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Addis Ababa Institute of Technology

School of Chemical and Bio Engineering

Partial Substitution of Malted Barley by Raw Barley in Brewing Technology

A Thesis Submitted to the School of Graduate Studies of Addis Ababa Institute of Technology, in Partial Fulfillment of the Requirements for the Degree of Master of Science in Chemical Engineering (Process Engineering Stream)

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Abstract

The effect of commercial enzymes on quality of wort developed from replacement of malted barley with raw barley as adjunct of 40 % and 60 % was investigated for yield of extract, Free Amino Nitrogen, Degree of fermentation, pH, VDK, colour…. The wort pH is relatively stable with no significant changes at all levels of replacements. The colour is lighter with increment in proportion of the raw barley as it appears as colour diluents. The wort produced tends to be turbid which affects the utilization of the wort. The total soluble nitrogen and free amino nitrogen value increased with the use of commercial enzymes while the yield increases with enzymes. The use of ceremix Plus and Ondea Pro have been found to be relatively more effective in the preparation of beer wort as observed for the result obtained in proportions of raw barley and malt barley. The use of high percentage raw barley as adjunct with commercial enzymes are found to be useful in production of high quality wort with low cost and profitability.

The use of barley instead of malt in the brewing process is interesting with respect to costs. However, in cost calculations it is important to consider that barley has a considerable enzyme deficit and requires the addition of microbially produced enzymes during the brewing process. Owing to the improving knowledge about brewing with barley using microbial enzyme cocktails during recent years, it is possible to brew beverages with a barley content of up to100 % without technological process problems in the brewery. However, there are different opinions with respect to the influence of unmalted barley on beer quality and flavour. Previous investigations showed a decrease in the colour, bitterness and free amino nitrogen (FAN) content and poor results in sensory analyses. Additionally, increasing b-glucan content was observed using barley. The aim of this study was to investigate the influences of unmalted barley on the brewing process and the quality of the resulting product, with the main focus on the Yield of extract & free amino Nitrogen. For the investigation, brews with 40 % & 60 % raw barley proportion brews with a colour malt addition, to compensate for a lower colour using barley, were produced. In general, it can be said that beers with a proportion of up to 54.57 % barley achieved a comparable or higher extract yield and final attenuation owing to the combined effectiveness of the malt and microbial enzymes. Although all analytical values were within the normal range according to EBC, a slight decrease in total polyphenols and free amino nitrogen content was observed (Table 10).

List of Acronyms

AC	Alcohol Content
AOAC	Association of Official Analytical Chemists
Ea	Apparent Extract
Er	Real Extract
ASBC	American Society of Brewing Chemists
BAC	Brewers Association of Canada
EBC	Europeans Brewing Convention
FAN	Free Amino Nitrogen
OG	Original Gravity
IOB	Institute of brewing
IBD	Institute of Brewing & Distilling
SSB	Scandinavian School of Brewing
WHO	World Health Organization

CHAPTER ONE

1. INTRODUCTION

1.1 Background

Beer is a very old drink the history of which, as a result of tomb excavations and other discoveries, can be traced back almost 5,000 years. Beer is the world's most widely consumed, likely the oldest alcoholic beverage and the third most popular drink overall, after water and tea. The oldest mention of beer is in Mesopotamian cuneiform writing, from the year 2800 BC, which describes the distribution of daily ration of beer and bread to the workforce. The production and dispensing of beer is regulated closely in the collection of laws of the Babylonian King Hammurabi. The production of beer is tied to three consecutive biochemical processes: the formation of enzymes in germinating grain, the breakdown of starch to sugar by these enzymes and the resulting fermentation of sugar to alcohol and CO_2. (Kunze, 2014).

Worldwide, the brewing industry is registering growth in both volumes and profits year on year. Ethiopia is not exception to this global phenomenon. Over the last two years; 2012 & 2013 G.C, the biggest brewing industries in Ethiopia: Heineken, Diageo, Bavaria, BGI Ethiopia, Dashen Breweries … have made enviable strides, both in volume turnover. The potential for the industry to grow has become unquestionable. The challenge however is how the industry can reduce production cost by the use of cheaper sources of raw materials. Ethiopia's per capita consumption of beer stands at 8 (eight) liters on 2014 and it is expected to reach 9 (nine) or 10 (ten) by the end of 2015/ 2016) (Fortune, 2015); which demands more raw materials with the limited resource the country has.

Over 30 years ago Novo Nordisk A/S (today Novozymes) introduced industrial, microbial produced enzymes for the brewing industry. The first products were a bacterial protease and a bacterial alpha-amylase. Enzymes are proteins, which act as catalysts. Enzymes lower the energy required for a reaction to occur, without being used up in the reaction. Many types of industries, to aid in the generation of their products, utilize enzymes. Examples of these products are; cheese, alcohol and bread. Fermentation is a method of generating enzymes for industrial purposes. (Michael Eblinger, 2009).

Fermentation involves the use of microorganisms, like bacteria and yeast to produce the enzymes. There are two methods of fermentation used to produce enzymes. These are submerged fermentation and solid-state fermentation. Submerged fermentation involves the production of enzymes by microorganisms in a liquid nutrient media. Solid-state fermentation is the cultivation of microorganisms, and hence enzymes on a solid substrate. Carbon containing compounds in or on the substrate are broken down by the microorganisms, which produce the enzymes either intracellular or extracellular. The enzymes are recovered by methods such as centrifugation, for extracellularly produced enzymes and lysing of cells for intracellular enzymes. Many industries are dependent on enzymes for the production of their goods. Industries that use enzymes generated by fermentation are the **brewing**, wine making, baking and cheese making.

Even for an old industry like beer brewing new industrial processes benefit from using enzymes developed from microbial sources. In the last years quality issues like flavor control, beer stability and general cost savings in the industry go hand in hand with efficient solutions of environmental problems. Future aspects focus on a wider application of enzymes to brew with high amounts of inexpensive raw materials like barley. Alternative beer processes for production of wort and beer with higher productivity and reduced amounts of waste and by-products are under development.

The limitations to raw material choice and process-ability have expanded significantly over the last years by the use of enzymes. Traditionally, high portions of well modified malted barley needed to dominate the brewing recipes to achieve sufficient yield, efficiency and quality. Enzymes are selected according to cereal-specific substrates and the relevant pH and temperature optima. Processing up to 100% under modified malt, barley or sorghum, as well as including more than 60% wheat, rice and maize (corn), are globally well established approaches today. However, raw material optimization is not only about including more un-malted cereal in the recipes, but rather about achieving high consistency and efficiency in production and beer specifications without compromising quality. In general, enzyme works to address customer needs and enable the brewing industry to drive a raw material agenda in using unmalted adjuncts.

The enzymes in malt can cope with up to 15 to 20 % barley adjunct without any problem. Barley may be used in the form of milled barley or barley flakes made from husked/ de-husked barley. The lower extract yield must be set against the lower price compared with malt. Problems may be

caused due to the absence of malting process, the α-glucan is not dissolved and is not sufficiently degraded during mashing. In such a case filtration problems are to be expected. (Kunze, 2014)

Nobody envisages a dramatic shift in grist materials used in the current beer market. Some brewers have shifted from sizeable use of adjuncts to grists that are largely composed of premium malted as they are convinced that this offers genuine quality. However, the remaining clear justification for many brewers to use adjunct materials, since they offer unique product attributes such as flavor and color. The quality attributes of some of the world's leading global beer brands are heavily based on the adjunct used in their formulation. (D. L. Goode, H. H. Wijngaard, E. K. Arendt, 2005) The foregoing underpins the employment opportunities offers to inhabitants in areas where brewing industries are vibrant and are endowed with cereals that are commonly used as brewing adjuncts.

Brewing adjuncts are materials other than malted barley that bring additional sources of carbohydrate and protein into wort. Adjuncts have largely been limited to cereals such as corn, rice, sorghum, wheat and barley with little contribution coming from cane sugar. Globally, the use of adjuncts from roots and tubers has however received relatively little attention. The German purity law (Reinheitsgebot) defines an adjunct as "anything that is not malt, yeast, hops or water". However the definition is much broader today. The United Kingdom food standards committee interprets adjuncts to be "Any carbohydrate source other than malted barley which contributes sugars to the wort". (Institute of brewing & Distilling, 2003). The latter definition seems to embrace roots and tubers alike that can provide sources of carbohydrate, which can meet the requirements for brewing.

It has been reported (Bamforth, 2003) that when the total cost of beer production is taken into consideration (from raw material purchase and processing through to packaging, sales and taxation), then malts' costs in general have been estimated to represent just approximately 3.5% of the total cost. Therefore, it becomes apparent that grain costs represent only a relatively minor contribution to the total cost of beer production. The foregoing raises the question, why replace malted barley with an unmodified substrate "adjunct"? In less developed countries, malting facilities and malting conditions are quite often less than optimal. Therefore, because of its lower price, locally produced adjunct material can be used to supplement malted barley grain (Grujic,

1999). Apart from the direct cost benefits of using cheaper raw materials, indirect costs (much greater than the direct costs) can also influence raw material selection.

In Kenya, for example, beer is made from malted grain, (Cege *et al.*, 1999). Kenyan brewers are therefore encouraged to develop beer from exclusively non-malted grain (mainly raw barley). Likewise in Japan, much lower rate taxation is applied to products containing high adjuncts levels (Happoshu Brewers Association of Japan; Shimizu *et al*., 2002). Therefore, Japan's brewers have great incentive to brew products from grists containing adjunct levels in excess of 50 %. Likewise, in Nigeria a 1988 government economic decision to ban the importation of malted barley forced local brewers to develop alternative brewing procedures to utilize locally grown sorghum and maize crops (Hallgren, 1995; Little, 1994). Additionally, factors associated with product quality, tradition and consumer product expectations can be the decisive reason to use adjuncts, such as the impact that rice has on flavor, color and colloidal stability of an American pale lager, or the role that wheat plays in the taste and appearance of a Belgian or German style wheat beer (Delvaux et al., 2001). Also the use of liquid adjunct materials in today's high gravity brewing culture can increase production output and significantly reduce production costs, whilst contributing to product character.

The first commercial beer with 100 % barley was brewed and sold in 1963. Due to the improving knowledge about brewing with barley using technical enzymes during the recent years, it is possible to brew beverages with a barley proportion up to 100 % without technological problems in the brewery. The use of barley instead of malt in the brewing process is interesting in an economic point of view since the material, energy and human resources for malting cause remarkable costs. However, in the cost calculation it is important to consider that unmalted barley has a considerable enzyme deficit against malt due to the lack of a germination process. Therefore, the addition of enzymes is required to compensate the enzyme deficit during the brewing process. Accordingly, the extract yields as well as the price difference between barley and malt is significant despite of the cost of enzymes. There are different calculations, which assume about 115 kg barley is required to get the same extract yield as from 100 kg malt. Concerning the difference in water content between barley and malt of about 11 %, approximately 104 kg barley is needed. This means that the yield based on the dry matter is only a few percent lower than from malt. (Thomas Kunz, August 2011, Vol. 64)

1.2 Problem Statement

In the past, the main drivers for the usage of brewing adjuncts have been cheaper cost of raw materials, together with opportunities of increasing product output capacity without the necessity of increasing brew house capacities (addition of syrups). In addition, usage of certain adjuncts has offered the brewer more control over product quality with regard to flavor, color and colloidal stability. In Ethiopia, malt barley is the only cereal used in the brewing industry. Currently beer brewing companies are booming. The beer market in the country has attracting international beer producers like the Heineken and the demand for beer is steadily growing, with high potential for both local and foreign markets. Beer production in Ethiopia has increased from 1 million hectoliters in 2003 to **7** million hectoliters in 2015 (The Ethiopian Herald, 2015). Nevertheless, much of the demand for malting barley is being covered *via* import as the existing single government owned malt factory (Asella Malt Factory) meets only 40 % of the demand and currently the local beer brewery industries are importing about 60 % of malt barley (AMF, 2014). To produce **7** million HL beer requires about **112,000** metric ton malt.

The ever increasing price of imported malt barley, limited local production due to climate variability and stiff competition of malt barley with food barley for land and other resources are some of the major constraints. The unnecessary mixing of the food barley with malt barley from the supplier's side (where the food barley has high beta-glucan which has negative consequences on the brewing industries) is also a series quality constraint.

This forced the brewers to import malt to solve their supply problem. But importing can never be sustainable solution. This practice has impact on an economic point of view since the material, energy and human resources for malting process cause remarkable costs. However, in the cost calculation it is important to consider that unmalted barley has a considerable enzyme deficit against malt due to the lack of a germination process. The addition of enzymes is required to compensate the enzyme deficit during the brewing process. Accordingly, the extract yield, quality of beer as well as the price difference between barley and malt is significant despite of the cost of enzymes.

Basically the application of the enzymes is the reason for using malt in the process of brewing instead of pure adjuncts. These enzymes are the most important ingredients to convert the complex sugars to simple sugars in the mashing process. But nowadays breweries use high gravity brewing

technology to produce more beer with lean resource utilization which needs additional enzymes to catalyze the bulky substrate and the additional adjuncts. By enabling flexible raw material use and lowering energy consumption, enzymes are a tool for breweries to reach their strategic business goals. Enzymes offer opportunities to secure processes that are right the first time and that enable the creation of tasty and inviting brews for beer lovers around the world. The shortage of local malt supply, because of lack of malt factories and the cost breweries incur by using 100 % malt is not feasible for the country. This is the problem that needs an immediate solution from experts in the field instead of thinking import as the solution.

1.3 Objectives of the Research

General Objective:

The general objective of this study is to evaluate the effect of unmalted Barley as an adjunct substitute for malted barley in Barley Malt beer quality.

Specific Objectives:

The specific objective of the research will be:

- To carry out brewing of unmalted barley mixed with malted barley in 40 % and 60 % proportions.
- To compare production cost of the treatments
- To measure the extract yield, Free Amino Nitrogen & fermentability of each treatments
- To assess the quality of beer produced from different levels of raw barley proportion
- To define optimum parameters to achieve maximum conversion without affecting quality of beer.

NB. Up to 20 %, raw barley can be used as an adjunct with malt without addition of enzymes (Kunze, 2014).

1.4 Significance of the Research

The purpose of this research is to support breweries to improve the beer in the production economy, process control or beer quality up on using adjuncts & microbial enzymes for brewing process. The individual enzyme products are developed to fill in what the natural enzymatic environment is lacking under the specific brewing conditions (substrate specificity, pH and temperature). The enzyme products are developed to work either in synergy with the existing enzyme systems in the various grains (barley, malted barley, wheat etc.), or to enable the degradation and utilization of cereals beyond the traditional malt-based enzyme configuration. To ensure optimal process-ability and ferment-ability, different enzyme products containing glucanases, xylanases, proteases, amylases, pullulanases (limit dextrinase) and lipase activities are optimally combined according to the properties of the relevant raw materials.

The findings of the study will help Ethiopian Breweries in innovating ways to:

- Solve malt supply problem,
- Save hard currency & time of transit, and
- Innovate processes in having alternative raw material advantages

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Raw materials of beer Production

Beer brewing is an intricate process encompassing mixing of four essential raw materials, including barley malt, brewing water, hops and yeast. Over time, different types of starchy plants have been used for brewing, including maize (in South America), soy (in India and Persia), millet and sorghum (in Africa) and rice (in the Far East). Nowadays, beer production using barley malt is the most common brewing process worldwide.

Barley Malt

Malt is one of the main ingredients and is obtained from barley, which is subjected to a process of germination under controlled conditions. This operation (called malting) causes, towards the end of the production process, the development of carbohydrates and nitrogenous substances by the enzymes formed during the germination process. Varying conditions during the malting process (temperature and humidity) allows different types of malt to be obtained, giving different colours and flavours to the beer.

Figure 1: Raw Barley

The criteria for a good malting barley are, apart from good agronomical properties & high yield:

- Large kernels
- High starch content (giving high extract yield)
- High vitality
- Good “enzyme potential”
- Mealy endosperm
- Moderate protein content (9.5% - 10.2%)
- Low β – glucagon content

Eg. Two-row barley

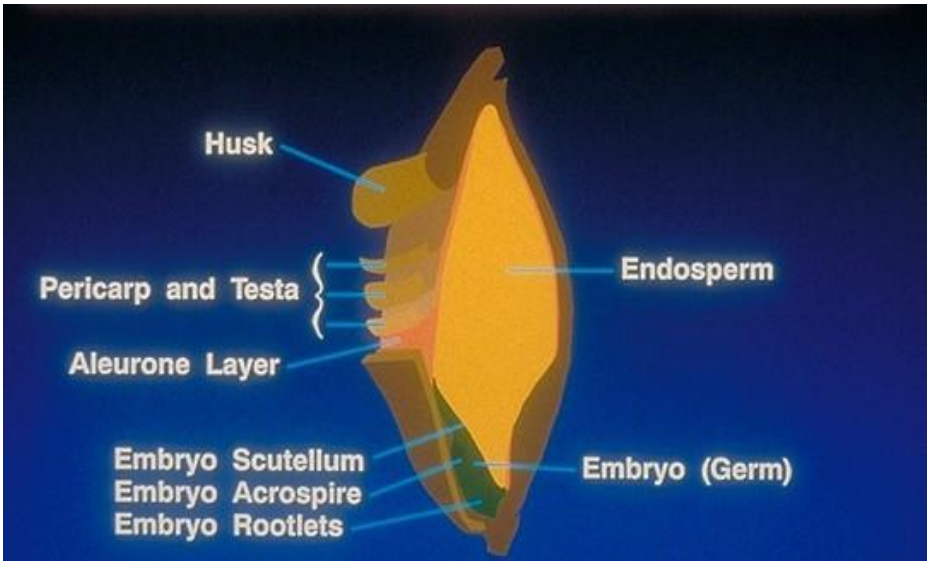


Figure 2: Structure of the Barley Kernel

Brewing water

Quantitatively water is the major raw material used in beer production. Only a part of the water required is used directly in the beer while another part is used for cleaning, rinsing & other purposes. Supply & preparation of the water is particularly important to the brewer because the water quality affects the quality of the beer produced. In general, the water chemistry contributes to both the efficiency of your mash and the flavor of the finished beer. In the mash, the chemistry of the water helps maintain the proper pH so that you can have optimal starch conversion to fermentable sugars. In the finished beer, the water chemistry helps accentuate malt and or hoppy flavors. (Kunze, 2014)

Hops (humulus lupulos)

The **hop** is an aromatic plant that gives beer its flavour and bitterness. It contributes to the formation of a good froth and protects the beer against contamination by microorganisms. Today, extracts from this plant are used industrially, obtained in such a way as to preserve its qualities. Hop varieties are classified in terms of bitterness and flavour, which vary according to the amount of resin and essential oils they contain.

Figure 3: Hop pellet

Hops determine to a great extent typical beer qualities such as bitter taste, hoppy flavor, and foam stability. Conversely, hop-derived bitter acids account for an offending light struck flavor, which is formed on exposure of beer to light. These various processes are presented in detail, while due emphasis is placed on state-of-the-art hop technology, which provides brewers with efficient means to control bitterness, foam, and light-stability thereby allowing for the production of beers with consistent quality.

Brewer's Yeast

Yeast is a unicellular micro-organism which can obtain the energy it needs: in the presence of oxygen (aerobic) by respiration and in the absence of oxygen (anaerobic) by fermentation. During beer production the sugar in the wort is fermented by yeast to alcohol & CO_2. Yeast is made up of many tiny, single-celled plants, which grow by budding, each bud breaking away from the parent cell and forming new buds. The conditions required for growth are warmth, moisture and food (starch plus a small amount of sugar). Refrigeration slows down the growth so that yeast can be kept for a limited period of time. When the yeast is used, the conditions and the utensils should be kept lukewarm to obtain the best results. As soon as the yeast has been added to the dough or

batter, the yeast begins to feed on the starch in the mixture, forming sugar, alcohol and carbon dioxide. The bubbles of CO_2 cause the dough to expand ("rise"). The dough must be "kneaded" thoroughly to distribute the bubbles evenly and then left to rise again, usually to about double its original volume. If the mixture is left too long, acid produced by the oxidation of the alcohol causes the product to taste sour.

Adjuncts

Several reasons have dictated the use of adjuncts in brewing. Prominent among these is the role it plays as a cheaper source of extract than malt (Grujic, 1999). The use of adjuncts in brewing has been determined by several factors among which availability, handling equipments, brew house operations are prominent (Coors, 1976). Adjuncts are sources of carbohydrate materials other than malted barley, which are employed in brewing to generally increase extracts and impart special characteristics to the final product. (Coors, 1976) has pointed out that adjuncts can be considered an essential ingredient in American beers because of consumer preference for the lighter, more drinkable and more stable beer they make. A wide variety of cereal starches and sugars could be used as brewing adjuncts but due to economic factors, availability and utilization in the brewing process, their use is limited to a small number of types (Coors, 1976). At present the volume of beer produced with adjunct throughout the world has indeed increased much more than those produced with malt and this tendency is growing constantly, not only because of economic factors but also as a result of advantages related to the quality in the finished beer (Glienke, J. and Edwards, W. R., 1965).

Figure 4: Barley before harvesting

Among the plethora of reasons why brewers employ the use of adjuncts for brewing are:

- Adjust or mask flavor balance and head retention
- Diluting wort produced from malts that have high levels of nitrogenous materials to forestall the formation of haze in the finished product
- Modify the body and the color
- Save on mash tun capacity
- Save on cost per degree of starting gravity and alcohol.

Gelatinization temperature of some cereal starches

Starch in the uncooked stage is insoluble in water. It forms a temporary suspension of large particles which are undissolved in the surrounding medium and will settle to the bottom of the container of liquid unless agitated (Vaclarik, and Christian, 2003). 10 % of starch breakdown occurs during malting and the remaining 90% occurs during mashing. Starch degradation occurs in three stages, the sequence of which is unchangeable but which merge into one another: Gelatinisation to Liquefaction & then to Saccharification. By gelatinization is meant the swelling and bursting of starch granules in hot aqueous solution. The starch molecules set free into this viscous solution are more easily attacked by amylases than ungelatinised starch.

In hot aqueous solution a large amount of water is incorporated into the starch molecules.

