily accessible to the brewer. [26]

2. ***Drying.*** *Once* the maltster determines that the grains, now called *green malt*, are sufficiently modified, they are moved to a kiln and carefully dried to about 4% moisture content. Drying takes place over a period of twenty-four to thirty-six hours at a temperature of 122°F to 158°F. For some types of malt this is the end of the process. These are called *base malts*

Figure 2.4. Malt drying step

3. ***Kilning*** – After drying some grains are heated in kilns at higher temperatures and for longer periods of time. This extended kilning gives these malts the unique colors and flavors. Lower temperature and shorter duration kilning results in light colored grains with more subtle flavor characteristics. Longer kilning times and higher temperatures result in dark colored malts with more intense flavors. Two chemical reactions are involved in the development of these colors and flavors, *caramelization* and *Maillard reactions*. Caramelization is the decomposition of sugar at high heat. It results in sweet flavors like toffee, molasses, and raisin. The Maillard reaction is the darkening that results from interactions of amino acids and sugars. It is the same reaction that makes toasted bread brown or creates grill marks on meat. Maillard reactions result in the bready, toasty, and biscuity flavors associated with baking. Malts that have undergone kilning or roasting are called *specialty grains*. [22]

### 2.3.2. Wort Production

A brewery has a brewhouse in order to produce wort in a process starting with the raw materials and terminating with wort cooling. This series of complex and costly procedures takes place to convert the raw materials, water, malt, adjuncts, and hops into fermentable liquid wort, which will become beer.

#### 2.3.2.1 Grist Preparation

The process objective is to mill the correct quantities of malts and adjuncts to produce grist with the optimum particle size distribution required for efficient wort creation and wort separation.

The husks should be kept intact with the application of a lauter tun to obtain a permeable filter cake. With excessive milling, the pores of the filter cake clog up very rapidly during lautering so that the permeability is also reduced due to decreased porosity. The consequences are longer run off times of the filtrate and the need to loosen the filter cake mechanically.

Currently, hammer mills and roller mills are mostly employed. Furthermore, there is a differentiation between wet milling and dry milling. The most used process is where the dried malt is ground by counter - rotating roller pairs. Depending if the rollers rotate at the same or at different speed, the grinding of the dry grist is carried out by applying pressure or by pressure and shearing. [18]

The production of wort in the brewhouse serves as a key element in the brewing process. An overview of the processing steps needed for beer production in a brewery will be seen later at the end of this chapter by flow diagram.

#### 2.3.2.2. Mashing

The process objective is to slurry the grist with hot water to dissolve the soluble malt components (grist hydration) and then to regulate enzymic activity to achieve the required wort composition (mash conversion). Mashing is the solubilization of malt components by enzymatic, physical and chemical solution processes. Starch, proteins and cell wall substances are the most important classes of substances for malting, which are dissolved by hydrolysis. Furthermore, during mashing organic phosphate is transformed into primary phosphates by phosphatases (phosphorolysis). This increases the buffering capacity of the mash and wort, and can influence the pH fall during fermentation. Lipids are decomposed by autoxidation as well as enzymatically to numerous products. Polyphenols undergo oxidation and polymerization processes, which result in the decrease of valuable antioxidants and consequently in a reduction of the taste stability. The used malt significantly affects the color and aroma. By enriching low molecular nitrogen compounds there is a possibility to introduce additional starting materials for the Maillard reaction into the mash. These can be transformed at high temperature (e.g. during wort boiling) into color and aroma rich compounds. The enzymatic breakdown during mashing can be controlled by the parameters of temperature, viscosity, pH value and time. [21]

Over the course of time many different mashing processes have been developed, which can in general be divided into infusion and decoction methods. [24]

**A. Infusion mashing**

This is mashing at a single temperature usually called the conversion temperature. The temperature of the mash in infusion is critical since changing it may involve serious dilution with water It has been established that the best mash temperature for this system of mashing is in the range of 62 – 67 $^{O}$C and usually is 65 $^{O}$C. Since infusion mashing employs a single temperature stand particularly for starch hydrolysis, protein and glucans may not be hydrolysed. [25]

**1. Single step infusion**.

The malt is combined with hot water to reach a temperature appropriate for starch conversion. This is the method of choice for fully-modified malts such as those used t brew British ales. It has the advantage of requiring a minimum of labor, equipment, energy and time, but prohibits the use of under modified malt or adjuncts.

**2. step-infusion mash** allows a little more flexibility by moving the mash through a series of temperature rests. The temperature is increased by external heat or the addition of boiling water. This requires more resources than a simple infusion mash, but under modified malts may be used.

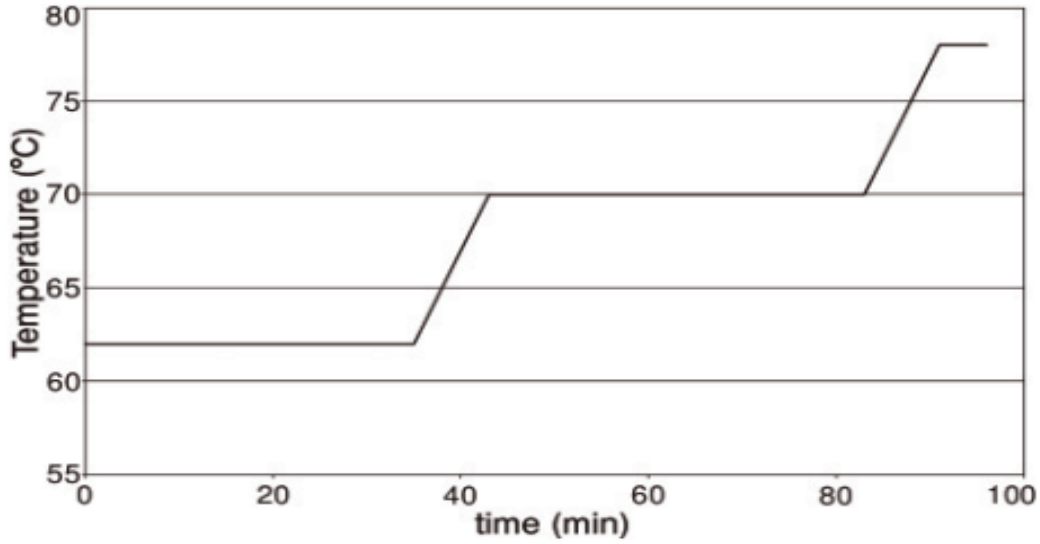



Figure 2.5**.** Infusion mashing method

**B. Decoction mashing**

Decoction mashing involves the removal of a thick fraction of the mash (usually quarter of) and running it through a brief saccharification rest at a relatively high temperature. It is then boiled it for 15-30 minutes before mixing it back into the main mash. This is repeated as many as three times, depending on the modification of the malt and the beer style. The decoction helps explode starch granules and break down the protein matrix in under-modified malt, improving the extraction efficiency, and also promotes the formation of melanoidins. These compounds are formed from amino acids and reducing sugars in the presence of heat and are responsible for the rich flavors in malty lagers. This mashing method is the most resource intensive, but is the traditional method for many lagers. A possible side-effect of the extended mash schedule is the extraction of higher levels of tannins and DMS precursors from the grain husks; though, this is not significant at typical mash pH levels. [17]

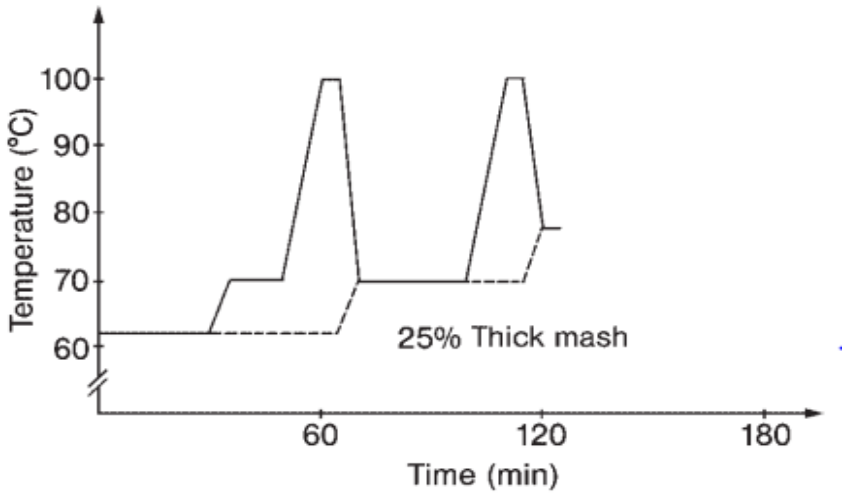



Figure 2.6. Decoction mashing method [29]

**C. Double mash**

Double mash can be viewed as a combination of infusion and decoction. As the name implies, it involves two separate mashes: a main mash consisting of crushed malt, and a cereal mash consisting of raw adjuncts and a small charge of crushed malt. The latter is boiled for at least an hour to gelatinize the starches and is then added to the main mash, which has undergone an acid rest.

**2.3.2.2.1. Gelatinization temperature**

The most important property of starch for brewing is gelatinization. Gelatinization is the water adsorption- induced swelling of the starch grains and the resulting irreversible loss of

the crystalline structure of the starch**.** The temperature at which gelatinization start is the gelatinization temperature (GT). [22]

Below the GT, only those starch grains are hydrolysed that were enzymatically attacked during malting or that were mechanically damaged during milling. Only those grains can also take up water below the GT and are thus enzymatically hydrolysable. The GT is hence of importance during mashing as in malt the bulk of starch grains are present in native form and consequently are only hydrolysed at temperatures above the GT. The gelatinization temperature of some cereals are as following. [7]

Table 2.1. Gelatinization temperature of some cereals [24]

| Cereal | GT ( $^{O}$C) | | |
|---|---|---|---|
| | minimum | maximum | average |
| Barley adjunct | 65 | 69 | 67 |
| Maize adjunct | 73 | 79 | 76 |
| Rice adjunct | 67 | 91 | 81 |
| Barley malt | 58 | 65 | 62 |

**2.3.2.2.2. Enzymatic rests**

In step-infusion and decoction mashing, the mash is heated to different temperatures at which specific enzymes work optimally. The table below shows the optimal temperature ranges for the enzymes brewers pay the most attention to and what material those enzymes break down. There is some contention in the brewing industry as to just what the optimal temperature is for these enzymes, as it is often very dependent on the pH of the mash, and its thickness. A thicker mash acts as a buffer for the enzymes. Once a step is passed, the enzymes active in that step are denatured by the increasing heat and become permanently inactive. The time spent transitioning between rests is preferably as short as possible. However, if the temperature is raised more than 1 °C per minute, enzymes may be prematurely denatured in the transition layer near heating elements. The optimal conditions of some cereals are listed as follow.[16]

Table 2.2.Optimal rest temperatures and conditions for major mashing enzymes [20]

| **Temp °C** | Optimum pH | **Enzyme** | **Breaks down** |
|---|---|---|---|
| 40–45 °C | 6.0 | β-Glucanase | β-Glucan |
| 50–54 °C | 5.5 | Protease | Protein |
| 62–67 °C | 5.5 | β-Amylase | Starch |
| 71–73 °C | 5.2 | α-Amylase | Starch |

The optimum conditions for an enzyme can be improved by other factors. These include:

- ✓ Mash thickness (water to grist ratio), thicker mash seems to "protect" the enzymes.
- ✓ Ionic composition (particularly Calcium ions). Calcium seems to "protect" the enzymes from heat
- ✓ Concentration of substrate, not normally a problem with a standard mash.

**2.3.2.3 Mash Filter**

In place of the lauter tun, several brewers employ mash filters to separate the mash into solids and liquid. The total mash is transferred into a vertically arranged filter press. The frames are covered on both sides with filter cloth made of synthetic material. At the same time, the air must escape quickly when the homogeneous mash is pumped into the chambers. After yielding the first wort, water is pumped in and the filter compartments of the filter press are pressed together with corrugated steel plates. Since very low volumes of sparging water (0.5 hl/100 kg) are necessary, this facility is very suited for high- gravity brewing.

**2.3.2.4. Wort Boiling**

Kettles are fitted with a heating system that heats the wort from mash temperature (65–78$^{O}$C) to boiling temperature, which is just above 100$^{O}$C (at sea level) due to dissolved solids. Boil length can range from 30 to 120 min. Liquid adjuncts and hops can be added at various points during boiling. Following boiling, the solid material precipitated is removed and the clear wort is cooled ready for fermentation. This process stabilizes the wort, removes unpleasant flavors, and extracts hop components that give beer its distinctive flavor.

Wort boiling has a number of objectives, which are summarized below:

- ✓ Evaporation, concentration, and removal of volatiles
- ✓ Destruction of enzymes
- ✓ Sterilization, killing of spoilage organisms
- ✓ Extraction and conversion of hop material (flavors and preservatives)
- ✓ Coagulation of proteins (hot break)
- ✓ Promotion of reactions between proteins and hop constituents
- ✓ Completion of salt reactions
- ✓ Caramelization of sugars, especially in dark wort
- ✓ Color and flavor formation and
- ✓ Cooking of nonfermentable extracts

The necessary steps of wort boiling can roughly be divided in two processes: hot holding and evaporation.

**2.3.2.4.1. Hot Holding**

During hot holding, different chemical reactions take place such as hop isomerization, development of aroma substances, development of color and dissolution processes, as well as inactivation of enzymes and sterilization. The inactivation of the malt enzymes is necessary as otherwise the result would be atypical taste profiles (e.g. uncontrolled over - fermented beers). Furthermore, during wort boiling the proteins and protein tannin complexes (break) need to be eliminated to obtain clarified wort. If the protein coagulation is too strong foam-positive high molecular proteins (10 and 40 kDa) are also precipitated that worsen the stability of the foam. This is particularly important if no foam - aiding additives are added (e.g. in accordance with the Bavarian Purity Law). Insufficient protein coagulation leads to colloidal unstable beers. For the customer turbid beer is a reason for reclamation. Turbidity - causing substances can, however, be partially removed by absorption before or during beer filtration using filtering aids. [24]

The Maillard reactions that occur at about 80 $^{O}$C generate new aroma substances. Of particular importance are Strecker aldehydes developed from amino acids that influence the taste stability of the beer. These are primary and secondary products of the Maillard reaction, and can be reduced depending on volatility, boiling system used and evaporation (i.e. boiled wort contains more of the aroma substances than unboiled wort). The a-acids in the hops are

extracted into the wort and isomerised into iso-α-acids, which provide the characteristic bitter taste of beer.

#### 2.3.2.4.2. Evaporation

Evaporation serves to remove undesired aroma substance such as myrcene from hops and different carbonyl as well as sulfur substances, especially dimethylsulfide (DMS). Aroma substances from lipid metabolism are also reduced by evaporation during wort boiling. [23] Some of these substances can be used as an analytical indicator of the evaporation efficiency of the boiling process as these are not reformed during boiling. Moreover, the original extract is adjusted. This is necessary to ensure the product constancy and legal requirements marketability of the beer. [20]

A high evaporation allows an effective leaching of the spent grain and thus saves malt. At the same time more energy is needed for the evaporation. In this process the thermal stress of the wort is increased, which has a negative effect on the taste stability of the beer. A high evaporation efficiency of the boiling system results in a good quality as the basic analytic markers of the boiling such as free DMS, thiobarbituric acid number ( TBN ) and coagulable nitrogen can be ideally adjusted. Consequently, the total evaporation can be reduced, so that the heat and time demand for the boiling process is reduced.

#### 2.3.2.5. Wort Cooling and Aeration

After whirlpool separation, wort is at a temperature of approximately $95^{O}C$ and must be cooled to between 8 and $22^{O}C$ depending on the intended fermentation temperature. The wort must also be aerated to aid yeast growth. Cooling should be carried out as quickly as possible after clarification to reduce formation of DMS and to prevent excess color formation. Care must be taken with the handling of cold wort as it, unlike hot wort, is highly susceptible to microbial contamination. Equipment used to handle cold wort must be kept sterile [15]

**Plate and Frame Heat Exchangers**

The method most frequently employed for wort cooling is the plate and frame heat exchanger. The heat exchanger plates are made of stainless steel and are thin (0.5 mm) to allow optimal heat exchange. The surfaces of the plates are embossed to cause turbulent flow and speed up heat transfer. It is difficult to find data for heat transfer, but Alfa Laval (Brussels, Belgium) claim a film coefficient of 3000–6000 W/moC for clean plates. Heat exchangers can be set up to give single-stage or two-stage cooling, with the flows of wort and cooling water arranged to flow in opposite directions. [15]

In single stage coolers, wort is cooled with cooled water in counter flow. The total hot liquor requirements of the brewhouse (6.0–7.3 hl water of 82$^{O}$C for 100 kg of malt) can be provided by the water at 79$^{O}$C from the wort cooler. Two-stage coolers have an initial water cooling stage, reducing the wort to around 16$^{O}$C, followed by an extra circuit using a refrigerant that further cools the wort to 7$^{O}$C. Two-stage coolers are used when the temperature of the cooling liquor is high or when lower wort temperatures are required. .

**2.3.2.6. Brewhouse Efficiency**

When measuring the efficiency of a brewhouse, the most important factor is the percentage extract recovery. This is a comparison between the potential extract from the raw materials and the amount of extract actually collected in the fermentation vessel. The calculation is based on laboratory analyses of the raw materials and the volume and gravity of the wort produced. The brewhouse yield depends on the raw material, the brewhouse equipment, the mashing process, the lautering process, and the overall operating methods. [17]

To calculate the brewhouse yield, the following parameters must be known:

- ✓ Weight of grist used in kg. This parameter is usually obtained from the automatic grist weighing device.
- ✓ Volume of the produced wort in liters.
- ✓ The extract content in kg/100 kg. This value is the reading from the hydrometer. For example, 11.4 means there are 11.4 kg extract in 100 kg wort and is the specific gravity of the cast wort. The specific gravity (kg/l) is obtained with the hydrometer reading and a conversion table.
- ✓ The contraction or correction factor. This factor compensates for the difference in the volume of wort at 100$^{O}$C where the wort volume is usually assessed, and at 20$^{O}$C where the extract content is measured. This factor is 0.96; meaning that from 100 l of casting wort at 100$^{O}$C, 96 l of cold cast wort at 20$^{O}$C can be obtained.

$$\text{Brewhouseyield} = \frac{\boldsymbol{volume\ of\ \ wort} * \mathbf{0.96} * \boldsymbol{extract\ content}(\boldsymbol{kg}/\mathbf{100}\boldsymbol{kg}) * \boldsymbol{specific\ gravity}}{\boldsymbol{weight\ of\ grist\ charge}} \quad \mathbf{2.1}$$

Where, 0.96 = the contraction or correction factor

**2.3.3. Fermentation, Maturation and Storage**

Transferring wort into beer is the third main step in brewing. Fermentation means to metabolize substrates into products by the activity of microorganisms and simultaneously to gain energy. In our case yeast transfers sugars to ethanol and $CO_2$. During this process we

have also the formation of fermentation byproducts, which have a considerable effect on the aroma profile and the taste of the resulting beer. Fermentation is started by aeration of cold wort and adding yeast to the wort a process called pitching. The main chemical reaction occur during fermentation is the following. [15]

$$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2 + \text{Heat}$$

**2.3.3.1. Aeration**

Yeast needs oxygen to multiply and thus wort must be aerated. Aeration can take place either on the hot or cold side of the wort cooler. Aeration on the hot side has the benefit of increased sterility and mixing, but will cause oxidation of polyphenols, which will darken the wort. Thus, air or oxygen is usually added to cooled wort. Some breweries inject sterile air or oxygen between two stages of a plate heat exchanger with the temperature at 10–15$^{O}$C. An intensive distribution with fine bubbles is achieved by aeration with sintered candles, venturi tubes, special nozzles or static mixers. By using air for aeration, no problems of over-aeration can occur. A value of 8– 10 mg $O_2$ per liter wort is optimal.

**2.3.3.2. Yeast Addition or Pitching**

The fermentation process is initiated by the addition of 0.5– 0.7 l of a heavy yeast slurry per hectoliter of wort, corresponding to 15 – 20 million yeast cells per milliliter of cold and aerated wort. After the addition of yeast, the wort is called young beer or simply beer. An alternative method measures the yeast load involving the combination of solids content and flow rate to give a measurement parameter expressing the solids content of a volume in kilogram dry weight. This yeast addition is independent of the consistency of the yeast slurry. Aeration and yeast addition can be performed together in one systemYeast addition is spread over the entire duration of wort addition; thus, yeast is distributed uniformly in the fermentation vessel. A weighing cell on the yeast storage tank assesses the total amount of yeast dosed and thus it is possible to add exactly the desired amounts of yeast and air required. The single yeast cells must quickly come in contact with the nutrients of the wort. Consequently, the yeast is injected continuously into the flow of the cold wort

Brewery yeasts are mainly two types, called top- fermenting (Saccharomyces cerevisiae) and bottom-fermenting yeast (Saccharomyces uvarum var. carlsbergensis and Saccharomyces bayanus). The Saccharomyces yeasts are facultative anaerobes, which means that they can easily adjust their metabolism from aerobic to anaerobic conditions [14]

Yeast doubles or triples its mass during fermentation. For the build - up of cell substances yeast needs mostly amino acids, which it either takes from the fermenting substrate or must synthesize by itself. Apart from proteins, lipids have to be synthesized for yeast propagation because they are important components of the cell wall and are also needed for the uptake of nutrients. Molecular oxygen is necessary for the synthesis of these lipids from acetyl coenzyme. Wort itself contains only few lipids. Finally, yeast also requires minerals for the stabilization of its enzyme systems [17]

**2.3.3.3. Changes during Fermentation**

The dissolved extract substances of the wort are fermented to ethanol and $CO_2$ by the activity of yeast enzymes. This metabolic pathway is exothermic and is called glycolysis. Yeast hydrolyses hexose and sucrose first (starting sugar), then takes maltose (main fermentation sugar) and afterwards maltotriose (secondary sugar). Fermentation byproducts are formed on metabolic sideways. They can be characterized into six groups: [17]

- ✓ Higher aliphatic and aromatic alcohols.
- ✓ Multivalent alcohols.
- ✓ Esters.
- ✓ Carbonyl compounds.
- ✓ Sulfur - containing compounds.
- ✓ Organic acids.

All these compounds have different taste and odor thresholds. Their combined contributions make up the flavor or off- flavor of the beer; the amounts produced can be influenced to some degree by brewing technology.

During the main fermentation, the pH decreases by one unit because volatile (acetic, formic) and non- volatile organic acids (pyruvic, malic, citric, lactic) are formed from amino acids by deamination. The final pH of the beer ranges from 4.3 to 4.6. The intensity and speed of acid formation is determined by the buffering capacity of the wort, amount of easily assimilated nitrogen, yeast strain and fermentation method used. The pH has a direct influence on the flavor and the sparkle of the beer.

Short - chain fatty acids are formed at the beginning of the main fermentation process: butyric, isovaleric, hexanoic, octaoic and decanoic acids. Their amounts can be controlled by the wort composition, aeration, yeast strain and general fermentation conditions. During

pressure fermentation, increased levels of these compounds can be expected. They cause a yeasty odor and impair head retention. DMS is not affected by yeast. DMS in beer depends on the amount present in wort. Glycerol, a multivalent alcohol, is formed during glycolysis; its concentration depends on the amount of fermented sugars (1300– 2000 mg/l) and is therefore proportional to the gravity of the wort. Aldehydes and ketones are responsible for the aroma of the green beer and for the stale flavor. Acetaldehyde is formed in the green beer during the first 3 days and gives beer an unripe, unbalanced taste. [16]

**2.3.3.4. Fermentation goal**

Fermentation is a decisive step to obtain a well- balanced and high - quality beer. The main goals of the fermentation are to reach:

- ✓ Constant fermentation times.
- ✓ Vigorous extract degradation and pH drop.
- ✓ A desired degree of fermentation.
- ✓ A constant beer quality.
- ✓ A long shelf - life.
- ✓ Maintain the analysis parameters.

**2.5.3.5. Factors Affecting Fermentation**

There many factors which affect the fermentation process some are listed as the following.

- Composition of the wort
  - ✓ original gravity FAN > 230 mg/l
  - ✓ Ca 10 – 20 mg/l
  - ✓ Mg > 40 mg/l
  - ✓ Zn 0.10 – 0.15 mg/l
- Temperature course:
  - ✓ speed of fermentation start influences the total fermentation time
  - ✓ fermentation byproducts
  - ✓ CO2 counter - pressure on top of the fermenter
- Yeast cell count:
  - ✓ yeast viability
  - ✓ yeast vitality
  - ✓ yeast strain

- Aeration:
  - ✓ rate of reproduction
  - ✓ sulfur dioxide formation

Sulfur dioxide is a highly antioxidative substance that is positive to the shelf - life of the beer. $SO_2$ has come into focus as an ingredient that can be an allergen to some people. Therefore, if a concentration above 10 mg/l is reached, it has to be declared on the label. Formation during fermentation depends mostly on the yeast strain. In addition, it can also be influenced by technological parameters during pitching. Lower aeration, high original gravity and poor yeast vitality lead to higher sulfur dioxide contents; however, yeast count at pitching is also important, as higher extract and lower oxygen per cell can increase sulfur dioxide. [2]

**2.3.3.6. Maturation**

The total diacetyl concentration is used to judge the maturity of fermenting beer and must be decreased below the flavor threshold by means of brewing technology. The diacetyl precursor 2- acetolactate is also called 'potential diacetyl ' because it transforms into free diacetyl only in the filtered, yeast- free beer and then cannot be broken down any further. After maturation, beer is lagered at temperatures of 0 to -2 ° C to clarify for 1-2 weeks. During this process its filterability and its colloidal stability are improved. The yeast cell count should be below 2 million cells/ml after conditioning beer in this way. During the propagation phase the yeast cells need numerous nitrogen compounds for the formation of yeast protein. Therefore, the yeast assimilates amino acids and peptides, and transfers $NH_2$ groups on α- ketoacids coming from carbohydrates. Diacetyl is formed during valine synthesis. This highly temperature - dependent step is catalyzed by yeast enzymes and occurs very slowly below $10^{O}$C. Diacetyl itself is present in very small quantities in fermentation samples and in green beer, because its reduction to acetoin is much faster than its formation. Bacteria, which may occur in the brewery as infections, are also likely to promote the formation of diacetyl. [17]

**2.3.3.7. Storage**

Beer must reach its desired $CO_2$ level during the phase of cold storage (up to 4.8g/l for draft beer, 5.0 g/l for canned beer and up to 0.55 g/l for bottled bottom fermented beer). This can be achieved in the conventional procedure by using a definite bunging over - pressure of 0.2 – 0.6 bar, depending also on the hydrostatic pressure and temperature. Beers produced with warm maturation require either higher pressure or $CO_2$ to be added during transfer from warm to cold storage tanks. During storage, the beer must clarify by allowing the yeast and

other haze - causing materials to settle, and its taste must refine and round-off. In order to achieve these requirements, the beer must be stored at 0 to -2 ° C for 1 – 2 weeks.

#### 2.3.3.8. Bottom fermentation

In many countries, bottom - fermented beer is designated as lager beer. Its extract of original wort varies according to the local laws (tax classification) from 7 to 14%. Lager beers are the most popular, with an average bitter substance content of 20 EBC bitter units.Bottom - fermented beer with an extract of original wort of 10 – 14% comprises an extraordinarily large variety of beer types, including pale and dark beers, export beers (more than 12% extract of original wort), special beers and festival beers (13 – 14% extract of original wort). Within these limits there are such different beer types as Pilsener, Dortmunder, Munich, as well as smoky flavor beer and cellar beers; these are, however, restricted to certain localities. The upper gravity limits for special beers differ from country to country between 13 and 15%. Strong beers range from 15/16% extract of original wort up to a maximum of 28%. [16]

#### 2.3.3.9. Top Fermentation

Top fermentation is the oldest method of beer production and was the only one used until about the middle of the nineteenth century. Top - fermented beers differ from bottom - fermented beers in their ingredients (more than 50% wheat malt or other malted cereals) and by their special aroma, which is primarily induced by the top - fermenting yeast strains of S. cerevisiae. The particular yeast strain employed has a higher optimum fermentation temperature, and therefore the fermentation proceeds between 15 and $24^{O}$C. During fermentation, the yeast rises and can be skimmed off the top. The number of yeast generations is as mentioned considerably greater. At the higher fermentation temperatures, the diacetyl is easily decreased. Owing to the fast rate at which fermentation proceeds a relatively low pH value of 4.1- 4.3 results. [16]

### 2.4. Filtration and Stabilization

Filtration processes can be described as a flow through of layers. The purpose of filtration is to preserve the beer so that no visible changes occur in the long run and the beer keeps its original appearance. Generally, the filtration steps fulfill two roles:

- ✓ To remove suspended materials from the green beer (the real filtration).
- ✓ To unhinge potential turbidity formers (stabilization).