This results in an increase in volume which causes the closely packed starch granules to swell and finally to burst. A viscous (sticky) solution is formed. The degree of viscosity depends on the extent of water uptake and is different for different types of cereal. For instance, rice starch swells much more than malt starch. This process, during which no chemical degradation occurs, is called gelatinization. It plays an important role in everyday food preparation (e.g. pudding cooking, thickening of soups or sauces).

Because the gelatinized starch is no longer held together in the solid starch granules, it can be directly attacked by the enzymes contained in the liquid mash. In contrast, degradation of ungelatinised starch takes several days. The gelatinization temperature is different for each type of cereal. Malt and barley starch gelatinize in the presence of amylases at 58-62°C. Rice starch normally gelatinizes at 75 to 85°C, but there is a big variation. Maize starch gelatinizes at a temperature around 68 to 75°C. (SSB, 2007)

The gelatinization temperature of the starch in these adjuncts is higher than that used for Saccharification in mashing. Therefore it is necessary to cook the adjunct prior to addition to the mash to ensure complete gelatinization and liquefaction (Bentley, 2006). Adjunct cooking is traditionally carried out using the addition of some malt into the cereal cooker along with the adjunct. The α-amylase of the malt has sufficient activity at the higher temperatures of the cooker to liquefy the starch. However a more efficient method is the use of a heat stable α-amylase (Bentley, 2006).

2.2 Overview of Ethiopian Malt Barley Production

Barley was the first cereal to have been domesticated by man dated back 7000 BC, and its cultivation probably originated in the Abyssinia highlands (Ethiopia) and Southeast Asia in prehistoric times (Zhou, 1995). It is the primary cereal widely used in the preparation of malt for brewing beer and other distilled liquors. This was perhaps due to its ability to produce α-amylase and β-amylase that take part in hydrolyzing starch, but also partly due to its morphology (tightly cemented lemma and palea which protects embryo and results in uniform germination) (Burger and Laberge, 1985).

In the year 2010, the global barley malt demand was projected to increase by 27% and most demand growth for malting barley in the subsequent five years would be in countries with rapidly expanding beer production including Africa. Nevertheless, the growth of malting barley demand in many countries has not been proportional to its production. As a result, beer breweries in many countries are being adjusting their production technique to incorporate other substituting and adjunct crop species (Burger and Laberge, 1985).

2.2.1 Use of barley

Barley is cultivated from the sub-arctic Scandinavian to near the equator; in the mountains of Ethiopia and in the South American; from below sea level near the Dead sea to great altitudes in the Andes and Himalayas; from normal temperature regions like western Europe to dry land areas in parts of Northern America to irrigated areas in deserts such as the Sahara (Hunter, 1962; Briggs, 1978; Rasmusson, 1985) Barleys will grow on a wide variety of soils. The English-type, low

nitrogen malting barleys are best grown on light soils with pH values about 6.5. Chalky sub-soils are suitable.

Usually fertilizers are used to supply major quantities of Nitrogen, Potassium and Phosphorus. Usually the application of nitrogenous fertilizers has the most dramatic effects on grain yield and quality. Barley is a usual material for making beer. It lacks friability for easy milling; it provides a highly viscous extract deficient in amino acids and **lacks the color and flavor** required for making beer (Lewis and Young, 1995). Barley for brewing is considered sound if it has well defined analytical, agronomic and physiological properties. It has to be dry, about 12% moisture content free of diseases, infestation and discoloration. It should be reasonably free of debris including dust, weeds and broken corns. The grain should preferably be plump since these types contain relatively less husk and hence more starch to increase brewers‟ extract (Lewis and Young, 1995).

To be used in the brewing industry, barley must fulfill main criteria:

- High germination capacity
- Purity (in the variety)
- graded grain i.e extract yield
- Low protein content

Currently, there is increased emphasis towards:

- High malt extract at low modification levels
- High diastatic (enzymatic) power levels
- Low malt color levels
- Uniformity of grain size

2.2.2 Types of Barley

Domesticated barley is classified as either six-row (6R) or two-row (2R), depending on the physical arrangement of the kernels on the plant; the winter (2R or 6R) and spring (2R). The advantage of winter barley is that it can benefit from fall soil moisture, which ensures higher yields as compared with spring barley. 2R barley has a lower protein content than 6R barley and is therefore more suitable for malt production. High protein barley is best suited for animal feed. The 2R barley variety is chosen for this experiment which are varieties found in the plots.

Traditionally, spring malting barley was produced in regions with moderate temperatures and adequate rainfall throughout the growing season (650 mm per year). Winter malting barley was mostly grown in the milder arid and semiarid regions of Europe. Due to its higher yield potential, winter malting barley is now increasing in acreage in traditional spring barley regions. New varieties of winter malting barley could provide brewers with better quality, namely higher fine extract content, better malt modification, and lower malt protein content. Barley is used commercially for animal feed, malt production (barley is one of the most important ingredients in beer production), and for human consumption.

Each of these uses is best met with specific barley varieties. The main use of barley is fodder for livestock. Only 13% of the barley produced worldwide is processed into malt.

Figure 5: 2 Row & 6 Row Barley

2.2.3 Malting barley specific parameters

Malt barley is one of the principal ingredients in the manufacture of beer. Brewers can either purchase malt barley to manufacture malt themselves or purchase malt from malting companies. In either case, malting quality barley must meet the special quality specifications shown below. Accepted malting barley varieties must allow malt production within parameters that meet brewers' specifications. The malting characteristics of barley also depend on growing conditions, harvesting conditions, and storage.

The quality specifications for the physical characteristics of malting barley have tightened. This has been driven by the demand of maltsters to address specific quality parameters, as breweries themselves have become more stringent in their requirements.

The aim of malting:

- To loosen up the endosperm by degrading the endosperm cell walls
- To produce enzymes for further degradation of the content of the endosperm cells
- Convert the large chains of *insoluble starch* in the endosperm to short chain *water soluble starches*
- Break down proteins in the barley by activating Proteolytic ("protein breaking") enzymes
- Activates the diastatic ("carbohydrate breaking") enzymes that will convert starches to sugars during mashing
- Cytases (hemicellulases & β-gluconsases) occur during germination: break down husk cell walls and allow the diastatic enzymes to work more easily during the malting process
- Dextrase: Break the large starch chains at the 1-6 links to make smaller polysaccharide chains
- Diastatic enzymes
    - **α-amylase**: breaks the 1-4 links in the middle of the α-glucans starch to make smaller starch chains
    - **β-amylase**: breaks the smaller starch chains into maltose sugar by breaking 1-4 links near the reducing ends of the smaller chains

Generally Malt Contributions to Brewing are:

- Source of modified carbohydrates
- Source of enzymes to reduce modified carbohydrates to fermentable sugar
- Source of soluble proteins
- Source of filter material for wort clarification
- Source of color and flavor

2.2.4 Problems associated with some Adjuncts

- Maize usually has some fats or undesirable lipids which may linger throughout the brewing process and finally into the packaged beer forming the basis of lipid auto oxidation leading to the formation of the undesirable trans-2-nonenal flavor.

- Barleys may provide solubilized ß-glucans in mashes which may give run-off and filtration difficulties but in our treatment, the high gravity is lowered to 15 from 16 degree plato.

The economies of using adjuncts are not simple, since many require special plants to be installed for cleaning, handling, storing and milling. Plants needed to cook grits, or handle flours, flakes, micronized, raw grains or syrups are specialized, and it is not feasible to change easily from using one type of adjunct to another. Consequently users must ensure that a continuing supply of good quality, competitively priced material is available before committing themselves to installing plant to allow it to be used. (Dennis, E. 1998)

2.3 Malting

2.3.1 Harvesting & Preparation

Harvesting: Traditionally, harvesting included the cutting of near-ripe plants, which were then bundled into sheaths and allowed to dry in the field. Threshing would occur later at the farmers' convenience. This technique was far from ideal as the cut grains were liable to microbial attack, which would reduce the quality of the grain. Today, harvesting and threshing are combined with the use of the modern combine harvester. As one process that occurs in the field, the mature plants are cut, the grain threshed, separated and retained, whilst the straw baled to be discarded or collected later. However, the slightest fault in thresher adjustment can lead to incomplete threshing and significant grain loss. If threshing is too close, this results in damaged grains. These grains are unacceptable for malting as there is increased susceptibility to infection during storage and uneven germination will also occur.

The most important factor during harvesting is the moisture content of the grain. Following harvest the crop must be sufficiently dried to prevent germination and reduce the risk of microbial infections occurring prior to use. If dried incorrectly the quality of the grain will deteriorate in store. Grain harvested from the field will vary in moisture content from around 15 % in a dry year

to 30 % in a wet year. Whatever the moisture at intake, the grain must be dried down to 12 % (or less) for safe storage.

Above 12 % moisture, barley is susceptible to insect attack and since the grain may be stored for up to 14 months it is obviously essential that this risk be removed. Even if the eggs of grain weevil or saw tooth beetle are present they are unable to grow and multiply if the moisture of the grain is less than 12 % and the temperature is below 15 ^{o}C**.** If moisture levels exceed 12 %, the grain will start to respire and generate heat and more moisture. If action is not taken to correct this, further heat build-up will occur and force further respiration. This accumulation creates concentrated areas of heat known as "hot spots" in the grain silo or store. Hot spots both harbor and promote microbial growth whilst the heat itself will kill the embryo making it unsuitable for malting. To avoid heat buildup, the grain must be aerated and turned regularly. However, if moisture levels fall below 12 % the grain will dehydrate and become more susceptible to skinning and breakage during handling.

Whilst safe storage is the principal reason for drying barley we also benefit in a second way. The process of drying barley accelerates the grain from its natural dormancy. The actual mechanism by which this works is still unknown but experience shows that barley that has been dried is ready for steeping sooner than that which has not. This phenomenon has been taken one stage further – research indicated that if the grain was stored warm (after drying) then the recovery from dormancy was accelerated further.

After drying, the grain is cooled and stored until it is ready for use. During storage the grain respires, albeit at a low rate, and must be kept fresh by aeration. All medium to long term barley silos are fitted with low volume fans for this purpose. As mentioned earlier the grain in store has to be protected against fungal growth and insect infestation. This is achieved by drying the grain to 12 % moisture and holding the temperature below 15 ^{o}C. Regular temperature monitoring and physical inspection of grain in store are essential to ensure that localized infestations are not occurring and pre-cleaning/ fumigation of silos/ stores is imperative.

Over-cooling of grain must be avoided, however, since it is possible to chill the barley back into dormancy. Timing of warm storage, cooling and aeration is important so that recovery from dormancy is optimized and pest free storage is guaranteed. As general rule barleys with

approximately 10 % moisture should be stored above 15 ° C and those with approximately 20 % moisture should be stored below 15 ° C.

The first main difference between a batch dryer and a continuous dryer is that the dryer is charged, fired, cooled and discharged in steps as opposed to continuously. The second difference, and perhaps the most important, is that normally the fan arrangement of the batch dryer is such that a much greater volume of air is available. This means that the actual air temperature required to dry the grain is much lower (typically 40 – 45 ^{o}C) and the risk of heat damage is much reduced. This means that the grain is dried, therefore, with a higher volume of cooler air than in a tower dryer.

Since batch dryers appear to have distinct advantages over tower dryers it begs the question of why there are so few batch dryers used in comparison with towers. The answer is the age old one of money. Compared with tower dryers the cost of building a batch dryer is extremely high, mainly due to extra conveying requirements, and they are less common.

Preparation: Barley and malt are stored in deep silos, some with capacities in excess of 3000 tones. As a living tissue, prolonged storage in such conditions can suffocate the barley grain. To prevent the cessation of grain respiration, the barley is moved periodically for aeration. This movement of the grain is often combined with cleaning and pest fumigation, in preparation for malting.

The grain first passes over revolving or vibrating screens and sieves, in combination with air jets and magnets. These act to remove any non-barley material that is not of equal size or weight. The material that is removed can contain twigs, leaves, straw, stone, pieces of metal and dust, and is referred to as **dockage**. Throughout malting, and indeed any grain-handling environment, great care is taken to remove dust and any material that could generate a spark. This is to prevent violent and dangerous dust explosions.

Separation takes place in long, rotating cylinders that remove all grains that are shorter than barley, including broken half grains and small weed seeds. The cylinder has pockets or indentations on its interior surface into which only the weed seeds and half grains will fit. Only debris is retained in these indentations.

2.3.2 Steeping

Malt is the major raw material used in brewing and malting is therefore, an influential process. Malt determines final beer quality.

Put simply, the process of malting is the forced growth of the barley grain, but the maltster will reason that it is more the directed manipulation of the grain to achieve the required modification. Endosperm modification is achieved by malting the grain. By allowing the grain to germinate under controlled conditions, the ability of the grain to produce hydrolytic enzymes can be manipulated.

Hydrolytic enzymes released during germination are required to partially degrade (or modify) the starchy endosperm during malting and later to release fermentable extract during mashing.

The processes that take place during steeping are:

- Moisture content of the grain is increased to 45 % - 47 %.
- Increased respiration rate.
- Initiation of enzymatic activity that will continue during the germination phase.
- Washing dust off and leaching of substances from outer layers of grains.
- Production of waste steep liquors with high biological oxygen demand.
- "Chitting" - the appearance of the coleorhiza, surrounding the first rootlet.

Grain modification is the overall enzymatic action in the endosperm during malting, which transforms the hard starchy endosperm of barley into friable (crushable) malt.

The malting process combines three separate stages: -

i. **Steeping**: Initiation of growth through forced grain hydration.
ii. **Germination**: Controlled growth of the grain to effect endosperm modification.
iii. **Kilning**: The termination of grain growth to fix extract potential and malt specifications through grain dehydration.

During steeping, barley grains are placed in vessels with capacities ranging from 60 - 500 tones. During the steeping process, the grains are submerged in steep liquor, which is changed as required and the barley aerated (known as an air rest) for the following reasons:

- To remove CO_2
- To remove toxic metabolites
- To remove competitive micro-organisms

- To discharge excess heat
- To replenish oxygen

Steeping can be carried out in dedicated conical or flat-bottomed vessels, combined steeping and germination vessels (SGV's) or steeping, germination and kilning vessels (SGKV's). Most modern malting utilize microprocessor control systems throughout the process.

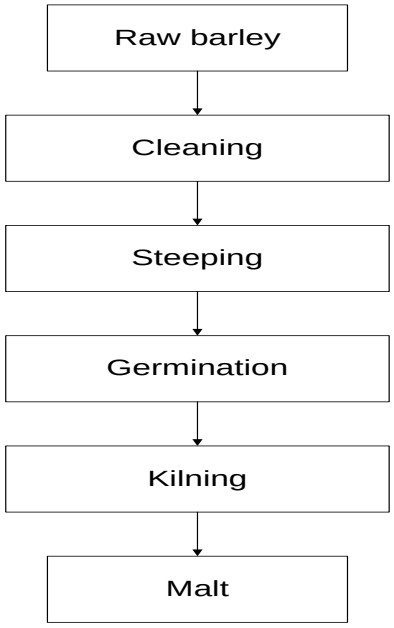


Figure 6: Malting Process Steps

During steeping, hydration rejuvenates the barley grain and respiration recommences, slowly at first, but quickly gaining vigor. Grain respiration (the production of energy to drive metabolic processes) produces heat, CO_2, and metabolites. If unchecked, the heat generated during steeping as the grain respires has a cumulative effect and forces the grain to respire more efficaciously. Primarily, increased respiration causes the grain to hydrate quicker, but in an irregular fashion throughout the grain population. The malt that results will be non-homogenous and poorly modified with the potential to cause brew house problems.

The steeping process physically washes and cleans the grain, leaving dirt suspended in the steep liquor. This can be removed when steep liquor is changed. The phenolic components leached from the barley husk are important as they contribute an astringent character to beer. If the steep liquor is not refreshed the phenol build up will taint the malt and be conveyed onto the beer. The maltster may use alkaline steep waters to combat the astringent contributions of leached phenolic.

Chalky, alkaline waters also check microbial growth, as well as extract the astringent phenols. Caution must be taken as highly alkaline treatments can kill the grain. The most effective methods involve the use of sodium carbonate or sodium hydroxide, used in short immersions at the start of steeping. These preliminary steeps also help to clean the grain, remove musty odors and lighten the grain color.

Barley is immersed in water at a given temperature in order to increase the moisture content and to initiate germination. It is important that the moisture content of the resulting germinating barley is distributed as evenly through each corn as possible. If this were not the case then uneven, partly modified malt may result. Also, the steeping of water sensitive grains must be considered.
These requirements can be met by the use of multiple steeping cycles where an air rest with good CO_2 extraction follows each immersion. In the case of water-sensitive grains, experience has shown that if the amount of water taken up by the grain in the early stages of steeping is limited then the water sensitive grains will begin to germinate.

If the grain moisture can be raised to a suitable level to initiate germination (30 - 33%) and then a long air rest is carried out, the grain will be able to accept more water without being damaged. If too much water is given too quickly the grain will be killed and then is useless to the maltster, economic losses also occur.

As a general rule, air rest temperature should not exceed 21 - 23 ^{o}C as damage to the embryo could occur and certain enzyme systems can be impaired – especially proteases that break down the protein matrix in which the starch grains are embedded. A consequence of this could be that less starch is available to the brewer leading to reduced soluble extract, increased viscosity leading to poor mash tun run-off – both of which would reduce the throughput of the brewery. Once germination has commenced it is important that steeping raises the moisture of the grain to a level that will promote the production, distribution and activation of the enzymes. Generally moisture of 45 - 47% is typical for malting but, as stated previously, it is important that this moisture is distributed as evenly as possible throughout each grain. Two typical examples of steeping cycles are shown below. The first programme is designed to cope with water sensitive barley and produce malt using a conical steep system. The second programme is designed for

fully mature vigorous barley to produce malt using a flat-bottom steep system. In example two the use of only two steeps also has the added benefit of economizing on water use.

From the outline on the previous page of practical steeping it is obvious that certain key parameters must be monitored and recorded in order to control the steeping process. The main considerations are temperature, moisture content and germination count.

The temperature of the incoming steep fill and re-fill water is monitored and controlled so that the initial mix temperature of the 1st steep is attained (12 - 16 ^{o}C) depending on individual barley requirements). The temperature at the end of each air rest is recorded, and also the time taken to attain this temperature in order to maximize these periods in subsequent batches. The laboratory analyses grain samples from the end of the 1st steep, end of the 2nd steep and cast stages of steeping to ensure that the moisture targets are being attained.

Growth counts are performed after casting to ensure the batch is germinating adequately and evenly. Generally more than 80 % of the grains should be chitting at this stage.

The germinating barley is transferred (cast) into the germinating vessel by gravity or conveyor, depending on the individual malting plant. The empty steeps are then cleaned to maintain adequate hygiene standards.

Moisture content of 45% - 47% is desired because this is the point at which germination can start. Achievement of this depends upon the rate and the quantity of water uptake by the grains. The following factors governing water uptake are critical to the process.

2.3.3 Germination

At the end of steeping, the grain moves into the second phase of growth, signaled by chitting. As the grain strives for further growth, its oxygen requirements to facilitate increased respiration jump dramatically. This demand for oxygen cannot be met in the steep tank. If the chitting grain is left submerged in the steep, it takes up too much water and effectively drowns. The grain is therefore, moved to germination chambers where its physical demands can be met more easily. There are various types of germination chambers in use around the world, but they all operate around similar principles:

- Controlling grain temperature
- Maintaining at least 40% grain moisture
- Turning and mixing of the grain
- Supplying sufficient oxygen to the grain

The grain can be transferred or "cast" between the steeping tank and germination chamber as a slurry, or dry with spraying, to prevent grain dehydration. The grain is then spread out to form an even bed, on average between 1-3 meters in depth.

Throughout germination, it is essential that the grain bed is kept moist. If the grain is allowed to dehydrate, modification will cease. If the grain dehydrates, hydrolytic enzymes will be unable to progress throughout the endosperm, resulting in an under modified malt of poor quality that will cause serious brew house problems. The problem of dehydration is overcome by lightly spraying the grain bed with water. In addition, the malt is aerated with cool air, saturated with moisture at 100% relative humidity. Although the circulating air is at 100% humidity as it enters the grain bed, it is warmed removing the heat generated by grain respiration. This warming of the air decreases its relative humidity and is sufficient to effect some water removal and therefore, dehydration of the grain.

Bed aeration serves several purposes. As well as helping to maintain bed hydration, circulating air replenishes the supply of oxygen for the grain and purges out any CO_2 that could stifle respiration. By maintaining air circulation, cooling of the grain bed is also accomplished.

Turning the grain bed using helical screws, also lifts the bed to allow easier passage of the circulating air and hence more efficient cooling and CO_2 removal. Germination will typically last between 3-5 days, at temperatures between 15-20 °C. As germination proceeds, respiration within the grain gains vigor, and by the end of the process the heat generated can result in the grain mass reaching temperatures of up to 22 °C.

The maltster aims to control the formation of α- and β-Amylase, proteolysis and degradation of β-glucans. These are summarized below.

(a) **α-Amylase**

- This does not exist in unmalted barley.
- The level increases with germination.
- The amount of α-amylase produced varies with barley variety.

It is stimulated by higher moisture content (e.g. 46 % moisture and a germination temperature of 12 ^{o}C favor synthesis).

(b) β-**Amylase**

- Exists in unmalted barley in an inactive form
- The amount of β-amylase produced varies with barley variety

- Activation requires proteolysis, is linked to embryo activity and respiration
- It is stimulated by an average moisture content (e.g. 43% moisture and lower germination temperatures).

(c) **Proteolysis**

- Higher moisture content and lower germination temperatures stimulate production.
- Elevated germination temperatures decrease activity.

(d) **Solubilisation and Breakdown of β-Glucans**

Breakdown of β-glucans cell walls is essential to achieve fast filtration of wort and to improve the filterability of the beer. The enzymes needed to solubilize and breakdown the β-glucans develop slowly during the malting process and they are sensitive to GA. An intensive malting process, for example, high moisture content and a germination temperature of 19 ^{o}C maintained for long periods, favors the degradation of the β-glucans.

It is worth performing a germinative energy test on barley prior to malting, as a high proportion of dead or dormant grains will lead to heterogeneous malt containing non-degraded β-glucans and problems in the brew house. An outline of b-glucans degradation in the cell wall is shown below.

2.3.4 Kilning

At the end of germination, the modified barley, now termed "green malt" (unkilned malt) has been manipulated to achieve the maltster's and brewer's specifications. These grain characteristics must now be fixed to avoid any deleterious alterations that may occur before the brewery can take delivery of their malt. Kilning effectively serves two purposes:

i. To halt and capture the biological activity of the germinating grain at a point of optimum enzyme yield and endosperm modification.
ii. To reduce the moisture content of the green malt to a level at which it can be safely stored to avoid deterioration and microbial infection.

Kilning reduces the moisture content of the grain, which, at the end of germination is between 42 – 48%. A final grain moisture level of approximately 3 – 5% is achieved by the end of kilning. Kilning of the grain is also responsible for the characteristic color and flavor of malt. This requires intense heating, which conflicts with the need to preserve essential enzymatic activity in the grain.

Enzymes are considerably more heat stable when the malt is dry.

Kilning objectives can therefore be accomplished with a drying regime that first excludes the majority of moisture at relatively subdued temperatures. Secondly, intense heating is used to obtain final moisture removal, and color/ flavor production.

Kilning consumes some 75% of the total energy utilized in malting and must therefore be operated at maximum efficiency. This dictates that the warm air (used to dry the grain) leaves the kiln satisfying its maximum moisture carrying capacity. This may not always be accomplished or even desired. Kilns are similar in design to germination chambers and in fact some malting utilize vessels in which the two process steps are carried out in the one vessel - Germination Kilning Vessels (GKVs).

Within the kiln, the green malt is loaded onto one or more perforated floors, through which heated air can be driven (by fan) upwards through the grain bed to effect drying. If there is more than one floor, the heated air from the lower level is rejuvenated with additional flow to heat the bed above, resulting in vast economic savings. As with germination, the grain bed is regularly turned securing homogenous drying and consistent quality malt. There are three stages incorporated in the drying process; Free Drying (or pre-break), Forced drying & Curing (or the equilibrium phase. In summary the main aspects of malt quality affected by kilning are: Enzyme activity, Color, Flavor & Nitrosamine content

2.3.5 Malt Quality Analysis

The moisture contents of malts are usually in the range 1.5 to 6 %, expressed on a fresh weight (fr. wt.) basis. Brewers normally specify an upper moisture limit; because malt is hygroscopic and it will normally have a lower value when dispatched, to allow for moisture uptake while in transit. Brewers also pay attention to the extract of the undried malt. However, for comparative purposes extracts are mostly given on a dry weight basis (on dry). Malt sample must not contain more than a certain percentage of thin corns, because thin corns are not broken up in mills with rollers set relatively far apart to achieve coarse grist. When malt is hammer milled this consideration does not apply. Extract is obtained in greater yields from finely grind malt and from coarsely grind malt. Extract difference shows the better the malt is modified. Because this value is the difference between two large numbers and is small relative to the errors involved in measuring the Extracts, the determination must be replicated to obtain a reliable value, which is Laborious. (Bourne and Wheeler, 1982; Briggs, 1998). The determination of extract, E, by the EBC and the very similar ASBC methods differs considerably from the IOB method. They were developed for traditional lager Brewers but the temperature programmed used does not resemble that of most old lager

Breweries or that of breweries which employ temperature programmed mashing. In the EBC method by attemperating the mashing bath at a temperature of 45 ^{0}C, with Continuous stirring. The temperature is then increased, at 1 ^{0}C /min., until it Reaches 70 ^{0}C. This temperature is now maintained and more water, also at 70 ^{0}C, is added. After one hour, during which the scarification time rate is determined, the mash is cooled and adjusted to 450 g. The specific gravity of the wort is determined. Using tables that relate the strengths of sucrose solutions with their specific Gravities, the weight of extract in the laboratory wort is calculated, assuming that the Dissolved extract solids change the specific gravity to the same extent as sucrose. The EBC method uses Plato's tables while the ASBC method uses Balling's tables the laboratory mashes differ from brewery mashes in a number of important ways. Unlike brewery mashing liquor the water used is distilled and contains No salts, nor is the mash pH adjusted. Also the grist is prepared by using mills that work differently from brewery mills. The laboratory mashes are dilute compared to brewery Mashes and at the end of mashing the grist is not sparged with hot water. Several attempts have been made to devise more `brewery-like' laboratory mashes, but they have not been accepted. Each brewer discovers the relationship between labs.' Extract and the Extract recovered from this malt in the brewery. The extract determinations described are applied to pale malts. Different methods are necessary for special, highly colored malts that lack enzymes. For example a 50:50 mix of a colored malt with an enzyme-rich pale malt may be mashed and the extract of the colored malt is calculated, making the assumption that the pale malt gives half of the extract it yields when mashed alone. The rate of wort filtration from a laboratory mash does not give a good indication of the brewery wort run off. Total soluble nitrogen (TSN) and free amino nitrogen (FAN) values are determined. The TSN needs to be sufficiently high so that the `body' and mouth-feel of the beer is adequate, and the beer foam (or `head') will be stable. The soluble nitrogen ratio (SNR; TSN/ TN) of the malt (or the soluble protein ratio or Kolbach Index of the ASBC and EBC methods (in each case soluble protein/total protein) serve as measures of modification and a value is often included in malt specifications. FAN values (chiefly amino acids and small peptides) must be sufficiently high to ensure that lack of nitrogenous yeast nutrients which does not limit fermentation. FAN has been determined by different methods, which gave different results, so it is essential that the method used is specified. In EBC analysis the time in minutes taken after the mash has reached 70 ^{0}C for samples to stop giving a positive iodine test for starch is recorded as the `Scarification time'. This is really a rough measure of the time taken for the starch to be dextrinized, and is largely dependent on the α-amylase content of the malt. The odor of the mash is noted as, less usually, is the flavor. Both should be normal for the type of malt being analyzed.

The appearance of the wort is noted, whether it is clear, opalescent or turbid. The activity of the mixture of the starch-degrading enzymes in malt is estimated as the `diastatic power', or DP. The enzymes are collectively referred to as diastase. In Principle, soluble starch is incubated with a malt extract and the degree of starch Breakdown is estimated after a period of incubation at a controlled temperature. The Results are not highly reproducible and represent the joint activities of several enzymes that are present in different proportions in different malts. Results are expressed in Different units including ^{0}L (degrees Lintner) and ^{0}W-K (Windisch-Kolbach units). The Values indicate to brewers if the enzyme content of malt is adequate. Analytically the ferment abilities of the HWE or E worts may be determined. However, the ferment ability of these worts increases with storage time as malt enzymes that have survived the mashing process continue to break down dextrins to simpler, fermentable sugars. Thus laboratory wort should be boiled to inactivate the enzymes. Then, it is inoculated with pure yeast and it is incubated under anaerobic conditions until fermentation is complete. The fall in the specific gravity of the wort allows the calculation of the attenuation limit of the wort and its ferment ability.

2.4 Microbial enzymes

2.4.1 History

The history of enzyme technology began in 1974 when the Danish chemist Christian Hansen produced the first specimen of rennet by extracting dried calves' stomachs with saline solution, which was the first enzyme used for industrial purposes. This significant event had been preceded by a lengthy evolution.

Although malting and mashing are physically separate processes and usually take place in different locations, malting has a profound influence on the subsequent release of sugars during mashing. During malting the barley corn is allowed to germinate where it produces enzymes which break down the cell walls in the corn and produces enzymes capable of releasing the energy stored as starch in the endosperm.

2.4.2 What are microbial enzymes?

Enzymes are proteins with a special structure capable of accelerating the breakdown of different substrates. They act as catalysts to increase the speed of a chemical reaction without themselves undergoing any permanent chemical change. They are not used up in the reaction or appear as reaction products. Microbial enzymes are produced by fermenting selected strains of bacteria or fungi in large fermentation tanks (up to 160 m^3). Most industrial enzymes are extracellular, so after

fermentation the biomass is separated from the liquid, which is then purified, concentrated, and adjusted to the required strength. In this way, liquid enzyme products are produced. When non-liquid products are required, a granulation process is introduced after concentration to produce safe, non-dusting, granulated products. The strains used for industrial enzyme production are nonpathogenic strains that have been improved with respect to yield and the absence of undesirable side activities by either classical microbiological techniques or modern recombinant DNA techniques.

Purity

The Novozymes product range offered to the brewing industry is food grade in the sense that the products comply with the FAO/ WHO recommendations for food-grade specifications.

Side activities

Enzymes for industrial use produced by fermentation and subsequent purification are not necessarily pure in the sense that they contain one enzyme activity only. Depending on the strain used for production, there may be one or more side activities present. Enzymes derived from sources other than malt may be used at various stages during Brewing, provided that this is allowed by local regulations (Bamforth, 1986; Briggs et al., 1981; Byrne, 1991; Godfrey and Reichelt, 1983). Enzymes are also used in the Production of some adjuncts and they should not contain viable microbes. The preparations available have a wide range of characteristics. Different suppliers describe their preparations in different ways so that it is difficult to make comparisons between them. The lack of standard analyses is a source of difficulties. The activities of `named' enzymes in preparations are standardized by suppliers. However, this is not true of other enzymes that may be present. The presence of these additional enzymes may be advantageous or harmful. For example, the presence of β-glucanase in preparations of bacterial α-amylase may be beneficial when added to a mash, particularly if under modified malt or barley or oats adjuncts are used in the grist. On the other hand, while the presence of protease activity may be an advantage if more FAN is needed, it is most undesirable if it elevates the levels of soluble nitrogen too far and/or if the degradation of protein leads to a reduction in foam formation or stability.

Why to use exogenous enzyme in brewing process

- Bad malt quality, High β-glucan, arbinoxylan, protein gel, low level of FAN, low level of β-amylase content,

- High content of adjunct: no enough enzyme, retrograding of starch,
- Malt with low level enzyme: Sorghum, oats or no enzyme in raw material,
- Regularity and performance of the process: improvement of the process, saving energy ,
- Degradation of sensible protein: colloidal stabilization of beer.

Using the full potential of exogenous enzymes you can create recipes with up to 100 % barley. However, any ratio of barley, wheat and malt can be processed efficiently. **Ondea Pro** enzyme enables brewers to brew maltose-based wort with standard fermentability and similar processability compared to using high portions of malt. The present pullulanase, amylase and protease activities in Ondea Pro ensures sufficient starch and protein degradation in synergy with the β-amylase and peptidases of the barley. The glucanase and xylanase components enable sufficient cell wall degradation and low viscosity. The lipase activity significantly improves the turbidity during filtration. Utilizing high amounts of under modified malt, or malt in combination with high portions of barley, rice or maize (corn) can impact sufficient FAN supply for the yeast as well as lead to limited diastatic power during mashing. This would lead to extract losses and poor fermentability. It is recommended to use approximately 0.25-0.70 kg of Ceremix plus MG per ton of barley. Depending on the malt quality an additional dosage of 0.25 kg **Ceremix plus MG** enzyme per ton of malt compensates a lack in malt modification and assures high processability and fermentability (NOVOZYMES, 2013).

Almost all processes in a biological cell need enzymes to occur at significant rates. Enzymes are selective for their substrates and speed up only a few reactions from many possibilities.

Enzymes are:

- ✓ Specific: particular enzyme works only on a small class of substrates
- ✓ Natural: made by growing microorganisms during fermentation processes
- ✓ Not alive: Although derived from living organisms, enzymes are not alive
- ✓ Not GMO$^{\alpha}$: Not organisms However, they may be derived from modified organisms to increase thermal stability, pH stability, co-factor dependence… (Kunze 2004).

2.4.3 Factors that influence the rate of an enzymatic reaction

The factors are Enzyme concentration, Substrate concentration, Temperature, pH and presence of stabilizers/ inhibitors, stay time (i.e. Enzyme amount used per brew, Mash concentration, optimum pH & temperature and duration of time at a given parameters are very important factors.)

Temperature: Increasing the mash temperature increases the rate of chemical and enzyme catalyzed reactions, accelerates the rates of denaturation and precipitation of proteins (including the inactivation of enzymes), accelerates dissolution and diffusion processes, accelerates mixing and, at least above a certain temperature, causes the gelatinization of starches and (at least during decoctions and adjunct boiling) disrupts the cellular structure of unmodified cereal endosperm tissues (Briggs *et al.,* 1988).

The activity of enzymes depends on the temperature. Mixtures of enzymes, which are active in mashing, have a range of widely different temperature sensitivities. It increases with increasing temperature and each enzyme reach's its maximum value at its own specific optimal temperature. At higher temperatures a rapidly increasing inactivation occurs as a result of unfolding the three-dimensional structure of enzymes (denaturing). The inactivation and destruction of enzymes activity is greater the more the optimum temperature is exceeded. Depending on the way in which the temperature is raised, mashing processes are divided in to two types; infusion processes and decoction processes (Kunze, 2004)

pH: Dependence of enzyme activity on pH, the enzyme activity reaches an optimal value, which is specific for each enzyme and decrease at higher or lower pH values. The effect of pH on enzyme activity in general is not as great as effect of temperature (Kunze, 2004).

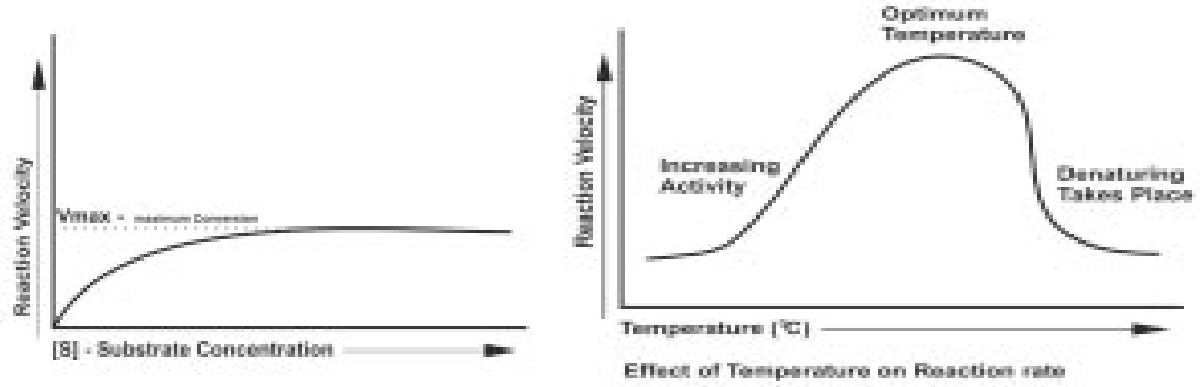


Figure 7: *The effect on limited enzyme-concentration on the rate of reaction*

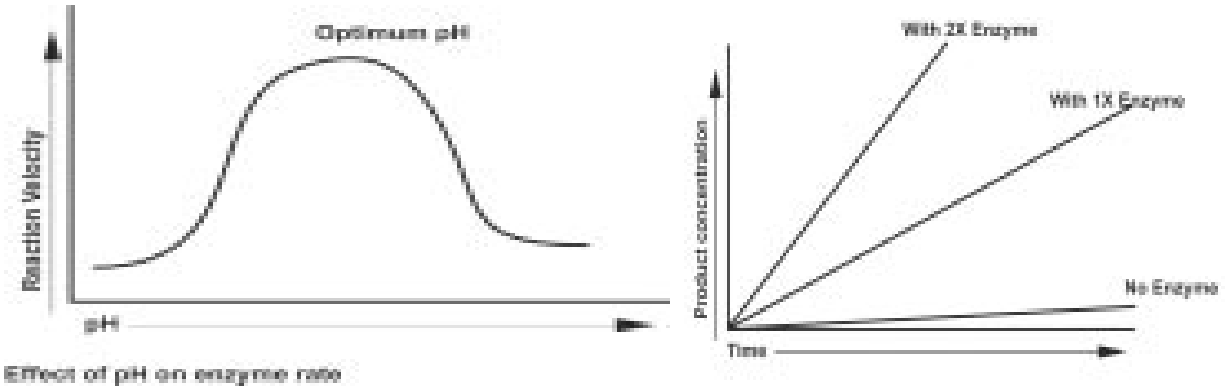


Figure 8: *Effect of increasing enzyme concentration on the rate of reaction with unlimited substrate*

α **GMO**: genetically modified organism: a plant or animal whose genes have been scientifically changed. (Kunze, 2004)

CHAPTER THREE

3 MATERIALS AND METHODS

3.1 Description of study area

The study is conducted at Raya Brewery S.C, Maichew town 660 Kilo meter from Addis Ababa to the north located at an altitude of 2,479 m in an area sewage treatment plant is available.

3.2 Materials

The major raw materials used in the study are almost from Raya Brewery S.C.; the Barley malt, Brewery yeast, Hops, Distilled & Brewing water. The unmalted barley was received from Alamata Agricultural Research Center and prepared exogenous enzymes, Ceremix Plus MG and Ondea Pro supplied from Novozymes biotechnological company from Denmark through Raya Brewery S.C.

3.3 Chemicals & Equipments

All the reagents & equipments used in this work for the various determinations of the samples were analytical grade & standard Laboratory tools respectively.

- **Chemicals/ Reagents:** Sulphuric Acid (H_2SO_4), Calcium Chloride ($CaCl_2$), Calcium Sulfate ($CaSO_4$), Iso-octane, O-toludine, EBT indicator, phenolphthalein (1%), Sodium hydroxide (NaOH), Phenol , EDTA (0.01%), Iodine solution, Zinc sulfate, etc
- **Equipments:** Equipments, which were used for experimental work, were:
  - Mash bath (Congress, temperature programmed, RS232, Czech),
  - Buhler-Miag disc miller (0.2-1.0 mm, DLFU-1980, Germany),
  - Spectrophotometer, HACH Large DR 3900
  - Kjeldahl apparatus (Gerhardt, Germany)
  - Magnetic Stirrer
  - Anton Paar DMA 450 digital density meter and alcoholyzer (Austria-Europe),
  - Beaker, oven, volumetric flasks, Pipette , burettes , desiccator
  - Analytical balance
  - Stop watch
  - Viscosity meter
  - pH meter

- Density meter
- Digital thermometer
- Goggles for eyes protection
- Protective gloves for hand
- Filter paper watt man
- Food grade acids for pH adjustment
- Haze meter

3.4 Methods

3.4.1 Reagent preparation

The experiment had two treatments with 40 % and 60 % raw barley as per the design to be compared with the 100 % Malted barley. The required process water, the malt (both local and imported), adjuncts for the mashing process, 0.01 N Iodine solutions, food grade salts like $CaCl_2$, $CaSO_4$, acids like H_2SO_4 and Enzymes based on the raw barley proportion are prepared.

3.4.2 Mashing process

The mashing process is carried out in two different scales.

- Laboratory scale

Using the laboratory scale and recipe of the brewing process, mashing is carried out under strict control and management. Based on laboratory scale pre-trials (mashing of pilot scale for the fixed proportions is done; mashing apparatus, filtration folded filters); the optimal mashing program is used and a specific combination of enzymes depending on the raw barley ratio are added to the mash (g per kg barley).

- Industry scale

As per the working instructions of the brewing process, three brewing treatments are done using adjuncts with the above fixed ratios.

Each treatment passed via the two scales in which both scales to be useful to observe the influence of raw barley on the yield extract & clarity of beer. To keep results approximately similar, raw materials (malt, hops, water) as well as yeast conditions are controlled.

3.4.3 Experimental Design:

Study factors:

- Amount of raw barley
- Ceremix Plus MG
- Ondea Pro

The three factors are analyzed against two levels (low [-] and high [+]) with 2^3 design; about (2 x 2 x 2 = 8) with three replicates totally 24 runs are performed. Analysis is done to know the interaction effects between the factors and their effect on the yield to be extracted, FAN gained & Degree of fermentation.

Measured responses:

- Yield of extract
- FAN
- Degree of fermentation

For the analysis of the data, Design Expert Software (version 7.0.0) is used.

Barley Malt beers with raw barley proportions of 40 % and 60 % is produced in the brewery consisting of hammer milling, mashing by infusion method in mash kettle, after being filtered using mash filter to separate the spent grain, the wort is transferred to wort kettle for boiling then after to the whirlpool for sedimentation. By cooling the wort, it is transferred to fermentation tanks it to be bottom fermented at 12 ^{o}c.

3.4.4 Framework of the experiment

Figure 9: Frame work of the Experiment

3.4.5 Raw material Quality

3.4.5.1 Moisture content of malted & raw barley (EBC Method 3.2, 1997).

By taking 20 g sample of barley and grinding finely at DLFU mill setting 0.2 mm, it was mixed thoroughly. 5g of ground sample was immediately taken & placed in a clean, dry moisture dish previously tarred to 0.001g and by closing the dish, the sample was weighed immediately to 0.001g. The cover was removed from the dish and placed the open dish and lid together in the preheated oven for 3 hours at the standardized temperature, starting timing from the point when the oven temperature is again between 103 and 105°C. The lid was replaced and removed from the oven. After allowing to cool in a desiccator to room temperature (Between 30 & 45 minutes), it was reweighed the dish and contents to 0.001g.

Moisture percentage of the sample was calculated using the formula:

$$\text{Moisture \% (m/m)} = ((M_0-M_1)/M_0) \times 100$$

Where: M_0 = mass in g of sample before drying and M_1 = mass in g of sample after drying

For sample above 17 % water, pre drying is necessary.

3.4.5.2 Germination Energy - *Shonfield Method. (EBC Method 3.6.3, 1997)*

Germination energy is the ability of barely to germinate fully. 500 grains of sample raw barley was transferred into a funnel standing in tap water (to ensure complete flooding of the grains) at 20 ^{o}C. The water was removed after steeping for 3 hours. The grains were covered with whatman No. 4 filter papers and the funnel itself covered with a glass plate. The steeping was repeated for 2 hours after 20 hours from the beginning of the test. The grains were again covered with filter paper and the funnel with glass plate. After 72 hours from the beginning of the test, the funnels were emptied and the number of non-germinated grains counted. Average result of the two counts (of the lots) after 72 hours was obtained. The formula below was used to calculate the Germination Energy:

Germination Energy (GE) = (500-N)/5

Where: N = number of non-germinated grains after 3 consecutive days.