Nowadays, kieselguhr is nearly exclusively used as a filter aid for beer clarification. Test installations for membrane filtration of beer show encouraging results. Research for

alternatives to kieselguhr by using regenerative filter aids has been prompted due to the increasing difficulties of disposing of the waste guhr. Filtrations can be classified as surface and depth filtration depending on the place of solid separation. In surface filtration, the particles to be separated are retained on the surface of the active media (filter material). By contrast, in depth filtration, the separation process takes place inside (in the depth) of the filter material. The process is called a cake filtration if a filter cake is built up by the separated solid materials during surface filtration and the outer layer of the filter cake takes over the separation.

In general, cake filtration and surface filtration are the main techniques available for beer clearing. An important representative of cake filtration is kieselguhr filtration. Surface filtration has not yet prevailed in beer clearing. The first large- scale plant with membrane filters seems to be working promisingly.

### 2.5. Packaging

The “package” is used primarily to move products through a distribution system to the consumer, through the use of attractive labels and colors. the consumer is drawn to select one container over another when confronted with two or more different choices of the same product. Modern packaging is constantly evolving with lighter weight metal containers, holograms to attract attention, composite materials to gain the best performance from each component, and the attempt to keep up with the ever-changing consumer attitude.

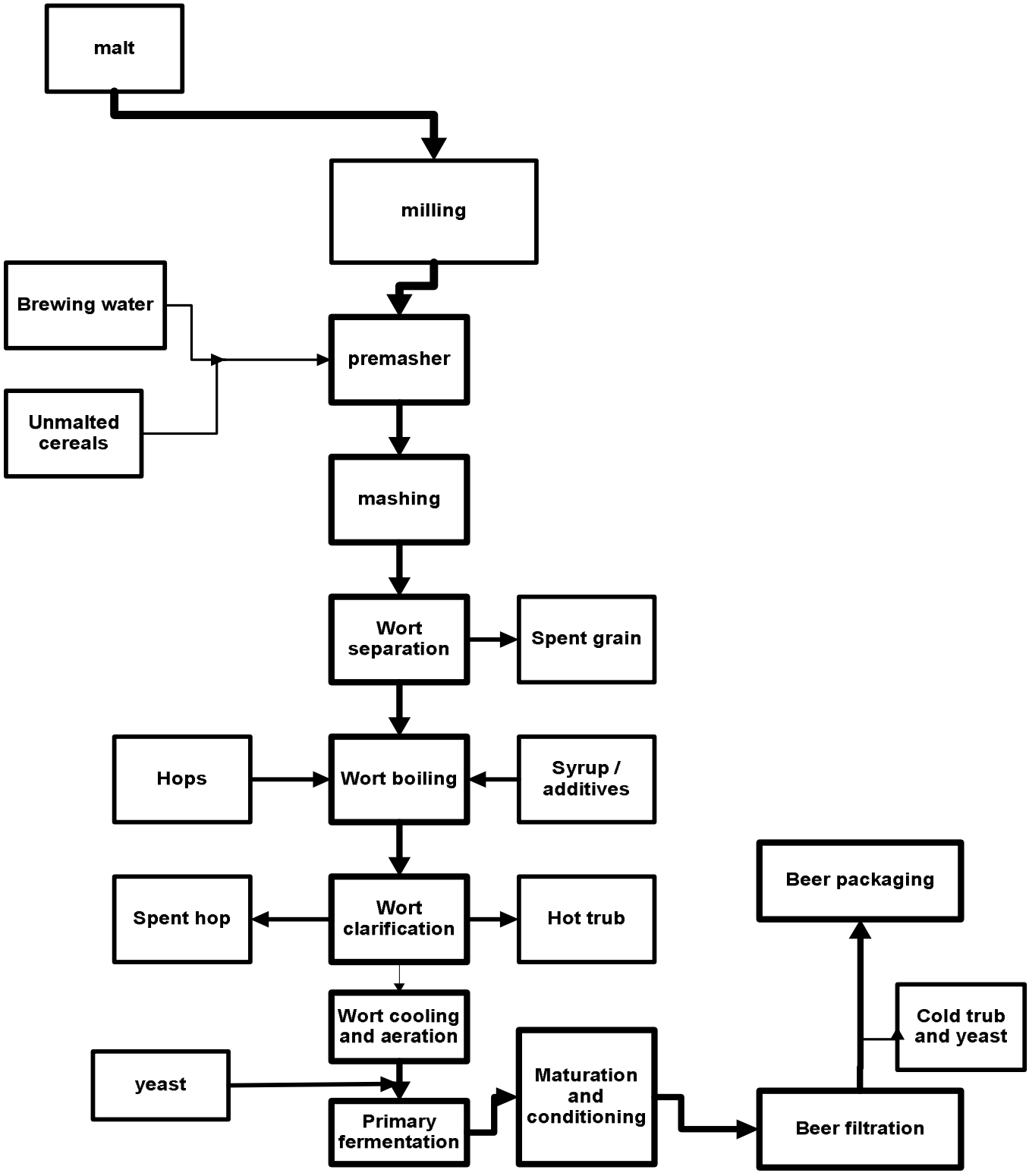



Figure 2.7. Basic flow diagrams for beer production

# CHAPTER 3

# MATERIAL AND METHOD

## 3.1. Material and Equipment used

There were a lot of materials used from data collection to final product. The main material and equipment used were explained next, while the chemicals and raw materials were shown under method.

### 3.1.1. Materials used to determine the physico-chemical characteristics of malted barley and maize

Miller A Buhler, Universal laboratory disc mill, Inframatic IM 9500, Oven (D-91107), digital balance, Different volume of glass beakers (100ml, 500ml, 1000ml), Test tubes 25ml, Desiccators and KJeldahl Digestion rack

### 3.1.2. Materials/equipment used to optimize wort and used for beer production

Mash bath fitted with mechanical stirring, Mashing beakers of 600ml stain less steel, Porcelain spot plate, Filter funnel (165mm plastic or 150mm glass diameter), Micro brewery plant, PH-meter, Lovis2000M (Micro viscometer) and Density meter (DMA2500)

### 3.1.3. Materials/equipment used to characterize beer

Density meter (DMA2500),$CO_2$-meter, Foam stability tester (Nibem-TPH), GENWSYS 10S UV-VIS Spectrophotometer, Anton paar alcoholyzer (PBA-B),Turbiditymeter (Vost Rota90/25), Automatic shaker (RY-002) and different size pipettes (2ml, 5ml, 10ml)

## 3.2. Methodologies

This section describes about the methodologies and approaches of how experiments were done in this research. It comprised of all steps procedures of the experiments and the whole picture of the work of this paper. For this paper there are two main works. The first one is laboratory level work. In this class there are two response variables. The first one is the yield of extract contents of wort or sugar contents of wort which the expected value was maximum. The other one is the free amino nitrogen that used for yeast growth which the expected result was targeted according to the literature. Here the response variables are optimized according to the design experiment. The second one is the production of beer from the optimized wort using Micro brewery pilot plant, at BGI Ethiopia plc and the characterization of the product. For this approach different physico-chemical measurements were taken for the raw materials, intermediate products and the final product or beer.

The main raw materials for this product are (maize, two row malted barley and six row malted barley), hop, water, and yeast. Maize and barley are available in our country at different areas. However, BH660 and BH661, BH 540 maize are collected particularly from western Shoa Bako research center. Then it was de-germed at addis ababa university .Two row barley and six row barley were collected from Arsi zone around Asela and malted at Assela malt factory, because the important equipment and technology are available there. Hop, yeast brewing water and other laboratory chemicals and equipments are obtained from BGI Ethiopia.

### 3.2.1. Determination the physico-chemical characteristics of malted barley and maize

The proximate analyses were done for the maize and malted barley by using different instruments and chemicals.

#### 3.2.1.1 Determination of moisture content, protein content, oil and extract content of barley and maize using inframatic machine 95000

First the Inframatic machine were made on and equilibrated for 15 minutes. Then the item program was selected and about 400gm of sample was given. Then the analyze key was pressed and the result was displayed in the screen.

#### 3.2.1.2. Determination of moisture content of maize and malt by drying method

The determination of moisture contents of all malt and maize by loss in mass of drying under specified condition.

**Method**

First the clean moisture dish was dried in oven and had been put in the desiccators to make it free from moisture. Part of the sample milled for mashing was taken and weighted in a clean moisture dish in duplication form. The weighted sample was dried in oven at $105^{O}$C 3 hour for malt and $135^{O}$C 4 hours for maize. When the sample was dried in the oven it had been allowed to cool for 20 minutes in the desiccators to $20^{O}$C. Then the contents of the dish were reweighted.

**Result Expression**

The moisture (M) contents are calculated as follow:-

$$M = \%(\ m/m) = \frac{W2-W3}{w2-w1} * 100$$

Where: W1 mass of the sample container in gram

W2 = mass in gram of sample and sample container before drying

W2 = mass in gram of sample and sample container after drying

The moisture contents of the maize and malt together are calculated as the following.

$$M_{Av} = \frac{(pmalt * Mmalt) + (Pmaize * Mmaize)}{Mmalt + Mmaize} * 100$$

Where:

$M_{Av}$ = Average moisture contents

$P_{malt}$ = percent of the maize used in the given sample

$P_{maize}$ = percent of the maize used in the given sample

$M_{malt}$ = moisture contents of the maize

$M_{maize}$ = moisture contents of the maize [5]

### 3.2.1.3. Determination of Total nitrogen contents both for malt and maize

**Principle**

The nitrogen compounds in the wort are digested with hot sulphuric acid in the presence of catalysts to give ammonium sulphate. The digest is made with NaOH solution and released ammonia is distilled into an excess of boric acid solution. [6]

**Method**

1.5g of finely ground sample was taken in the kjeldahl flask and 10 g of powder catalyst will be added to the flask. Then 20ml of 98 % $H_2SO_4$ was added and swirled to mix. The solution was boiled until the brown color disappears for 30minutes.The digest was allowed to cool and the solid ammonium sulphate was formed and the digest was diluted with 250ml distilled water, antibumping agent was added and 70ml of NaOH solution was added and finally two layers are formed.The trap was fitted and the condenser unit was connected to flask, while the exit tube from the condenser dips below the surface of the boric acid solution. Then the contents of the flask were swirled to ensure the rapid heating and mixing. The ammonia will be distilled into an excess 20g/litre of boric acid solution which is about 25m and containing 0.5ml of screened catalyst. 180ml distillate will be collected and titrated with standard acid to grey end point. The blank estimation on the reagent was made by using 1000g of sucrose in place of the substrate. The moisture contents of the powder were determined.

**Expression of the Result**

The total nitrogen content in the dry sample is calculated by using the following formula.

$$\text{Total nitrogen \% (m/m)} = \frac{T-14}{W}(100 - M) \quad (3.1)$$

Where:

T = standard acid required to neutralize ammonia after subtracting reagent blank, in ml

W = weight of sample taken in gram

M = Moisture contents of the sample in %( m/m).

**3.2.1.4. Determination of diastatic power of malt**

The determination of combined activity of α and β-amylase under standardized conditions only for malt [5]

**Method**

A 20g of malt was added into the mash beaker in duplicate form. The mashing bath had been attemperated at 40oC and 480ml of water was added to each beaker. The beakers were stirred with glass rod to avoid balling and placed in mashing bath by stirring for 1 hour. Then the extracted solution was cold to room temperature and the contents of the beaker are allowed to 520g by adding distilled water. The content of each mash beaker was filtered and he first 200ml was discarded. The next 50ml of the filtrate was used. After this100ml of the starch solution of each beaker was pippeted into 200ml volumetric flask and 5ml of acetate buffer was added at 20oCand stayed for 30 minutes. 4ml of NaOH was added to inactivate the enzyme and was increased to 200ml with water. The alkalinity was checked by adding drop of thymolphthalein solution and the color o the solution was blue. For blank sample 100ml of the starch solution was pippeted into 200ml volumetric flask and 2.35ml of NaOH solution was added for blank test. 5ml of malt extract was be added to malt extract and made up with water to 200ml. After this 50ml of the aliquot of the digest was transferred to the 150ml Erlenmeyer flask and 25ml of iodine solution and 3ml of NaOH solution was added and stand for 15min**.** 4.5ml of $H_2SO_4$ was added and titrated with thiosulphate until the blue color disappears.

**Result Expression**

The amount of maltose produced under the hydrolysis condition according to the formula was calculated.

$$DP_1(WK) = F(V_B - V_T) \quad (3.2)$$

$$DP_2(WK) = \frac{DP*100}{100-M} \quad (3.3)$$

Where, $DP_1$ = Diastatic power of the sample in Windisch-kolbacha units

$DP_2$ = Diastatic power of dry malt in Windisch-kolbacha units

$V_B$ = Titration value of the uncreated iodine in blank portion in ml.

$V_T$ = Titration value of the unreached iodine in test portion in ml.

F = Correction factor to obtain the result per 100g of malt used for the extraction

( F = 34.2), M = moisture contents of malt in % (w/w)

### 3.2.2. Method of wort optimization and production of beer

Before production of beer wort was optimized to highest yield of extract and targeted free amino nitrogen contents of the wort.

#### 3.2.2.1. Method of wort optimization

During milling the two rows and six row malted barley are mixed to give 1:1 ratio. Because two row barley is relatively rich in starch while, six row barley is rich in protein and this can contribute FAN. Therefore, in this case when we say malted barley we understand that it contains 50% two row and 50% six row. In this optimization process 70 gram of sample was taken and mashed with water at an appropriate maize percentage, mashing temperature mashing time and pH to get optimum yield of extract and free amino nitrogen. According to EBC (European Brewing Convection) method the yield of extract and FAN were mainly measured from the obtained wort. In other case all important physico-chemical analysis was done for the final wort produced and for individual sample.

##### 3.2.2.1.1 Milling of malt and maize

Mill Buhler universal laboratory disc ( DLFU) was used. The required 0.2mm gap setting of the mill was adjusted to grind 0.2mm fine grist of malted barley and maize

##### 3.2.2.1.2. Pre-mashing (maize gelatinization)

First, 21gm, 24.5gman 28gm of milled maize in three level was mixed with 2gm of malt and added to the cereal cooker (mashing beaker).Then an appropriate amount of maize to water ratio (150ml of water for each beaker) and finally the weight of the total solution was measured. The mashing beaker had been put in the mash bath and the bath temperature was held at $50^{o}$C for 10 minutes. When the portion of solution was stirred for 10 minutes the temperature was increased in the cereal cooker to 90°C in 15 minutes and was held for 25min. Following this, the weight of the solution after cooking was measure to calculate the amount of water recovered during boiling and it was kept to mix with the prepared grist solution in 8 minutes. After this the amount of water escaped during cooking was added.

##### 3.2.2.1.3. Mashing

The cooked mash from cereal cooker was mixed with the grist solution (made up of appropriate grist in three level i.e 49gm, 45.5gm, 40gm and 150ml of distilled water) to achieve $55^{O}$C of final solution. At this temperature holding time was 15 minute. On the completion of this, the resulting mash was derived to an appropriate mashing temperature by the rate of $1^{O}$C/min to ($64^{O}$C, $65^{O}$C and $66^{O}$C ) and mashing time was 8min, 9min and

10min respectively. Next, the temperature was increased ($1^{O}C$/ min) to$73^{O}C$ and rest for 22min, 21min, and 20min respectively. Finally, mashing-off temperature (78°C) was achieved and the whole portion of mash was transferred for filtration by using 260ml of water during filtration for dilution and rinsing.

**3.2.2.1.4. Mash filtration and calculation of extract**

The contents of the mash beaker thoroughly with a glass rod and empty immediately and completely into filter.The filtrate wort solution was accurately collected and injected to the DMA. .

**Expression of results [**18]

Calculate the extract contents of wort

$E_1$(% m/m) = P(M + 800)/100-P (.3.4)

$E_2$(%m/m) = (E1*100)/100-M (3.5)

Where

E1 = the extract content of sample in % (m/m)

E2 = the extract content of dry malt in % (m/m)

P = the extract content of wort in % (m/m)

M= the moisture contents of the malt in % (m/m)

800 = the amount of distilled water added into the mash to 100gm of malt in ml

**3.2.2.1.5. Determination of Free Amino Nitrogen in Wort by spectrophotometer**

**Principle**

The determination of the free amino nitrogen content of wort using colorimeter with (ninhydrin).The method gives an estimate of amino acids, ammonia and, in addition the terminal amino nitrogen group of peptides and proteins..The sample and a standard solution are heated in the presence of ninhydrin at PH 6.7 and the absorbance at 570nm is measured against reagent blanck. [4]

**Reagents**

*Color reagent*(Dissolve 100g $Na_2HPO_4.12H_2O$, 60 $KH_2PO_4$, 5g ninhydrin and 3g of fructose in water and diluted to 1 liter), *Diluting solution* (Dissolve 2 g $KIO_3$ in 600 ml water and add 400 ml of 96%v/v ethanol), *Glycine standard solution*(Dissolve 0.1072 glycine in water and diluted to 100 ml)

**Apparatus**

Test tubs, 16x150 mm, Glass balls, 20 to 25 mm diameter, Pipettes with automatic dispensers, Boiling water bath, Water bath at $20^{o}$c and Spectrophotometer to determine the absorbance at 570 nm**.**

## Method

The wort sample was diluted with water to contain 1 to 3 mg amino nitrogen/liter. It was diluted 1 ml to 100 ml. 2ml of the diluted sample was added in a test tube and 1 ml of color reagent was added and Stopper with a glass ball later placed in a boiling water bath for exactly16 min and then cold in a water bath at $20^{O}$C for 20 min. After this time 5 ml of diluting solutions was added and the absorbance was measured at 570 nm in a 10 mm cell against a reagent blank prepared from the reagents plus 2 ml of water in place of diluted wort.

**Calculations**

The free amino nitrogen (FAN) content of the sample was calculated by using the formula.

$$\text{FAN (mg/liter)} = \frac{2A1*d}{A2} \qquad (3.6)$$

Where, $A_1$=absorbance of test solution at 570 nm in 10 mm cell.

$A_2$=mean absorbance of standard solution at 570 nm in 10 mm cell.

d=dilution factor (e.g. 100 if dilution was 1 ml 100 ml)

**3.2.2.1.6. Determination of Viscosity of Wort**

The determination of the viscosity of the wort using viscometer was applied.

**Reagents: -** Di-ionized water,

**Apparatus: -** Viscometer, the glass capillary, Water bath at $20^{O}$C, Syringe 5 ml

**Method**

The instrument was standardized by taking the viscosity of water as 1.002mPa's at 20 $^{O}$C. 5 ml of the prepared filtered wort was taken and the viscometer was filled. The measurement was repeated twice more and the mean value was recorded. [1]

**Expression of Results**

The viscosity of the wort was calculated in mPa's according to the formula given for the instrument

### 3.2.2.2. Method of beer production from the optimized wort

In this work, beer is produced in the form of small scale from the optimized wort using Micro brewery pilot plant and the final product was analyzed. During this all important physico-chemical analysis was conducted. Beer is produced through the following steps:-

- ✓ Milling the grain to an appropriate grist and grits particle size
- ✓ mashing the grist and grits solution
- ✓ filtration of the mashed solution to get wort
- ✓ wort boiling with tetra hop
- ✓ wort cooling pitching with yeast and
- ✓ Fermentation of the wort.

### 3.2.3. Product characterization.

Product characterization is the comparing of the obtained product with the standard and similar product of different brands. The initial composition of wort is the basic measure of the final product or beer. The obtained final composition of beer specially related with the initial extract content of wort. Extract is a weight percent of dissolved material in the wort solution. For example if we have 5 Kg of sugar in the solution that weight 100 Kg of total, the solution is weight 5% sugar and this is called $5^{O}P$. A simple formula that is good approximate and applicable is:-

$$OE = \frac{1000(SG-1)}{4} \quad (3.7)$$

Where, SG =specific gravity

OE = original extract

If we measure the specific gravity before fermentation and converts to extract (number of degree plato) it is called original extract. But if we measure the specific gravity after fermentation and converts to number of degree plato it is called apparent extract.

**Attenuation**

Attenuation is the percentage of sugar that has been converted o alcohol. There are two types of attenuation. They are apparent attenuation and real attenuation. The apparent attenuation is calculated by using apparent extract.

$$A.A = \frac{(OE-AE)}{OE} * 100 \quad (3.8)$$

Where, A.A = Apparent attenuation

In this work higher apparent degree of fermentation will not be expected but it must be in the world standard point. The reason of this is free amino nitrogen value in the wort is around the lower limit and this will limit the propagation of yeast.

**Alcohol contents**

Alcohol contents are the alcohol percentage of the final beer. The alcohol content of the final beer is directly proportional to the difference in the original and final (real) extract values.

$$C_6H_{12}O_6 \longrightarrow C_2H_5OH + 2CO_2$$

180amu 92amu 88amu

This would give us an equation for the alcohol percent by weight:-

$$(A\%w/w) = (OE - RE) * \frac{92}{180} \quad (3.9)$$

If we insert that $OE = \frac{1000(SG-1)}{4}$ we will get the following formula.

$$\text{Alcohol } \%w/w = \frac{76.08(OG-FG)}{1.775-OG} \quad (3.10)$$

$$\text{Alcohol } \%v/v = A\%w/w \left(\frac{FG}{0.794}\right) \quad (3.11)$$

Where, 0.794 is SG of ethanol

**3.2.3.1. Determination of seven parameters by Antan paar alcoholyzer**

For most parameters analysis the Alcolyzer (Anton paar) device was used. Since this device is the most accurate and used widely in brewery companies in the world. Before measuring the parameters the Anton paar device was calibrated and cleaned. Then100ml of sample was filtered and injected to the device while the result was displayed in the screen after five minutes. This device was used to measure the alcohol value %w/w, alcohol value %v/v, specific gravity, original gravity, apparent extract, real extract and apparent degree of fermentation

**3.2.3.2. Determination of vicinal diketones (VDK)**

**Principle** [17]

This is to determine the vicinal diketones, mainly diacetyl and 2, 3- pentanedione in beer by ultraviolet spectrophotometry. The vicinal diketones, diacetyl and pentanedione, are distilled

from the beer and then combined with O-phenylenediamine(OPD) to form derivatives of quinoxaline. The amount of these compounds is measured by the absorbance at 335 nm.

**Apparatus**

Spectrophotometer for making measurement at 335 nm, Photometric cells of quartz, 500 ml round bottom flasks with conical ground joint ,Measuring cylinders, Pipettes, 2 ml, 10 ml, Glass with glass stopper,50ml, filter paper

**Reagents**

Hydrochloric acid 4 mol/l and O-phenyl diamine 1% (OPD)

**Method**

100ml of the filtered sample was distilled in round bottom flasks with conical ground joint. 10ml of the distillate sample was added to the 100ml Elmer flask and 0.5ml of 1% OPD was added. In the same way 10 ml of distilled water was taken and adde 0.5ml of OPD was added for blank sample. Then for further reaction the sample was stayed in the dark room for 20minutes. After 20 minutes 2ml of 4N HCl was added to each flask

**3.2.3.3. Determination of Bitterness [**17]

Beer is degassed without the loss of foam. The bitter substances are mainly iso-α-acids, that are extracted from acidified wort or beer with isooctane and the absorbance at 275 nm is measured.

**Apparatus**

Spectrophotometer with deuterium lamp suitable for making measurement at 275 nm
Photometric cells in quartz, optical path length 1.000 cm, Mechanical shaker, Conical flasks with glass stopper, 50 ml, Volumetric pipette 10 ml, Centrifuge, Separatory funnel, 1000 ml

**Reagents**

Isooctane (2, 2, 4-trimethylpentane) spectrophotometer grade, Hydrochloric acid 3 mol/l.
Octyl alcohol reagent grade

**Method**

First the 300ml sample was stirred by magnetic starrier for five minute and10ml of sample was taken into 1the 100ml flask. Then 1ml of 3N HCL and 20ml standardized of iso-octane

was added. The final solution was mechanical shaker for 15minutes. Finally the shaked solution was rested for 10minutes and the bitterness measured by using spectrophotometers at 320nm. The finally the spectrometer display give us 13.65. The expected result was 14.75 this can be tolerated and can adjusting by addition of tetra hop.

**Expression of Results**

Bitterness (BU) = 50*ABS275

**3.2.3.4. Polyphenol**
**Principle**

The treatment of the sample with a solution of Carboxymethyl cellulose and EDTA. The Reaction of polyphenols with ferric ions in alkaline solution. Measurement of the absorbance at 600nm of the read coloured solution against a blank solution [17]

**Apparatus**

Spectrophotometer, Visible range, Cuvettes, 10mm, Centrifuge, 25ml volumetric flask graduated with glass stoppers, Graduated Pipettes 0.5ml, 1 ml, 10ml, and 25ml

Carboxymethyl cellulose/ethylene diamine tetra acetic acid (CMC/EDTA) or 10gm/lit **CMC**, containing 2gm/lit EDTA, ferric reagent(5.6gm/liter of fe3+) 25%ammonia reagent

**Method**

10 ml of the sample beer and 8ml of CMC/EDTA reagent were pipetted in to a 25 ml volumetric flask. Stopper and thoroughly mix the contents. 0.5ml ferric reagents was added to the measurement sample only and thoroughly mixed. 0.5ml ammonia reagent was added and the solution was mixed. The whole solution was made 25ml with water and mixed. The same procedure for the blank sample but ferric reagent is not added. After 10 minutes the absorbance in a 10 mm cuvette using a spectrophotometer at 600 nm was measured.

**Expression of Results**

Calculate the content of polyphenols using the formula: P = A*820*F

The result in mg/lit to the nearest whole number.

Where, P = Polyphenol content (mg/litre, A = Absorbance at 600nm, F = Dilution factor (F = 1)

### 3.2.3.5. Determination of calcium ion ($Ca^{2+}$)

**Apparatus** Glass titration burette, conical flask Erlenmeyer 250 ml, volumetric pipette, 3 ml, 10 ml, Stainless steel spatula and Magnetic stirrer, [17]

**Reagents**

Potassium hydroxide 8 N, EDTA 0.01 mol/l (N/50), Cal Red indicator (calcium indicator: prepared from Calconcarboxylic acid 1 g and Potassium sulphate 99 g)

**Procedure**

10 ml of sample was Pipetted into a conical flask. Distilled water was added until 100 ml and 3 ml of potassium hydroxide solution was added. The solution was mixed well with magnetic stirrer for 5 minutes. Then 1 pinch of Cal-Red indicator was added and titrated with 0.01 mol/l EDTA.

**Expression of results**

Calcium, ppm = $40.08*V_1*0.01*1000$/ml of sample. Where, 40.08 is factor.

Where, $V_1$ = the volume of EDTA consumed

### 3.2.3.6. Determination of foam stability value

To determine the foam stability of beer using NIBEM T / TPH foam stability tester. Beer,is attemperated to 20 + 0.5 $^{O}$C in a sealed container, is dispensed through foam flashing device (Flasher) in which beer is forced under carbon dioxide pressure through an orifice. This produces a standard glass of beer /foam. [16]

## 3.4. Design of the Experiment

Data analysis has performed by Design Expert software 7.00 and suitable model equation was gained. In this optimization process the process variable such as maize percentage, pH,

temperature and mashing time are considered. From the above factors the pH was kept constan while, the three factors were seen in three levels General factorial design method was used to conduct this experiment and optimization of yield of extract content and free amino nitrogen of wort. Three replications, three factors, and three levels ($3x3^{3)}$ were designed in combinations for this work. Analysis of variance (ANOVA) was carried out to sort out the effect as a result of using different conditions, and to understand the main effect and the interaction effects. The Significance was accepted at 0.05 level of probability ($p<0.05$). Mean separation was performed by each Pair Student's t" for multiple comparisons of means.

# CHAPTER FOUR

# RESULT AND DISCUSSION

### 4.1. Determination the physico-chemical characteristics of malted barley and maize

Determination of the physico-chemical characterstics and the process designed was the main tools for the optimization of the described parameters.

#### 4.1.1. Determination the physico-chemical characteristics of malted barley and maize using Inframatic 9500 machine

Different literature indicate that the average oil contents of maize is 4.5 to 5.0 for this case the measurement from the Inframatic machine indicate that the BH660 are more appropriate than the other because relatively BH661 and BH540 it contains low oil contents. Therefore in this experiment only BH660 was considered and used.

Table 4.1 proximate analysis result of different types of maize

| Type of sample | Moisture contents (%) | Protein contents(%db) | Extract content FG (%db%) | Oil content(%db) |
|---|---|---|---|---|
| Two row malted barley | 6.65 | 10.2 | 82.25 | - |
| Six row malted barley | 5.5 | 10.8 | 80.70 | - |
| BH660 Maize before de-germed | 9.2 | 7.5 | - | 4.2 |
| BH661 Maize before de-germed | 8.95 | 7.70 | - | 4.40 |
| BH540 Maize before degermed | 9.10 | 7.9 | - | 4.30 |
| BH660 Maize after de-germed | 9.30 | 6.8 | - | 0.95 |

Initially the proximate analysis indicates that BH660 maize type had relatively less percentage of oil. It was selected for wort preparation. From this table that we understand the oil content of the BH660 has been minimized to 0.95% and this is tolerant.