NB: result is reported as = a % (schonfield method 3 days).

After incubation in a germination room (20 ± 0.5 °C), germinated grains are counted after

- 24 ± 0.5 h, 48 ± 1 h, 72 ± 1h

Germination percentage = $((n_{24}+n_{48}+n_{72}) / 400) \times 100$

Mean germination time (MGT) = $(n_{24}+2n_{48}+3n_{72}) / (n_{24}+n_{48}+n_{72})$; Germination Index =10 / MGT

3.4.5.3 *Germination Capacity (EBC Method 3.5.2, 2004)*

This is an attempt to quantify the percentage of viable grains within a sample. 200 uniform sized and clean grains were handpicked and steeped in 500 ml beaker containing 200 ml of 0.75 % hydrogen peroxide (H_2O_2) solution and incubated for two (2) days at a temperature of 21 ^{O}C. At the end of the 2 days, the grains were strained and steeped again in 200 ml of H_2O_2 also at 21 ^{O}C for further 24 hours. The germinated grains were then counted and the germination capacity calculated using the formula:

Germination Capacity = (200 - n)/2,

Where: N = grains that did not show roots.

Hectoliter Weight (HLW)

A measure of grain bulk density was determined on dockage-free samples using a standard laboratory hectoliter weight apparatus (EASY-WAY hectoliter weight test machine) as described in the AACC (2000) Method No 55-10. The values were adjusted to 12.5 % moisture basis as described below:

$$\text{HLW (12.5\% M basis)} = \text{HLW measured} \times \frac{100\text{-}12.5}{100 - \%\,\text{moisture measured in the grain}}$$

3.4.5.4 *Total Nitrogen/ Protein: Kjeldahl Method (EBC Method 4.3.1, 2004).*

Finely milled 0.1 g of sample was weighed in a small weighing disk, and transferred quantitatively into a dry Kjeldahl flask. 10 g of catalyst mixture (a tablet) consisting of TiO_2, $CuSO_4.5H_2O$ and K_2SO_4 were added. The content was thoroughly mixed by gently adding 25 ml of conc. H_2SO_4 and some anti-bumping agent. The flask was then heated. The heating continued for 45 minutes after the solution turned bright green. The Kjeldahl flask and contents were then cooled to room temperature. Two drops of methyl red indicator was added to Erlenmeyer flask into which had been pipetted 20 ml of 0.1N H_2SO_4. The flask was connected to the distillation apparatus such that the outlet tube of the condenser dipped beneath the H_2SO_4 in the receiving Erlenmeyer flask. After Kjeldahl flask and content had been cooled to room temperature, 150 ml of distilled water was cautiously added and mixed. The solution was cooled to below 25 °C. Anti-bumping agent was added to prevent bumping. 100 ml of 0.1N NaOH was then added slowly until two distinct layers were formed. The Kjeldahl flask was connected to the distillation apparatus soon after the addition of NaOH solution. The flask was heated smoothly for 5 minutes and then strongly until liquid

began to distil. Distillation continued until Kjeldahl flask begun to bump. The excess H_2SO_4 in the receiving flask was titrated with standardized 0.1N NaOH, using methyl red as indicator. Simultaneously with the test, a blank determination on reagents was carried out in which 20ml instead of 25ml of 0.1N H_2SO_4 was added and without the addition of the test sample. This method was used for all malted and unmalted varieties. The Total Nitrogen/Protein content in the dry samples were then calculated and expressed as a percentage

$$\text{Total protein content (\%)} = \frac{(T-B)*14*N \text{ of acid}*6.25}{\text{Wt of sample}*D*M}$$

$$\text{Soluble protein content} = \frac{(T-B)*14*1000}{V}$$

Kolbach index = % soluble Protein / % of total protein

3.4.5.5 *Extract Content (EBC Method 4.5.1, 2004).*

The determination of the potential of malt for producing wort soluble by a standard mashing programme. This procedure is also used for the determination of Saccharification time, odour, speed of filtration, P^H of wort, colour, viscosity of wort, total soluble nitrogen content and free amino nitrogen content.

For the mashing process, 55 g of malted and 55 g of unmalted sample were milled with a Disc Mill (Böhler, Braunschweig, Germany) set at 0.2 mm. Each sample of ground 55 g malt was transferred into a beaker and well mixed with spatula. 5g of mixed sample based on proportion was taken for moisture content according to Method 3.3 above while the remaining 50 g and again based on design ratio was measured into the mash beaker. 200 ml of distilled water at a temperature of 46 ^oC was placed into each beaker and stirred with a glass rod to avoid formation of lumps. The mash bath has to be pre-heated to 45 ^oC. The temperature of the mash bath was then maintained at this temperature for 30 minutes. The temperature of the mash was then raised at 1 ^oC per minute to 70 ^oC rest 10 minute then 100 ml of distilled water at 70 ^oC was added. Saccharification (complete hydrolysis of starch) was determined from the time the 100ml distil water at 72 ^oC was added. This was done by transferring a drop of the mash into a spot on a white porcelain plate and a drop of iodine solution added. This was done in the 5 minutes intervals for a maximum of 1 hours. Complete Saccharification or starch conversion was indicated by a clear yellow spot. The response to the iodine test was then recorded as color or positive and negative. If Saccharification is not

complete after 1 hour, the test must be stopped. The result should be expressed in minutes and reported in periods of five minutes, e.g. under 10 minutes, 10 to 15 minutes and so on. By maintain the temperature of 70 °C for 1 hour, the mash was cooled to room temperature in 10 to 15 minutes with cold tap water in the mash bath. The stirrer must be washed with a small amount of distilled water, drying the outside of the beaker and the content of the beaker was adjusted to 450 ± 0.1 g by addition of distilled water.

By stirring the contents of the beaker thoroughly with a glass rod and emptying immediately and completely to a pleated filter paper, it was ensured that the filter paper did not project over the edge of the funnel. The first 100 ml of the filtrate was returned to the funnel. Few portion was taken for color measurement and returned it to the flask after color measurement. Filtration was stopped when the cake appears dry, or, with slow filtration after 2 hours.

The odour of the mash was noted organoleptically to express the odour as "normal" when it corresponds to the type of malt analyzed but there was no any foreign odour to be noted. Determination of specific gravity of wort is using Anton Paar DMA 35 but alternatively it can be done using Pycnometer as given in the laboratory work instruction **or** saccharometer as third option laboratory work instruction.

Figure 10: Anton Paar DMA 35

To find out the extract content of the wort according to the determined specific gravity from the official sugar table in grams of extract in 100 g of wort.

Calculating the extract content of malt according to the formulae:

$$E_1 = \frac{P*(M+800)}{100 - P}$$

$$E_f = \frac{E_1*100}{100 - M}$$

$$E_2 = \frac{P*(M+800)}{100 - P}$$

$$E_c = \frac{E_2*100}{100 - M}$$

Where: E_1 = the extract content of malt sample taken, in % (m/m)

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

E_2 = the extract content of coarsely grinded malt sample taken, in % (m/m)

E_c = the extract content on dry malt after coarse grinding, in % (m/m)

P = the extract content in wort, in g of extract per 100 g of wort (% Plato)

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

800 the amount of distilled water added into the mash to 100 g of malt

- Expressing the result in % (m/m) to 1 decimal place.

⇨ Evaluation of Different Grist Proportions

Using selected mashing program, three grist combinations with raw barley to malted barley proportions of 0:100, 40:60 and 60:40 were assessed and evaluated based on the selected main response.

⇨ Evaluation of Commercial Enzymes on 60% Raw Barley Substrate

In the following experiments, mashes consisting of 60 % unmalted barley and commercial enzyme additions were assessed. Unless otherwise stated, the commercial enzymes were added at mashing. *Evaluation of the mixed & prepared enzymes u*sing EBC mashing program, the enzyme was added at rates ranging from 0.025 to 1% vol/wt of grist added at mashing.

3.4.5.6 *Colour (Spectrophotometer Method-EBC Method 4.7.1)*

Colour measurement was done based on European Brewery Convention method (4.7.1) (fifth edition, 1998) using the spectrophotometer. 50 ml samples of the filtered wort produced was taken and re-filtered using whatman number 4 filter paper. The first 20 ml was discarded and remaining was collected. The spectrophotometer was set at 430 nm wavelength. Blank test was first done with distilled water and used to adjust the absorbance to 0.0. After rinsing the cuvette with the

bright wort, the absorbance of the sample was determined at 430 nm. The colour of the sample was then calculated using the equation below:

C= 25*A *F

Where,

C = the colour in EBC units

25 = the multiplication factor

A = absorbance at 430 nm in 10 mm cuvette

F = dilution factor.

3.4.5.7 Attenuation Limit

i. Wort Boiling

The filtrate was boiled in wort kettle for 30 minutes under atmospheric pressure. 7 g of hop extract, 1g of $ZnSO_4$ was added during boiling for flavoring. The rate of evaporation during boiling was 4.0 % per Brew. The wort was then cooled by placing in a cooling system to 9 °C. Whirlpool was installed for break removal. It was the most preferred method for hot break removal and was the least costly alternative of all trub removal methods.

ii. Wort cooling

Hot wort was cooled to reduce its temperature from close to boiling point to a temperature where yeast can be safely added without being destroyed by heat. It was also cooled so that proteins which are sensitive to lower temperatures would be separated out of solution and could be removed from the wort.

iii. Wort aeration & Pitching

Wort fermentation in beer production is largely anaerobic, but when the yeast is first pitched into wort, some oxygen must be made available to the yeast. Indeed, it is now evident that this is the only point in the brewing process where oxygen is beneficial. Oxygen must be excluded, as far as it is possible, for all other parts of the process because it will have a negative effect on beer quality. Specifically, it will promote beer flavour instability. Oxygen has a profound influence on the activity of yeasts and particularly on yeast growth. Certain yeast enzymes only react with oxygen

and it cannot be replaced by other hydrogen acceptors. This applies to the oxygenases involved in the synthesis of unsaturated fatty acids and sterols, which are vital components of cell membranes. Pitching was meant the addition of the yeast to the cold wort (pitching wort) and thus starting of fermentation. Activation of the yeast occurs not simply as a result of the introduction of oxygen but also quite specifically because of the uniform distribution of the yeast in the wort with a few yeast clumps as possible. The individual yeast cells must rapidly come into contact with the nutrients in the wort. This was important for the intensity of the initial fermentation, consequently in modern plants the yeast was dosed uniformly into the flowing stream of the wort.

The *Saccharomyces cerevisiae* strain Cell concentration was counted under microscope and the volume needed was calculated to inoculate into 10 L wort at $12x10^6$ cells/ml. The needed inoculum was centrifuged 1,000 g for 10 minutes and the supernatant was discarded then 400 g of yeast was added. Finally, yeast was added into the 500 ml wort used for fermentation and then mixed with rest part in the fermenter.

$$\text{Quantity of yeast} = \frac{\text{Theoretical volume of fermenter} * \text{Pitching rate}}{\text{Consistency} * \text{Viability}}$$

iv. Fermentation

Fermentation was carried out in a 2000 ml Erlenmeyer flask designed as laboratory scale fermenter. Yeast cells were pitched at a rate of 1% of wort volume and 80 % yeast slurry thickness (Esslinger et al., 2005) in to one liter cold wort (12.00 ^{0}P - 12.20 ^{0}P) and maintained at 12 ^{0}C. Wort was oxygenated with frequent agitation. Specific gravity or sugar drop was measured by using an Anton Paar DMA 5000 digital density meter in every 24 hr to calculate fermentability (attenuation limit).

The wort sample (250 g) was quickly brought to boil in a conical flask. After boiling for 10 minutes, the sample was cooled to room temperature and the weight was re-adjusted to the initial 250 g with distilled water. The extract was then determined after which the wort was transferred into a Schott fermentation bottle. 1.0g Brewers of yeast was then added and thoroughly mixed with l00 ml of the wort. The fermentation bottle was fitted with fermentation lock filled with water and was left to ferment at ambient temperature. The fermenting wort was swirled periodically. The residual wort extract (non-fermentable sugars) after the fermentation period was then determined by measuring the wort extract periodically in the course of 72 hours.

Apparent Attenuation Limit, which is the percentage of fermentable sugars in the total extract, was determined as follows:

Apparent Attenuation Limit (%) = (Initial extract - Residual Extract) × 100

Initial Extract

3.4.6 Finished Product Quality

3.4.6.1 Physicochemical Analysis

i. The Original gravity (OG), specific gravity (SG), color, P^H, apparent extract, real extract, attenuation and alcohol content (v/v), (w/w) were measured using the Anton Paar Beer analyzer. The colour, pH, VDK & polyphenol were measured as directed in the European Brewing Convention method (EBC, 1975). All the analysis was carried on the filtered sample.

Figure 11: Beer Analyzer

ii. Color is determined using the procedure mentioned in EBC Method 4.7.1

By adjusting the distilled water to zero absorbance at 430 nm, the beer sample is measured with the same cuvette at the same wavelength.

iii. *Bitterness (Spectrophotometer Method-EBC Method 4.9.3)*

Figure 12: Samples for Bitterness

10ml of the samples were filled into test tubes, which had previously been filled with 20ml 2, 2, 4-trimethylpentane (iso-octane) and covered. Samples were acidified with 1ml of 0.1N HCl and shaken vigorously for 20 min. The supernatant organic phase was carefully decanted into cuvette and the optical densities read at 275 nm wavelength. The optical density was expressed in terms of European Brewing Convention bitterness units as follows,

Bitterness units (BU) = 50 x ABS_{275} (EBC, 1998).

Figure 13: Spectrophotometer

iv. VDK (Vicinal Diketones), (Spectrophotometer Method-EBC Method 4.8.4)

Figure 14: Distillation apparatus

Add 100ml beer into the distillation flask and start distillation. Control the heating rate carefully to prevent over foaming. The available period under gentle heating should be at least 6 min until the first drop is collected into the receiving cylinder. And the collect 25 ml of the distillate within 6 to 8 min and mix thoroughly, pipette 10ml of the distillate into 50ml flask with glass stopper, then adding 0.5ml % 0-phenylenediamine (reagent).mix and place the flask 20-30minutes in the darkness. After adding 2ml 4N HCL (reagent) mix the sample thoroughly and measure within 20 min against the blank

VDK, ppm= $2.7*ABS_{335}$

v. *Total Polyphenol (Spectrophotometer Method-EBC Method 4.7.5)*

10ml of the beer sample was pipette out and 8ml of CMC/EDTA reagent into a 25 ml volumetric flask Stopper and thoroughly mix the contents. Then adding 0.5ml ferric reagent to the measurement sample and thoroughly mix, and 0.5ml ammonia reagent and mix was done make up to 25ml (or 50ml) with water and mix.After kept the measurement sample for 10minutes measurement the absorbance was done in a 10ml cuvette using a spectrophotometer at 600nm.ensure that the solution to be measured was cleared.

Blank Sample

Mix 10 ml of the sample of beer and 8 ml CMC/EDTA reagent in a 25 ml or 50 ml of volumetric flask and 0.5 ml of ammonia reagent was added and mix well. Allowed to stand for 10 minutes and measured the absorbance.

Calculate the content of polyphones using the formula

P (ppm) =A*820*F

Where: P = polyphenol content (mg/liter), A = absorbance at 600 nm & F = dilution factor

vi. Pasteurization

The finished product were packaged in bottles to volumes of 330 ± 5 ml and pasteurized above 60 °C for about 42 to 45 minutes using the bottles' Krones Tunnel pasteurizer at a pasteurization unit of 19 – 23 PU.

3.4.6.2 Microbiological Analysis

Pitching yeast, fermenting beer, lagered beer, pasteurized beer

i. *SACHAROMYICES WILD YEAST*

Standard medium: kleyns' agar

Procedure

- Place 20 ml of fermenting beer (or 0.5 g of yeast in 20 ml of beer) in a 25 ml test flask with a tight lid in a water bath at 46 ± 0.3 ^{o}C for 18 min.
- Cool the sample immediately to room temperature and decant the beer leaving approx. 2 ml of Suspended yeast in the bottom of the flask.
- Add 10 ml of wort; add 0.5 ml of 96 % ethanol for inhibition of bacteria.

- Plug the flask with cotton and incubate at 27 ^{o}C for 48 hours
- Decant the supernatant liquid and transfer a drop of the yeast to a Petridis, previously prepared with hardened Kleyns' agar.
- Incubate the Petridish for 48 hours at 27 ^{o}C.
- Examine the yeast cells from Kleyns' agar for spores under the Microscope.
- In bright field microscopy wild yeast spores are refracting, i.e. they become bright when the focusing is slightly altered by the fine adjustment knob.
- In phase contrast microscopy the spores are visible as dark particles.
- Spores of saccharomyces wild yeast can be distinguished from particles in the ascus by being present in numbers of 2, 3 or 4 in the ascus, and by being globe -shaped and equal of size.
- Spores of brewer's yeast may interfere with the results from time to time. They can be distinguished from wild yeast spores by being large so that they fill out the whole ascue. They don't refract light in bright field microscopy.
- Do not stain the spores as this is likely to cause interference by prephasial brewery yeast spores.

ii. *TOTAL COUNT*

Standard medium: UBA

Procedure

Standard Techniques

- Membrane: Methods Laboratory membrane filtration or sampling through membrane filters. Filter membrane growth technique.
- Sample size 100 ml (see note)
- Incubation Aerobic
- Time 3 days
- Temperature 27 ^{o}C
- Examination Colony counts under Microscopy

Expression of Results: Number and type of Colonies per 100 ml of sample

iii. *WORT BACTERIA*

Reagents

Standard medium: UBA + 10 ppm actidione

Procedure

Standard Techniques

- Plating Surface spread
- Sample size 0.1 ml
- Incubation Aerobic
- Time 3 days
- Temperature 27 ^{o}C
- Examination Colony count

iv. *LACTIC BACTERIA*

PRINCIPLE

- Membrane filtration followed by anaerobic incubation on a selective medium containing actidione
- Take the samples aseptically

Notes:

- ✓ For general purpose the present method is less suitable than the method for detection of beer spoilage organisms as it will exclude most yeast

Reagents

Standard medium: UBA + 10 ppm actidione

Procedure

Standard Techniques

- Membrane methods: Laboratory membrane filtration, or Sampling through membrane filters.

Filter membrane growth technique

- Sample size 100ml
- Incubation Anaerobic (CO_2 - catalysed)
- Time 7 days
- Temper 27 ^{o}C
- Examination: Colony counts, Microscope, Catalase and gram- reaction if necessary

v. *PROCEDURES FOR PREPARATION OF THE MEDIA*

UBA MEDIUM

Reagents

- UBA medium
- NaOH 35 % / HCl 20 %

Procedure

- Rapidly suspend 62 g of the UBA powder (reagent 1) in 750 ml of demineralised water.
- Mix thoroughly. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
- While the medium is still hot, add 250 ml commercial beer (not degassed) and mix well.
- Dispense into the laboratory bottles, at the rate of 200 ml per bottle preferably.
- Autoclave for 10 min. at 121 °C

WORT AGAR

Reagents

- Wort agar
- NaOH 35 % / HCl 20 %

Procedure

- Rapidly suspend 48.3 g of wort agar (reagent 1) in 1 litre of demineralised water containing 2.35 g Glycerol.
- Mix thoroughly, heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
- Dispense into the laboratory bottles, at the rate of 200 ml per bottle preferably.
- Autoclave 15 min. at 121 °C.
- Avoid over heating which could cause a softened medium. However, to prepare a more solid medium suitable for streaking, add 5 g Agar- Agar prior to boiling and autoclaving.

vi. WLN MEDIUM

Procedure

- Rapidly suspend 77.2 g of the WLN powder (reagent 1) in 1 liter of demineralised water or in a mixture of 400 ml clarified tomato juice and 600 ml demineralised water by heating in a boiling bath or in a current of steam. Adjust the pH to 6.5 if desired.
- Dispense into the laboratory bottles, at the rate of 200 ml per bottle preferably.
- Autoclave for 15 min. at 121 °C

3.4.6.3 Organoleptical Analysis

Figure 15: Beer in a taste glass for sensory Tasting zones

An organoleptic profile (aroma, taste, color, mouth feel, bitterness & foam stability) tasting are conducted on 9 beer samples i.e 0%, 40 % and 60 % raw barley substitute with its triplicate. The sensory analysis was conducted using 3 member-trained panelists using the sensory evaluation form in appendix B. The panel judges constituted 3 males & myself who are staff and currently have been working in Raya Brewery S.C having brewing experience and are familiar to beer drinking. Training in descriptive analysis of beer was conducted in three separate days for three consecutive days about the range of sensory attribute with reference beers. The performance of panelist were checked by comparing the sensory analysis result and instrumental result. The samples were served at 12 ^{0}C so that panel members could easily pick flavor notes. Samples were served in clean and odorless drinking glasses. All assessors had one score sheet for four samples and tasted all samples within 15 minutes. The scale was designed as three score levels for the evaluation of external appearance (foam, color intensity and turbidity), smell (hop, malt, toasted, caramel, odour intensity), mouth feel (carbon, viscosity, astringency) and gustatory sensations (sour, bitter and sweet taste, intensity and persistence). Panelists were instructed to rinse their mouths with water before starting and between sample tasting. Parameters for evaluation are shown in the questionnaire in appendix B. The ratings of each sensory attribute were converted to numerical scores and the numerical scores were collected for statistical analyses. The organoleptic taste of testing was carried out on May 3 & 5, 2016. Only one taste panelist gave good result on the appearance but all result scores are excellent. (Table 10)

CHAPTER FOUR

4 RESULTS & DISCUSSION

At the end of the research all the above quality parameters with 40 % and 60 % Unmalted Barley produced beer are analyzed and compared with the normal beer produced using 100 % Malted Barley. Barley traditionally and technically can be considered the most important. In its malted form barley provides the carbohydrate, protein and vitamins for yeast growth. Hydrolytic (reducing) enzymes derived from the malt release and convert these latter substances into fermentable forms for the yeast to metabolize. Malt also provides beer with its colour and flavour, whilst contributing a proportion of essential polypeptides and polyphenols to generate beer's characteristic foam. Hop products impart the bitter and related aroma effects and can confer microbial and flavour stability, whilst also supplying foam stabilizing polyphenols.

Barley has been the basic raw material for brewing (Yonkova et al., 2007) for both barley and non-barley producing countries. Its malt is known to be rich in protein and enzymes and has a high β-glucan and pentosan level when compared to other local grains (Malomo et al., 2011). Its high diastatic power and high level of maltose producing β −amylase, a key enzyme in breaking down malt starch into fermentable sugars ensuring efficient Saccharification, than other cereals makes it brewers' choice for beer production.

However, skyrocketing prices of malted barley due to strong global demand and high shipping costs have forced a re-think by most African countries to invest in other method as a malting substitute (KUNZE, 2004)

In the present study's laboratory analysis, different proportion unmalted barley and malted barley are analyzed using design software presented in Table 7.

4.1 Malt Analysis

Three plot areas near to Raya Brewery are selected for genotype Holker from Alamata Agricultural Research Center are as shown below in Table 1**.** The slight difference in quality parameters is not unusual for malts due to various factors (cropping, malting, and processing, instrumental and human factor) or geographical location. Barley has an extremely wide geographic range and it is widely used amongst farmers with limited and poor resources in less favorable climate and soil conditions. Ethiopia is the top barley producer in Africa and eleventh

in the world (USDA, 2014). Although there was some variation in some quality parameters all the three malts were within the specification. Three types of genotypes are observed Holker, Bekoji & Sabini. On the basis of results, Holker variety was chosen for this research as control malt due to its better yield and its lower protein content as well as higher diastatic power. The evaluation was carried out because the barley seeds are collected from different plots.

Table 1: Analysis results of Holker barley malts at different plots

Parameter	Unit	EBC STD	Different Plots		
			Korem	Ashenge	Mekhan
HLW	Kg/hL	> 65	65.70	67.64	66.60
Germination capacity	%	> 98	99.81	99.22	99.42
Diastatic Power	W.K.		110.02	144.80	114.7
Total Protein Content	%	9 - 11.5	9.68	10.18	10.34
Total Nitrogen	%	1.6 – 1.8	1.6	1.6	1.7
Moisture Content	%	3 - 5.8	4.69	4.81	4.59

In this study, the HLW of the three plots are ranged from 65.70 kg/hL (Korem) to 67.64 kg/hL (Ashenge). In terms of HLW the varieties meet most of national and international requirements for malt barley production; there is no significant difference between varieties ($p < 0.05$).

4.1.1 Grain Moisture content

The difference in moisture content of the grains among the plots (Table 1) ranged from 4.59 to 4.81 %. The moisture level in the grain is influenced by field moisture & drying conditions. Barley grain was dried to almost similar low moisture level are within the safe moisture range (< 5.8 %) which is required for malt barley storage (Edney and Brophy, 2004). High moisture content encourages microbial growth and allows increment in metabolic rate microorganisms, which depletes the extract content. According to Okon and Uwaifo, (1985), this problem of high moisture obtained in the study, however, could be overcome by optimizing the kilning regime.

4.1.2 Total nitrogen content

All the three samples of the plots studied had suitable nitrogen content. The total nitrogen content ranged between (1.6 - 1.8%). In spite of the ideal range stated above, it has been argued that Total

Nitrogen (TN) values greater than 1.9 % will usually give run-off problems during filtration and make the beer less stable, due to the formation of chill haze. On the other hand, levels must be high enough to impart body to the beer, good head formation as well as healthy fermentation (O'Rourke, 2002). In general the mean difference among the plots in terms of total nitrogen content was significant ($p < 0.05$). The effect of malting on nitrogen content (measured in terms of protein) on different variety was not significant ($p < 0.05$).

4.1.3 Total protein content

The observed protein content of Holker was significantly low compared to the standard. The desired protein content of malt barley lies in the range of 9 to 12 % (MacLeod, 2004). High protein content in malt barley is not desirable because it leads to a reduction of malt extract caused by proportionally lower carbohydrate content (Kent and Evers, 1994 and Hoseney, 1998). It also leads to a longer time of grain modification during malting, resulting in more rootlet development, greater respiratory and metabolic losses (Weston et al. 1993; MacLeod, 2004). The malt from high-protein barley can also contain relatively more soluble protein compared to low- protein barley. This soluble protein will pass into the extract, forming haze, possibly impairing the quality of the beer. On the other hand, a too low level of protein is not desirable because it limits the nutrient required for yeast growth in the course of the brewing process (Weston et al. 1993; Hoseney 1998). Malting barley varieties possess high levels of several enzyme systems that are necessary to bring about the desired chemical changes within the kernel during malting. They also have other characteristics necessary for the efficient production of quality malt, and high quality beer. Most varieties of feed barley lack one or more of the necessary enzyme systems or have other features that make them unsuitable for malting purposes.

4.1.4 Germination Capacity

Germination capacity (GC %) showed no significant variations (< 0.05) due to condition (Table 1). Percentage GC for the plots ranged from 99.22 to 99.81 %. Diastatic power ranged from 110.02 to 144.80 .The diastatic power for HOLKER variety is higher than others which indicates the presence of high enzymatic power than other varieties. In the course of malt production, germination is required to mobilize the endogenous hydrolytic enzymes (dominantly α- and β-amylases) of the grain. These enzymes modify the structure of the grain, so that it will be readily solubilized during the brewing process to produce fermentable wort of desirable characteristic flavor and color with minimum loss of dry matter.

4.1.5 Mash & wort analysis

The Holker malt was used to brew lager beer using an infusion-congress: the Saccharification time, color, extract content (^{o}P) are all in good range. Its soluble protein and kolbach index are important for yeast fermentation, are also in the required standards according to EBC (1998).

Table 2: The analysis results of Mash & Sweet Wort for 60 % raw barley Beer

Parameters	Unit	EBC STD	Values
Mash pH		5.2 – 5.6	5.4
Sweet wort Extract	^{o} P	14 – 16	15.2
Sweet wort color	EBC	13 – 15	12.0
Saccharification time	min.	≤ 10	10

4.1.6 Fermentation

The fermenting worts were monitored twice on daily basis for its extract & population (for industrial scale extract, temperature, population & pressure). The primary aim of wort production was to produce a consistent and fermentable wort product that provides sufficient nutrients to support yeast fermentation and allow for its subsequent attenuation. The apparent attenuation limit (degree of fermentation) was used as a parameter to assess the fermentability and quality of the wort. The wort produced was found to have an attenuation limit of 3.0 and subsequent fermentation studies showed complete attenuation after 120 h. The details are given in Table 5.

Table 3: Fermentation of 60 % unmalted barley brew (Average of three treatments) with Maximum Enzyme usage compared to the 100 % Malted Barley.

Time (hr)	Original gravity		pH	
	60 % Raw barley	100 % Malt	60 % Raw barley	100 % Malt
0	15.5	16.0	5.20	5.3
24	12.6	13	4.80	4.9
48	6.8	7.1	4.66	4.7
72	4.0	4.3	4.64	4.6
96	3.2	3.0	4.63	4.59
120	3.0	2.8	4.57	4.55

Table 4: Characteristics beer produced from the average of 60 % unmalted barley with Maximum Enzyme usage compared with 100 % malt beer.

Parameters	RB STD	Beer from 60 % Raw barley	Beer from 100 % Malt
OE, % by mass	11.6 – 12.0	11.82	11.85
pH	4.4 - 4.6	4.51	4.5
Color	8 - 10	8.0	9.0
EA , % by mass	2.0 - 2.4	2.25	2.26
ADOF	75 - 85	79.5	80.1
Alcohol % v/v	4.9 – 5.1	4.95	4.97

4.1.7 Evaluation of commercial enzymes on different proportion of raw barley

In the following experiment, mashes consisting different proportion of raw barley and commercial enzyme addition were assessed using the following experimental design.

Table 5: Design summary: experiments 24, Block Number

Factors, the corresponding ranges and levels:

Factors	Unit	Range	Levels	
			Low (-)	High (+)
Raw barley Proportion	%	40 - 60	40	60
Ceremix Plus MG	g/ Kg	0 – 0.5	0	0.5
Ondea Pro	g/ Kg	0 – 1.5	0	1.5

Table 6: Response summary

Response	Unit	Minimum	Maximum	Model
Yield of Extract	% dry wt.	75.1	81.0	2F1
FAN	mg/l	70	156	2F1
Degree of fermentation	%	71.0	79.8	2FI

Table 7: Experimental Results

STD	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
	Raw Barley (%)	Ceremix Plus (g/Kg)	Ondea Pro (g/Kg)	Yield of Extract (%)	FAN (mg/L)	Degree of ferment. (%)
1	40	0.0	0.0	77.3	79.0	75.6
2	40	0.0	0.0	77.4	79.6	75.8

3	40	0.0	0.0	77.2	80.0	75.0
4	60	0.0	0.0	75.1	70.0	71.0
5	60	0.0	0.0	75.4	70.2	71.2
6	60	0.0	0.0	75.8	73.0	70.9
7	40	0.5	0.0	77.8	135	78.5
8	40	0.5	0.0	77.5	138	78.1
9	40	0.5	0.0	77.8	132	77.9
10	60	0.5	0.0	76.0	128	75.4
11	60	0.5	0.0	75.8	126	75.1
12	60	0.5	0.0	76.0	129	75.8
13	40	0.0	1.5	78.4	124	78.1
14	40	0.0	1.5	78.6	122	78.0
15	40	0.0	1.5	78.5	120	78.2
16	60	0.0	1.5	76.3	113	74.6
17	60	0.0	1.5	76.4	100	74.3
18	60	0.0	1.5	76.5	112	74.6
19	40	0.5	1.5	80.8	156	79.8
20	40	0.5	1.5	81.0	150	79.8
21	40	0.5	1.5	80.0	154	79
22	60	0.5	1.5	79.3	142	78.4
23	60	0.5	1.5	79.8	148	78.5
24	60	0.5	1.5	79.5	141	77.9

4.2 Development of Empirical Models

From the study, empirical models for the output responses; extract recovery (% dry wt.), FAN (mg/l) and Degree of fermentation in terms of the process parameters in coded factors were developed by using the RSM.

The mathematical model describing the relationships among the process dependent variables and independent variables in second order equation were developed (1983).

The Model F-value of 152.72 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, B, C, AB, BC, ABC 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 mathimatical models obtained for the response yield of extract after mashing different proprtion of unmatled barely.

Final Equation in Terms of Coded Factors:

Yield of Extract = +77.67 -0.86 * A +0.76 * B +1.07 * C + 0.13 * A * B + 0.050 * A * C + 0.53 * B * C + 0.11 * A * B * C

Final Equation in Terms of Actual Factors:

Yield of Extract = +81.03333 - 0.093333 * Raw Barley + 0.40000 * Ceremix Plus MG + 1.11111 * Ondea Pro + 0.010000 * Raw Barley * Ceremix Plus MG - 7.77778E-003 * Raw Barley * Ondea Pro - 0.044444 * Ceremix Plus MG * Ondea Pro + 0.057778 * Raw Barley * Ceremix Plus MG * Ondea Pro (1)

This mathematical models are polynomials having several variables with correlation coefficients $R^2 = 0.9853$, adjusted $R^2 = 0.9788$, Predicted $R^2 = 0.9668$ and Press 2.25. The "Pred R-Squared" of 0.9668 is in reasonable agreement with the "Adj R-Squared" of 0.9788. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 35.796 indicates an adequate signal. This model can be used to navigate the design space.

Three tests were carried out to determine the adequate of model for all response. These included Sequential model Sum of Squares, Lack of Fit Tests and Model Summary Statistics. Select the highest order polynomial where the additional terms are significant and the model is not aliased. P-value < 0.0001.

It was found that the two factor model was found to be the fit model for the response variable Yield of extract, FAN & degree of fermentation although the quadratic model had high R^2, adjusted R^2, predicted R^2 and low PRESS, quadratic or cubic models are not chosen because the model was aliased.

The "Lack of Fit Tests" table compares residual error with "Pure Error" from replicated design points. The two factorial model, identified earlier as the likely model for all response, does not show significant lack of fit (Appendix table A).

4.3 Adequacy Check for the Developed Models

The adequacy of the model was checked by analysis of variance (ANOVA) and some diagnostic plots. Analysis of variance (ANOVA) is employed to test the significance of the developed models. The detail ANOVA for the three responses is given at Appendix table A1. The F-value is measure of variation of the data about the mean. Generally, the calculated F-value should be several times greater than the tabulated value, if the model is a good prediction of their experimental results and the estimated factors effects are real. Also the high F-value and a very low probability indicates that the present models are in a good prediction of the experimental results. The p-value serves as a tool for checking the significance of each of the coefficients. The pattern of interaction between the variables is indicated by these coefficients. The Model F-value of 152.72 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise and Values of "Prob > F" less than 0.0500 indicate model terms are significant.

Additionally, the developed models has been checked by using residual analysis. Residuals are usually considered as components of variations, imprecisely fitted to the model and subsequently it is predicted that they behave according to a normal distribution feature. For the evaluation of normality of the residuals, a graphical visualization of the normal probability plot is considered as the proper method.

Residual analysis and Diagnostic plot guide

A) Diagnostic plot

Shape is linear so is works.

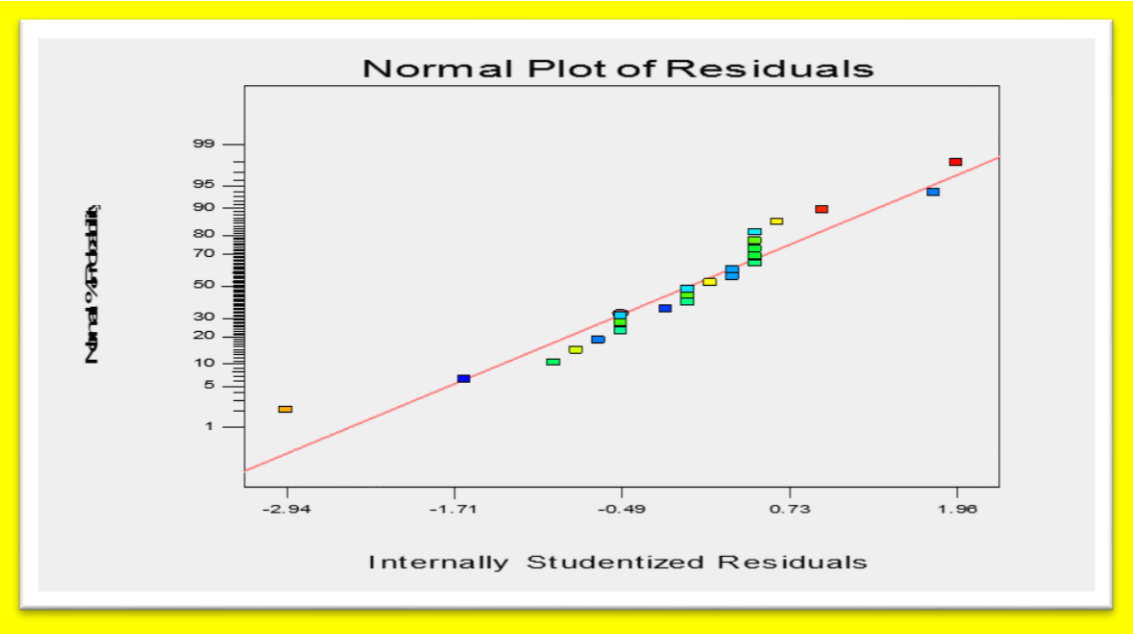


Figure 16: Normal Plot of Residuals for the response Yield of extract

The normal probability plot of the residuals from a nice straight line with no outliers or grouping in the data. This implies that the residuals are normally distributed, describing the experimental noise.

B. Residuals vs predicted plot: randomly scattered

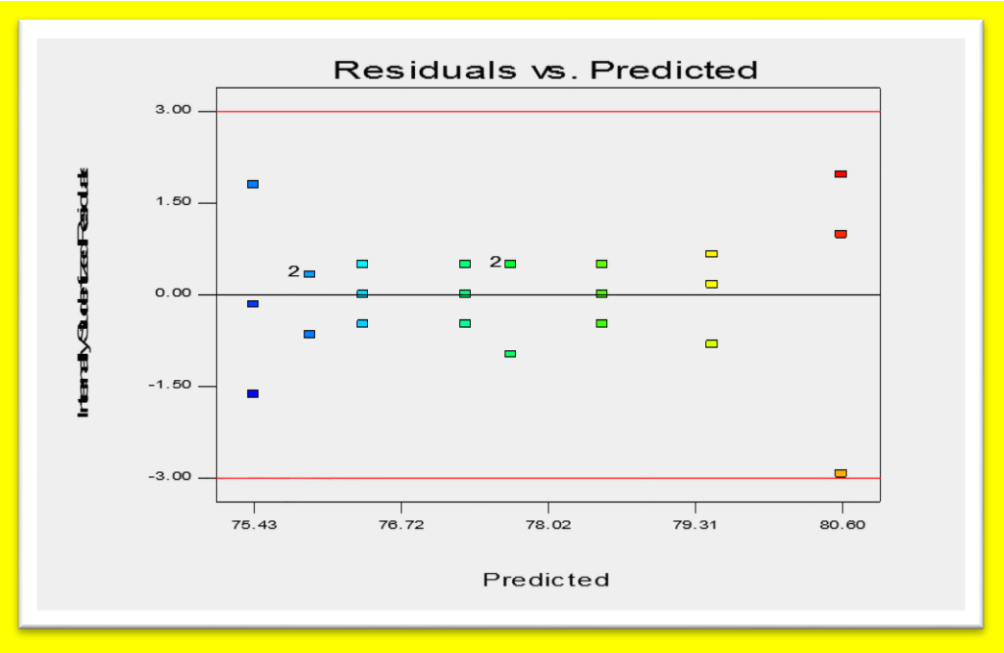


Figure 17: Residuals vs Predicted

Conclusion so for the residuals are normally distributed with no outliers or grouping in the data, therefore the residual standard deviation can be used as the measure of the random variability of the process.

C) Predicted vs Actual: Should be randomly scattered under 45 ^{0}

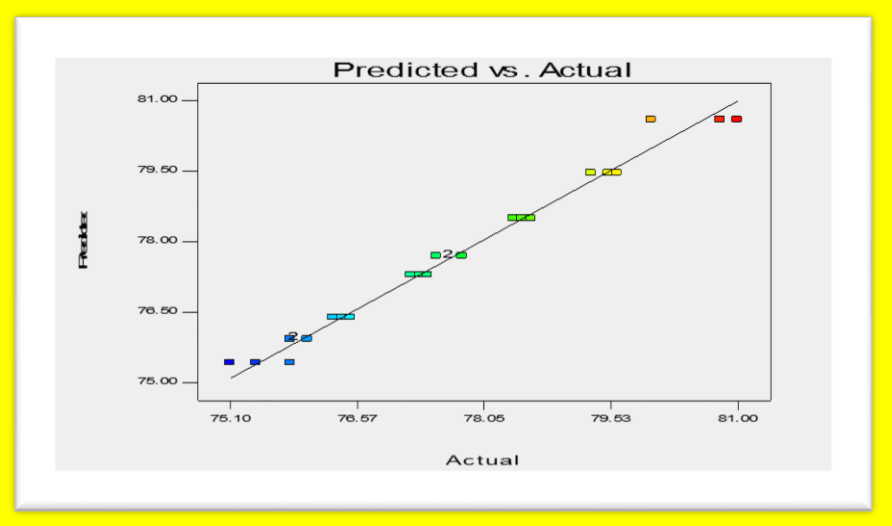


Figure 18: Predicted vs Actual

4.4 Effect of Ceremix Plus MG on yield of extract

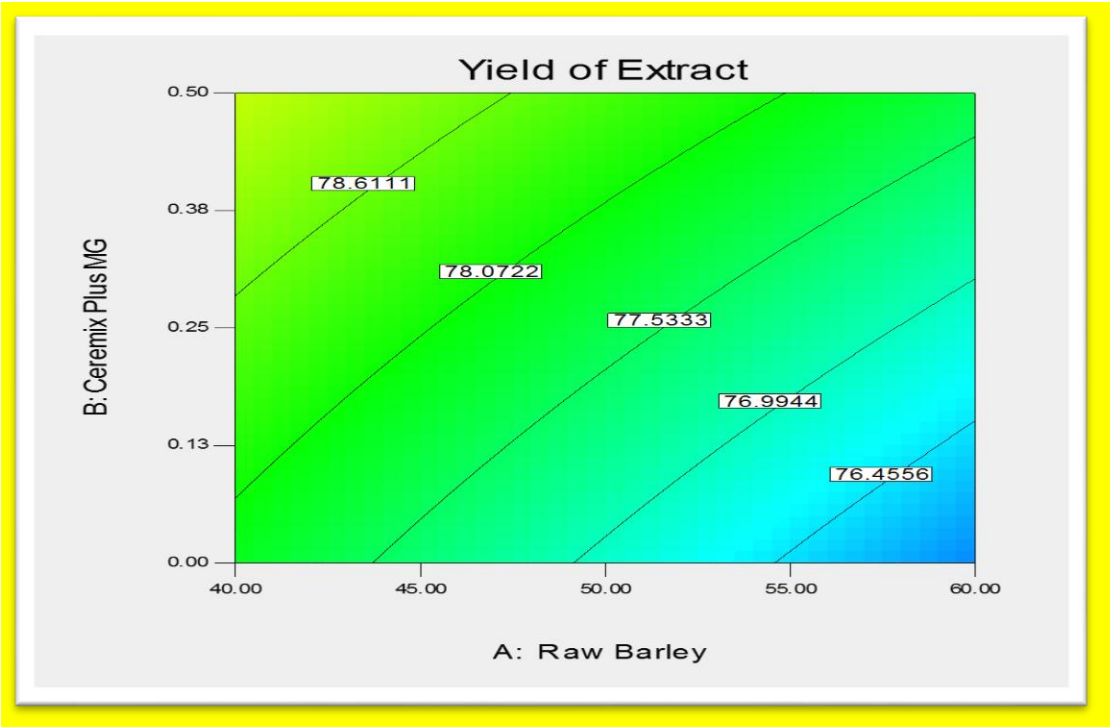


Figure 19: The impact of Ceremix Plus MG on yield of extract of wort

Yield of extract of wort decreased with increasing concentration of enzyme. Statistical analysis also showed that this contribution was significant ($p = 0.0001$). From the mathematical model, it was shown that in its linear form (A) in the appendix table A1 $P < 0.05$ was significant, in its quadratic form (A^2) remained statistically significant ($p = 0.0131$) which is less than 0.05. This confirmed the above biologically supplement during mashing is important.