### 4.1.2. Determination of diastatic power and Total nitrogen

The diastatic power is the important parameter to obtain optimum yield of extract. If the diastatic power is lower, the enzymatic action during mashing to break the starch to simple sugar (fermentable sugar) will be limited and this will characterize lower yield of extract. In other case the amount of total nitrogen indicate the protein content of the grain and the result is as the following.

Table 4.2. Determination of diastatic power and total nitrogen

| SN | Type of grain | Diastaic power (Windisch-kolbacha units | Total nitrogen (w/w |
|---|---|---|---|
| 1 | Two row | 238.50 | 1.67 |
| 2 | Six row | 275.48 | 1.84 |
| 3 | BH660-maize | - | 1.38 |

Here from the above table the diastatic power of the six row barley were relatively higher than the two row, this is true while the diastatic power of the BH660-maize was not measured because it is not malted and the value of diastatic power is assumed to be zero. This value is supported by different literature that distatic power of six row barley is greater than two row barley. In other case the total nitrogen value of six row is higher than the two row barley and BH660-maize. But the average total nitrogen content of the three grain is 1.63. However, the contribution of optimum free amino nitrogen is depends on the soluble nitrogen compounds and this is obtained during FAN analysis which the result obtained was tolerated.

## 4.2. Wort optimization and production of beer

Optimum wort is basically the first thing we have to do before we think about the qualified beer. Optimum wort is the type of wort that characterized maximum yield of extract and enough free amino nitrogen for the growth of yeast.

### 4.2.1. Wort optimization

Different literature say that 7-10% of malt is must be mixed with maize to decrease the viscosity of the gelatinized maize solution during cooking. Here 2gm of malt was mixed with maize during cooking and this was perfectly observed that the solution was non viscous and easily agitated without forming balling. All the calculation mentioned in the section of method was used to calculate the yield of extract and free amino nitrogen. The expected yield of extract was obtained but the amino nitrogen is not yet. During mashing the effect of independent variables was seen both on the yield of extract and free amino nitrogen. The maize percentage was the main effect seen on the value of the two response variable, but it is not the only independent variable to decide the optimum point. Therefore to decide the optimum points the Design expert® 7 software was used.

The resulting data obtained during mashing was discussed using Design expert® 7 software to decide the optimum operating parameters of maize percentage, mashing time and mashing temperature. The dependent variable used as a response parameter was the yield of extract of wort and free amino nitrogen (FAN). All experiments were carried out in a randomized order to minimize the effect of unexpected variability in the observed response due to extraneous factors.

To determine whether or not the quadratic model is significant, it was crucial to perform analysis of variance (ANOVA). The probability (P-values) values were used as a device to check the significance of each coefficient, which also show the interaction strength of each parameter. The smaller the P-values are, the bigger the significance of the corresponding coefficient. The following table is formulated and input to soft ware.

Table 4.3. Experimental design formulated for wort optimization

| Std order | Run order | Block | Factor1 A: maize percentage (%) | Factor2 B:mashing time (minute) | Factor3 C:mashing temperature ($^{O}C$) | Yield of extract Percent (%) | FAN(ppm) |
|---|---|---|---|---|---|---|---|
| 22 | 1 | Block1 | 35 | 10 | 64 | 84.6 | 114.45 |
| 1 | 2 | Block1 | 30 | 8 | 64 | 84.34 | 118.25 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 48 | 3 | Block1 | 30 | 10 | 65 | 84.26 | 117.24 |
| 25 | 4 | Block1 | 40 | 10 | 64 | 84.45 | 110.25 |
| 4 | 5 | Block1 | 35 | 8 | 64 | 84.5 | 114.25 |
| 7 | 6 | Block1 | 40 | 8 | 64 | 84.84 | 109.56 |
| 59 | 7 | Block1 | 35 | 8 | 66 | 84.56 | 114.87 |
| 47 | 8 | Block1 | 30 | 10 | 65 | 84.26 | 117.52 |
| 13 | 9 | Block1 | 35 | 9 | 64 | 84.37 | 114.23 |
| 62 | 10 | Block1 | 40 | 8 | 66 | 84.7 | 111.04 |
| 77 | 11 | Block1 | 35 | 10 | 66 | 84.36 | 114.22 |
| 54 | 12 | Block1 | 40 | 10 | 65 | 84.14 | 116.22 |
| 41 | 13 | Block1 | 35 | 9 | 65 | 84.71 | 114.96 |
| 38 | 14 | Block1 | 30 | 9 | 65 | 84.35 | 116.98 |
| 67 | 15 | Block1 | 35 | 9 | 66 | 84.43 | 113.25 |
| 64 | 16 | Block1 | 30 | 9 | 66 | 84.12 | 117.64 |
| 9 | 17 | Block1 | 40 | 8 | 64 | 84.74 | 110.25 |
| 19 | 18 | Block1 | 30 | 10 | 64 | 83.84 | 118.08 |
| 53 | 19 | Block1 | 40 | 10 | 65 | 84.94 | 110.12 |
| 39 | 20 | Block1 | 30 | 9 | 65 | 84.32 | 117.16 |
| 81 | 21 | Block1 | 40 | 10 | 66 | 84.67 | 108.88 |
| 52 | 22 | Block1 | 40 | 10 | 65 | 84.89 | 108.62 |
| 3 | 23 | Block1 | 30 | 8 | 64 | 84.26 | 117.68 |
| 70 | 24 | Block1 | 40 | 9 | 66 | 84.62 | 108.55 |
| 75 | 25 | Block1 | 30 | 10 | 66 | 84.08 | 117.08 |
| 78 | 26 | Block1 | 35 | 10 | 66 | 84.49 | 114.27 |
| 8 | 27 | Block1 | 40 | 8 | 64 | 84.69 | 111.55 |
| 5 | 28 | Block1 | 35 | 8 | 64 | 84.6 | 115.08 |
| 17 | 29 | Block1 | 40 | 9 | 64 | 84.54 | 109.94 |
| 79 | 30 | Block1 | 40 | 10 | 66 | 84.64 | 110.32 |

Continues of table 4.3

| Std order | Run order | Block | Factor1 A: maize percentage (%) | Factor2 B:mashing time (minute) | Factor3 C:mashing temperature | Yield of extract Percent (%) | FAN(ppm) |
|---|---|---|---|---|---|---|---|
| 61 | 31 | Block1 | 40 | 8 | 66 | 84.8 | 110.22 |
| 65 | 32 | Block1 | 30 | 9 | 66 | 84.14 | 116.22 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 35 | 33 | Block1 | 40 | 8 | 65 | 85.06 | 109.11 |
| 71 | 34 | Block1 | 40 | 9 | 66 | 84.7 | 109.36 |
| 51 | 35 | Block1 | 35 | 10 | 65 | 84.6 | 114.82 |
| 23 | 36 | Block1 | 35 | 10 | 64 | 84.18 | 115.05 |
| 6 | 37 | Block1 | 35 | 8 | 64 | 84.45 | 114.94 |
| 43 | 38 | Block1 | 40 | 9 | 65 | 85.05 | 107.94 |
| 14 | 39 | Block1 | 35 | 9 | 64 | 84.38 | 113.35 |
| 80 | 40 | Block1 | 40 | 10 | 66 | 84.66 | 108.4 |
| 16 | 41 | Block1 | 40 | 9 | 64 | 84.47 | 111.46 |
| 37 | 42 | Block1 | 30 | 9 | 65 | 84.35 | 116.98 |
| 10 | 43 | Block1 | 30 | 9 | 64 | 84.06 | 116.5 |
| 27 | 44 | Block1 | 40 | 10 | 64 | 83.84 | 118.08 |
| 45 | 45 | Block1 | 40 | 9 | 65 | 84.95 | 109.26 |
| 73 | 46 | Block1 | 30 | 10 | 66 | 83.94 | 118.24 |
| 57 | 47 | Block1 | 30 | 8 | 66 | 84.2 | 118.28 |
| 15 | 48 | Block1 | 35 | 9 | 64 | 84.35 | 114.74 |
| 55 | 49 | Block1 | 30 | 8 | 66 | 84.23 | 118.56 |
| 2 | 50 | Block1 | 30 | 8 | 64 | 84.19 | 116.56 |
| 76 | 51 | Block1 | 35 | 10 | 66 | 84.45 | 115.01 |
| 24 | 52 | Block1 | 35 | 10 | 64 | 84.52 | 108.43 |
| 50 | 53 | Block1 | 35 | 10 | 65 | 84.67 | 113.98 |
| 12 | 54 | Block1 | 30 | 9 | 64 | 84.09 | 117.68 |
| 18 | 55 | Block1 | 40 | 9 | 64 | 84.42 | 110.52 |
| 72 | 56 | Block1 | 40 | 9 | 66 | 84.67 | 108.96 |
| 46 | 57 | Block1 | 30 | 10 | 65 | 84.35 | 116.68 |
| 34 | 58 | Block1 | 40 | 8 | 65 | 85.13 | 111.5 |
| 74 | 59 | Block1 | 30 | 10 | 66 | 84.03 | 117.21 |
| 26 | 60 | Block1 | 40 | 10 | 64 | 84.52 | 108.3 |

Continues of table 4.3

| Std order | Run order | Block | Factor1 A: maize percentage (%) | Factor2 B:mashing time (minute) | Factor3 C:mashing temperature | Yield of extract Percent (%) | FAN(ppm) |
|---|---|---|---|---|---|---|---|
| 11 | 61 | Block1 | 30 | 9 | 64 | 84.35 | 114.74 |
| 40 | 62 | Block1 | 35 | 9 | 65 | 84.84 | 109.56 |
| 20 | 63 | Block1 | 30 | 10 | 64 | 84.38 | 113.35 |
| 60 | 64 | Block1 | 35 | 8 | 66 | 84.49 | 114.23 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 29 | 65 | Block1 | 30 | 8 | 65 | 84.52 | 108.3 |
| 58 | 66 | Block1 | 35 | 8 | 66 | 84.44 | 115.1 |
| 30 | 67 | Block1 | 30 | 8 | 65 | 84.48 | 117.56 |
| 69 | 68 | Block1 | 35 | 9 | 66 | 84.48 | 114.45 |
| 63 | 69 | Block1 | 40 | 8 | 66 | 84.83 | 109.56 |
| 33 | 70 | Block1 | 35 | 8 | 65 | 84.89 | 114.94 |
| 32 | 74 | Block1 | 35 | 8 | 65 | 84.72 | 114.86 |
| 68 | 75 | Block1 | 35 | 9 | 66 | 84.38 | 115.02 |
| 42 | 76 | Block1 | 35 | 9 | 65 | 85.05 | 107.94 |
| 44 | 77 | Block1 | 40 | 9 | 65 | 85.01 | 110.05 |
| 66 | 78 | Block1 | 30 | 9 | 66 | 84.05 | 117.25 |
| 56 | 79 | Block1 | 30 | 8 | 66 | 84.15 | 117.53 |
| 31 | 80 | Block1 | 35 | 8 | 65 | 84.82 | 115.25 |
| 28 | 81 | Block1 | 30 | 8 | 65 | 84.36 | 118.45 |

From the above table an appropriate point of maize percentage, mashing temperature and mashing time to give maximum yield of extract and targeted free amino nitrogen was seen in detail for each response variables.

#### 4.2.1.1. Data Analysis of Response1 (yield of extract)

The resulting data obtained during mashing was discussed using Design expert® 7 software to decide the optimum operating parameters of maize percentage, mashing time and mashing temperature. The optimization of yield of extract can be seen in different dimension.

##### 4.2.1.1.1.The analysis of variance or ANOVA

The analysis of variance or ANOVA to show the effect of each independent variable individually and the interaction between each variables to obtain maximum yield of extract by significant model was seen in detail.

Table 4.4. Analysis of variance (ANOVA) for Quadratic Model

| Source of model | Sum of squares | df | Mean squares | F -value | p-value prob>F | Remark |
|---|---|---|---|---|---|---|
| Model | 5.99 | 9 | 0.67 | 33.68 | <0.0001 | Significant |
| A-maize percentage | 3.33 | 1 | 3.33 | 168.19 | <0.0001 | |
| B-mashing time | 0.70 | 1 | 0.70 | 35.20 | <0.0001 | |
| C-mashing | 0.056 | 1 | 0.056 | 2.84 | 0.0966 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Temperature | | | | | | |
| AB | 0.053 | 1 | 0.053 | 2.68 | 0.1063 | |
| AC | 0.15 | 1 | 0.15 | 7.37 | 0.0083 | |
| BC | 0.071 | 1 | 0.071 | 3.60 | 0.0620 | |
| A2 | 0.13 | 1 | 0.13 | 6.38 | 0.0138 | |
| B2 | 2149E-003 | 1 | 2149E-003 | 0.11 | 0.7426 | |
| C2 | 1.52 | 1 | 1.52 | 76.76 | <0.0001 | |
| Residual | 1.40 | 71 | 0.020 | | | |
| Lack of Fit | 0.24 | 17 | 0.014 | 0.67 | 0.8200 | Not significant |
| Pure Error | 1.16 | 54 | 0.021 | | | |
| Cor Total | 7.40 | 80 | | | | |

The Model F-value of 33.68 implies the model is significant. There is onlya 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, AC, $A^2$, $C^2$ are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve our model. The "Lack of Fit F-value" of 0.59 implies the Lack of Fit is not significant relative to the pure error. There is a 87.95% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good. Because we want the model to fit.

The coefficient of Variation, the standard deviation expressed as a percentage of the mean; Predicted Residual Error Sum of Squares, which is a measure of how the model fits each point in the design; the R-Squared, measure of the amount of variation around the mean explained by the model; Adj R-Squared that is a measure of the amount of variation around the mean explained by the model, Pred R-Squared, a measure of the amount of variation in new data explained by the model, and Adequate Precision, this is a signal to disturbance ratio due to random error, presented in the table 4.4, below, are used to decide whether the model can be used or not.

Table 4.5. Model adequacy measures

| | | | |
|---|---|---|---|
| Std. Dev. | 0.14 | R-Squared | 0.8102 |
| Mean | 84.48 | Adj R- Squared | 0.7862 |
| C.V.% | 0.17 | Pred R- Squared | 0.7471 |
| PRESS | 1.89 | Aeq Precision | 20.783 |

The regression coefficients and the corresponding 95% CI (Confidence Interval) High and Low were presented in table 4-3 below. If zero was in the range High and Low 95% Confidence Interval, the factors has no effect. From the 95% CI High and Low values of each model term, it could be concluded that the regression coefficients of maize percentage and the interaction terms of mashing temperature time & maize percentage have highly significant effect in wort product

Table 4.6. Regression coefficients and the corresponding 95% CI High and Low

| Factor | Coeffecint of Estimate | Standard Error | 95%CI Low | 95%CI High |
|---|---|---|---|---|
| Intercept | 84.74 | 0.041 | 84.66 | 84.82 |
| A- maize percentage | -0.25 | 0.019 | 0.21 | 0.29 |
| B-mashing time | -0.11 | 0.019 | -0.15 | -0.075 |
| C- mashing temperature | 0.032 | 0.019 | -5.930E-003 | -0.070 |
| AB | -0.038 | 0.023 | -0.085 | 8.394E-003 |
| AC | 0.064 | 0.023 | 0.017 | 0.11 |
| BC | 0.044 | 0.023 | -2.283E-003 | 0.091 |
| A2 | -0.084 | 0.033 | -0.15 | -0.018 |
| B2 | -0.011 | 0.033 | -0.077 | 0.055 |
| C2 | -0.29 | 0.033 | -0.36 | -0.22 |

By the designed experimental data from table 4.6, the quadratic polynomial model equation for wort production will be shown as below:

**Quadratic polynomial model equation**

Final Equation in Terms of Coded Factors:

**yield of extrac**t = +84.74 +0.25* A -0.11* B +0.032* C -0.038 * A* B +0.064 *A* C +0.044 *B* C -0.084 * $A^2$ -0.011*$B^2$ -0.29 *$C^2$ (4.1)

Final Equation in Terms of Actual Factors:

**yield of extract** = -109.34293-0.47394*maize percentage -2.53741*mashing time +36.93509*mashing temperature -7.66667E-003*maize percentage*mashing time +0.0122722*maize percentage*mashing temperature +0.044444* mashing time* mashin temperature-3.34815E-003*maize percentage$^2$ -0.010926*mashing time$^2$

-0.29037*Temperature$^2$

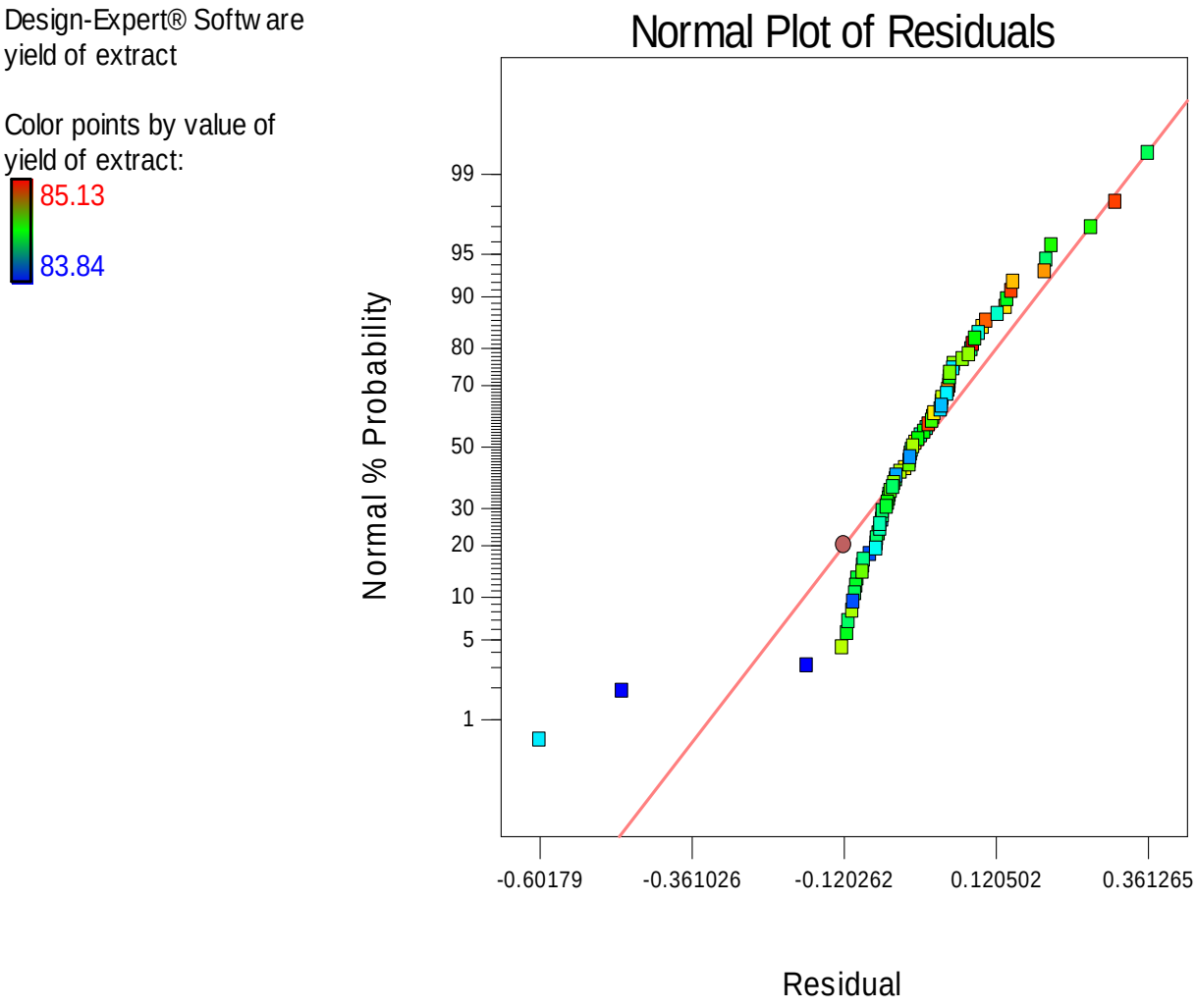



Figure 4.1 Normal plots of residuals for yield of extract

From the plot as shown above, the normal probability plot indicates the residuals following a normal distribution, in the case of this experiment the points in the plots shows almost fit to a straight line in the figure, this shows that the quadratic polynomial model satisfies the assumptions analysis of variance (ANOVA) i.e. the error distribution is approximately normal. But the result of response variable is very close and this shows that the effect of variables on the response variable is small.

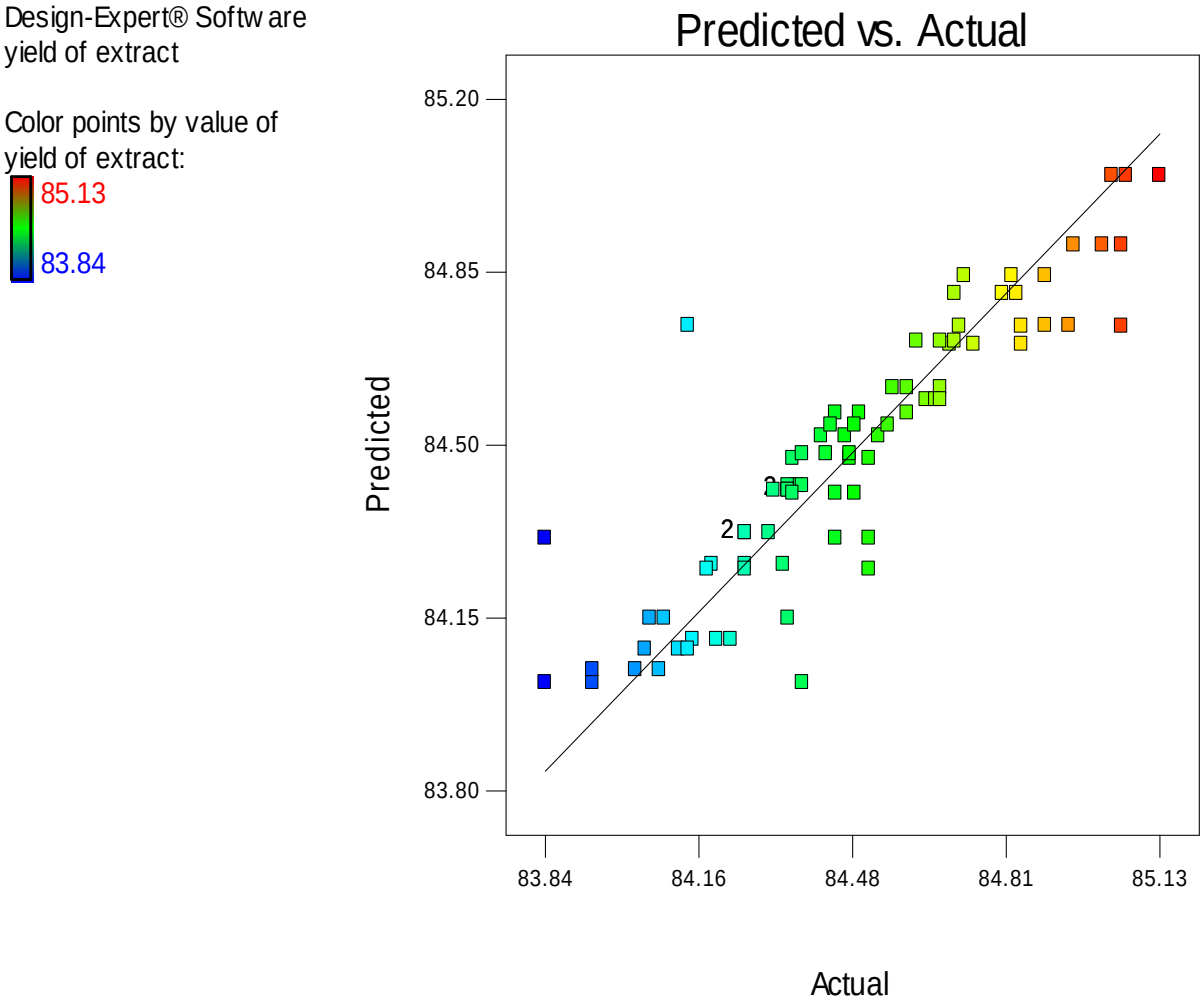



Figure 4.2. Predicted versus Actual for yield of xtract

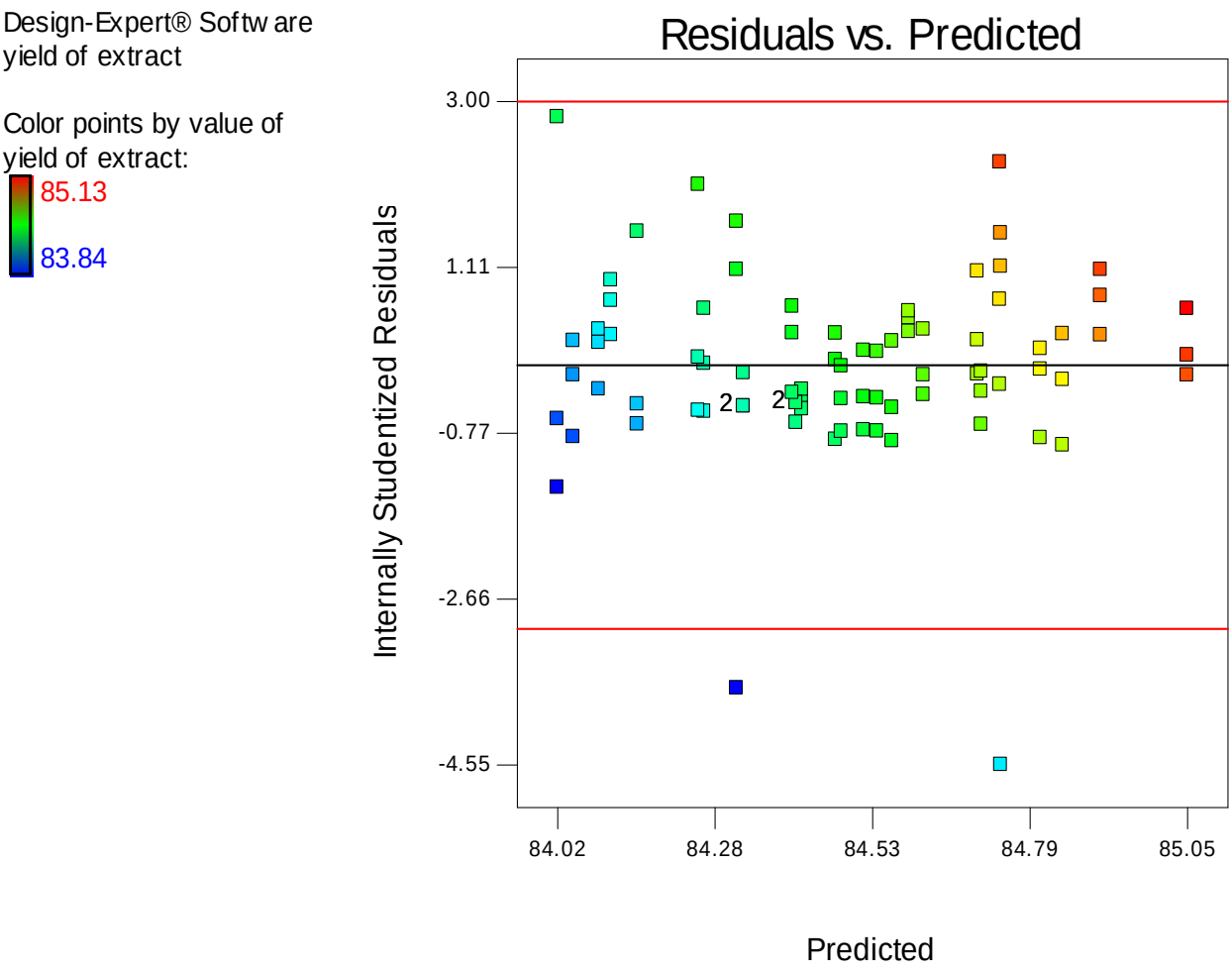


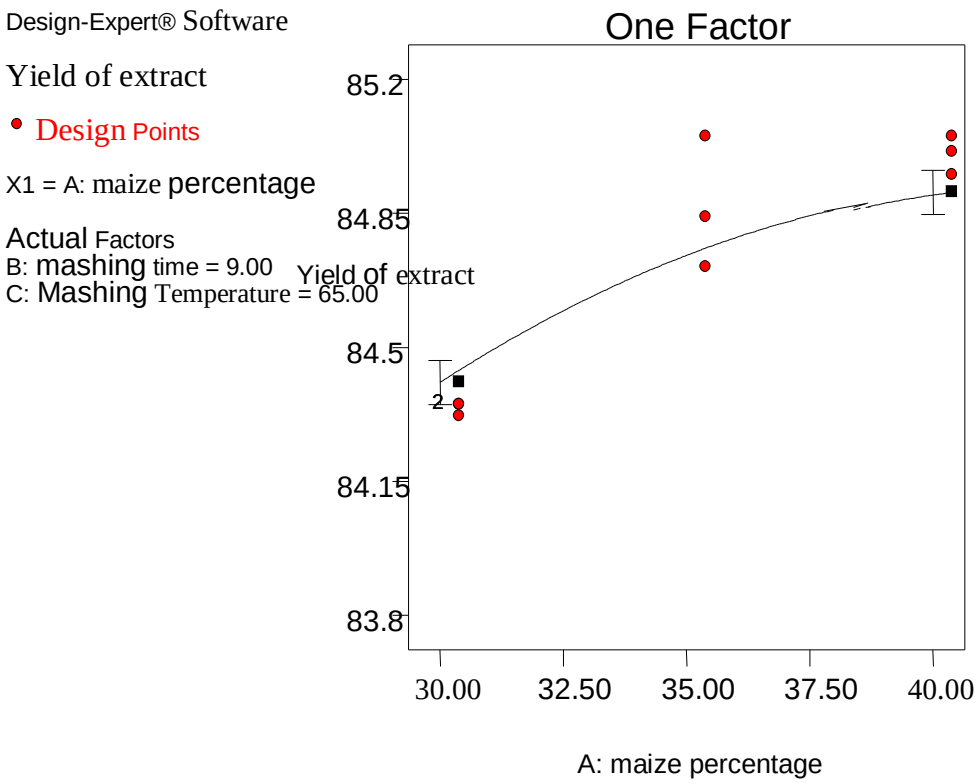

Figure 4.3 Residual versus predicted values for yield of extract

If the model is correct and the assumptions are satisfied, the residuals should be structure less. They should be unrelated to any other variable including the predicted response. A simple check is to plot the residuals versus the fitted (predicted) values. A plot of the residuals versus the rising predicted response values tests the assumption of constant variance. The plot shows random scatter which justifying no need for an alteration to minimize personal error.