4.4 Development of empirical model for the response % extract recovery

Both statistical tests in the appendix table 2 ANOVA for the response % of extract recovery tell us that the current model is adequately describing the process. The variation described by the model (regression) is significantly larger than the variation not captured (residual). In other words, R^2 value and the model as whole is significant. The model error (residuals excluding replicate variation) is not significantly greater than the replicate error in other word the model has no lack of fit.

Additionally, the developed models for cause and effect has been checked by using residual analysis. Residuals are usually considered as components of variations, imprecisely fitted to the model and subsequently it is predicted that they behave according to a normal distribution

feature. For the evaluation of normality of the residuals, a graphical visualization of the normal probability plot is considered as the proper method.

A. Diagnostic plot

Shape is linear so it's good

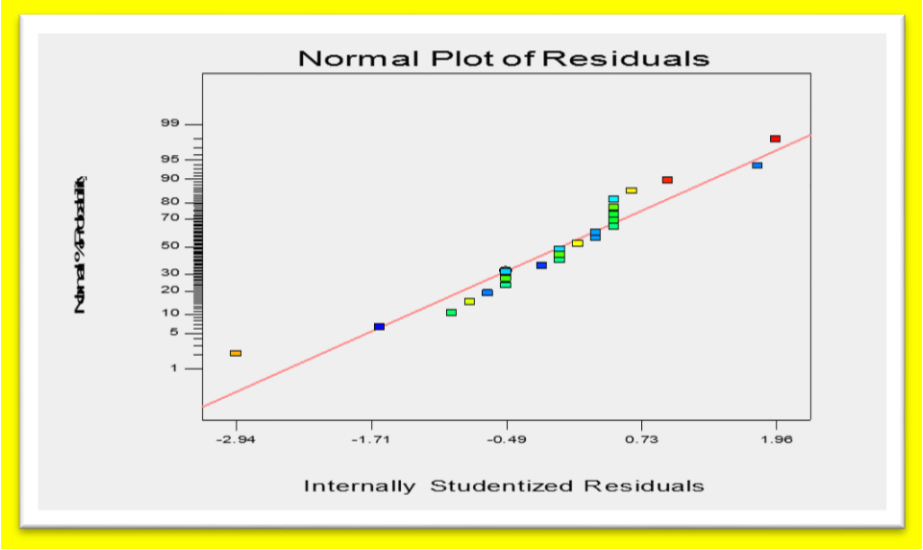


Figure 40: Normal Plot of Residuals

B. Residuals vs predicted plot: randomly scattered

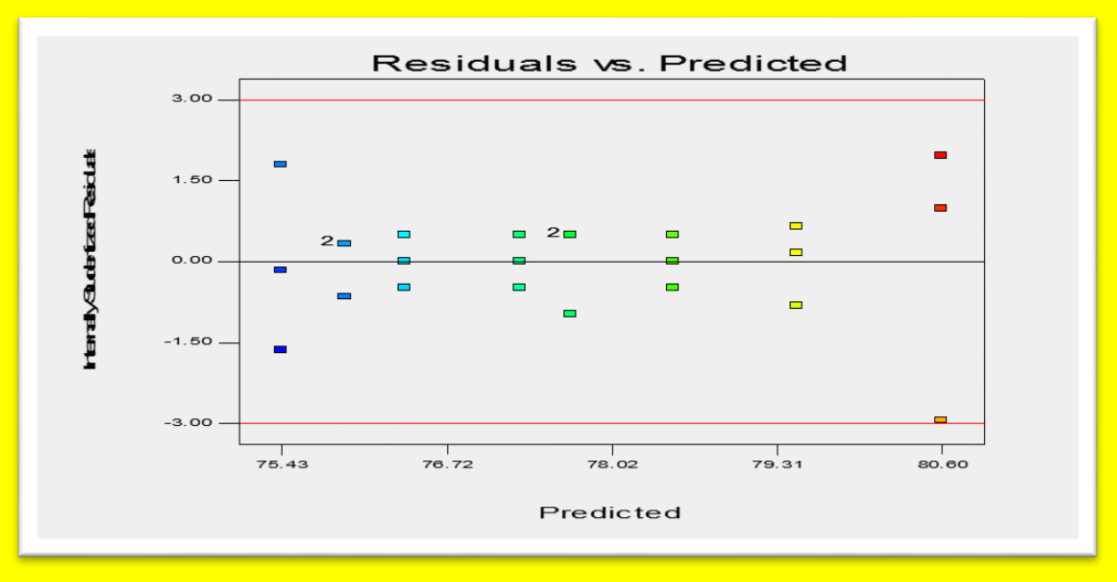


Figure 25: Residuals vs Predicted

C) Predicted vs Actual

Should be randomly scattered under 45 ^{0}

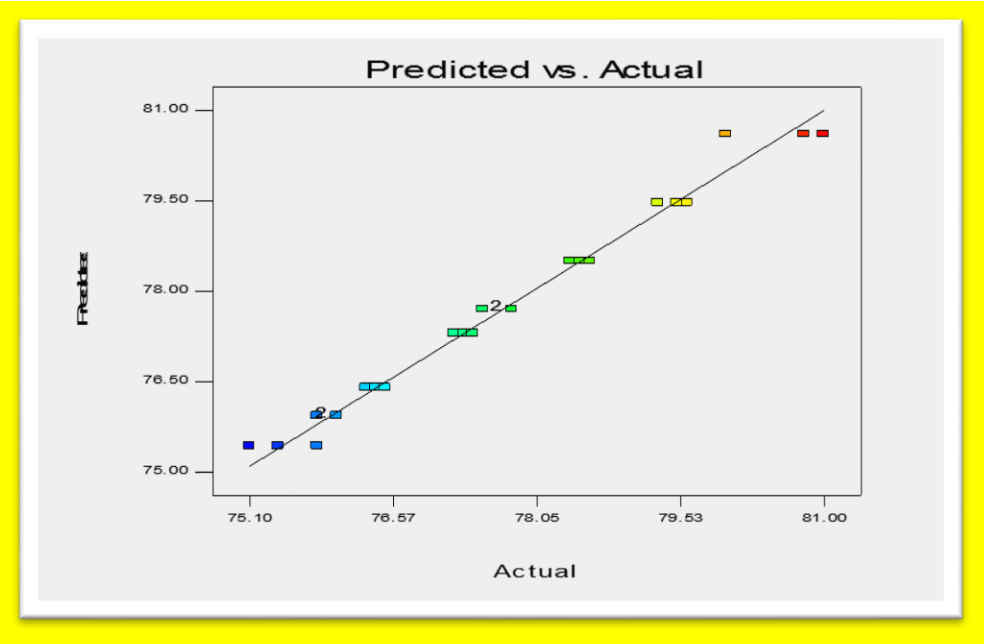


Figure 22: Predicted vs Actual

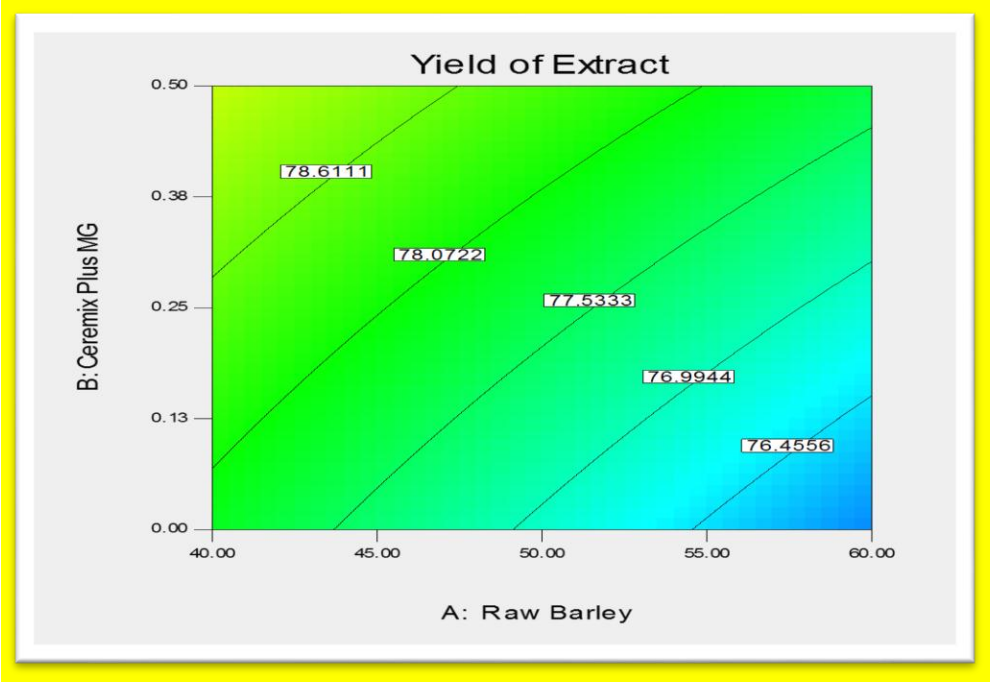


Figure 23: Effect of Ceremix Plus MG as sole mashing enzyme on increasing % of Extract Recovery

The impact of Ceremix enzyme Plus MG as sole mashing enzyme on mashing of different proportion malted and unmalted barley on the response % of extract recovery was increased with increasing concentration of enzymes.

Statistical analysis also showed that this contribution was significant (p =0.0001). From the mathematical model, it was shown that in its linear form (C) in the appendix table A1 P = 0.0001 was significant. This confirmed the above biologically supplement during mashing has no significant impact in excess use it shows it has limit.

The Model F-value of 152.72 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise.

Final Equation in Terms of Coded Factors:

FAN = +117.83 - 4.64 * A + 22.09 * B + 14.51 * C - 5.92 * B * C (2)

Where: A - Raw barley, B - Ceremix Plus and C - Ondea Pro

Final Equation in Terms of Actual Factors:

FAN = + 98.50833 - 0.46417 * Raw Barley + 112.06667 * Ceremix Plus MG + 27.24444

* Ondea Pro - 31.60000 * Ceremix Plus MG * Ondea Pro

Both statistical tests in the appendix A for ANOVA response FAN tell us that the current model is adequately describing the process. The variation described by the model (regression) is significantly larger than the variation not captured (residual). In other words, R^2 value and the model as whole is significant. The model error (residuals excluding replicate variation) is not significantly greater than the replicate error i.e. the model has no lack of fit.

Additionally, the developed models for FAN has been checked by using residual analysis. Residuals are usually considered as components of variations, imprecisely fitted to the model and subsequently it is predicted that they behave according to a normal distribution feature. For the evaluation of normality of the residuals, a graphical visualization of the normal probability plot is considered as the proper method. Figure ..., (a), (b) and (c) are the plot of the residuals calculated against the order of experimentation.

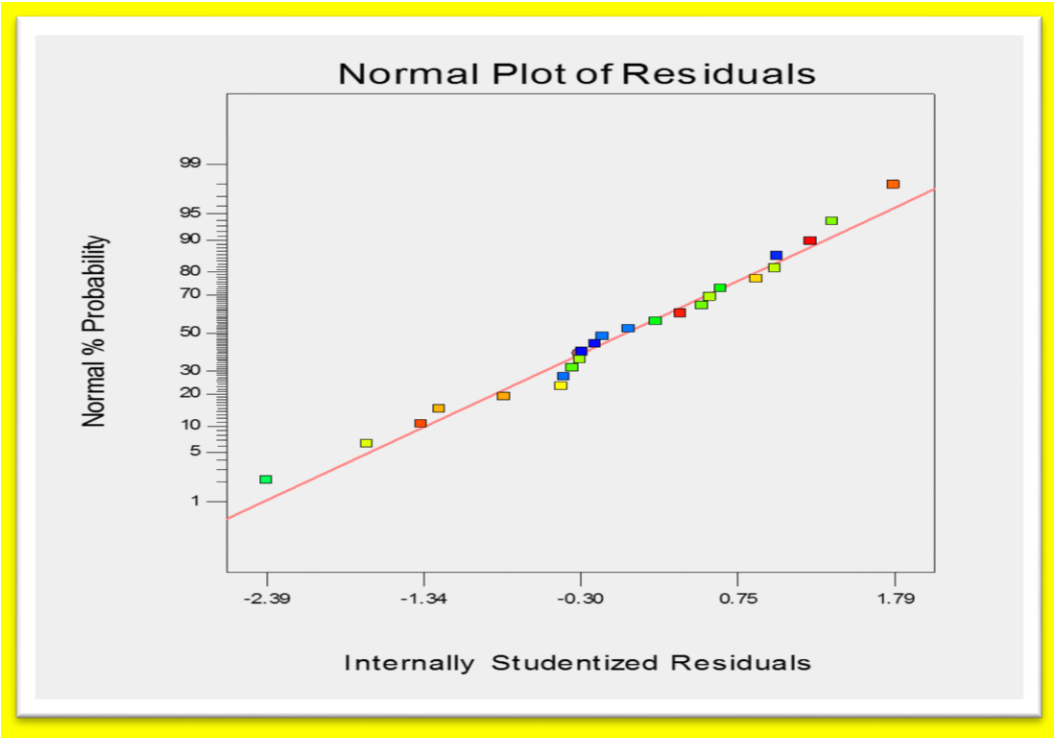


Figure 24: Normal Plot of Residuals

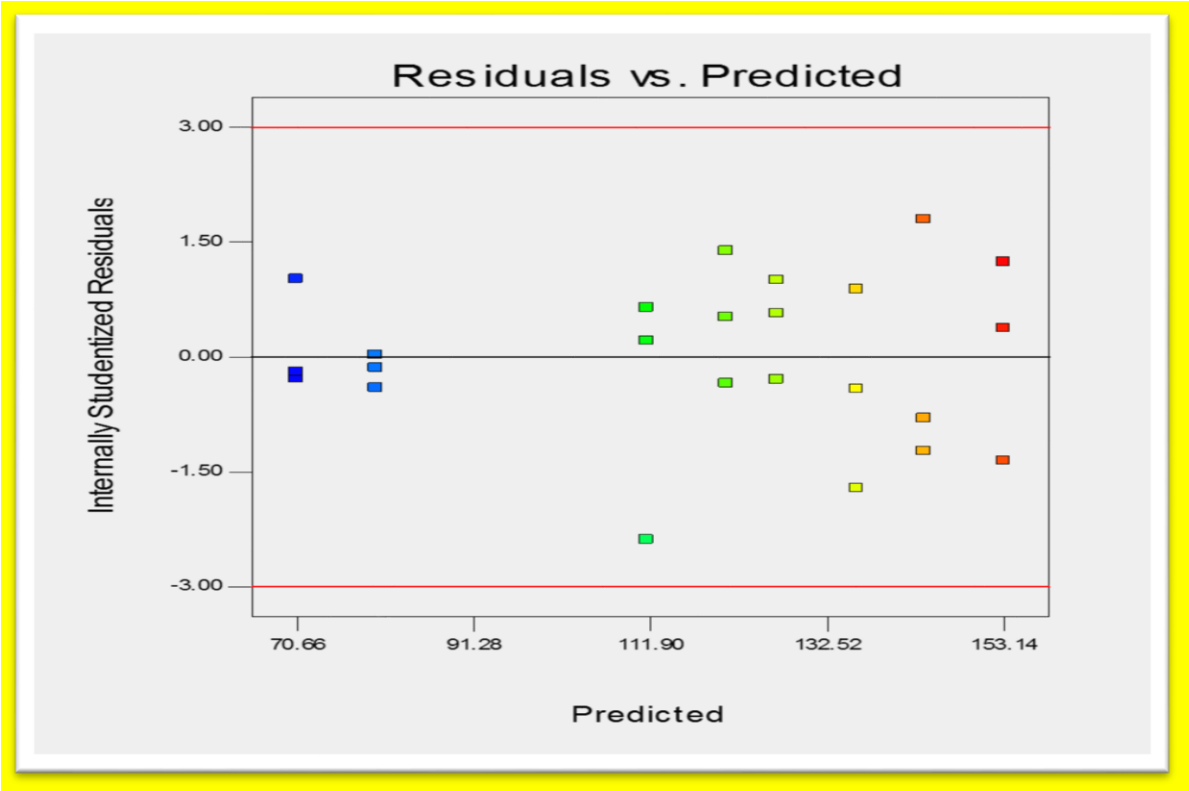


Figure 6: Residuals Vs Predicted

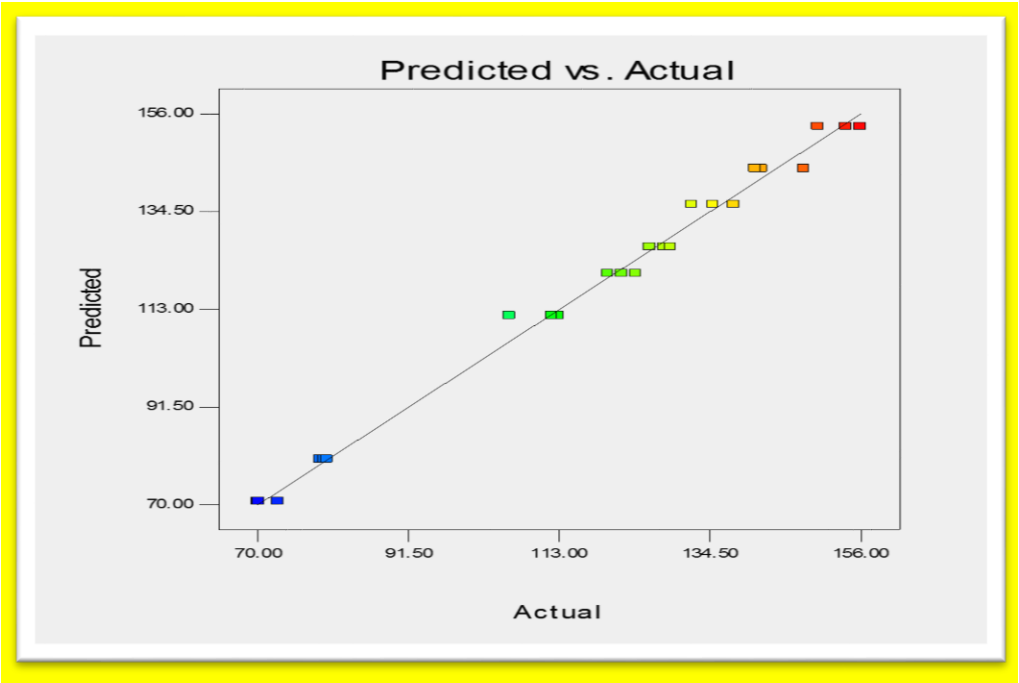


Figure 26: Predicted Vs Actual

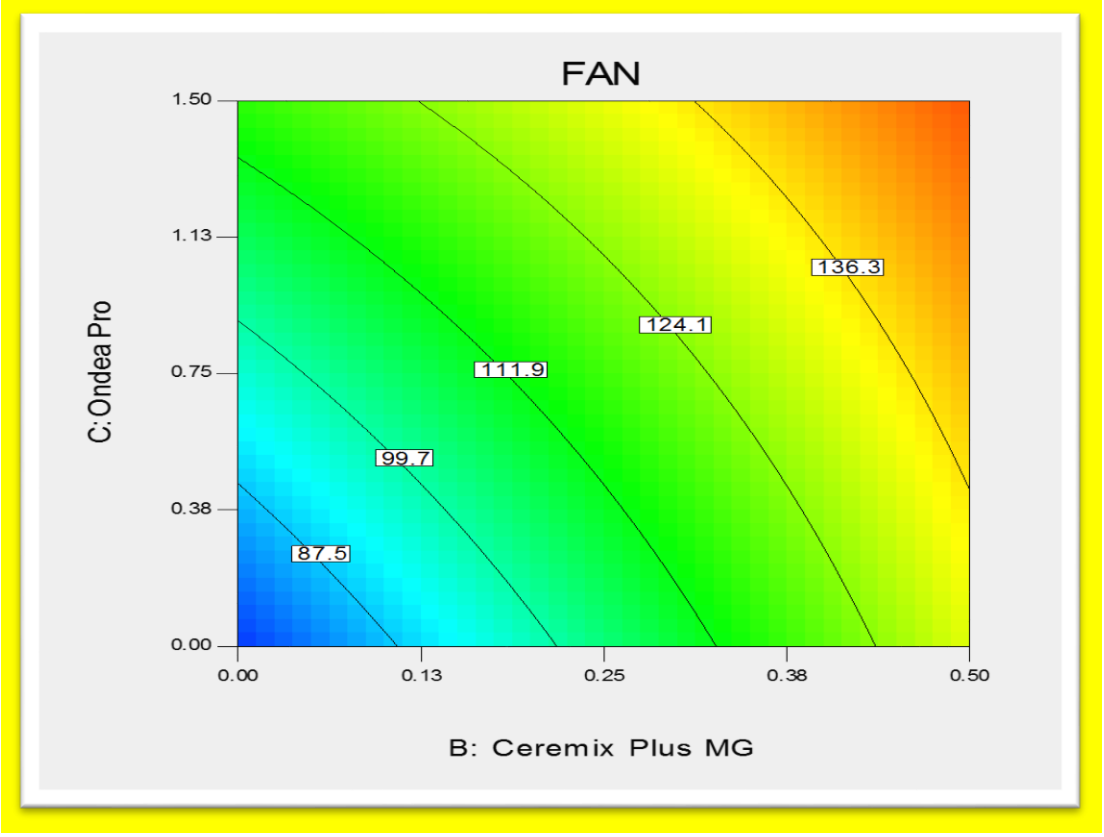


Figure 27: **Effect of Ceremix Plus & Ondea Pro on 50% Unmalted barley ratio mashing on increasing free amino nitrogen content of wort**

Conclusion so for the residuals are normally distributed with no outliers or grouping in the data, therefore the residual standard deviation can be used as the measure of the random variability of the process.