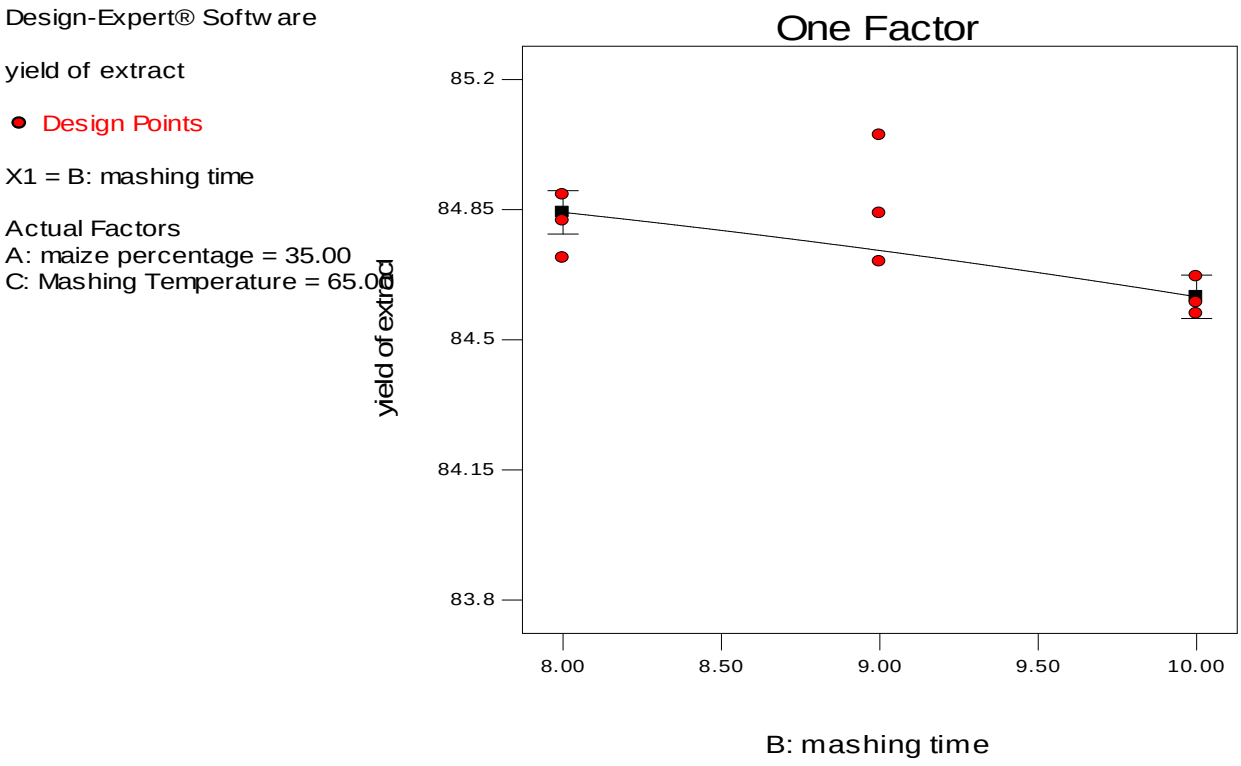
#### 4.2.1.1.2. Effects of Experimental Variables on yieldf of extract

Figures below show the response of yield of extract versus individual variable and two independent variables. Maize percentage is the most significant factor for yield of extract among the three variables and the effect of the three independent variables together are not significant while the effect of the two independent variables on the yield of extract are little.

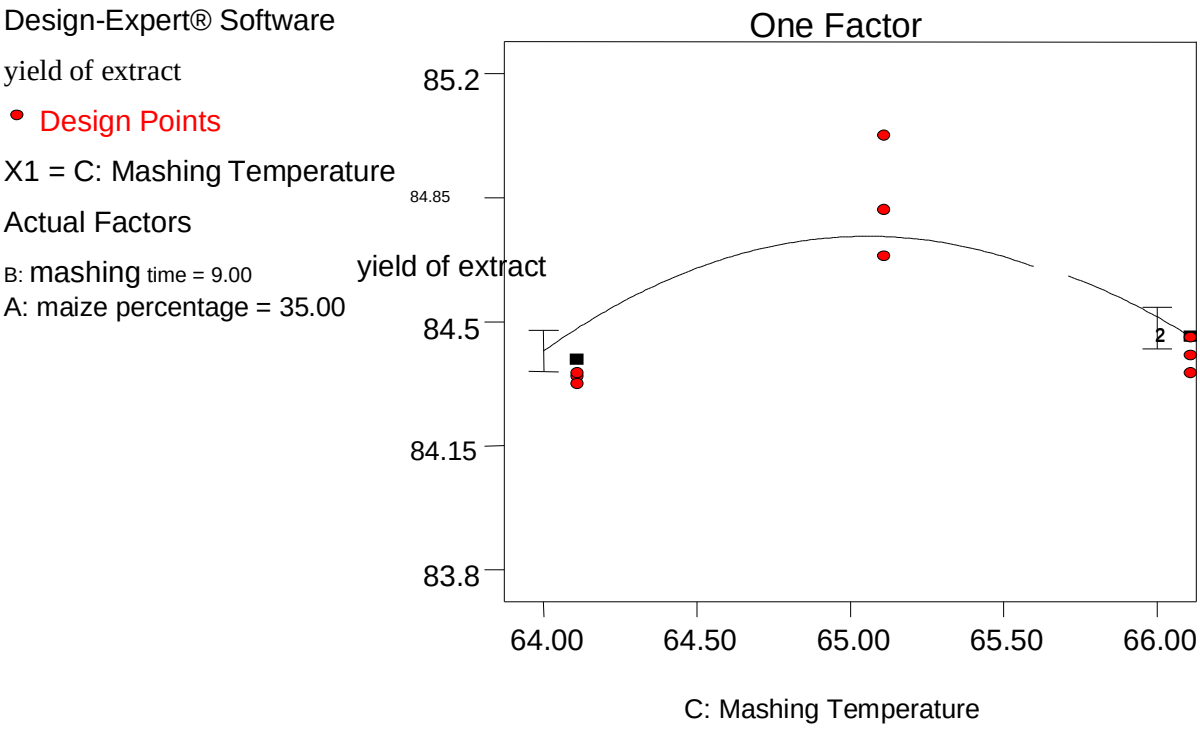
**A. The effect of individual variable on yield of extract**




a) Effect of maize percentage on yield of extract




b) Effects of mashing time on yieldf of extract

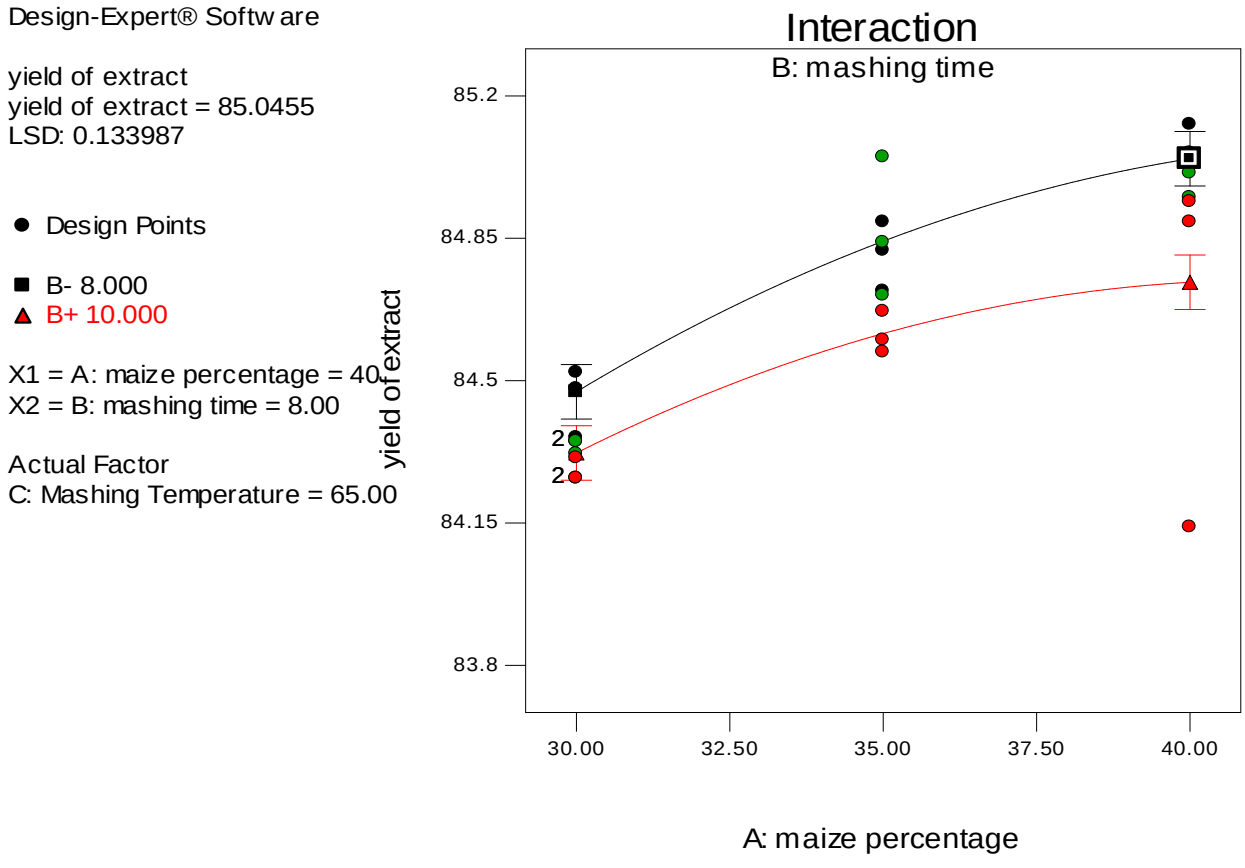



c) Effects of mashing temperature on yieldf of extract

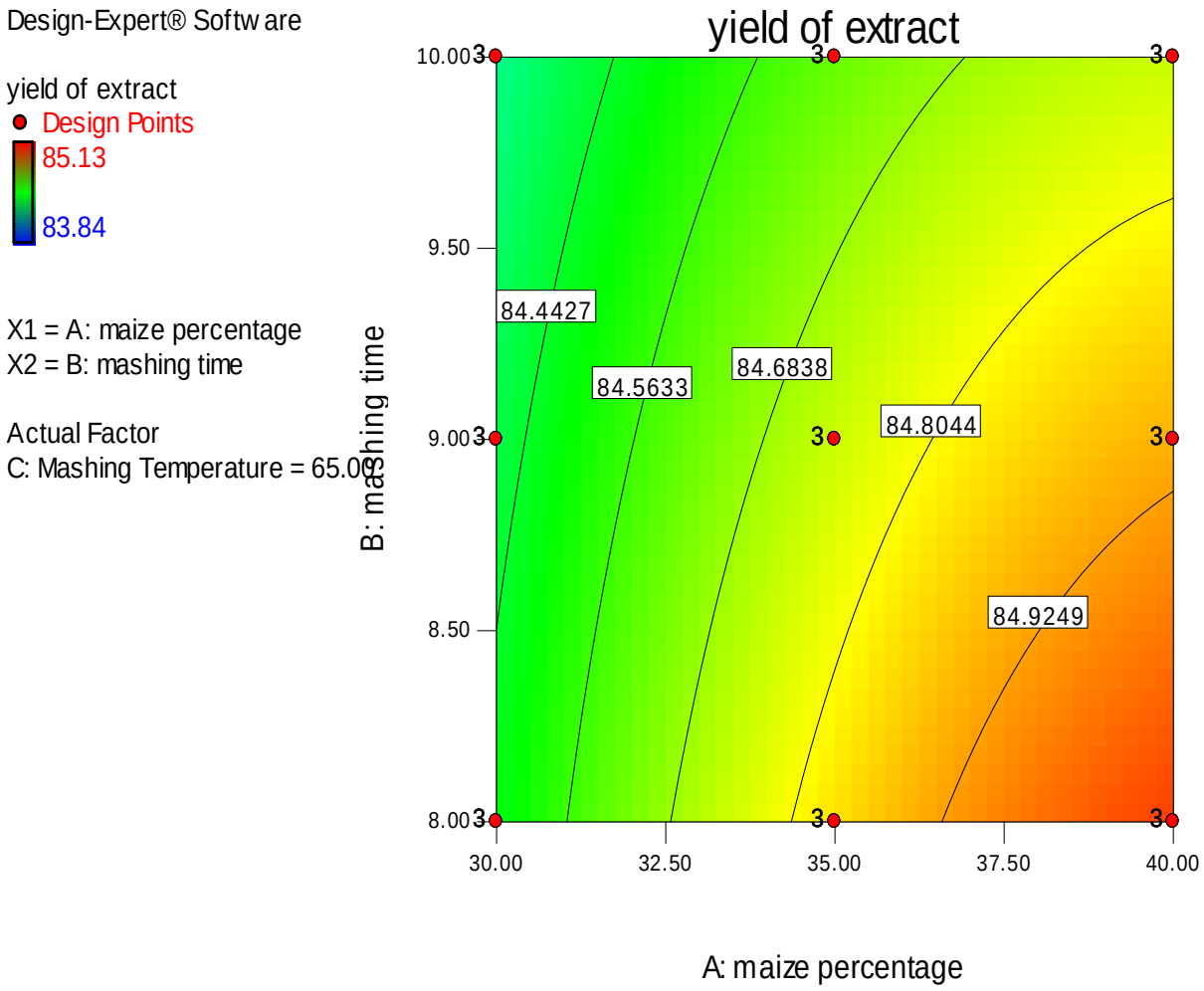
Figure 4.4. the effect of individual Variables on the yied of extract

The above picture a) show that the maize percentage have significant effect in which as maize percentage increase the yield of extract will be increased. In case the gragh shows that the increasing yield of extract cannot continue because enzyme in the malt will be limited for saccharification of the total starch in the grain .Therefore the diastatic power of the malt must be high to use high percentage of malt. In the case of picture b) the effect of mashing temperature is not as much significant but when mashing temperature is increased the yield of extract will be decresed. This because β-amylase enzyme is complete the break down of starch to maltose in prefered time(short time) than long time. The effect of mashing temperature on the yield of extract observed from the picture c) above the best optimum temperature for the β-amylase enzyme is 65$^{O}$C because at this temperature high yield of extact was seen.

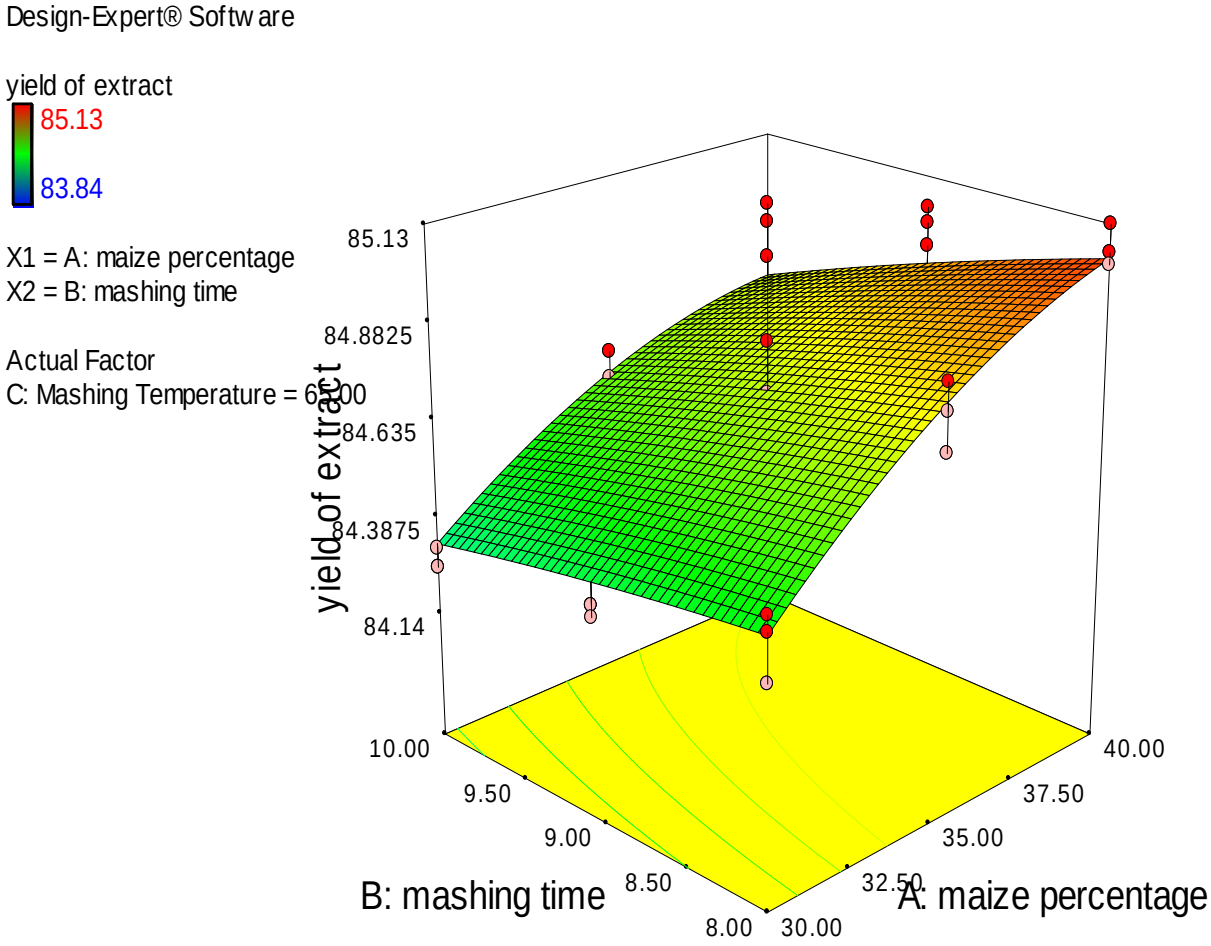
**B. The effects of mashing time (fixed) and maize percentage on the yield of extract of wort**




a) The effects of mashing time (fixed) and maize percentage on the yield of extract of wort, when the mashing temperature is at the center point




b) Contour plots of the effects of mashing time and maize percentage on the yield of extract of wort




C) Response surfaces plot of the effects of maize percentage and mashing time on the yield of extract

Figure 4.5. The effects of mashing time and maize percentage on the yield of extract of wort

Here from this plot the effect of the two independent variables on the yield of extract is parallel and opposite effect. That means at low mashing time the yield of extract is low while at higher maize percentage higher yield of extract was observed. But the curved graph indicates that the activity of enzyme is limited at high percentage of maize.

**C. The effects of mashing temperature (fixed) and maize percentage on the yield of extract**

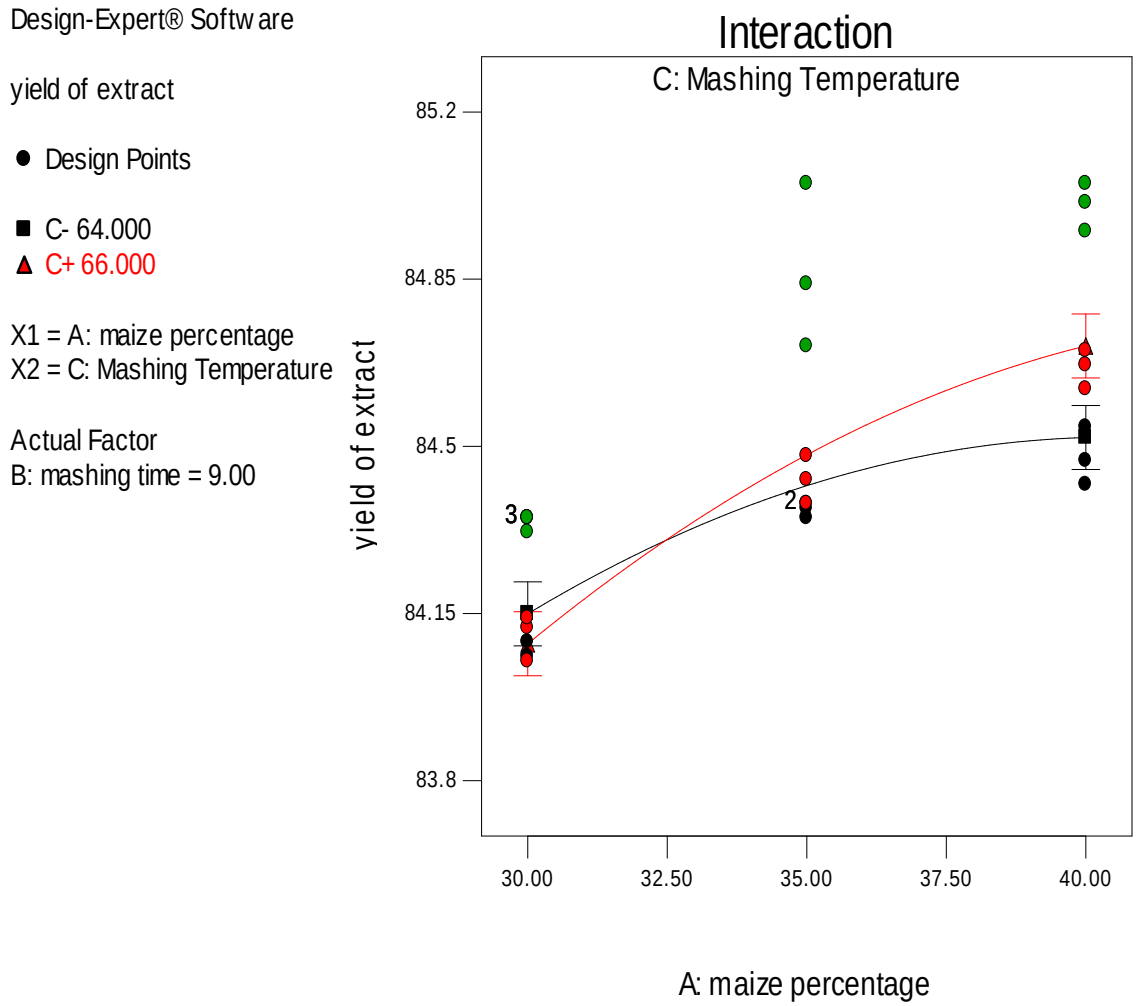



Figure 4.6. The effects of mashing temperature (fixed) and maize percentage on the yield of extract when mashing time is at the center point

From the above plot we understand that at lower mashing temperature the yield of extract is higher at the low percentage of maize and at higher mashing temperature the yield of extract is higher at high percentage of maize. This is due to the activity of β-amylase at lower temperature and higher temperature. β-amylase is not very active at low temperature and damaged at higher temperature. As a result of this low yield of extract is observed.

**D. The effects of mashing temperature (fixed) and mashing time on the yield of extract**

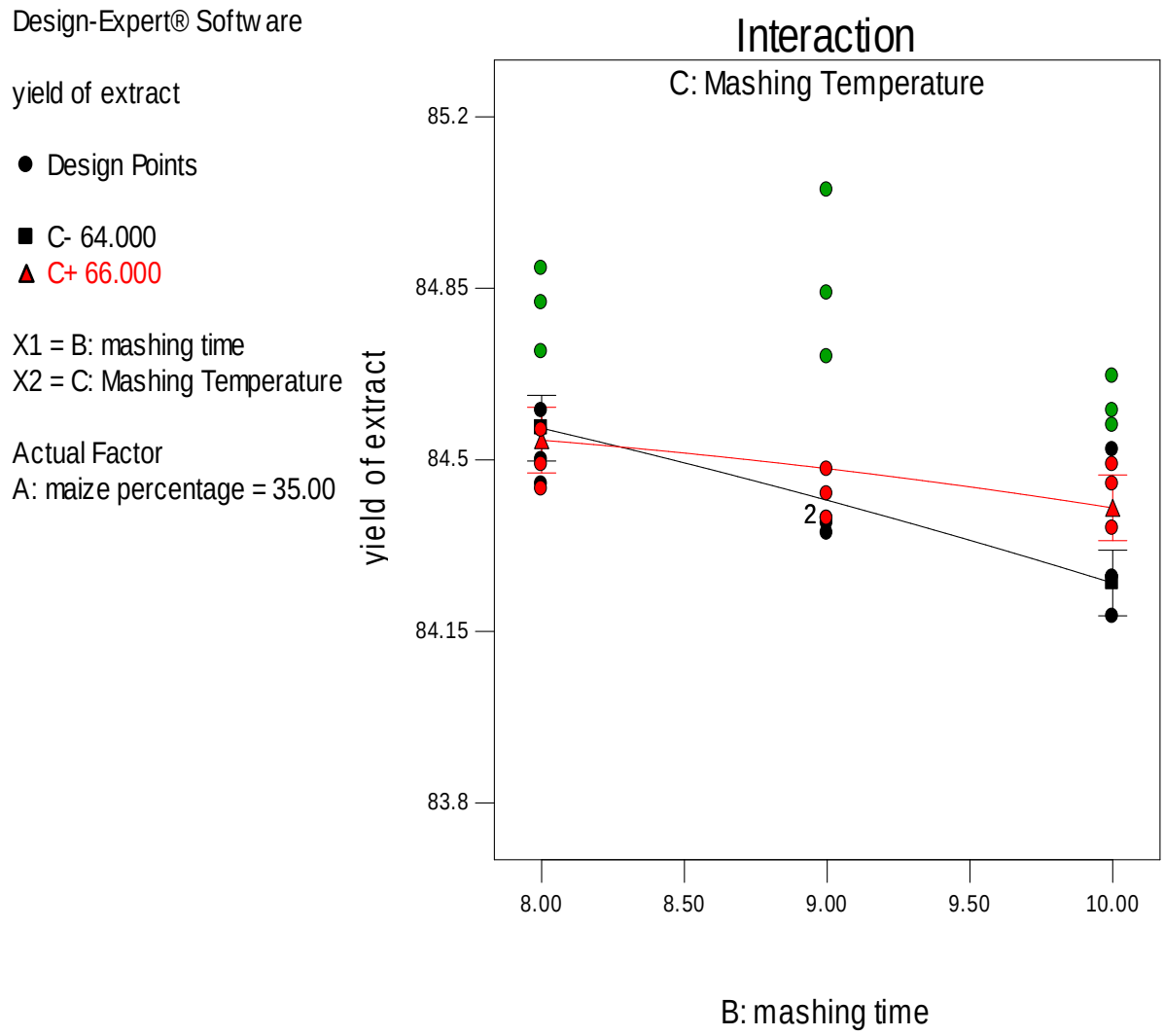



Figure 4.7. The effects of mashing temperature and mashing time on the yield of extract when mashing time is at the center point

From the above plot we understand that at higher mashing temperature and time the yield of extract are significantly decreased. This may because higher temperature and time limit the breakdown of starch to maltose and create short time for the rest time for α-amylase.

#### 4.2.1.2. Data Analysis for Response 2 (Free amino nitrogen)

Free amino nitrogen was the other response variable which mainly affected by the three variables. The detail of this response variables will be explained as the followings.

##### 4.2.1.2.1.Analysis of variance (ANOVA)

Table 4.7. Analysis of variance(ANOVA) in Quadratic Model

| Source | Sum of squares | df | Mean square | F-value | p-value | Remark |
|---|---|---|---|---|---|---|
| Model | 623.04 | 9 | 69.23 | 15.94 | <0.0001 | Significant |
| A- maize percentage | 588.79 | 1 | 588.79 | 135.58 | <0.0001 | |
| B-mashing time | 0.019 | 1 | 0.019 | 4.350E-003 | 0.476 | |
| C-mashing temperature | 0.019 | 1 | 0.019 | 4264E-003 | 0.9481 | |
| AB | 0.77 | 1 | 0.77 | 0.18 | 0.6741 | |
| AC | 13.37 | 1 | 13.37 | 3.08 | 0.0836 | |
| **BC** | 0.046 | 1 | 0.046 | 0.010 | 0.9188 | |
| **A2** | 2.62 | 1 | 2.62 | 0.60 | 0.4402 | |
| **B2** | 12.76 | 1 | 12.76 | 2.94 | 0.0908 | |
| **C2** | 4.65 | 1 | 4.65 | 1.07 | 0.3044 | |
| **Residual** | 308.33 | 71 | 4.34 | | | |
| **Lack of fit** | 65.97 | 17 | 3.88 | 0.86 | 0.6154 | Not significant |
| **Pure Error** | 242.36 | 54 | 4.49 | | | |
| **Cor Total** | 931.37 | 80 | | | | |

The Model F-value of 15.94 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob >F" less than 0.0500 indicate model terms are significant. In this case A are significant model terms.Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. The "Lack of Fit F-value" of 0.86 implies the Lack of Fit is not significant relative to the pure error.There is a 61.54% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good.we want the model to fit.

Table 4.8. Model adequacy measures

| | | | |
|---|---|---|---|
| Std.Dev | 2.08 | R-Squqred | 0.6690 |
| Mean | 113.69 | AdjR-Squared | 0.6270 |
| C.V.% | 1.83 | Pred R-Squared | 0.5672 |
| PRESS | 403.07 | Adeq precision | 12.057 |

The "Pred R-Squared" of 0.5672 is in reasonable agreement with the "Adj R-Squared" of 0.6270.

Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. our ratio of 12.057 indicates an adequate signal. This model can be used to navigate the design space.

**Quadratic polynomial model equation**

Final Equation in Terms of Coded Factors:-

$$FAN = +113.04 - 3.30 * A + 0.019 * B - 0.019 * C + 0.15 * A * B - 0.61 * A * C - 0.036 * B * C - 0.38 * A^2 + 0.84 * B^2 + 0.51 * C^2 \quad (4.2)$$

Final Equation in Terms of Actual Factors:-

FAN = +2044.78451 +8.06600 * maize percentage -13.85352 * mashing time -61.49167 * Mashing Temperature +0.029333 * maize percentage * mashing time -0.12189* maize percentage * Mashing Temperature -0.035556 * mashing time * Mashing Temperatur - 0.015252 *maize percentage$^2$ +0.84204*mashing time$^2$ +0.50815*Mashing Temperature$^2$

**4.2.1.2.2. Effects of Experimental Variables on free amino nitrogen**

Figures below show the response of FAN versus individual variable and two independent variables. Maize percentage was the most significant factor for FAN contents among the three variables and the effect of the three independent variables together were not significant while the effect of the two independent variables on the yield of extract are little.

A. **Effect of individual variables on the free amino nitrogen (FAN)**

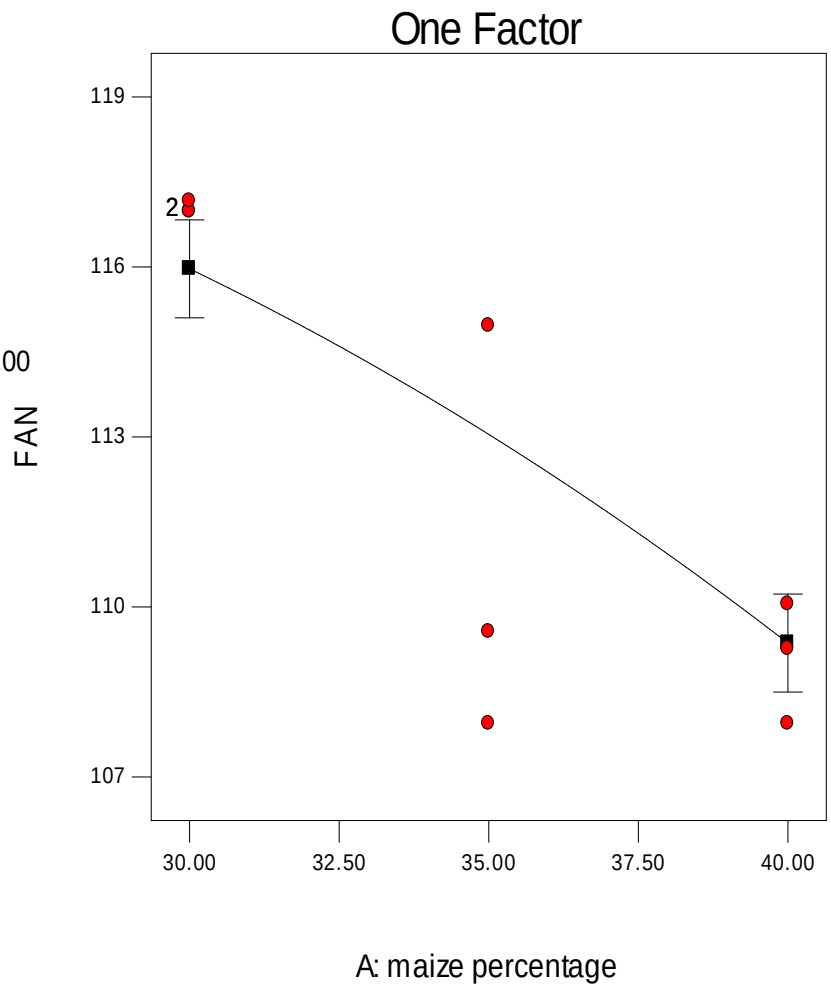
Design-Expert® Software

FAN

Design Points

X1 = A: maize percentage

Actual Factors
B: mashing time = 9.00
C: Mashing Temperature = 65.00



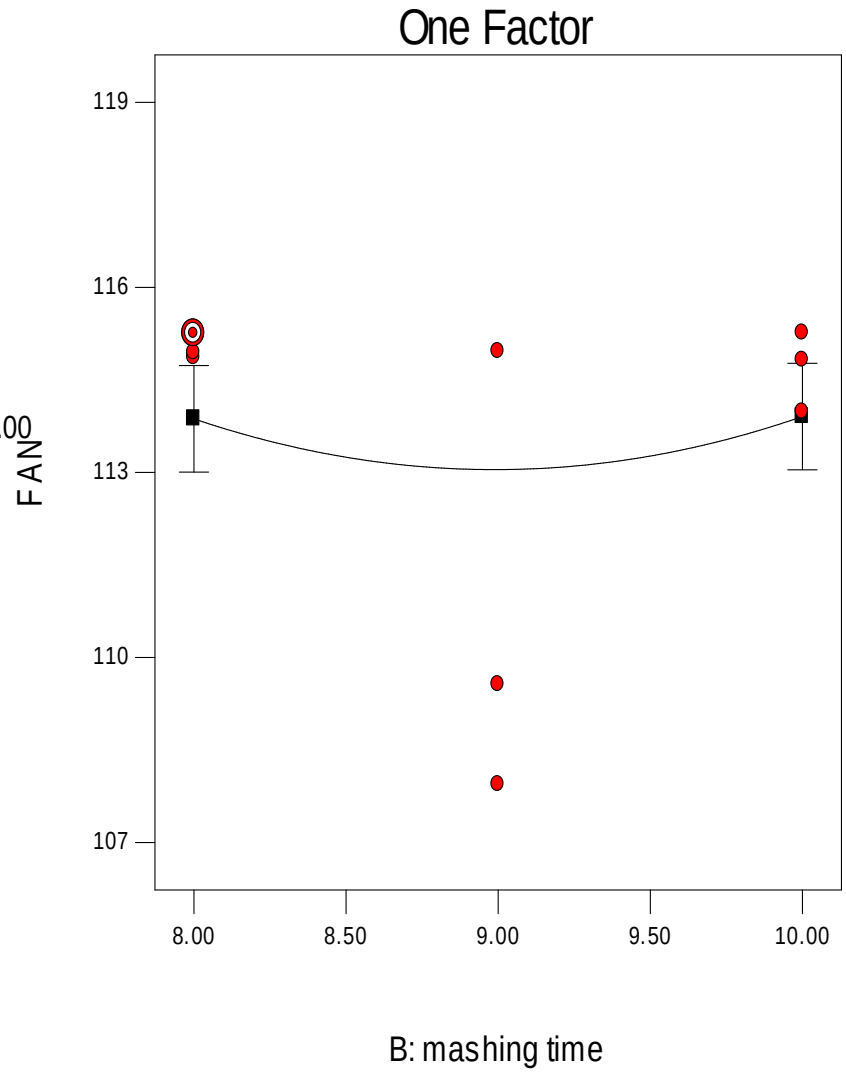

a)

Design-Expert® Software

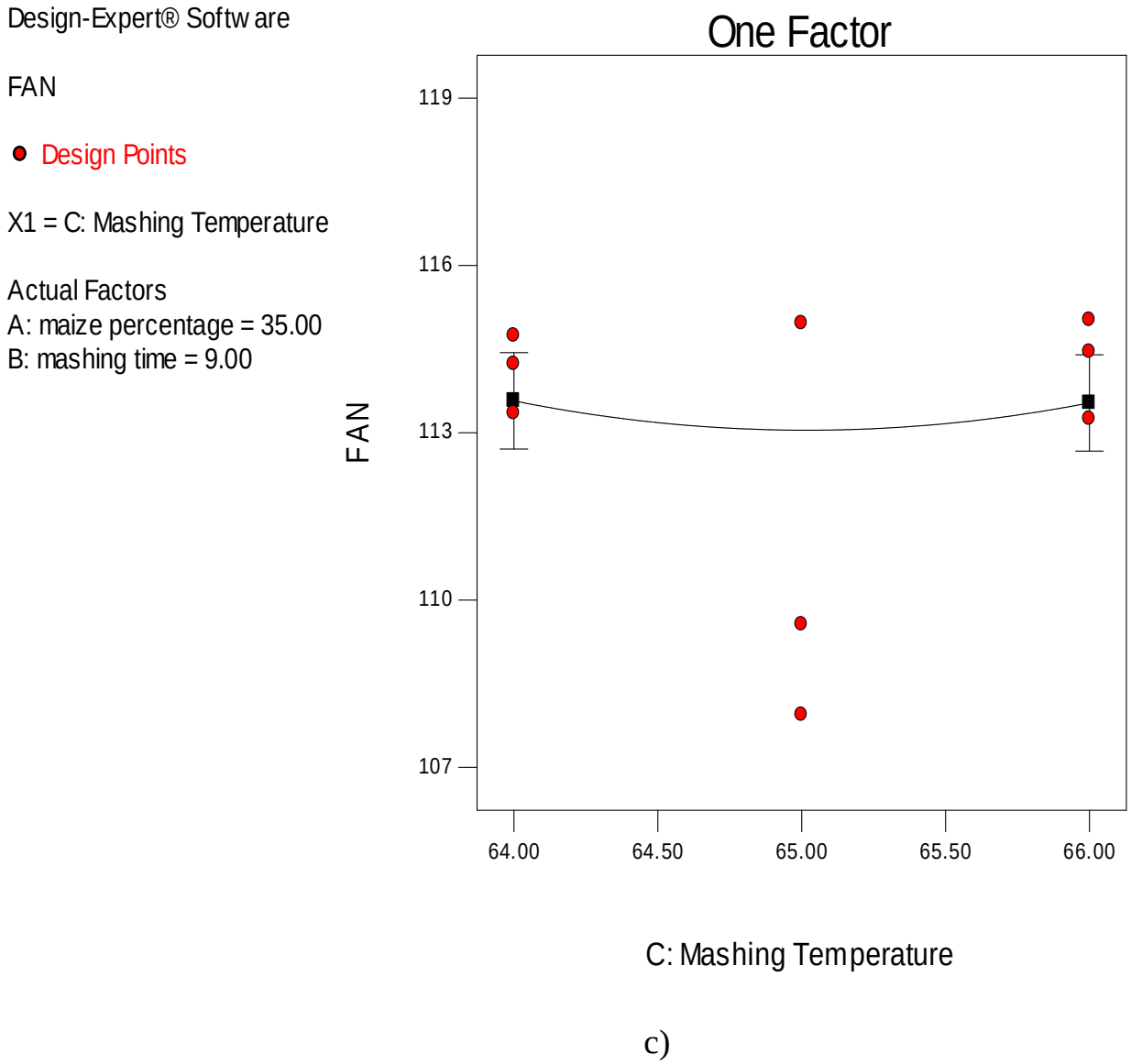
FAN
FAN = 115.25
Std # 31 Run # 80
Design Points

X1 = B: mashing time = 8.00

Actual Factors
A: maize percentage = 35.00
C: Mashing Temperature = 65.00




b)




c)

Figure 4.8. The effect of individual Variables on the yied of extract

In the above plot( a,b,and c) the free amino Nitrogen is significantly affected by maize percentage. FAN is highly generated during malting grain and in low manner during mashing. In this condition maize is not malted and the probability it contiribute FAN before mashin is almost assumed to be zero. High percentage of maize will decrease the whole percentage of the FAN of the wort .Therefore percentage of maize must be limited when we use the adjunt maize grits..

**B. The effect mashing time(fixed) and maize percentage on the free amino nitrogen when the when mashin temperature is at the center point**

The effect of mashing time and maize percentage when the mashing temperature constant was indicated by the following graphs.

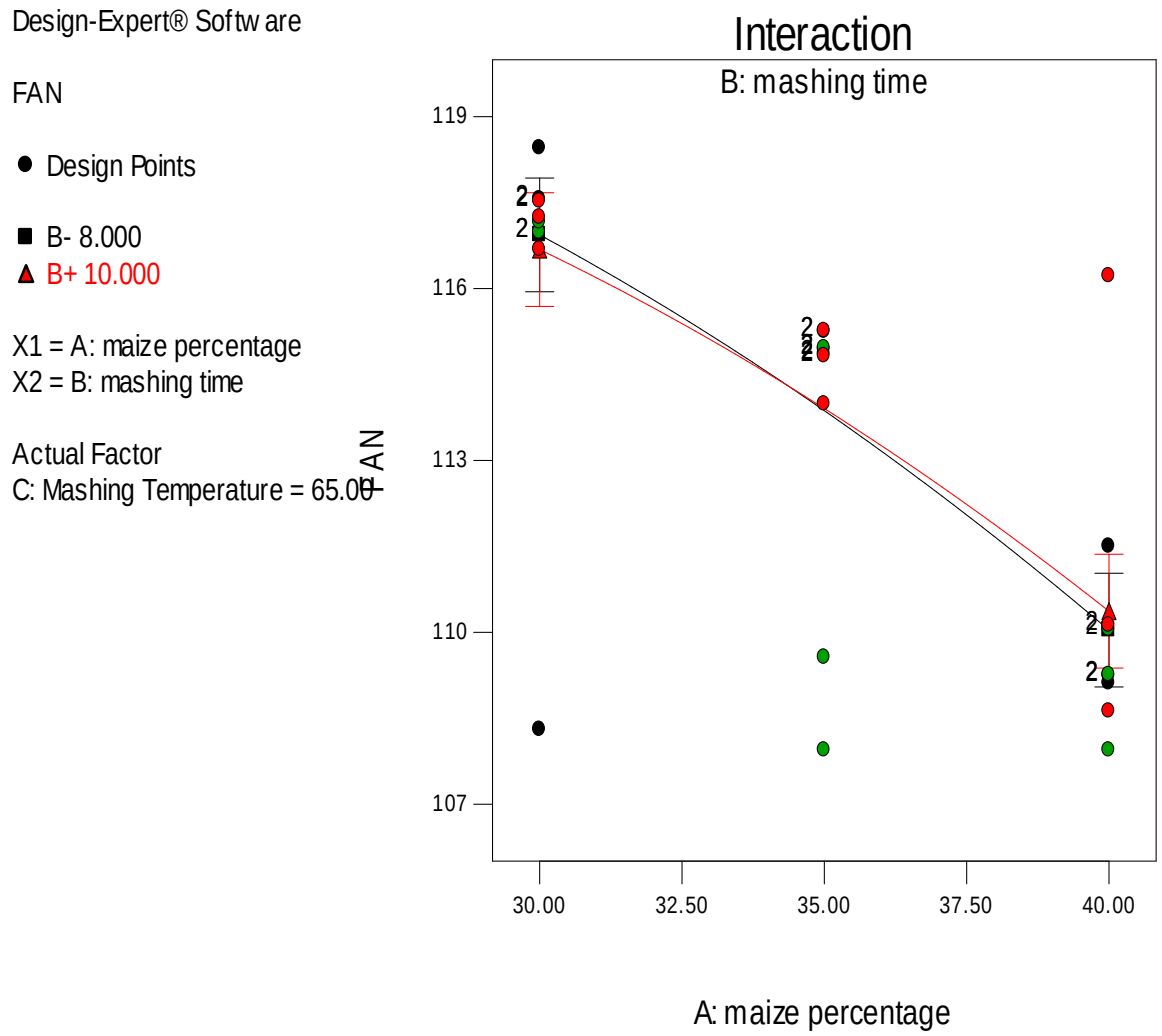



Figure 4.9. The effect of mashing time(fixed) and maize percentage on the free amino nitrogen b) when the when mashin temperature is at the center point

In above plot the interaction effect of the maize percentage with mashing time is not as much and the two effects is not significant. Because at relatively short and long mashing time the free amino nitrogen content depends mostly on maize percentage.

**C. The effect mashing temperature(fixed) and maize percentage on the Free amino nitrogen when the mashin time was at the center point**

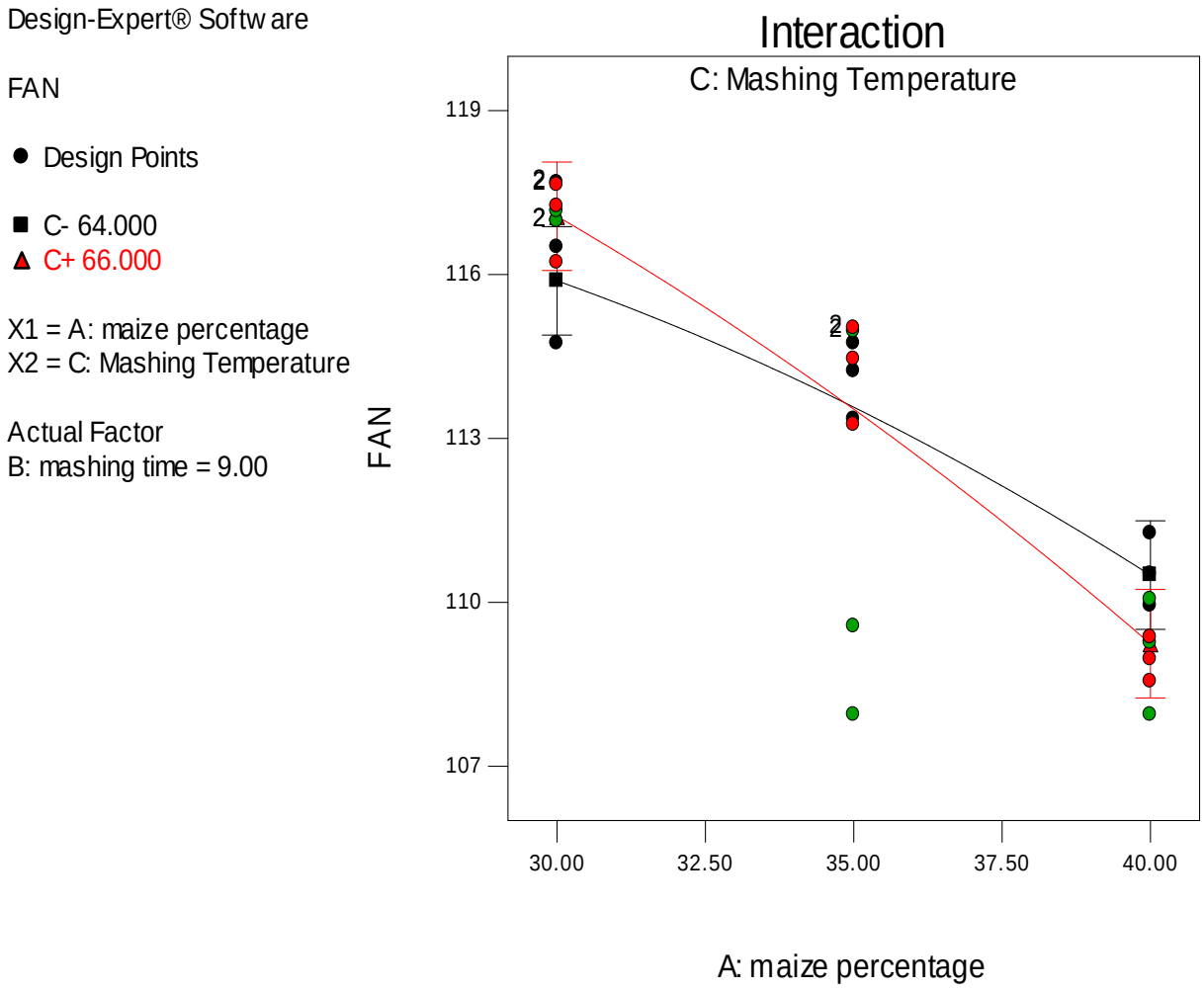



Figure 4.10 The effect of mashing temperature(fixed) and maize percentage on the Free amino nitrogen when the mashin time was at the center point

In above plot the effect of maize percentage and mashing temperature on the FAN content is insignificant. Threfore, the above plot tell us FAN content of The wort is low at high mashing temperature and high maize percentage or vice versa. But the change of temprature is not that much the case for free amino contentent and contribution.

**D.The effect mashing temperature(fixed) and mashing time on the free amino nitrogen when the maize percentage is at the center point**

In this case the free amino nitrogen value is almost not affected at specified mashing temperature. Because the line the graph at high temperatute and low temperature is seems on

the same point. but litte effect was seen when time of mashing changed. The effect of this these factors will shown as thee following graphs.

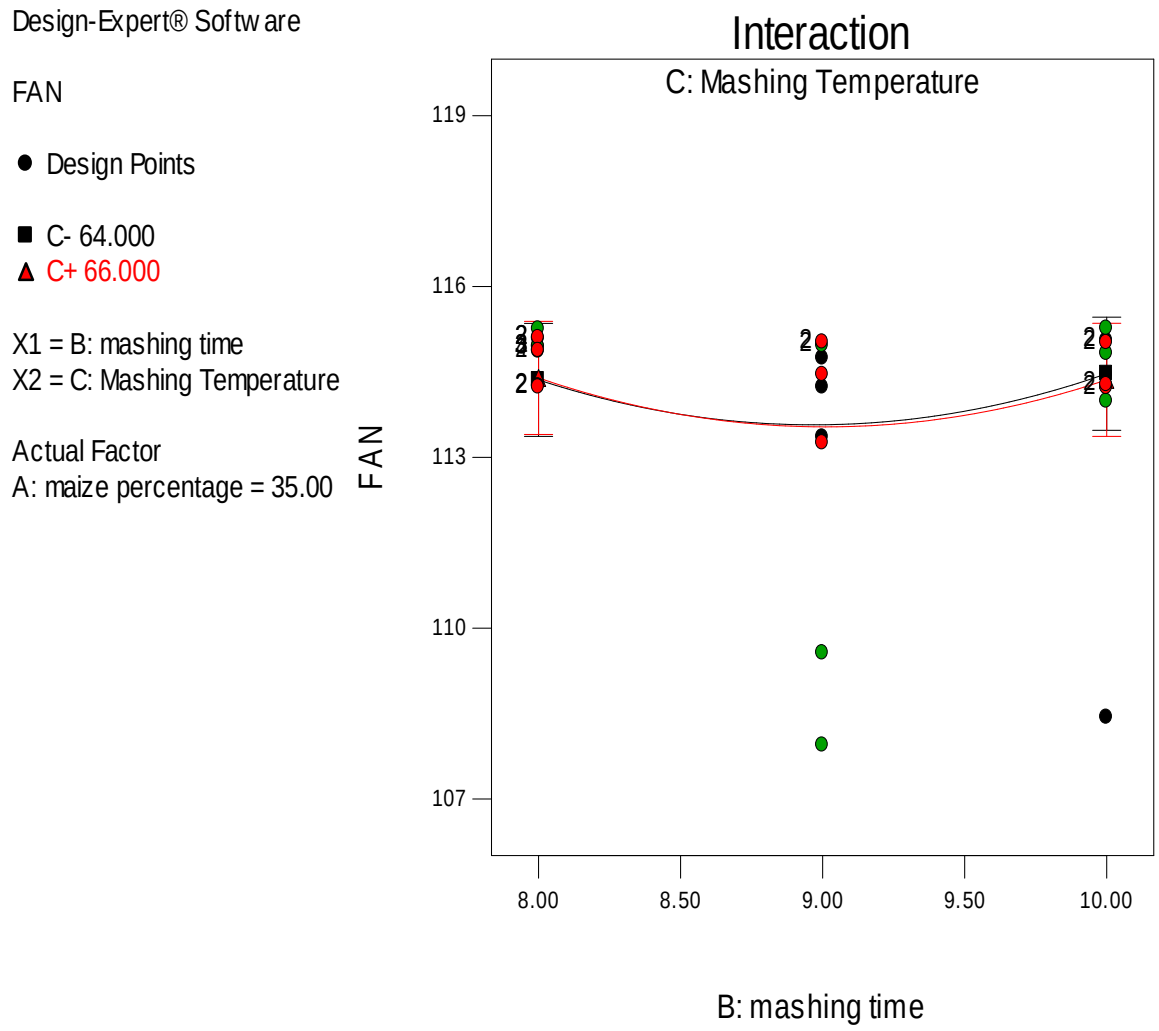



figure 4.11. The effect mashing temperature(fixed) and mashing time on the free amino nitrogen when the maize percentage was at the center point

From the above picture we can summurize that the contribution of maize in generating FAN is very small and percentage of maize in wort production will affect the yeast growth and therefore, this is the main problem to use high percentage of maize.

### 4.2.1.3. Numerical optimization of both independent variables

The best optimum parameters point is depends on the goal and criteria of each parameter to give the most important and acceptable response variables. It must communicate with simple productio way and low cost expenditure to get high quality product.