The Model F-value of 671.30 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, B, C, BC 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 0.73 implies the Lack of Fit is not significant relative to the pure error. There is a 54.89% 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. The "Pred R-Squared" of 0.9888 is in reasonable agreement with the "Adj R-Squared" of 0.9915. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 69.557 indicates an adequate signal. This model can be used to navigate the desig n space.

Final Equation in Terms of Coded Factors:

Degree of Fermentability = +76.25 - 1.53 * A + 1.62 * B + 1.37 * C + 0.47 * A * B + 0.22 * A * C - 0.17 * B * C + 0.15 * A * B * C (3)

Where: A Raw barley proportion, B – Ceremix Plus MG and C – Ondea pro

Final Equation in Terms of Actual Factors:

Degree of Fermentability = + 83.43333 - 0.20667 * Raw Barley + 0.80000 * Ceremix Plus MG + 1.60000 * Ondea Pro + 0.12667 * Raw Barley * Ceremix Plus MG + 8.88889E-003 * Raw Barley * Ondea Pro - 4.88889 * Ceremix Plus MG * Ondea Pro + 0.080000 * Raw Barley * Ceremix Plus MG * Ondea Pro

Both statistical tests in the ANOVA table tell us that the current model is adequately describing the process. The variation described by the model (regression) is significantly larger than the variation not captured (residual) i.e. R^2 value and the model as whole is significant. The model error (residuals excluding replicate variation) is not significantly greater than the replicate error or the model has no lack of fit. Additionally, the developed

models has been checked by using residual analysis. Residuals are usually considered as components of variations, imprecisely fitted to the model and subsequently it is predicted that they behave according to a normal distribution feature. For the evaluation of normality of the residuals, a graphical visualization of the normal probability plot is considered as the proper method.

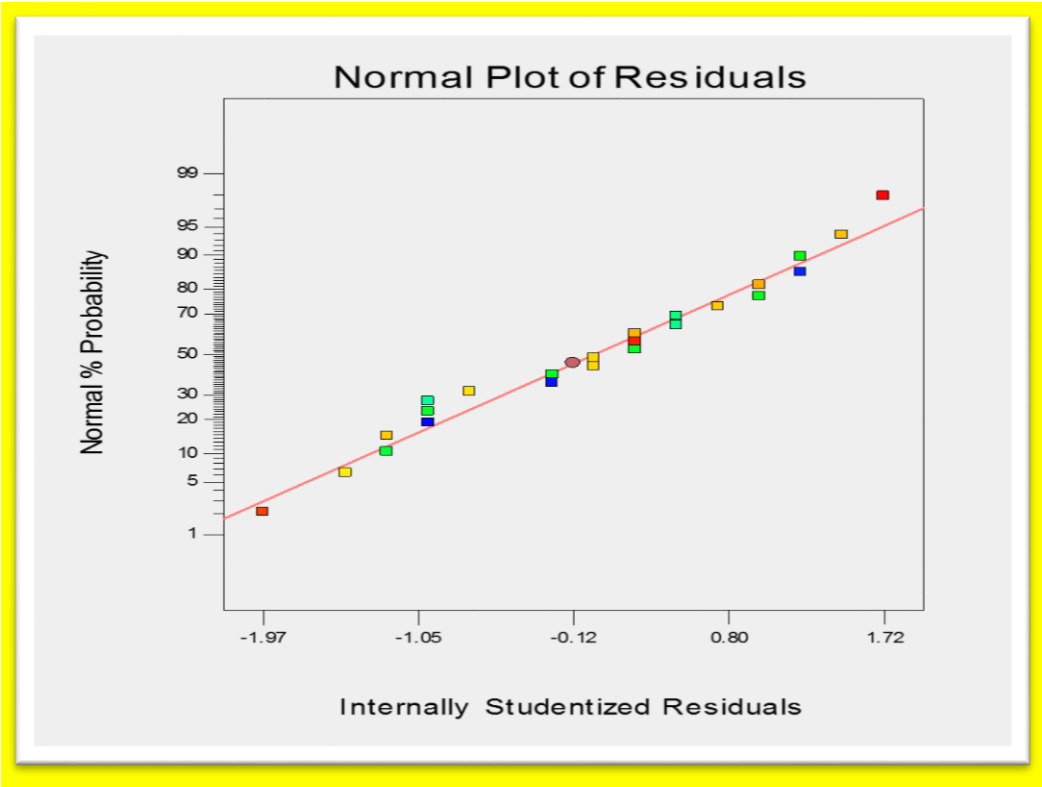


Figure 7: Normal Plot of Residuals

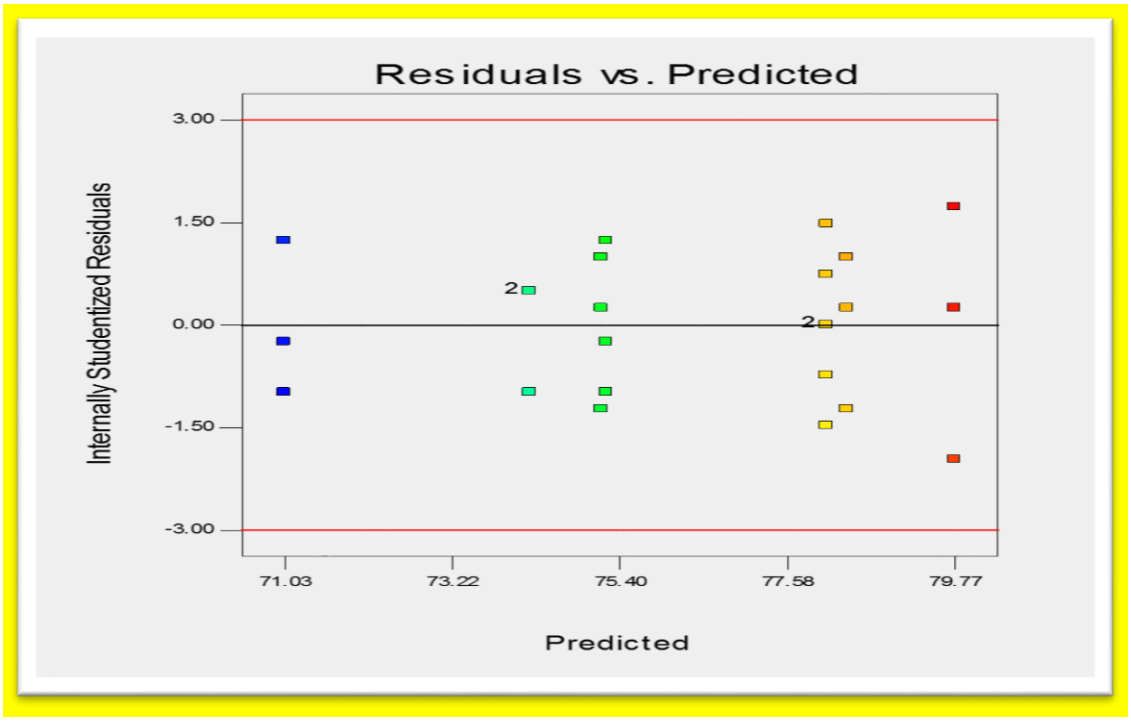


Figure 8: Residual vs Predicted

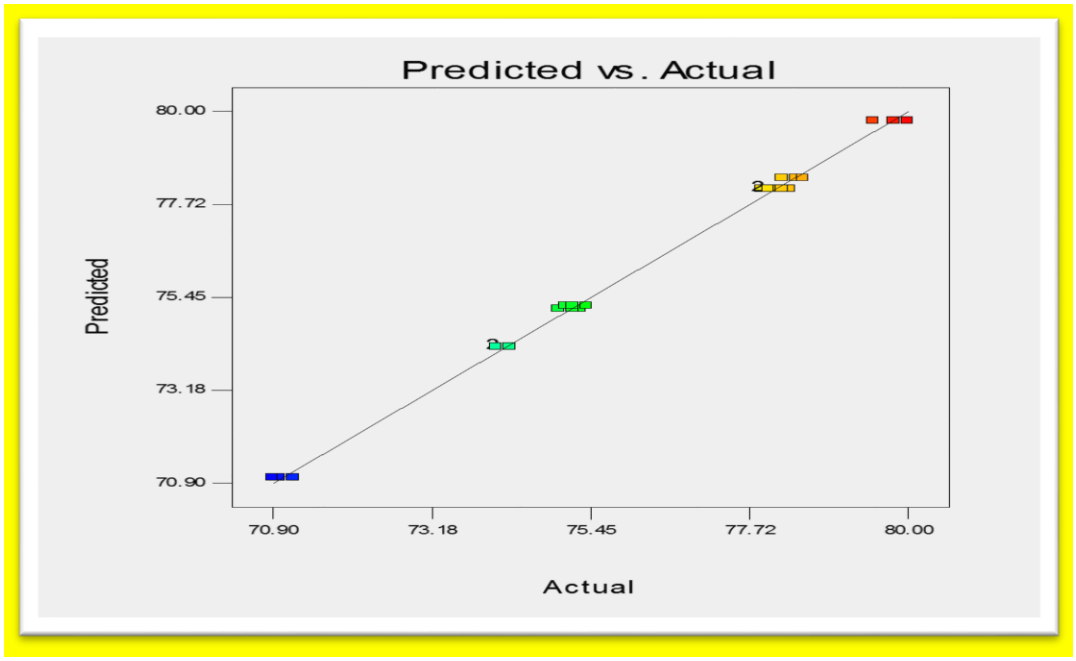


Figure 30: Predicted vs Actual

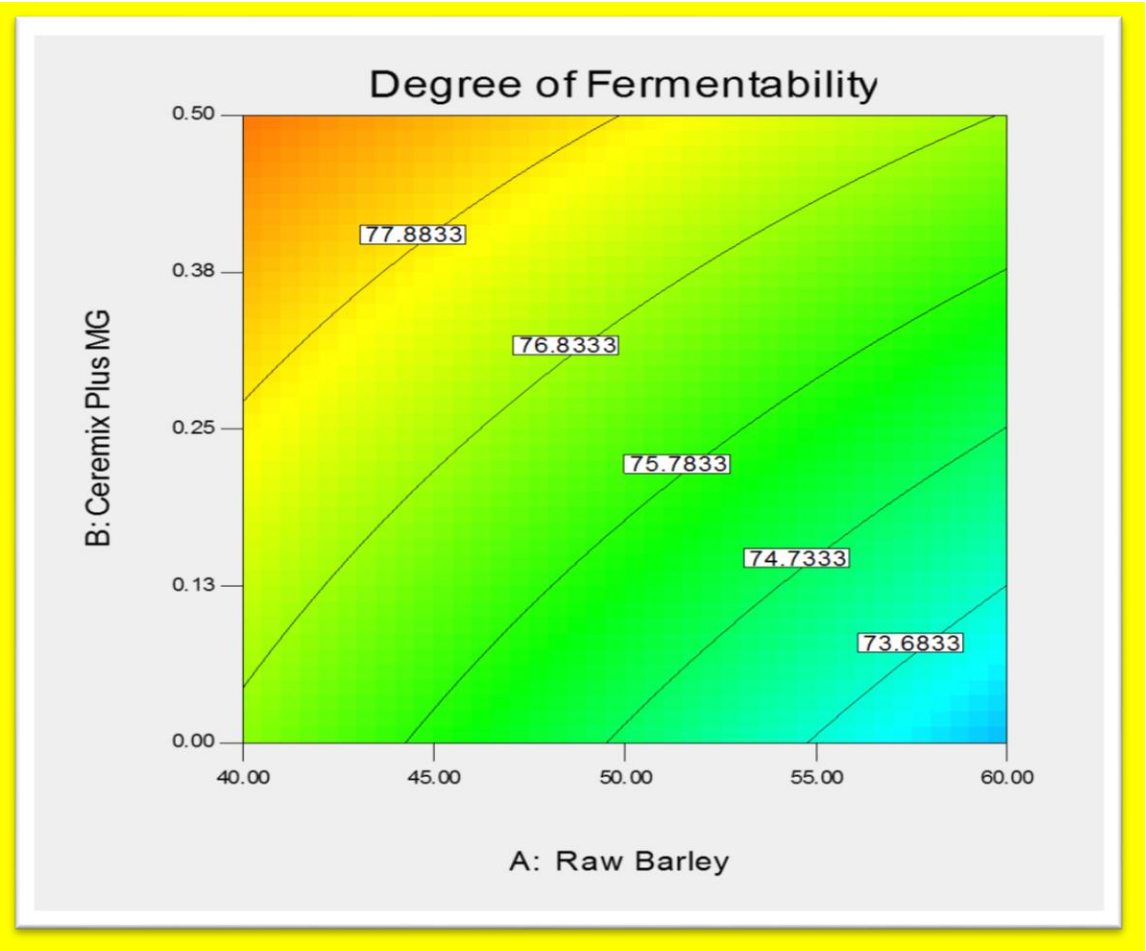


Figure 91: Counter plot Ceremix Plus vs raw barley proportion

Conclusion so far the residuals are normally distributed with no outliers or grouping in the data, therefore the residual standard deviation can be used as the measure of the random variability of the process.

Optimization of Process Condition by Response surface methodology

The process condition were optimized using DOE software in contrast with classical optimization process, which may lack the effectiveness of different combination for different parameters.

Table 8: Constraints applied for optimization

Name	Goal	Lower limit	Upper limit	Upper weight	Lower weight	Importance
R.B Propor. %	Maximum	40	60	1	1	3
Ceremix Plus MG	Minimize	0	0.5	1	1	3
Ondea Pro	Minimize	0	1.5	1	1	3
Yield of Extract (%)	Maximize	75	85	1	1	5
FAN (mg/l)	Maximize	70	156	1	1	5
Fermentability (%)	Maximize	75	85	1	1	5

Table 9: Selected optimum solution

S. Nr	Raw Barley prop. (%)	Ceremix Plus (Kg)	Ondea Pro (Kg)	Yield of Extract (%)	FAN (Kg)	Fermentability (%)	desirability	Optimum Selection
1	54.57	0.37	1.03	78.17	130.50	76.94	0.38	selected

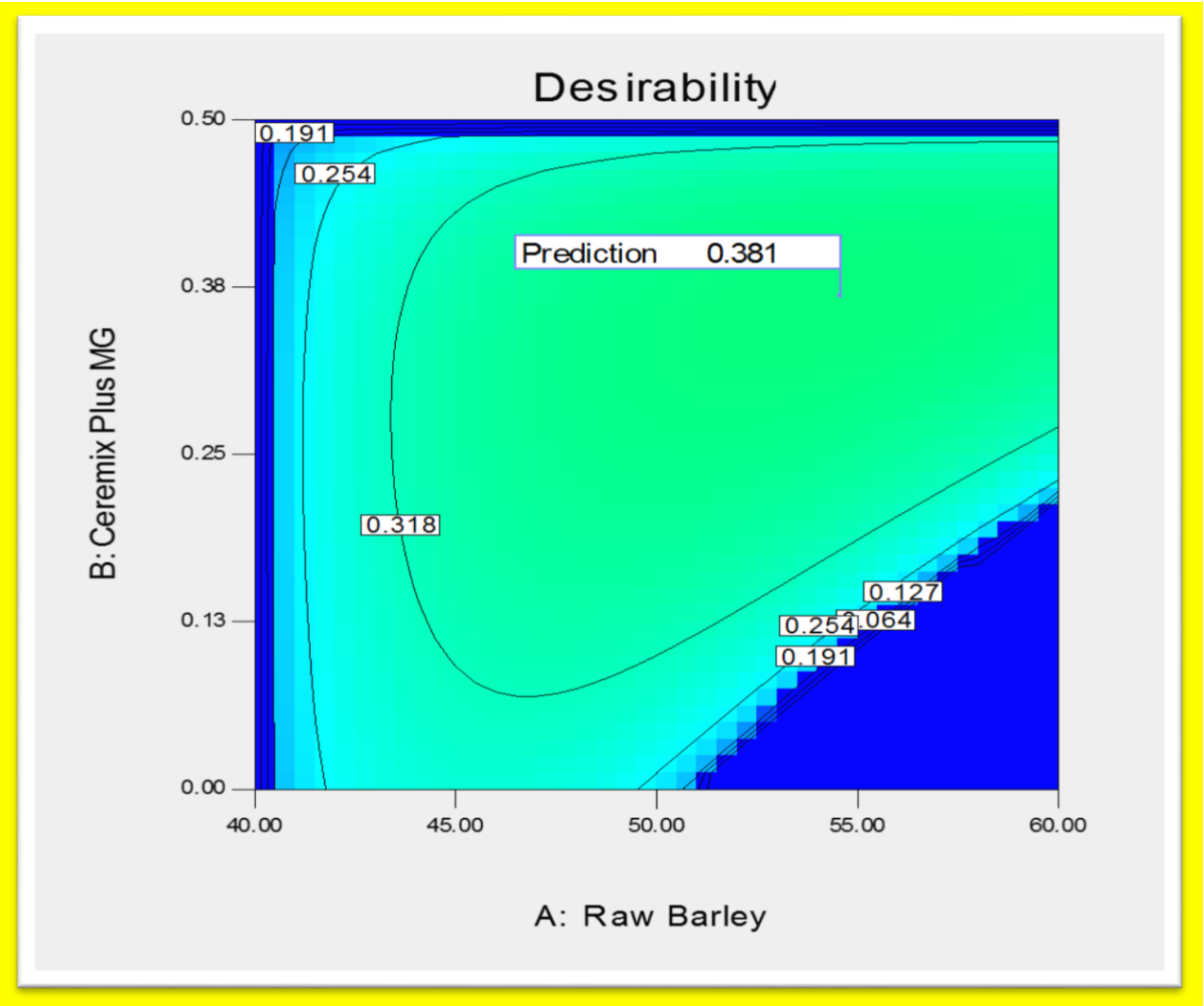


Figure 310: **Counter diagram for desirability**

Validation of the Developed Models

Optimization of the mashing enzymes action on the three responses:

The results obtained for the action of the enzymes on increasing the yields after mashing on the basis of the models, were optimized to define a satisfactory domain of compromise for the action of the mashing enzymes. This domain was obtained for Yield of Extract recovery (dry wt.) 78.17 %, FAN 130.30mg/l, % attenuation 76.94

Table 10: Validation of optimal combination (Comparison of products)

Parameters	Unit	54.57 % Raw barley	100 % malted barley
ADOF	%	76.94	80.1
Color	EBC	8.0	9.0
VDK	ppm	0.06	0.05
Polyphenol	ppm	120	110
FAN	mg/l	130.30	150.0
Appearance		+1 (3), 0 (1)	+1 (4)
Smell		+1 (4)	+1 (4)
Taste		+1 (4)	+1 (4)

In key research conducted by Johns and piece in 1964 is assumed that brewing yeast is only capable of assimilating simple amino acids and peptides but not proteins. Therefore, the amount of free amino nitrogen (FAN) is important to guarantee a stable fermentation process. For a conventional wort FAN levels of ~150 mg/L are reported to be necessary for a healthy fermentation (Dl Goods 2005). In the current study, as the level of malted barley was increased, the amount of total soluble nitrogen (TSN) and FAN fractions also increased.

The addition of malted barley was found to bring in higher levels of assimilable nitrogenous material than was unmalted barley. Since unmalted barley contains little endo-proteolytic activity, it is important to consider whether the endogenous malt proteolytic enzymes can bring extra assimilable nitrogen from the raw barley substrate. With regard to the total level of amino acids, the malt proteolytic enzymes were found to have a negligible impact on bringing extra amino acids into the wort. The rate of protein hydrolysis during mashing is regulated by the activities of endo-proteinases, which are known to be rate-limiting enzymes. The presence of these endo-proteinases is not sufficient, however, to ensure that the barley proteins are rendered soluble. This is because there are low-molecular-weight proteins in both raw barley and malted barley.

The total amino acid content of wort is important in determining the extent of yeast growth, while the individual amino acid spectrum of the wort influences beer flavor. Therefore, the lower amounts of amino acids soluble in worts containing low levels of malted barley may result in fermentation difficulties (affecting the extent of yeast growth), while the altered spectrum of amino acids may result in the production of beers of a different flavor and aroma.

Table 7 reveals that when the mixed enzyme dosage level was increased, resulted in the increase of extract recovery levels (Fig. 11). The experimental values of extract recovery, FAN & Ferment ability obtained under different amounts of enzymes are presented.

CHAPTER FIVE

5 TECHNICAL & ECONOMICAL FEASIBILITY

5.1 BASIC PPROCESS UNIT OPERATIONS

5.1.1 Wort Production

It is the formation & dissolving of soluble fermentable sugars with the aid of enzymes from the initially insoluble components in the malt.

- **Malt Handling & Milling**

For the purpose of the malt pre-treatment, malt delivered to the brewery is stored in silos until use. These silos are not aerated since the malt no longer respires. Before acceptance, quick tests are performed to check that the batch conforms to the company's specifications. Before processing, the malt is freed from impurities & the amount required for the brew is weighed out.

Malt components to be rapidly extracted and converted, the malt is milled to obtain coarse flour. The other unmalted cereals are also milled to varying degrees. In order to give the malt enzymes the opportunity, during mashing, to act on the malt contents & break them down, the malt must be broken into small fragments.