The optimization of wort production criteria from malted barley and maize are summarized as follows:

Table 4.9. Optimization criteria for optimum yield of extract and FAN

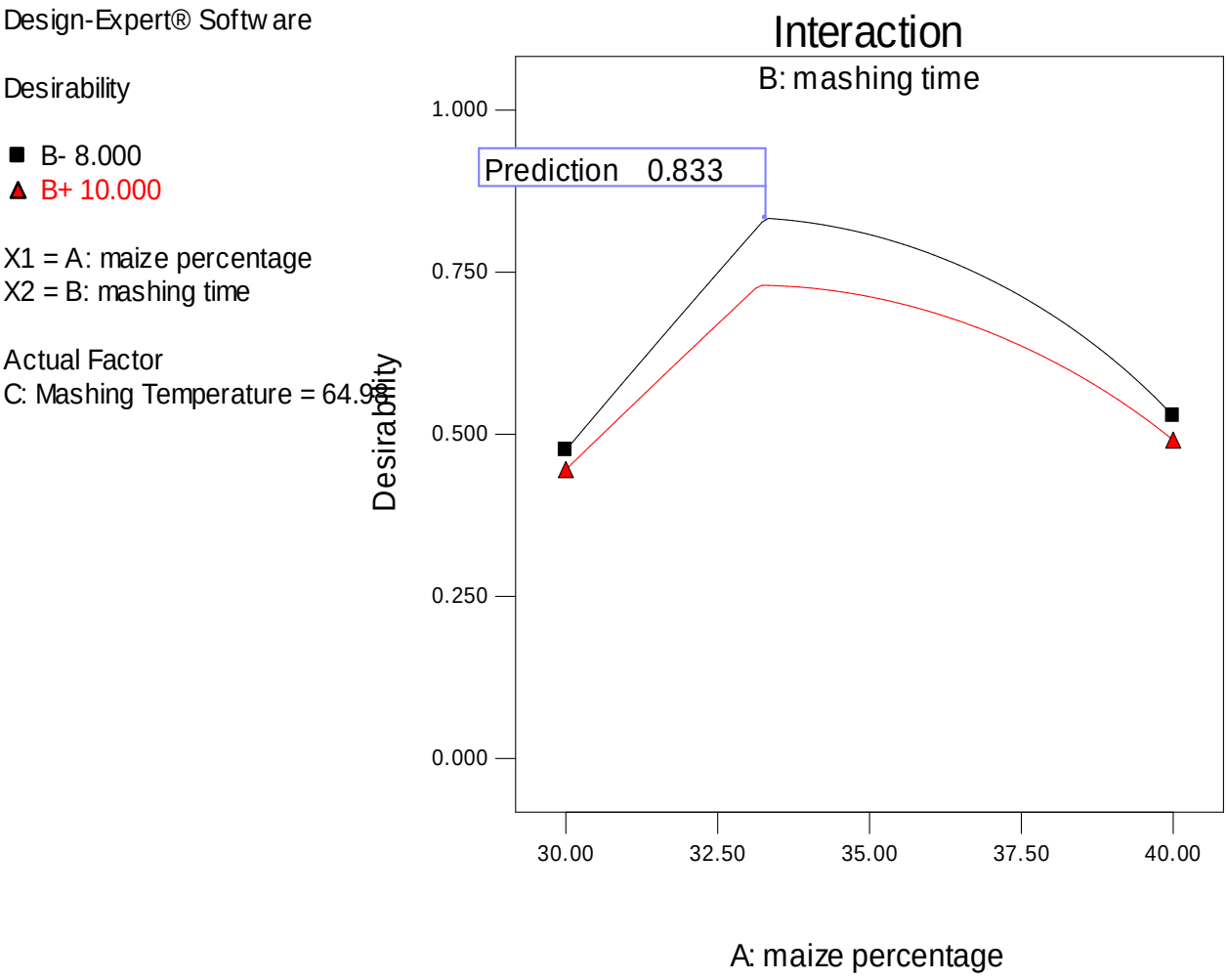
| **Parameters** | goal | Lower limit | Upper limit |
|---|---|---|---|
| Maiz percentage | In range | 30 | 40 |
| Mashing time | In range | 8 | 10 |
| Mashing temperature | In range | 64 | 66 |
| Yeild of extract | maximum | 83.84 | 85.13 |
| FAN(ppm) | target | 107.94 | 118.56 |

The optimum possible solutions in wort production for different variables for beer production and corresponding surface plot were presented in the following table.

Table 4.10. The desirable optimization solution

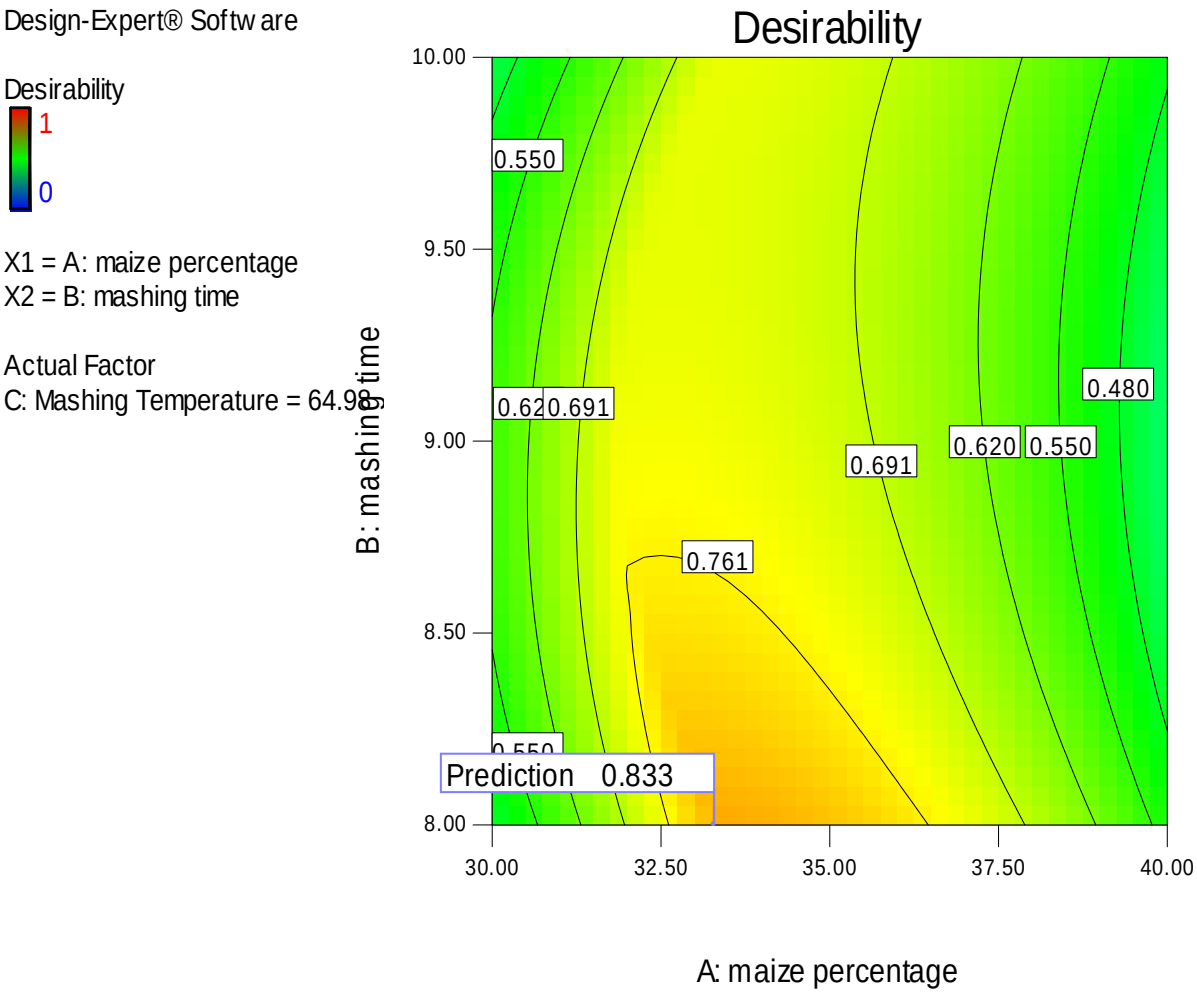
| Number | maize percentage (%) | Mashing time (minutes) | Mashing temperature ($^{O}$C) | Yield ofextract (%) | FAN (ppm) | Desirability | Remark |
|---|---|---|---|---|---|---|---|
| 1 | **32.29** | **8.00** | **64.98** | **84.7354** | **115** | **0.833** | **Selected** |
| 2 | 33.30 | 8.00 | 65.01 | 84.7352 | 115 | 0.833 | |
| 3 | 33.30 | 8.00 | 64.96 | 84.7362 | 114.991 | 0.833 | |
| 4 | 33.29 | 8.00 | 65.00 | 84.7349 | 115 | 0.833 | |
| 5 | 33.31 | 8.00 | 65.04 | 84.7346 | 115 | 0.833 | |
| 6 | 33.34 | 8.00 | 65.10 | 84.7321 | 115 | 0.832 | |
| 7 | 33.57 | 8.00 | 65.01 | 84.7537 | 114.824 | 0.831 | |
| 8 | 33.36 | 8.00 | 65.14 | 84.729 | 115 | 0.830 | |
| 9 | 34.08 | 8.00 | 64.74 | 84.7735 | 114.493 | 0.820 | |
| 10 | 33.24 | 10.00 | 65.14 | 84.5395 | 115 | 0.733 | |
| 11 | 3334 | 10.00 | 65.12 | 84.5395 | 114.933 | 0.733 | |
| 12 | 33.27 | 10.00 | 65.20 | 84.5327 | 115 | 0.733 | |

Therefore, 12 best solution found are selected here from the 12 best solution the first one is selected and considered for beer production .

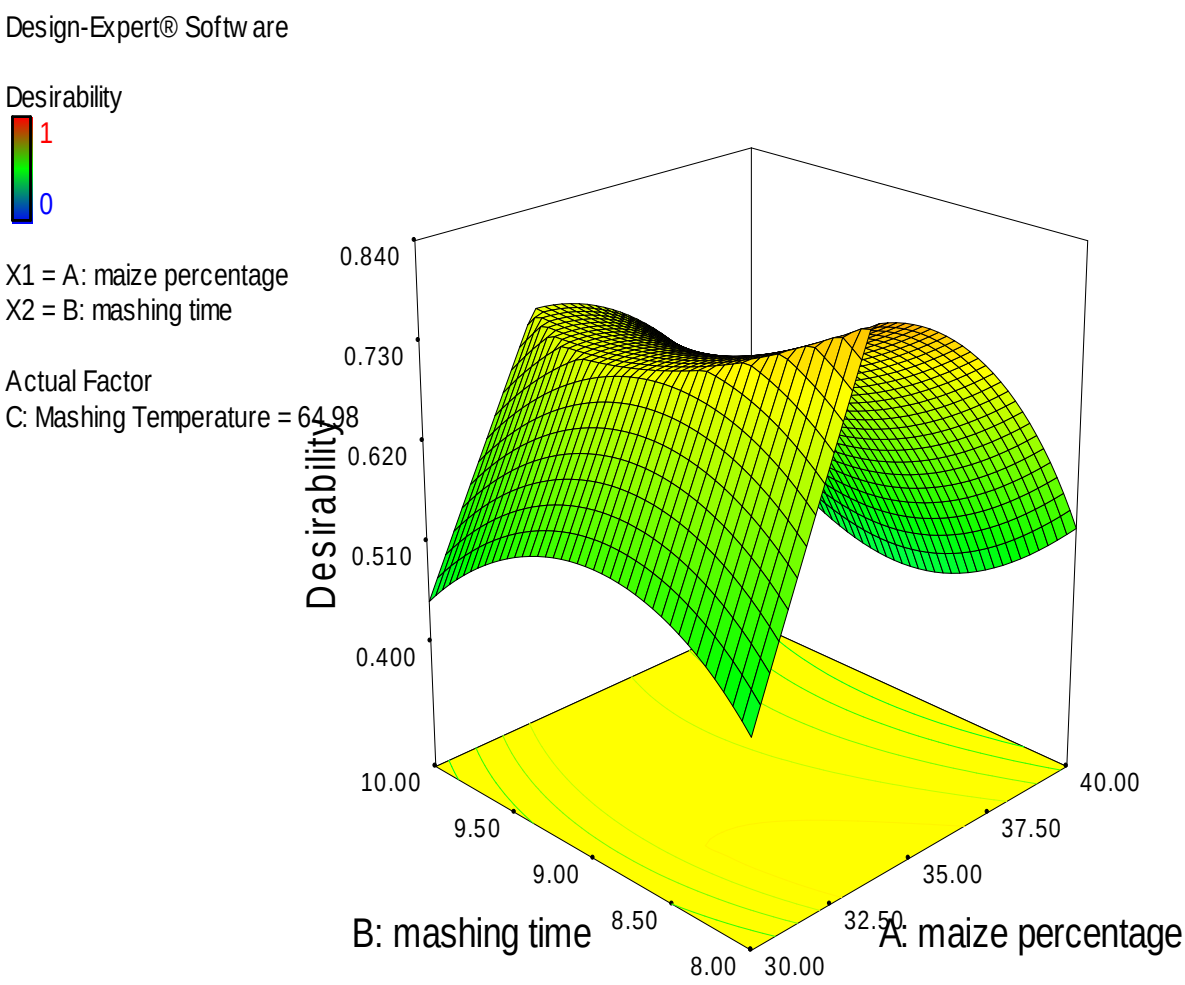



a) Optimization of interaction of maize percentage and mashing time plot in wort production when the mashing temperature was 65$^{o}$C

From the above graph we can understand that when mashing temperature is 64.98 or approximated to 65$^{o}$C and mashing time is 8 minute, the maize percentage must be 32.29 to keep the desirability of 0.833. This tells us the interaction effect of the three factors to optimize the response variables. This point is considered that the most optimum point for the production of beer. However, as the above analysis predicted there is 12 most desirable optimum points.




b) Optimization of wort production in contours plot form




c) Surfaces plot (3D) of possible optimum solutions

Figure 4.12. Optimization plot of wort production to show desirability

### 4.2.2. Production of beer from optimized wort

Beer is produced from the optimized wort using Micro brewery plant. During this all important physico-chemical analysis was conducted. The equipment used during this time is Disc mill, mash tun, mash filter, CO2 cylinder, wort kettle, automatic cooler machine, fermentation tank, Air compressor, oxymeter and CO2-meter, thermometer, and other. There are several steps to produce beer from grain milling to fermentation step. In this case in the same way the beer was produce through these steps and finally the name of this product is called "***BH660 beer.***"

**Step 1:-Malt and maize milling**

Mill Buhler universal laboratory disc ( DLFU) was used. The required 0.2mm gap setting of the mill was adjusted to grind 0.2mm fine grist of malted barley and maize

**Step2:- pre-mashing of maize grits**

1.50KG of maize was cooked with 0.1 kg of barley malt by using 5 litre of water. The maize was cooked for 25 minutes at $90^{O}C$ before mashing. After 25 minutes, the cooked grits solution temperature was cooled to $65^{O}C$. Finally 35% or 1.75 litre of water was added to replace the evaporated water during cooking and mixed with the prepared grist Solution at $50^{O}C$ to achieve $55^{O}C$.

**Step 3:- Mashing**

Mashing is the production of wort that serves as a key element in the brewing process. Mashing was undergoing at different mashing temperature and time as the following procedure. 3.15 Kg of malt grist was added with 7 liters of water and mixed with the cooked grit solution and the mixed solution was rested at $55^{O}C$ for 15 minutes, while 6gm of CaCl2 was added during this rest time. After 15 minutes rest the temperature of the solution was increased to $65^{O}C$ ($64.98^{O}C$) by $1^{O}C$/min and at $65^{O}C$ the solutions was rest for 8 minutes. After 8 minutes rest the temperature of the solutions were derived to $73^{O}C$ and rest for 22 minutes. At $73^{O}C$ iodine test was checked and after 22 minutes the temperature was increased to $78^{O}C$ to mash off step and rest for 10 minutes. The pH value and temperature of the mash was seen during each rest time and the result will be explained in the following tables

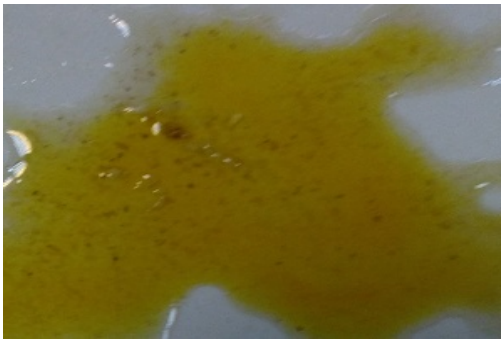


Figure 4.13. Iodine test for mashed solution at the 73 $^{O}C$

This yellow color indicates that the starch saccharification was occurring during mashing time. But the yellow color had seen was not very clear and this indicate that there was no full conversion of starch to simple sugar.

Table 4.11. Analysis of mashing solution

| Sample | pH | Temperature ($^{O}$C) | Iodine test |
|---|---|---|---|
| Mashing water | 3.95 | 55 | - |
| Gelatinized grits solution at 90$^{O}$C | 5.52 | 90 | - |
| Malt grist solutions | 5.48 | 55 | - |
| Mixed solution at 65$^{O}$C | 5.49 | 65 | Positive (Not yellow) |
| Mixed solution at 73$^{O}$C | 5.28 | 73 | Negative (yellow) |
| Mashed off solution | 5.29 | 78 | Negative (yellow) |

Here, the yellow color at 73$^{o}$C indicates the starch conversion was complete at the α-amylase rest temperature.

**Step 4:- mash filtration**

The mashed solution was filterer by using $CO_2$ gas to compress the whole solution and increase the rate of filtration. During this time 11 litre of sparging water is used to wash the husk and dilute the wort solution. Different measurement was taken as the following table.

Table 4.12. Analysis of filtered mashed solution (first wort)

| Sample | pH | Color (EBC) | Extract Degree plato ($^{o}$P) | dynamic Viscosity (m Pa.S) |
|---|---|---|---|---|
| First wort | 5.35 | 25.55 | 13.57 | 1.887 |
| Start of boiling | 5.37 | 17.58 | 11.25 | 1.735 |
| Last running | 5.58 | 3.55 | 2.05 | 1.164 |

**Step5:-Wort boiling**

During wort boiling 10gm hop, 55gm $CaCl_2$ and 20mg $ZnCl_2$ were added while the wort was boiled for 90 minutes. The following measurement was obtained during this step:-

Table 4.13. Analysis of boiled wort or final wort

| Number | measurement | Result |
|---|---|---|

| | | |
|---|---|---|
| 1 | pH | 5.32 |
| 2 | Color (EBC) | 21.4 |
| 3 | Extract ($^{o}$P) | 11.84 |
| 4 | dynamic Viscosity (m Pa.S) | 1.759 |
| 5 | Bitterness (BU) | 36.55 |
| 6 | Polyphenol (PPm) | 211.45 |
| 7 | $Ca^{2+}$ (PPm) | 104.21 |

**Step 6:- wort clarifying and cooling**

At this step the boiling was stopped or the steam line valve was switched off and kept rest. The hot trups were separated at the bottom of the wort kettle, while the clear solution was cold to 11$^{O}$C later. Then the cold wort was mixed with oxygen and pumped to fermentation tank by pitching with yeast.

Table 4.14. Analysis of cold wort

| Number | Measurement | Result |
|---|---|---|
| 1 | pH | 5.30 |
| 2 | Color (EBC) | 21.44 |
| 3 | Extract ($^{o}$P) | 11.76 |
| 4 | dynamic Viscosity (m Pa.S) | 1.745 |
| 4 | Bitterness (BU) | 34.75 |
| 5 | Polyphenol (PPm) | 206.4 |
| 6 | $Ca^{2+}$ (PPm) | 100.20 |
| 7 | FAN ( ppm) | 168.4 |

In all above steps 1-6 or wort preparation steps the major finding was summarized in table 4.14 and these measurements were the final measured parameters before fermentation. The values of the fermented beer were affected by these measurements.

**Step.7 Fermentation**

The amount of fermented extract determines the attenuation of wort, which is the main parameter indicating the course of fermentation. In this work the extract contents of the

fermenting wort was measured periodically (after 24 hours) and mashing temperature is the main parameter indicating course of fermentation, while fermentation diagram was prepared and recorded. A primary fermentation was achieved according to the information from the fermentation diagram in about 8 days. The fermentation average temperature was ($12.0^{O}C$). Finally the temperature of the beer was increased for three day to $16^{O}C$ and VDK was measured to indicate maturation.

As we can see from the next table the exothermic nature of the fermentation process was increasing the whole temperature of the system and during his the extract contents of the fermenting solution was decreasing. In other case the yeast population which was initially little in number becoming to increasing during primary fermentation and decreasing from time to time after primary fermentation. This is because the main nutrients for yeast growth had been become to minimum at this time.

Table 4.15. Fermentation diagram follow up

| Day | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Date | May 9 | May 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
| Extract($^{o}$p) | 11.76 | 9.85 | 8.57 | 6.95 | 5.42 | 4.08 | 3.85 | 3.40 | 3.18 | 2.85 | 2.53 | 2.40 | 2.36 | 2.34 |
| Population $*10^{7}$ | 1.2 | 3.29 | 4.48 | 6.25 | 5.55 | 5.34 | 4.65 | 3.27 | 1.25 | 0.98 | 0.85 | 0.85 | 0.82 | .80 |
| Temperature $^{O}C$ | 11.40 | 11.0 | 11.8 | 11.60 | 12.20 | 12.0 | 12.0 | 12.1 | 11.9 | 15.20 | 16.10 | 15.70 | 3.0 | 3.0 |

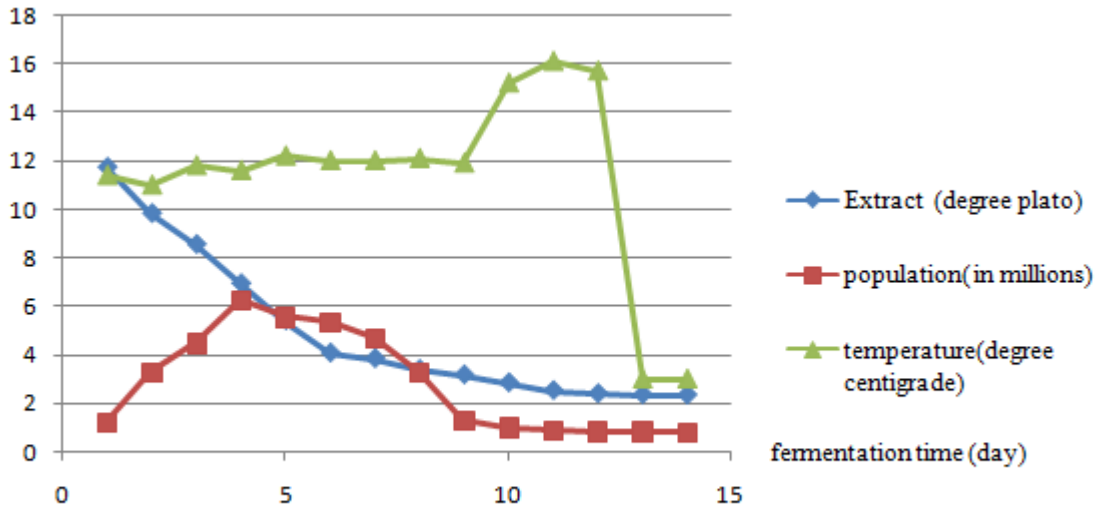



Figure 4.14. fermentation diagram indicating the interaction of extract, temperature and population with time (day)

### 4.2.3. Product characterization

The product characterization was done at BGI Ethiopia laboratory. Physico-chemical analysis of the product was analyzed by using different chemical and instruments that calibrated checked daily. Finally the result of analysis was compared with the product of other brewery companies.

Specifically for this product the following analysis was done. Alcohol content, Specific gravity, original gravity, apparent extracts real extract color value.VDK value, bitterness value, polyphenol, $Ca^{2+}$content, foam stability $CO_2$ content and pH value

#### 4.2.3.1. Determination of seven parameters by Antan paar

For most parameters analysis the Alcolyzer (Anton paar) device was used. Since this device is the most accurate and used widely in brewery companies in the world. Before measuring the parameters the Anton paar device was calibrated and cleaned. Then100ml of sample was filtered and injected to the device while the result was displayed in the screen after five minutes. This device was used measure the alcohol value %w/w, alcohol value %v/v, specific gravity, original gravity, apparent extract, real extract and apparent degree of fermentation

Table 4.16. Seven parameters determination by of alcoholyzer

| Number | parameters | Unit of the parameters | Value of the parameters |
|---|---|---|---|
| 1 | alcohol value %v/v | percent | 4.29 |
| 2 | alcohol value %w/w | percent | 3.36 |
| 3 | Specific gravity | No unit | 1.00864 |
| 4 | Original gravity | $^{O}P$ | 10.37 |
| 5 | Real extract | $^{O}P$ | 3.79 |
| 6 | Apparent extract | $^{O}P$ | 2.22 |
| 7 | Apparent degree of fermentation | percent | 78.60 |

For this product apparent degree of fermentation is obtained that 78.60% and this is very good if it compared to other brewery product.

From the above table the apparent degree of fermentation 78.60% tell us that the conversion of the fermentable sugar to alcohol is in the standard that most brewery company product characters. The specific gravity of the cold wort was 1.04737, while the specific gravity of the beer was 1.00864 this is the indicative that enough conversion was occurring during fermentation. The rest parameters are depends on the original extract (the original extract of cold wort was 11.76$^{O}$P and the results obtained was in the expected gap.

**4.2.3.2. Determination of other parameters by different method**

These are analyzed by the method explained in the materials and method chapter using an appropriate, calibrated instruments and standard chemicals. The summary of these parameters are as the following tables.

Table 4.17. Determination of other parameters by different methods

| parameters | Unit of the | Value of the |
|---|---|---|

| | parameter | parameters |
|---|---|---|
| VDK | ppm | 0.34 |
| Bitterness | BU | 13.65 |
| Polyphenols(ppm) | Ppm | 149.20 |
| $Ca^{2+}$(ppm) | Ppm | 64.124 |
| Haze (EBC) at 20$^{o}$C (90$^{o}$) | EBC | 1.55 |
| Haze (EBC) at 20$^{o}$C (25$^{o}$) | EBC | 0.55 |
| Foam stability (second) | Second | 208 |
| Color value(EBC) | EBC | 11.18 |
| pH value | pH | 4.42 |

#### 4.2.3.3. Validation of BH660 beer by comparing with other brewery product

When we compare this product with other product we have to consider the basic parameters that have commonly compared between beers of different companies (brands). Those are apparent degree of fermentation, VDK, foam stability, flavor, aroma, and foreign gas contents mainly oxygen and etc. The rest parameters can be specified according to plant plan of the company by fixing the ratio of water. In other case the original extract obtained during wort boiling and cooling were the main factor for the rest parameters. All result obtained in above tables are tolerated except VDK which the expected value is less than 0.20 ppm. However, the VDK value seems to be high because of the disturbance occur during production. Since the fermentation tank temperature controlling was challenging because of the exothermic reaction during fermentation and the less efficient cooling system.

Table 4.18. Composition of BH660 beer and comparing with other brands of beer

| S.No | Parameters | BH660 | St.George | Dashen | Walia | Meta | Raya |
|---|---|---|---|---|---|---|---|
| 1 | alcohol value( %v/v) | 4.29 | 4.69 | 5.01 | 4.84 | 4.84 | 4.94 |
| 2 | alcohol value (%w/w) | 3.36 | 3.67 | 3.93 | 3.78 | 3.80 | 3.87 |
| 3 | Specific gravity | 1.00864 | 1.0082 | 1.0075 | 1.0113 | 1.0069 | 1.0081 |
| 4 | Original gravity($^{O}$p) | 10.37 | 10.97 | 11.39 | 11.98 | 10.95 | 11.40 |
| 5 | Real extract($^{O}$p) | 3.79 | 3.80 | 3.74 | 4.64 | 3.54 | 3.87 |
| 6 | Apparent extract($^{O}$p) | 2.22 | 2.10 | 1.92 | 2.89 | 1.77 | 2.07 |
| 7 | Apparent degree of fermentation (%) | 78.60 | 80.90 | 83.12 | 75.91 | 83.80 | 78.84 |
| 8 | VDK (ppm) | 0.34 | 0.08 | 0.10 | 0.090 | 0.210 | 0.090 |
| 9 | Bitterness (BU) | 13.65 | 16.50 | 17.50 | 17.65 | 18.95 | 16.84 |
| 10 | Polyphenols(ppm) | 149.20 | 206.4 | 194.2 | 197.5 | 185.5 | 208.5 |
| 11 | $Ca^{2+}$(ppm) | 64.124 | 56.12 | 52.4 | 60.12 | 68.5 | 48.10 |
| 12 | Haze (EBC) at 20$^{o}$C (90$^{o}$) | 1.55 | 0.91 | 0.78 | 0.97 | 0.47 | 1.05 |
| 13 | Haze (EBC) at 20$^{o}$C (25$^{o}$) | 0.55 | 0.05 | 0.50 | 0.03 | 0.68 | 0.49 |
| 14 | Foam stability (second) | 208 | 204 | 235 | 202 | 212 | 225 |
| 15 | Color value(EBC) | 11.18 | 9.05 | 8.68 | 10.70 | 12.8 | 9.00 |
| 16 | pH value | 4.42 | 4.68 | 4.44 | 4.48 | 4.36 | 4.70 |

From the above table most parameters had been compared with different brand of beer and the result obtained are almost tolerated. As we observed from fermentation diagram the deviation of the actual temperature from the set point was relatively large because of the cooling system was not perfect to control the temperature. The important thing of this product is the isolated and attractive flavor it characterizes. Therefore, this can add an asset to increase the acceptance of this product.

# CHAPTER FIVE

# MATERIAL AND ENERGY BALANCE

Material and energy balances are fundamental to the control of processing, particularly in the control of yields of the products. Material balance were based on mass flow rates, e.g., kg/h. volumetric flow rates, (L/h or m3/h) are converted to mass flow rates, using the appropriate mass density of the material (kg/L) as (Coulson and Rechardson, 2005) stated. International Units (SI) are used throughout the study. While, Energy balances are used in the examination of the various stages of a process. The energy balance determinations are also made to determine the energy requirements of the process, the heating, cooling and power required.

### 5.1. Material balance

All material and energy balance starting from maize processing was performed. While barley malting process was not performed, because it was supposed to bought from malt producing company. The oil is located in the germ of the maize corn. Because of the damaging effect of oil on beer foam, maize is degermed before further processing. Degerming the maize reduces the oil content to around 1%. Maize is screened to remove debris. It separates the material into four groups by density: -

1. Stones and other heavy impurities
2. Whole grains
3. Low density and damaged kernels
4. Dust

Only whole grains are allowed to pass for processing. Broken grains are sold as animal feed. The embryo or germ is removed from the whole grain. The grain is wetted before this, to make the degermination process easier. The germ and bran are separated from the grain. The degermed maize can be goes on to be processed for beer production.

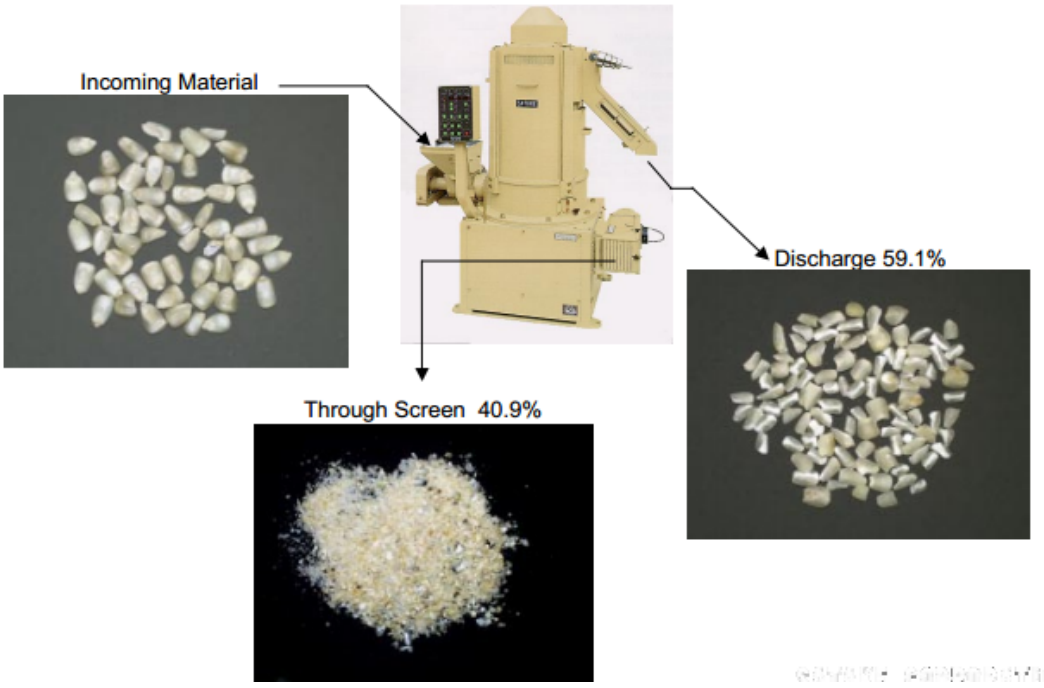



Figure 5.1. Alternative Maize De-germer

The plant capacity was 21 of wort L /batch and six batch per day. According to different literatures, most brewery companies have their own grist to water ratio. But most breweries to produce 320hl of wort about 7000kg of malt was used and this ratio will be applied. In this case to produce 21L of wort, 4.65Kg of grain was used for which 32.29% or 1.50 Kg was maize and 67.71% or 3.15Kg was malted by mixing two rows and six rows to the ratio of 1:1.

### 5.1.1. Material balance for maize grits production

During the production of maize grits from 10Kg of maize by using disc mill for de-germination, 7.43Kg of maize grits was obtained from this point the material balance was done as the following.

Moisture 14.5 % → Maize (10kG)

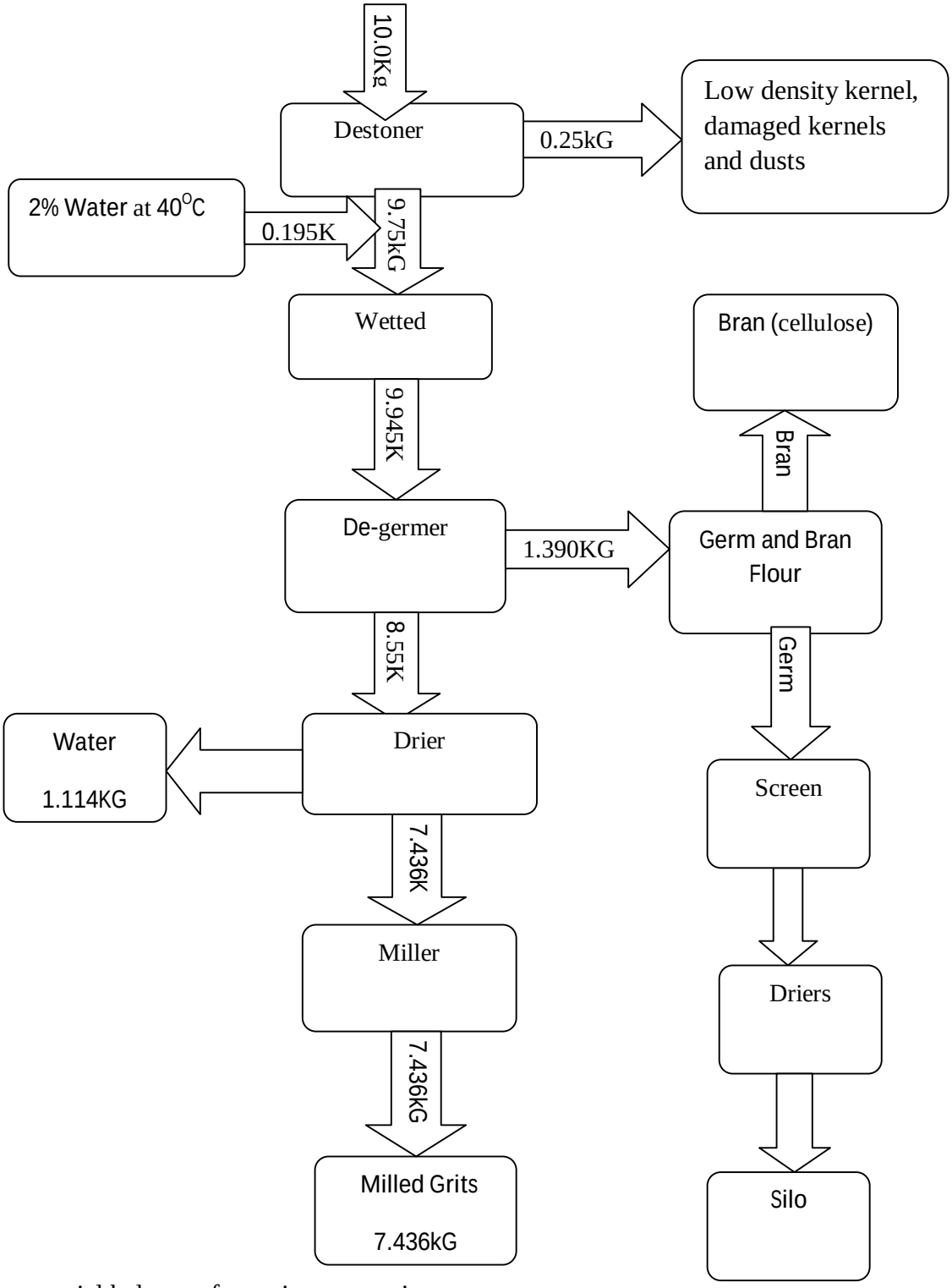
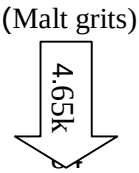
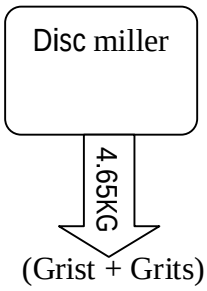



Figure 5.2 .material balances for maize processing

### 5.1.2. Material balance for hammer Mill (both for maize and malt)

Assumption: there is a little loss in the mill, we neglect it.





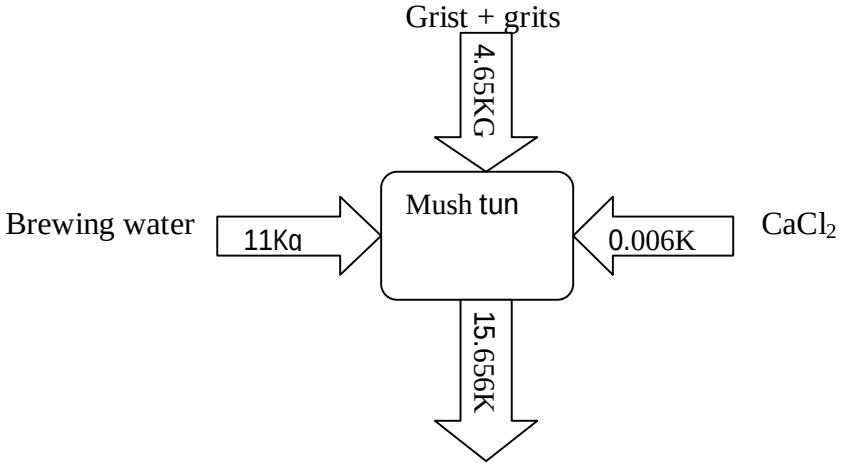

We have six batches (brews) per a day. The total (grist +grits) per a day will be:-

4.65KG/batch *6 batch/day = 27.9 Kgday

### 5.1.3. Material balance for mash tun

Assumption: there is 4.65Kg (grist+grits) and total 11 litre of water consumed during mashing.

Particularly here 35% of water was evaporated during maize cooking and this water will be replaced during mashing and the effect will be zero.




The mashed solution

The mass of mash solution obtained = Brew water + Grist + additives = (4.656+0.006+11) **=15.656Kg**

### 5.1.4. Material balance for Mash filter

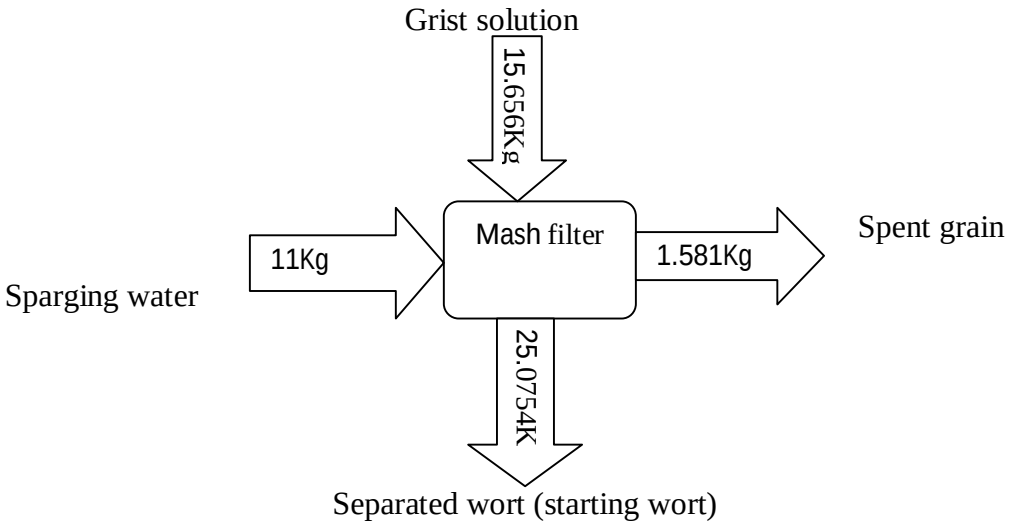
Assumption: - For one kilogram of grist solution, 0.101Kg spent grain is obtained.

Spent grain amount that leave the filter = 0.101*15.656 = **1.581Kg**

11L of sparging water were used during filtration needed to a kilogram of mash.

Mass of wort that separated = (Grist solution) + (Water sparging) - (Spent grain)

= 15.656KG + 11kg - 1.581kg = 25.075 kg of wort




### 5.1.5. Material balance for wort kettle

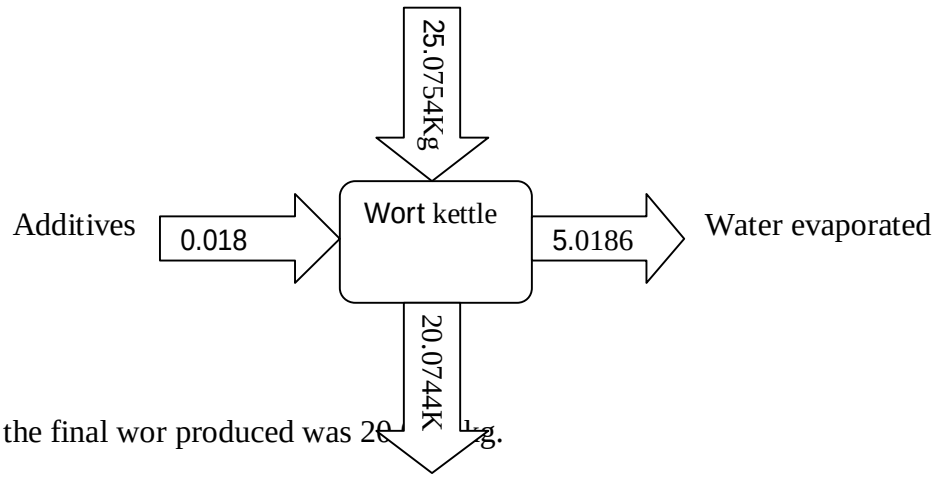
Wort boiling is performed in the wort kettle. 0.01kg of tetra hop ($CO_2$ extract), 0.005Kg Caramel and 0.003Kg of $CaCl_2$ were added. Due to heat addition in the wort kettle some of the water in the wort should be evaporated in order to achieve the required concentration of wort. According to most literature the mass of the final wort will be decreased by 20% due to evaporation of water during wort boiling.

Water evaporated = 0.2*(25.075 +0.01+0.005+0.003) Kg = 0.2*25.093Kg = 5.0186Kg

The total final wort will be = (starting wort + Additives) – evaporated water

= 25.093 – 5.0186 = **20.0744Kg**

Starting of boiling wort




The final wort the final wor produced was 20[illegible]g.

### 5.1.6. Material balance for fermentation tank

During the production of beer the fermentable sugar in the wort must be fermented by enzymes in the yeast to ethanol and carbon dioxide

$C_6 H_{12} O_6$ + yeast $\longrightarrow$ $2C_2H_5OH + 2\ CO_2$ + Heat

The dosing rate (DR) of the yeast to the cold wort was 8gm/litre. The amount of yeast used is calculated as the following.

Volume of wort = $\frac{Mwort}{\text{dwort}} = \frac{20.0744Kg}{1.04545\text{Kg/lit}}$ = **19.202 litre**.

Quantity of yeast used = 8gm/lit * 19.202lit = **153.61gm**

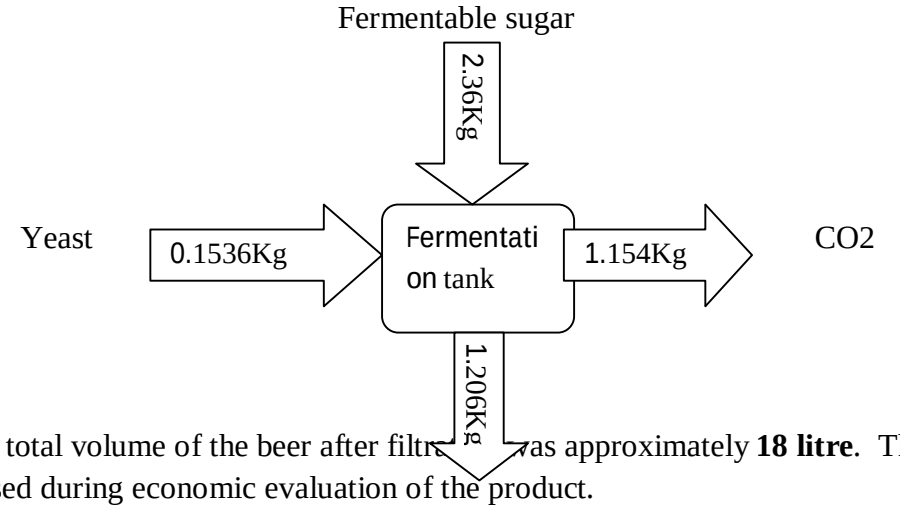
Since 20.0744kg of cold wort was obtained, the amount of sugar in the wort becomes

11.76/100(20.0744Kg) = 2.36 kg of extract. Where, 11.76% is the original extract of the cold wort. Now, we can calculate the amount of alcohol and $CO_2$ formed by mass-mass relation ship

| 2.36 | | X | | Y | |
|---|---|---|---|---|---|
| $C_6 H_{12}O_6$ + yeast | $\longrightarrow$ | $2C_2 H_5OH$ | + | $2CO_2$ | + heat |
| 180 | | 92 | | 88 | |

$X = \frac{92*2.36}{180} = 1.206Kg$ $\quad$ $Y = \frac{88*2.36}{180} = 1.154$ Kg

Where "X" is the mass of $C_2H_5OH$ and "Y" is he mass of $CO_2$




Finally, the total volume of the beer after filtration was approximately **18 litre**. This was basically used during economic evaluation of the product.

### 5.2. Energy Balance

The energy is utilized in two forms, thermal (steam) and electrical. Electrical energy is used to run machinery with moving parts or motors such as pumps, centrifuges, and mills while

steam is used for boiling and temperature adjustment during production. In this context the steam energy source will be considered for balancing

### 5.2.1. Energy balance on mush tun

**Qin = Qout = UAT** **(**6.1)

$m_c C_{pc} (T_{c,out} - T_{c,in}) = m_{steam} C_{psteam} (T_{steam\ in} - T_{steam,out})$

$m_{in}$=15.656 kg $C_{p,steam}$=1.996kj/kg k

$p_c$=O.27kj/kg k $T_{steam}$,out=52$^O$C

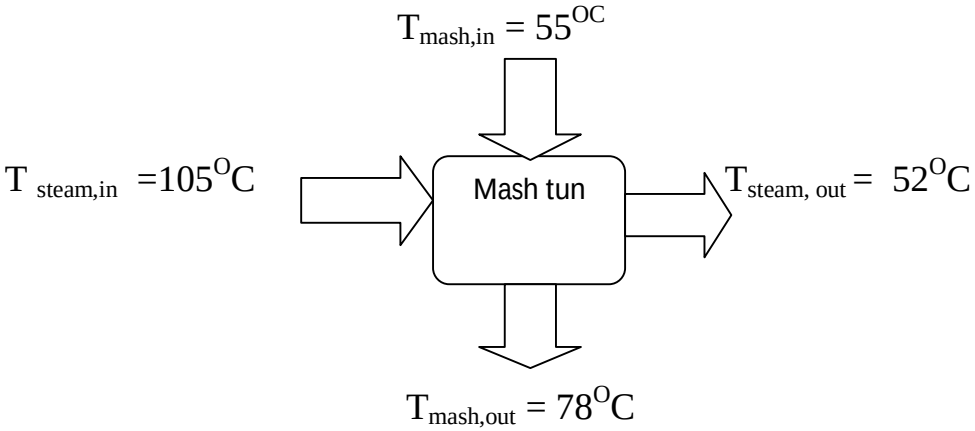
Tin = 55$^O$C Tsteam in 105$^O$C

**$T_{C,out}$=78$^O$C** $m_{steam}$ = ?

$$m_{steam} = \frac{mcCpc\ (Tc,out - Tc,in)}{Cpsteam\ (Tsteam\ in - Tsteam,out)}$$

$$m_{steam} = \frac{15.656(78-55)}{1.996(105-52)} = 3.403Kg$$

Q = mcCpc (Tc,out – Tc,in = 15.656* 0.27KJ(78-55) = **97.22KJ**




### 5.2.2. Energy balance for wort kettle

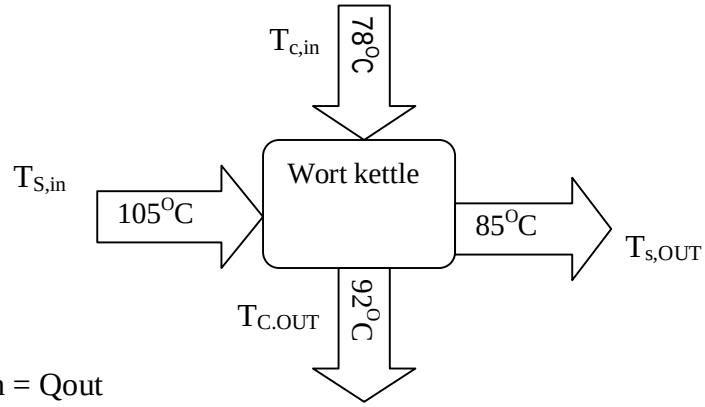
In this case steam energy is used to increase the temperature of wort in the kettle from 78$^O$Cto 92$^O$C

$M_{c,in}$ = 25.093Kg $m_s$ = ?

$C_P$ = 0.27kJ/kg.k Cp.s = 1.996 kJ/kg .K

Tin = $78^{O}C$ $T_{S,in} = 105\ ^{O}C$

$T_{c.out} = 92^{O}C$ $T_{S.OUT} = 85^{O}C$




Qin = Qout

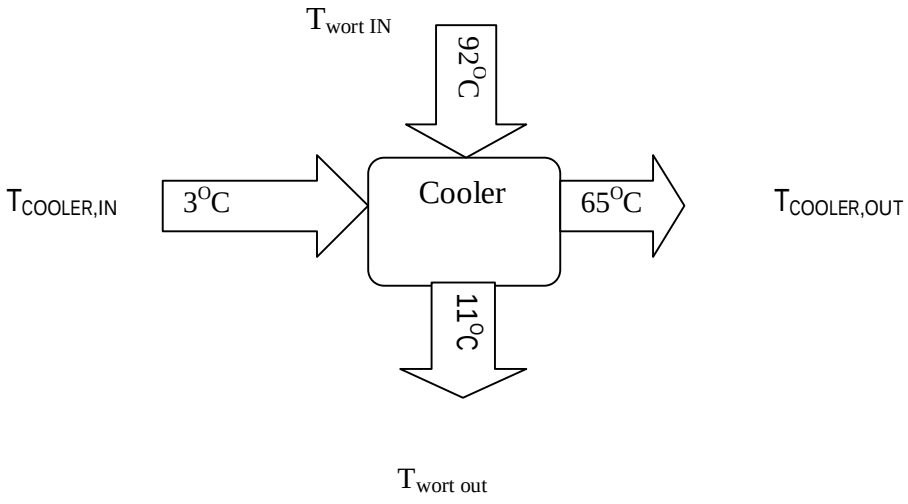
$Mw,Cp,C\ (Tc,out - Tc,in) = M_S\ Cps\ (Ts,in - Ts,out)$

$$m_{steam} = \frac{Mw,Cp,C\ (Tc,out - Tc,in)}{Cps\ (Ts,in - Ts,out)} = \frac{25.093*0.27*(92-78)}{1.996*(105-85)} = 2.376Kg$$

$Q = M_S\ Cps\ (Ts,in - Ts,out)$ =2.376*1.996(105-85) = **94.852KJ.**

### 5.2.3. Energy Balance on Heat Exchanger

The wort that leaves the kettle at $92^{O}C$ must be cooled to attain the desired temperature $11^{O}C$ for fermentation. The type of cooler that I selected was water: $C_{p.c}$ = 4.189 kJ/kg .k




From the above, Qin = Q out

$m_{water}\ C_{water}\ (T_{water,\ out} - T_{water,in}) = m_{wort}\ Cp.c\ (T_{wort,\ in} - T_{wort,out}) =$

$m_{water}(4.189)\ (65-3) = 20.0744(0.27)\ (92-11)$

$m_{water}$ = **1.690KG** .

$Q = m_{water}\ C_{water}\ (T_{water,\ in} - T_{water\ ,out}) = 1.690*4.189*(65-3)$ = **439.03KJ**

### 5.3. Equipment Design and Selection

If the cost of a piece of equipment or plant of size or capacity $q_1$ is $C_1$, then the cost of a similar piece of equipment plant size of capacity $q_2$ can be calculated according to the following equation. [22]

$$C_2 = C_1 * (\frac{q2}{q1})^n \quad 6.1$$

In this material design and selection, all the main equipments had been used was considered and the capacity of each equipment was assumed to increased by 100 times. (Scale 1:100).

#### 5.3.1. Hammer mill

From the material balance, the amount of grain which enters to the miller is 4.65kg/batch. If this production capacity is increased to 100 times, the amount of grist and grits together will be 465kg/batch. If the flow rate of the hammer Q= 6 $m^3$/hr and Density of the malt grist is 670kg/$m^3$. The mass of malt and maize milled will be = 6.0 $m^3$/hr * 670 kg/$m^3$ = 4020 kg/hr

Depend on this the specification of the miller will be:-

**Specification [**http://www.matche.com/Equip Cost/index.htm]

**Capacity** = 4020kg/hr **Material:** - carbon steel

**Pressure** = atmospheric **price** = 31300 US $

To mill 465Kg of grain the time taken will be = 465/4020Kg/hr = 0.1156 hrs = 6.94minutes

#### 5.3.2. Degermer machine

**Specificati**on **[**http://www.matche.com/Equip Cost/index.htm]

**Capacity** = 500kg/hr **motor power** = 1kw

**Dimension** = (HLD) = 2000*500*1100mm **price** = 9500 US$

#### 5.3.3. MashTun

The volume of the grist solution obtained was 15 litre/batch. If this capacity wiil be inceased to 100 times the volume will be 1500 litres. The volume of the mash tun used during beer production was 25 litre with safety factor 60%. If this volume is increased to 100 times, it will be 2500 litre with the same safety factor

**Specification [**http://www.matche.com/Equip Cost/index.htm]

**Type: -** jacketed and agitated **Materia**l: cabon steel

**Internal pressure: -** 300 PSI **Volume:** - 550 galon, **Cost** 27400 US $

**5.3.4. Wort kettle**

Since, from the material balance the total volume of wort solution was 21 litres. If this capacity is increased to 100times, the new volume will be 21000lire. For this the volume of wort kettle if the safety factor is 75%, the volume of the kettle will be 2100/0.75 = 2800litre.

**Specification [**http://www.matche.com/EquipCost/index.htm]

**Type: -** Jacketed and non-agitated **Material**: - stain less steel

**Internal pressure**: - 300 PSI **Volume:** - 616 gallon

**Cost 28500 US $**

**5.3.5. Heat exchanger (cooler)**

**Specification [**http://www.matche.com/EquipCost/index.htm]

**Type: -** cooling tower induced graft **Material**: - carbon steel

**Internal pressure**: - atmospheric **cooling load =** 0.25 million BTU/hr.

**Cost 42500 US $**

**5.3.6. Fermentation tank**

Since the volume of the cold wort produced after wort boiling was 19.202litre and if this capacity is increased to 100 times the volume of total cold wort solution will be 1920.2litres. The fermentation tank with 75% safety factor for six batches will be:-

6*1920.2/0.75 = 12801litre.

For this production capacity, the number of fermentation tank will be 15, because the duration of fermentation will be 14-15 days.

**Specification**

**Type: -** Jacketed and non-agitated **Material**: - stain less steel

**Internal pressure**: - 150 PSI **Volume:** - 3379 gallon

**Cost 28500 US $/Tanker**

# CHAPTER SIX

# FINANCIAL ANALYSIS

The evaluation determines whether the project is economically and financially feasible. The project is economically feasible, when it is more profitable than other competing projects and financially feasible when the capital is raised for its implementation. For this project the capital investment will be borrowed from bank and the *Interest* is considered to be the compensation paid for the use of borrowed capital. A fixed rate of interest is established at the time the capital is borrowed and it is 10% of the total investment cost when production started.

## 6.1. Purchased equipment cost

Table 6.1. Purchased equipment cost

| Number | Equipment | Cost( US $) |
|---|---|---|
| 1 | Maize degermer | 9500 |
| 2 | Hammer mill | 31300 |
| 3 | Mash tun | 27400 |
| 4 | Wort kettler | 28500 |
| 5 | Heat exchanger(Cooler) | 42500 |
| 6 | Fermentation tank | 28500*15 = 427500 |
| 7 | Boiler | 45,000 |
| 8 | Storage tank | 25,000 |
| Total | | **636700** |

Table 6.2. Estimation costs: direct, indirect and total capital investment

| Number | Direct cost | % of purchased cost | Purchased cost(US $) |
|---|---|---|---|
| 1 | Total Purchased Cost | 100 | 636700 |
| 2 | Purchased Equipment Installation | 25 | 159175 |
| 3 | Instrumentation and Control | 10 | 63670 |
| 4 | Electrical Installation | 10 | 63670 |
| 5 | Building (Including Services) | 14 | 89138 |
| 6 | Service Facilities | 20 | 127340 |
| 7 | Piping | 5 | 31835 |
| Total | | 184 | **1107858** |
| **Indirect cost** | | | |
| 8 | Engineering and supervision | 10 | 63670 |
| 9 | Construction and expense | 10 | 63670 |
| 10 | Contingency | 5 | 31835 |
| Total direct cost | | | **159175** |
| | Fixed capital investment = direct cost + Indirect cost | | **1267033** |
| 11 | Working capital | 10 | **126703** |
| 12 | Total capital investment= TCI = FCI + WC | | **1393736** |

When we express the dollar value in ETB birr it will multiplied by 21.45 birr/dollar

FCI = 1267033US dollar*21.45birr/dollar = 27,177,858birr

TCI = 1393736 dollar*21.45birr/dollar =29,895,637 birr

WC = 126703dollar*21.45birr/dollar = 2,717,779 birr

### 6.2. Variable Operating Costs

Variable operating costs, which include raw materials, waste handling charges, and by-product credits, are incurred only when the process is operating. Quantities of raw materials used and wastes produced were determined using the material balance. These are listed below

**1. Manufacturing cost**

Manufacturing cost are all expenses directly connected with the manufacturing operation or the physical equipment of a process plant itself.

A. **Direct production cost**

Table 6.3. Direct production cost (variable cost)

| **S.N** | Item | | Cost in birr per Kg | Total cost in birr |
|---|---|---|---|---|
| **1** | Raw materials | Malt | 19.50birr/Kg*18.9*100*300 | 1105650 |
| | | Maize (BH660) | 6.50birr/kg*9*100*300 | 175500 |
| | | Tetra hop($CO_2$ extract) | 1850birr/kg*0.06*100*300 | 333000 |
| | | Yeast (saccharomysis cervisae) | 550birr/kg *15.36*300/90 | 28160 |
| | | Water | 0.1birr/kg*20*100*300 | 60000 |
| | | Additives | 200birr/kg*0.108*100*300 | 64800 |
| **2** | Operating labor(L) | | 0.1 FCI | 272412 |
| **3** | Direct supervisors and clerical labor | | 0.1TPC | 299694 |
| **4** | Utilities(electric & water cost) | | 0.1TPC | 29969 |
| **5** | Maintenance and repair | | 0.02TCI | 59939 |
| **6** | Operating supplies | | 0.05TCI | 69688 |
| **7** | Laboratory changes | | **0.**01FCI | 27241 |
| Total direct production cost | | | | **26,260,230** |

**B. Fixed charges**.

Fixed charge include Depreciation, Local tax 35%, Insurance and rent

Table 6.4. Fixed operating costs

| SN | Item | Approximation | Cost (ETH Birr) |
|---|---|---|---|
| 1 | Depreciation | 0.2FCI | 7,473,909.25 |
| 2 | Local taxes | 0.02FCI | 543557 |
| 3 | Insurance | 0.005FCI | 13589 |
| 4 | Rent | 0.08(land + building) | 152960 |
| Total fixed operating cost | | | **8,184,015.25** |

**C. Plant overhead costs**

It is about 60% of operating labor

Plant over head cost = 60% of operating labor = 0.6*271412 = **163,447 birr**

**Manufacturing cost** = direct cost +fixed charge + plant overhead cost

= 26,260,230 + 8,184,015.25+ 163447 = **34,607,692.25 birr**

**2. General expense**

In addition to manufacturing cost, other general expense are involved in any company's operations. These general expenses are includes:- Administration cost, about 10% of total product cost, Distribution and selling costs, about 10% of total product cost, Research and development cost, about 2% of total product cost, Financing (interest), about 10% of capital investment

Table 6.5. General expense

| SN | Item | Approximated factor | Cost (ETH Birr) |
|---|---|---|---|

| | | | |
|---|---|---|---|
| 1 | Administrative | 0.5*lobar operating cost | 136206 |
| 2 | 2 Distribution and sell | 0.1*labor operating cost | 27241 |
| 3 | Research and development Not | | Not applicable |
| 4 | Financing interest | 10% | 2,989,563.7 |
| Total general expense | | | **3153010.7** |

**3. Total product cost** = manufacturing cost + general expenses

Total production cost = TPC = 34607692.25+ 3,153,010.7 = 37,760,702.95 birr

If the annual l production capacity = 18L*6*100*300 = 3,240,000 Litre

Unit production cost = TPC/ annual production capacity = 37,760,702.95/3240000

**= 11.65 birr/litre**

### 6.3. Economic evaluation

#### 6.3.1. Net income and return on investment

Gross earn of current price of the beer is 20-25 Birr/lit. However, the price is based on the specification of the beer. Based on this I planned to price the cost of my product to 20 birr/ litre.

Total selling price (revenue) = 20birr/Litre * 3240000Litre = 64,800,000birr

Gross profit = 64800000 – 37,760,702.95 birr = 27039297.05

Income tax on gross profit (35%) = 0.35*27039297.05= 9,463,753.97 birr

Net income (profit) = 27039297.05- 9463753.97 birr = 17,575,543.08 birr

% profit = net income /annual production cost = 17,575,543.08 /37,760,702.95 *100 = 46.54%

#### 6.3.2. Discounted Cash Flow Return (DCFR)

The discount flow rate of return is the return obtained from an investment in which all investment and cash flows are discounted. It is determined by setting the NPV equation equal to zero and solving for the discount rate that satisfies relation (Timerhouse, 2007). I consider the plant capacity starting with 80% capacity at the first year and 90% capacity in the second year and with 100% capacity for the remaining project life. Detail manipulation of the first three year is shown below:-

**During first year**

Percent rate of operating time = 80%

Product rate = 80%*annual production = 0.80*3,240,000 Litre = 2,592,000Litre

Variable cost = 80%*variable cost = 0.80*26,260,230= 21,008,184 birr

Fixed charges + plant over head cost = 8,184,015.25 +163447 = 8,347,462.25 birr

General expense = 3153010.7

Total product cost = Variable cost + Fixed cost + General expense

= 21,008,184 + 8347462.25+3153010.7= 32508656.95birr

Annual Sale = Price per liter * Annual product = 20*2,592,000 = 51,840,000 birr

Gross profit = Annual sale – Total product cost = 51,840,000 – 32508656.95= 19,331,343.05 birr

Net income = Growth profit - Income tax = 19331343.05 - (0.35*19331343.05)

= 12565372.9825birr

Cash flow = Net income + Depreciation = 12565372.9825+ 7,473,909.25 = 20039282.23 birr

Assumption, IRR = 0.12

Discount factor = $(1 + IRR)^{-n} = (1+0.12)^{-1} = 0.893$

Present Value = Cash flow *Discount factor = 20039282.2325*0.893 = 17895079.03 birr.

**During Second year**

Percent rate of operating time = 90%

Product rate = 90%*annual production = 0.90*3,240,000 Litre = 2916000Litre

Variable cost = 0.90%*variable cost = 0.90*26,260,230= 23634207 birr

Fixed charges + plant over head cost = 8,184,015.25 +163447 = 8,347,462.25 birr

General expense = 3153010.7

Total product cost = Variable cost + Fixed cost + General expense

= 23634207+ 8,347,462.25 +3153010.7 = 35134679.95 birr

Annual Sale = Price per liter * Annual product = 20*2916000= 58,320,000 birr

Gross profit = Annual sale – Total product cost = 58320000– 35134679.95= 23185320.05 birr

Net income = Growth profit –Income tax = 23185320.05– (0.35*23185320.05)

= 15,070,458.03 birr

Cash flow = Net income + Depreciation = 15070458.03 + 7,473,909.25 = 22544367.28 birr

If IRR = 0.12

Discount factor = $(1 + IRR)^{-n} = (1+0.12)^{-2} = 0.797$

Present Value = Cash flow *Discount factor = 22544367.28*0.797 = **17,967,860.72** birr

**During third year**

Percent rate of operating time = 100%

Product rate = 100%*annual production =1.0*3,240,000 Litre = 3,240,000 Litre

Variable cost = 100%*variable cost = 100*26,260,230= 26,260,230 birr

Fixed charges + plant over head cost = 8,184,015.25 +163447 = 8,347,462.25 birr

General expense = 3153010.7

Total product cost = Variable cost + Fixed cost + General expense

= 26,260,230 + 8,347,462.25 +3153010.7 = 37760702.95 birr

Annual Sale = Price per liter * Annual product = 20*3,240,000 = 64,800,000 birr

Gross profit = Annual sale – Total product cost = 64,800,000– 37760702.95= 27039297.05 birr

Net income = Growth profit –Income tax = 27039297.05– (0.35*27039297.05)

= 17575543.0825 birr

Cash flow = Net income + Depreciation = 18900462.29 + 7,473,909.25 = 26,374,371.54 birr

If IRR = 0.12

Discount factor = $(1 + IRR)^{-n} = (1+0.12)^{-3} = 0.712$

Present Value = Cash flow *Discount factor = 26374371.54*0.712 = **18,778,552.54** birr

For the remaining periods of the project life, summary of economic data detail is given in the following cash flow table.

Table 6.6. Cash flow chart considering interest

| year | -1 | 0 | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|---|
| Capacity utilization% | - | - | 80 | 90 | 100 | 100 | 100 |
| **I. Cash inflow** | - | - | 51,840,000 | 58,320,000 | 64,800,000 | 64,800,000 | 64,800,000 |
| Annual Sale (revenue) | | | 51,840,000 | 58,320,000 | 64,800,000 | 64,800,000 | 64,800,000 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Salvage value | | | | | | | |
| **II.Cash outflow** | 29,895,637 | - | 35498225 | 37376852 | 39245485 | 38508094 | 30286793 |
| TCI | 29,895,637 | - | - | | | | |
| Direct production cost | - | - | 21008184 | 23634207 | 26260230 | 26260230 | 26260230 |
| Total fixed cost except dep. | | | 710106 | 710106 | 710106 | 710106 | 710106 |
| Factory overheads | | | 163,447 | 163,447 | 163,447 | 163,447 | 163,447 |
| Total general expense | | | 3153010.7 | 3153010.7 | 3153010.7 | 3153010.7 | 3153010.7 |
| Depreciation | | | 7,473,909 | 7,473,909 | 7,473,909 | 7,473,909 | - |
| Interest | | | 2,989,568 | 2242172.8 | 1494781.9 | 747391 | - |
| **Gross Profit (I-II)** | | | 16,341,775 | 20943148 | 25554515 | 26291906 | 34513207 |
| Tax (35%) | | | 5719621 | 7330101.8 | 8944080 | 9202167.1 | 12079622 |
| Net profit | | | 10622153 | 13613046 | 16610434 | 17089739 | 22433584 |
| Cash flow | | | 18096068 | 21086955 | 24084343 | 24563648 | 22433584 |
| Discount factor = $(1 + IRR)^{-n}$ | | | 0.893 | 0.797 | 0.712 | 0.636 | 0.567 |
| PV | | | 16159784 | 16806303 | 17148052 | 15622480 | 12719842 |

### 3.2.3. Payback period calculation

| | 0 | 1 | 2 | 3 |
|---|---|---|---|---|
| Cumulative cash flow | -29,895,637 | -1927348 | -5660438 | +10949996 |
| Yearly cash flow | | 10622153 | 13613046 | 16610434 |

Payback period = $2 \text{ years} + \frac{5660438}{16610434}$ = 2.34 years or about 2 years and 4 months

From this evaluation we can summarize that this project is economically and financially feasible and profitable. The payback period is short and this can minimize the amount of interest compensated. The main reason is as we have seen from the economic evaluation of the project the cost of maize was cheapest compared to the cost of malted barley and this can minimize the production coast.

Table 6.7 Cost benefit by using maize

| SN | Items | Cost of items birr/kg | Operation cost /kg | total cost/kg | cost benefit /kg |
|---|---|---|---|---|---|
| 1 | Two row barley | 12.00 | 7.50 | 19.50 | 0 |
| 2 | Six row barley | 9.50 | 7.50 | 17.0 | 2.50 |
| 2 | BH660 Maize | 6.50 | 5.50 | 12.0 | 7.50 |

- ✓ 32.29% maize and 33.86% six row were used while the annual grain consumption was = 837000kg/annual
- ✓ Cost if two row only = 19.50birr/kg*837000kg = **16321500birr**
- ✓ Cost if the three grain is used = 0.3229*12birr/kg*837000 + 0.3385*17birr/kg *837000 + 0.3386*19.50birr/kg*837000 = **13.586,184 birr**

Total cost benefit/year = 16321500-14296127 = **2,735,316 birr**

Percent of cost benefit /year = 16.76**%**. **Therefore, cost benefit is significant**

# CHAPTER SEVEN

# CONCLUSION AND RECOMMENDATION

## 7.1. Conclusion

Even the physico-chemical characteristic of the raw materials are an appropriate and the main precondition for the production of beer, the method or the condition of the production process is not little. The proximate analysis shows that the BH660 maize has relatively less percentage of oil and selected for wort preparation. The diastatic power of the six row barley were relatively higher than the two row, while the diastatic power of the BH660-maize was not malted and the value of diastatic power is assumed to be zero. The excess diastatic power of the six row barley was assumed to be contributing for the BH660 maize while the two row has excess enzyme for starch hydrolysis or saccharification.

There are many variables which affect the response variables. But the three variables which considered are maize percentage, mashing time and mashing temperature have significant effect. The optimization of wort for high yield of extract and enough FAN was interpreted. ANOVA shows that the effect of each independent variable individually and the interaction between each variables to give maximum yield of extract and targated free amino nitrogen was the main tools for decision making during optimization. The Model F-value of 33.68 for yield of extract and the Model F-value of 15.94 for free amino nitrogen implies the model is significant. There is 0.01% chance this could occur due to noise. Values of "Prob > F" less than 0.0500 for both response variables indicate model terms were significant. The value Coefficient of Variation, the standard deviation, the R-Squared were and adequate Precision, were decide the model can be used. From the three factors the percentage of maize was contribute large effect on the two response variables. The beer was produced according the desirability of the most favorable solution. Finally the produced beer was characterized and which all parameters are tolerated except VDK value. The cost of production by using maize was proved that it is less than beer which is produced by using only malted barley.

### 7.2. Recommendation

There is variety of different maize which has been obtained from the research center. Further researches have to be done to improve the production of high quality beer from the other maize type. The main drawback to produce beer from maize is the highest percentage of oil in the germ parts of the maize. To solve this creating new technology to separate the germ part from the whole part of the grain must be developed. Otherwise, the degerming machine can bought from the market.

The other information I have to summarize is during germ separation there was germ and bran flour which can be used for different purpose and this can cover the cost of the operation. Therefore, further research must be done on this title.

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## Appendices

**Appendix A:** Laboratory analysis and result at T1=64$^{O}$C, t1=8min, T2 = 73 $^{O}$C t2=22min

| Std Order | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| Run Order | 2 | 50 | 23 | 5 | 28 | 37 | 6 | 27 | 17 |
| mass of maize in gram | 21 | 21 | 21 | 24.5 | 24.5 | 24.5 | 28 | 28 | 28 |
| mass of malt for mash cooking in gram | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| mass of malt in gram (50+50) | 47 | 47 | 47 | 43.5 | 43.5 | 43.5 | 40 | 40 | 40 |
| Moisture content of sample(%) | 6.96 | 6.96 | 6.96 | 7.12 | 7.12 | 7.12 | 7.28 | 7.28 | 7.28 |
| specific gravity | 1.03781 | 1.03775 | 1.03778 | 1.03787 | 1.03789 | 1.03785 | 1.038 | 1.03794 | 1.03796 |
| wort extract in g/100g | 9.4625 | 9.4475 | 9.455 | 9.4775 | 9.4875 | 9.4725 | 9.51 | 9.495 | 9.5 |
| sample extract E1(%) | 84.34 | 84.19 | 84.26 | 84.5 | 84.6 | 84.45 | 84.84 | 84.69 | 84.74 |
| dry sample extract E2 (%) | 90.65 | 90.49 | 90.56 | 90.98 | 91.09 | 90.09 | 91.5 | 91.34 | 91.39 |
| FAN(ppm) | 118.25 | 116.56 | 117.68 | 114.25 | 115.08 | 114.94 | 109.56 | 111.55 | 110.25 |

**Appendix B:** Laboratory analysis and result at T1=64, t1=9min, T2=73, t2=21min

| Std Order | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
|---|---|---|---|---|---|---|---|---|---|
| Run Order | 40 | 61 | 54 | 9 | 39 | 48 | 41 | 26 | 55 |
| mass of maize in gram | 21 | 21 | 21 | 24.5 | 24.5 | 24.5 | 28 | 28 | 28 |
| mass of malt for mash cooking in gram | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| mass of malt in gram (50+50) | 47 | 47 | 47 | 43.5 | 43.5 | 43.5 | 40 | 40 | 40 |
| Moisture content of sample(%) | 6.65 | 6.65 | 6.65 | 6.825 | 6.825 | 6.825 | 7 | 7 | 7 |
| specific gravity | 1.03771 | 1.03775 | 1.03773 | 1.03781 | 1.03783 | 1.03782 | 1.03786 | 1.03789 | 1.03784 |
| wort extract in g/100g | 9.4375 | 9.4475 | 9.4425 | 9.4625 | 9.4675 | 9.465 | 9.475 | 9.4825 | 9.47 |
| sample extract E1(%) | 84.06 | 84.16 | 84.09 | 84.37 | 84.38 | 84.35 | 84.47 | 84.54 | 84.42 |
| dry sample extract E2 (%) | 93.03 | 91.55 | 90.08 | 90.55 | 90.56 | 90.53 | 90.82 | 90.9 | 90.52 |
| FAN(ppm) | 116.5 | 118.05 | 117.68 | 114.23 | 113.35 | 114.74 | 111.26 | 109.94 | 110.53 |

**Appendix C:** Laboratory analysis and result at T1=64 $^{O}$C, t1=10min, T2=73 $^{O}$C, t2=20min

| Std Order | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 |
|---|---|---|---|---|---|---|---|---|---|
| Run Order | 18 | 63 | 73 | 1 | 36 | 52 | 4 | 60 | 44 |
| mass of maize in gram | 21 | 21 | 21 | 24.5 | 24.5 | 24.5 | 28 | 28 | 28 |
| mass of malt for mash cooking in gram | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| mass of malt in gram (50+50) | 47 | 47 | 47 | 43.5 | 43.5 | 43.5 | 40 | 40 | 40 |
| Moisture content of sample(%) | 6.68 | 6.68 | 6.68 | 6.86 | 6.86 | 6.86 | 7.04 | 7.04 | 7.04 |
| specific gravity | 1.03761 | 1.03769 | 1.03765 | 1.03778 | 1.03775 | 1.03778 | 1.03785 | 1.03788 | 1.03784 |
| wort extract in g/100g | 9.415 | 9.4325 | 9.425 | 9.455 | 9.4475 | 9.455 | 9.4725 | 9.48 | 9.47 |
| sample extract E1(%) | 83.84 | 84.01 | 83.94 | 84.26 | 84.18 | 84.26 | 84.45 | 84.52 | 84.42 |
| dry sample extract E2 (%) | 89.8414 | 90.0236 | 89.9486 | 90.466 | 90.3801 | 90.466 | 90.8455 | 90.9208 | 90.8133 |
| FAN(ppm) | 118.08 | 116.91 | 117.65 | 114.45 | 115.05 | 113.21 | 110.25 | 108.43 | 109.45 |

**Appendix D:** Laboratory analysis and result at T1=65 $^{O}$C, t1=8min, T2=73 $^{O}$C t2=22min

| Std Order | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 |
|---|---|---|---|---|---|---|---|---|---|
| Run order | 81 | 65 | 67 | 80 | 74 | 70 | 58 | 33 | 72 |
| mass of maize in gram | 21 | 21 | 21 | 24.5 | 24.5 | 24.5 | 28 | 28 | 28 |
| mass of malt for mash cooking in gram | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| mass of malt in gram (50+50) | 47 | 47 | 47 | 43.5 | 43.5 | 43.5 | 40 | 40 | 40 |
| Moisture content of sample(%) | 7.12 | 7.12 | 7.12 | 7.29 | 7.29 | 7.29 | 7.46 | 7.46 | 7.46 |
| specific gravity | 1.03781 | 1.03785 | 1.03786 | 1.03799 | 1.03795 | 1.03802 | 1.03812 | 1.03809 | 1.03808 |
| wort extract in g/100g | 9.4625 | 9.4725 | 9.475 | 9.5075 | 9.4975 | 9.515 | 9.5375 | 9.53 | 9.5275 |
| sample extract E1(%) | 84.36 | 84.45 | 84.48 | 84.82 | 84.72 | 84.89 | 85.13 | 85.06 | 85.03 |
| dry sample extract E2 (%) | 90.8269 | 90.9238 | 90.9561 | 91.4896 | 91.3817 | 91.5651 | 91.9927 | 91.917 | 91.8846 |
| FAN(ppm) | 118.45 | 118.52 | 117.56 | 115.25 | 114.86 | 114.94 | 111.5 | 109.11 | 109.24 |

**Appendix E:** Laboratory analysis and result at T1=65 $^{O}$C, t1=9min, T2=73 $^{O}$C, t2=21min

| Std Order | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 |
|---|---|---|---|---|---|---|---|---|---|
| Run Order | 42 | 14 | 20 | 62 | 13 | 76 | 38 | 77 | 45 |
| mass of maize in gram | 21 | 21 | 21 | 24.5 | 24.5 | 24.5 | 28 | 28 | 28 |
| mass of malt for mash cooking in gram | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| mass of malt in gram (50+50) | 47 | 47 | 47 | 43.5 | 43.5 | 43.5 | 40 | 40 | 40 |
| Moisture content of sample(%) | 7.29 | 7.29 | 7.29 | 7.46 | 7.46 | 7.46 | 7.62 | 7.62 | 7.62 |
| specific gravity | 1.0378 | 1.0378 | 1.0378 | 1.0379 | 1.0379 | 1.038 | 1.0381 | 1.0381 | 1.038 |
| wort extract in g/100g | 9.46 | 9.455 | 9.4575 | 9.49 | 9.495 | 9.4975 | 9.528 | 9.524 | 9.5175 |
| sample extract E1(%) | 84.35 | 84.3 | 84.32 | 84.66 | 84.71 | 84.74 | 85.05 | 85.01 | 84.95 |
| dry sample extract E2 (%) | 90.9826 | 90.9287 | 90.9503 | 91.4848 | 91.5388 | 91.5712 | 92.0654 | 92.0221 | 91.9571 |
| FAN(ppm) | 116.98 | 118.07 | 117.16 | 114.56 | 114.96 | 114.32 | 107.94 | 110.05 | 109.26 |

**Appendix F:** Laboratory analysis and result at T1=65 $^{O}$C, t1=10min, T2=73 $^{O}$C, t2=20min

| Std Order | 46 | 47 | 48 | 49 | 50 | 51 | 52 | 53 | 54 |
|---|---|---|---|---|---|---|---|---|---|
| Run Order | 57 | 8 | 3 | 71 | 53 | 35 | 22 | 19 | 12 |
| mass of maize in gram | 21 | 21 | 21 | 24.5 | 24.5 | 24.5 | 28 | 28 | 28 |
| mass of malt for mash cooking in gram | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| mass of malt in gram (50+50) | 47 | 47 | 47 | 43.5 | 43.5 | 43.5 | 40 | 40 | 40 |
| Moisture content of sample(%) | 7.15 | 7.15 | 7.15 | 7.325 | 7.325 | 7.325 | 7.5 | 7.5 | 7.5 |
| specific gravity | 1.03779 | 1.03777 | 1.03777 | 1.03789 | 1.03793 | 1.0379 | 1.03801 | 1.03803 | 1.03804 |
| wort extract in g/100g | 9.4575 | 9.4525 | 9.4525 | 9.4825 | 9.4925 | 9.485 | 9.5125 | 9.5175 | 9.52 |
| sample extract E1(%) | 84.31 | 84.26 | 84.26 | 84.57 | 84.67 | 84.6 | 84.89 | 84.94 | 84.96 |
| dry sample extract E2 (%) | 90.8024 | 90.7485 | 90.7485 | 91.2544 | 91.3623 | 91.2868 | 91.773 | 91.827 | 91.8486 |
| FAN(ppm) | 116.68 | 117.52 | 117.24 | 115.26 | 113.98 | 114.82 | 108.62 | 110.12 | 109.4 |

**Appendix G:** Laboratory analysis and result at T1=66 $^{O}$C, t1=8min, T2=73 $^{O}$C, t2=22min

| Std Order | 55 | 56 | 57 | 58 | 59 | 60 | 61 | 62 | 63 |
|---|---|---|---|---|---|---|---|---|---|
| Run Order | 49 | 79 | 47 | 66 | 7 | 64 | 31 | 10 | 69 |
| mass of maize in gram | 21 | 21 | 21 | 24.5 | 24.5 | 24.5 | 28 | 28 | 28 |
| mass of malt for mash cooking in gram | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| mass of malt in gram (50+50) | 47 | 47 | 47 | 43.5 | 43.5 | 43.5 | 40 | 40 | 40 |
| Moisture content of sample(%) | 7.53 | 7.53 | 7.53 | 7.685 | 7.685 | 7.685 | 7.84 | 7.84 | 7.84 |
| specific gravity | 1.03774 | 1.03771 | 1.03773 | 1.03782 | 1.03787 | 1.03784 | 1.03796 | 1.03792 | 1.03797 |
| wort extract in g/100g | 9.445 | 9.4375 | 9.4425 | 9.465 | 9.4775 | 9.47 | 9.5 | 9.49 | 9.5025 |
| Sample extract E1 (%) | 84.23 | 84.15 | 84.2 | 84.44 | 84.56 | 84.49 | 84.8 | 84.7 | 84.83 |
| Dry sample extract E2 $^{O}$C (%) | 91.089 | 91.0025 | 91.0566 | 91.4694 | 91.5994 | 91.5236 | 92.0139 | 91.9054 | 92.0464 |
| FAN(ppm) | 118.56 | 117.53 | 118.28 | 115.1 | 114.87 | 114.23 | 110.22 | 111.04 | 109.56 |

**Appendix H:** Laboratory analysis and result at T1=66 $^{O}$C, t1=9min, minute, T2=73 $^{O}$C, t2=21min

| Std Order | 64 | 65 | 66 | 67 | 68 | 69 | 70 | 71 | 72 |
|---|---|---|---|---|---|---|---|---|---|
| Run order | 16 | 32 | 78 | 15 | 75 | 68 | 24 | 34 | 56 |
| mass of maize in gram | 21 | 21 | 21 | 24.5 | 24.5 | 24.5 | 28 | 28 | 28 |
| mass of malt for mash cooking in gram | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| mass of malt in gram (50+50) | 47 | 47 | 47 | 43.5 | 43.5 | 43.5 | 40 | 40 | 40 |
| Moisture content of sample(%) | 7.23 | 7.23 | 7.23 | 7.385 | 7.385 | 7.385 | 7.54 | 7.54 | 7.54 |
| specific gravity | 1.03771 | 1.03772 | 1.03768 | 1.03783 | 1.03781 | 1.03785 | 1.037895 | 1.03793 | 1.03792 |
| wort extract in g/100g | 9.4375 | 9.44 | 9.43 | 9.4675 | 9.4625 | 9.4725 | 9.485 | 9.4925 | 9.49 |
| Sample extract E1(%) | 84.12 | 84.14 | 84.05 | 84.43 | 84.38 | 84.48 | 84.62 | 84.7 | 84.67 |
| Dry sample extract E2 (%) | 90.6759 | 90.6974 | 90.6004 | 91.1623 | 91.1084 | 91.2163 | 91.5207 | 91.6072 | 91.5747 |
| FAN(ppm) | 117.64 | 116.22 | 117.25 | 113.25 | 115.02 | 114.45 | 108.55 | 109.36 | 108.96 |

**Appendix I:** Laboratory analysis and result at T1=66 $^{O}$C, t1min=10, T2=73 $^{O}$C, t2=20min

| Std Order | 73 | 74 | 75 | 76 | 77 | 78 | 79 | 80 | 81 |
|---|---|---|---|---|---|---|---|---|---|
| Run Order | 46 | 59 | 25 | 51 | 11 | 26 | 30 | 40 | 21 |
| mass of maize in gram | 21 | 21 | 21 | 24.5 | 24.5 | 24.5 | 28 | 28 | 28 |
| mass of malt for mash cooking in gram | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| mass of malt in gram (50+50) | 47 | 47 | 47 | 43.5 | 43.5 | 43.5 | 40 | 40 | 40 |
| Moisture content of sample(%) | 7.42 | 7.42 | 7.42 | 7.59 | 7.59 | 7.59 | 7.76 | 7.76 | 7.76 |
| specific gravity | 1.03762 | 1.03765 | 1.03768 | 1.03783 | 1.03779 | 1.03784 | 1.0379 | 1.03789 | 1.03791 |
| wort extract in g/100g | 9.4167 | 9.426 | 9.43 | 9.4675 | 9.4575 | 9.47 | 9.485 | 9.49 | 9.4875 |
| sample extract E1(%) | 83.94 | 84.03 | 84.08 | 84.45 | 84.36 | 84.49 | 84.64 | 84.66 | 84.67 |
| dry sample extract E2 (%) | 90.6675 | 90.7647 | 90.8188 | 91.3862 | 91.2888 | 91.4295 | 91.7606 | 91.7823 | 91.7931 |
| FAN(ppm) | 118.24 | 117.21 | 117.08 | 115.01 | 114.22 | 114.27 | 110.32 | 108.4 | 108.88 |