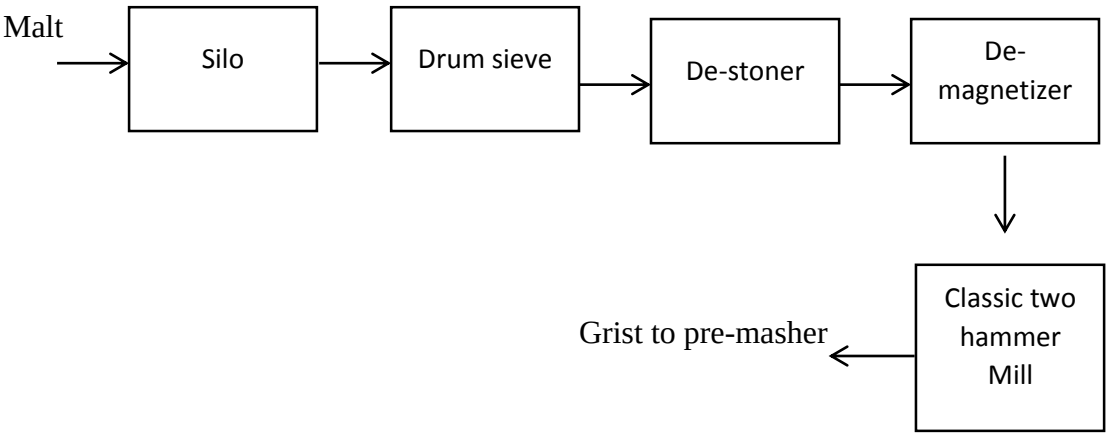


Figure 11: Malt handling and cleaning flow sheet

- **Mashing**

The flour from the cereals (malt and other unmalted cereals) is mixed with water and subjected to certain processes to obtain a wort of a suitable composition for the kind of beer being produced (varying times, temperatures and pH). These conditions encourage the development of complex starch molecules and proteins in other simpler ones by means of enzymes formed during the production of the malt. Mashing lasts 2 to 3 hours and finishes with a temperature of approximately 76 ºC.

- **Filtration of the wort**:

After mashing, the whole volume is filtered in order to separate the spent grains (which is an excellent animal feed) from the wort itself. This is done by passing water through the mash at the right temperature in a Mash filter press, which lasts around 2 – 2.5 hours, conducted at a temperature of 75 - 80 ºC.

- **Boiling the wort**:

The diluted and filtered wort is boiled for around 70 minutes. Hops are added at this stage.
The purpose of boiling is to:

- Transform and make soluble the bitter substances in the hops;
- Eliminate undesirable volatile substances;
- Sterilise the wort;
- Provoke the precipitation of proteins of high molecular weight;
- Establish the final concentration of wort.

After boiling, it is necessary to separate the precipitated protein and the insoluble hop components from the hot wort. Separation may be carried out in a decanter using gravity or with centripetal force in a "whirlpool".
Before the hopped wort goes into the fermentation tanks, it is cooled to a temperature of around 9 ºC and aired in sterile conditions.

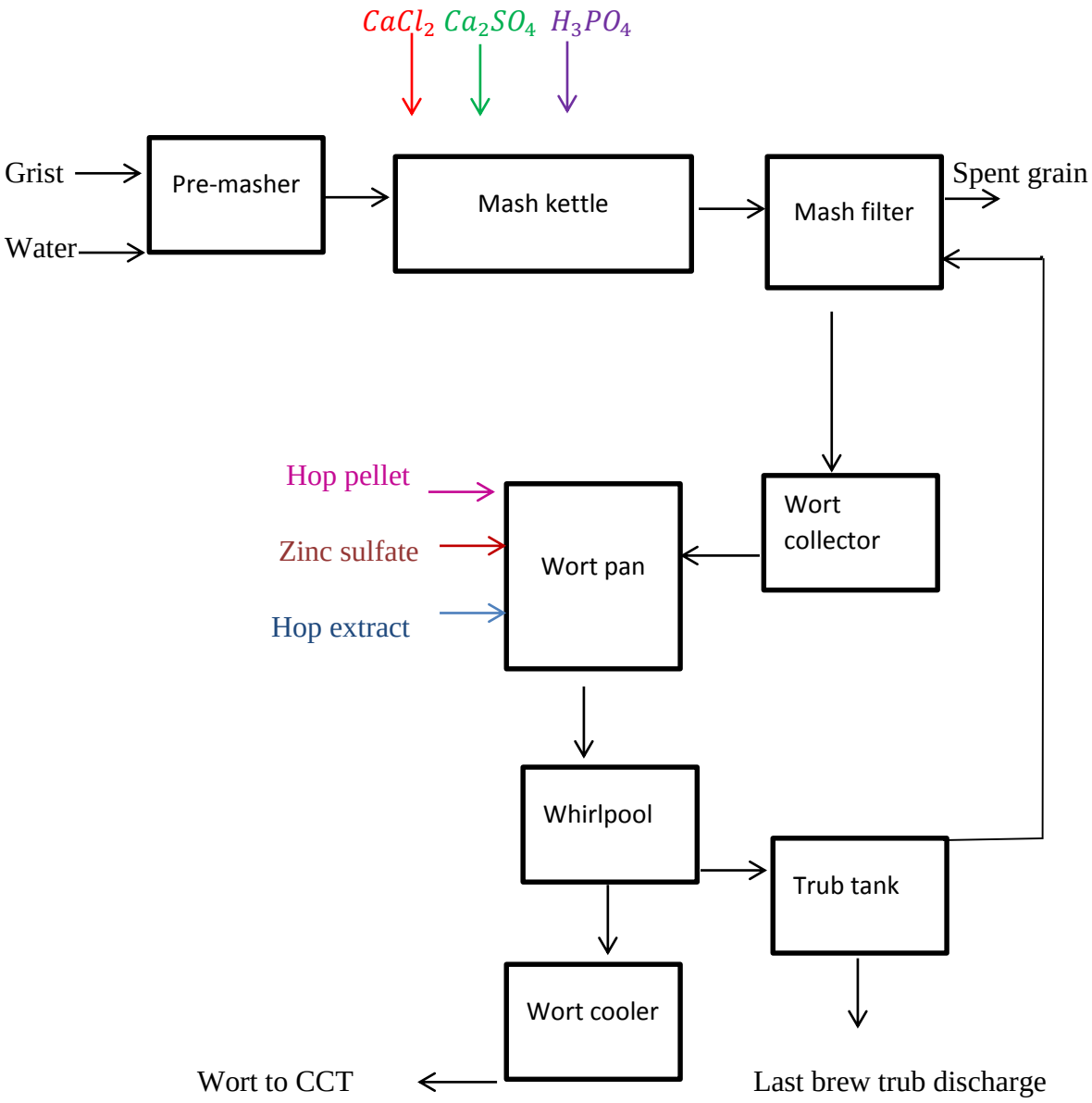


Figure 34: Wort production flow sheet

5.1.2 Beer Production

Fermentation: During fermentation, the wort sugars are converted by the transformation of yeast into alcohol and carbon dioxide. Fermentation begins when yeast of a culture selected for the type of beer being produced is added to the cooled oxygen-saturated wort. Fermentation takes place at controlled temperatures and lasts around 7 days. At first it is quite violent, then slows down gradually until the yeast is deposited on the bottom of the tank.

$$\tfrac{1}{2}\, C_{12}H_{22}O_{11} + \tfrac{1}{2}\, H_2O \rightarrow C_6H_{12}O_6 \rightarrow 2\, C_2H_5OH + 2\, CO_2$$

Maltose + Water → Glucose → Ethanol + Carbon dioxide

Maturation: The phase after fermentation, is the period in which the beer is allowed to rest at suitable temperatures in order for the undesirable volatile components, which might affect the final «bouquet» of the beer, to be released.

Stabilisation: This consists of letting the beer stabilise at temperatures of between 0 ºC and – 2 ºC, to permit colloidal stabilisation.

Clarification/ Filtration: It is the operation that gives the beer its clear limpid quality, eliminating the last remaining traces of clouding still in suspension. It consists of pumping the liquid through a suitable filter. The filtered beer is then stored in tanks called BBTS (Bright Beer Tanks), now ready for packaging.

The final stage of the beer production process is transferring the beer into different kinds of containers (bottles, barrels, kegs, cans etc.). Before or after bottling, the beer needs to be biologically stabilised. This operation may be carried out cold (sterilising filtration) or hot (using pasteurisation, which may be done either immediately before - flash pasteurization - or after the drink is introduced into its container - tunnel pasteurisation). During packaging the beer is packed into different forms (bottle, kegs, barrel, can etc.) to enable it to be appreciated with moderation.

5.1.3 Utility section unit operations

5.1.3.1 Carbon dioxide plant

Carbon dioxide is one of the product produced during fermentation. The large amount of Carbon dioxide produced is recovered and collected in the form of liquid in the CO_2 tanker. The purpose of recovering the CO_2 is, it to be used internally for beer carbonation, Draught dispensing purpose, pushing beer via pipes, … & to protect the environment from CO_2 pollution.

Process steps in CO_2 Recovery:

- Foam Separator: separates foam from CO_2 via washing by cold water & send to the balloon.
- Balloon: It is used for temporary storage for CO_2 and balance separator & booster function.
- Booster: It is used to produce pressure to move CO_2 into gas washer.
- Gas Washer: In this case the CO_2 is washed by cold water in order to remove water soluble substance like sugar, caramel etc.
- Compressor: It sucks the gas and compresses it in to liquefying pressure (17-18 bar). Because the gas becomes very hot as a result of this, it must be cooled again in a cooler coupled to the compressor.
- Active Carbon Filter: It is used to remove moisture and any flavor in the CO_2.
- Preheater: It uses to heat CO_2.
- Condenser: It is used to liquefying carbon dioxide by the help of ammonia due to the reason that to decrease volume.
- CO_2 tanker: It is temporary storage of liquid CO_2 and its capacity 20 tons
- Evaporator: It is used to evaporate liquid CO_2 into gas, then distribute into the system. Fan is used to evaporate the liquid CO_2.
- Air Compressor: It is used to suck air from the atmosphere and clean it.
- Air Filter: It is used to filter & remove moisture.
- Dryer: It is used for drying the air and distributes into the system.

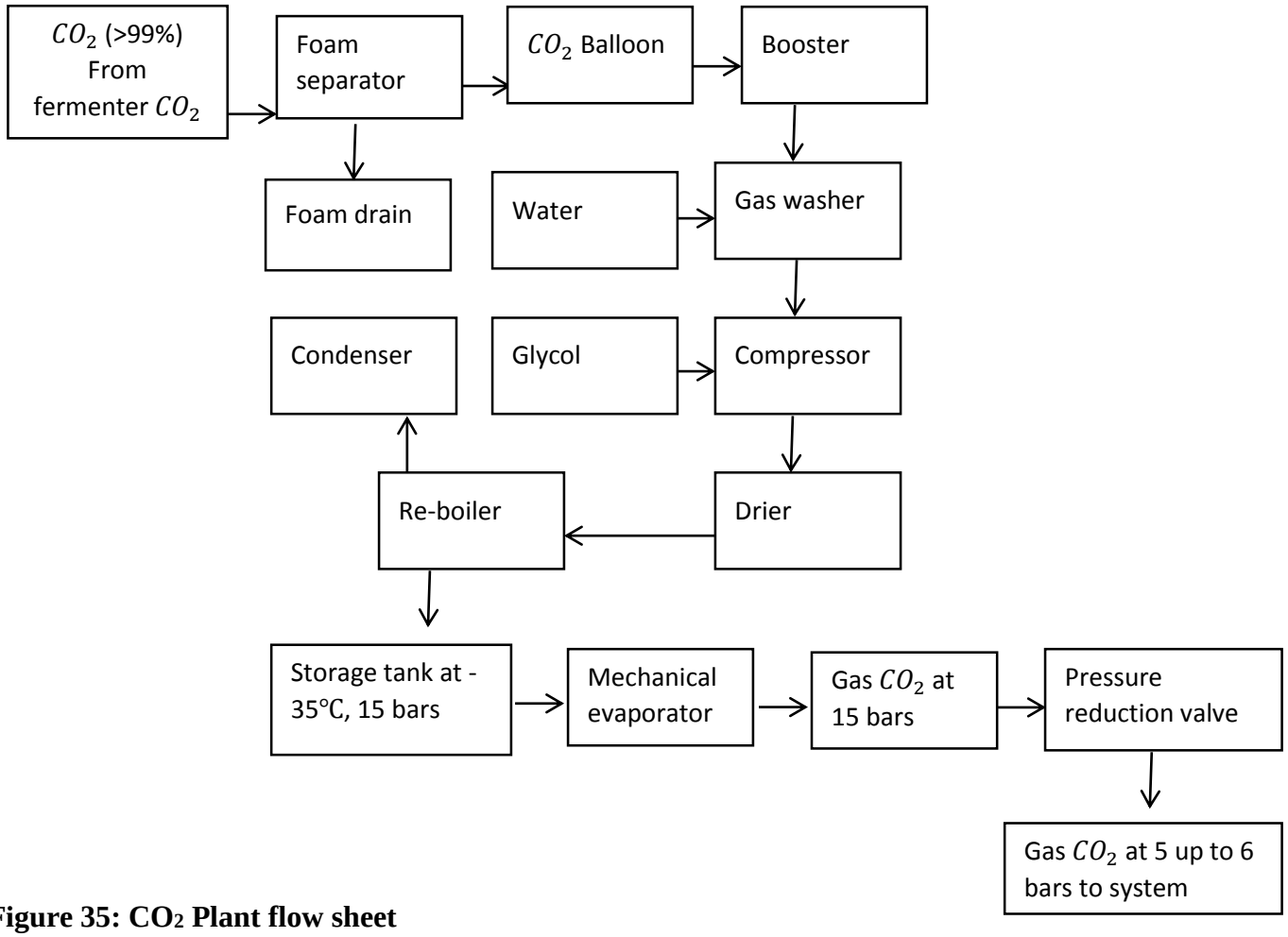


Figure 35: CO_2 Plant flow sheet

5.1.3.2 Cooling Plant

The cooling agents are ammonia and propyl glycol. The glycol & ice water are cooled by ammonia. Then, glycol uses for beer cooling, ice water for wort cooling etc. The ice water and the degased water are cooled in this plant. Glycol is a cooling chemical that is used in overall cooling of the sections which are located.

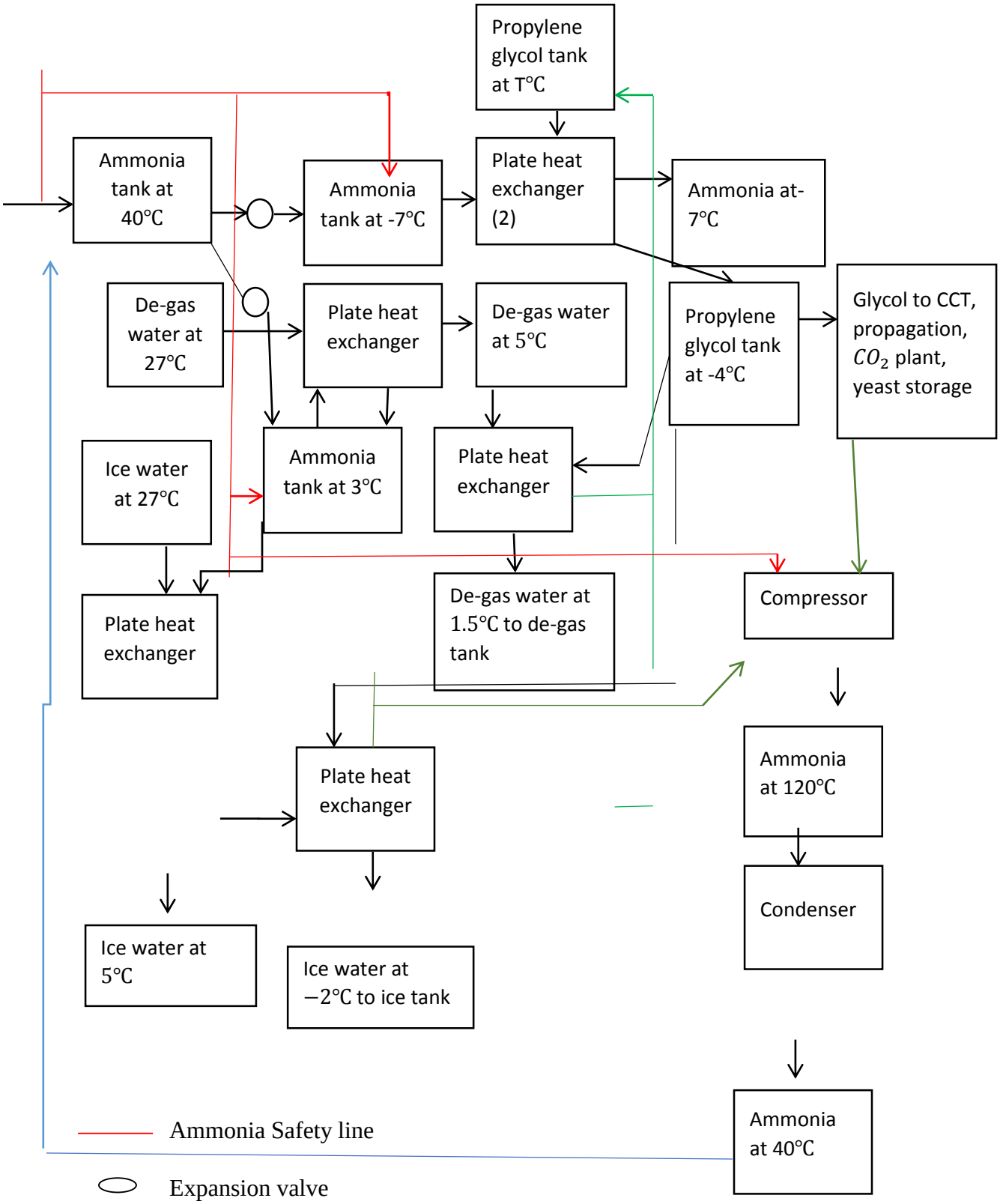


Figure 36: Flow sheet of cooling plant

5.1.3.3 Wastewater Treatment Plant

Waste water is a water collected from all the processing units after cleaning or discharging.

Waste water Treatment process steps are described as the solid wastes are mechanically separated by size, pH adjustment is done in the first tank i.e. Equalization tank & the other biological rectors are done in the Denitrification, Aeration & Sludge tanks.

De nitrification: Primary settling: separates fine solids by gravity and density difference and sent the sludge to sludge tank. De nitrification part: a point where Nitrogen gas is removed from nitrates where active sludge (active microbes and nitrates) is mixed here.

- Aeration (Nitrification): A process by which air is supplied for Chemical oxidation & Biological decomposition.
- Sludge tank: sludge is collected here in primary settling and Dewatering process will continue to remove water sludge by the addition of flocculants which are used to form flocks of sludge and make easy for pressing.

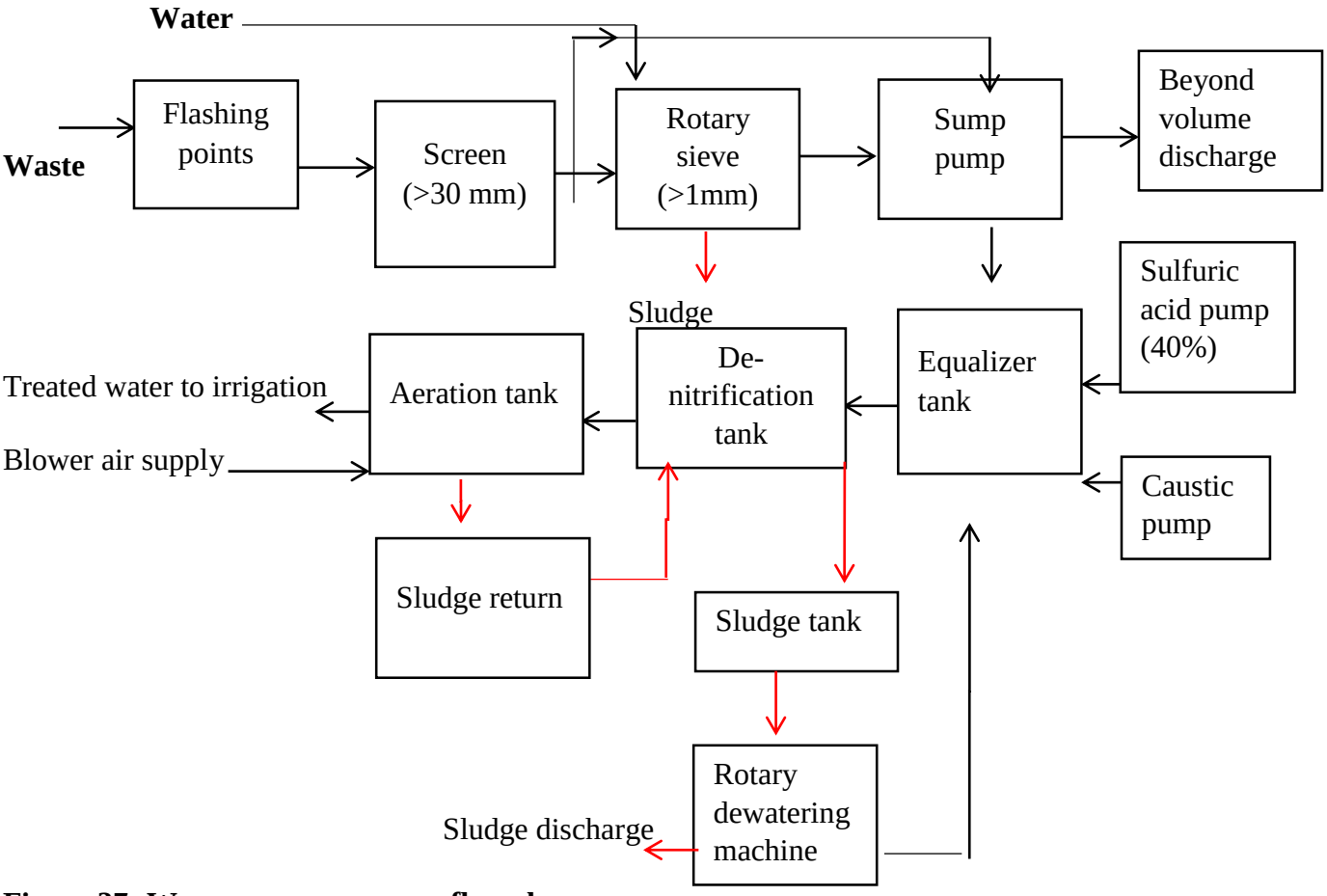


Figure 37: Wastewater treatment flow sheet

5.1.3.4 Water Treatment Plant

In water treatment plant, water is treated in the way of removal of suspended matters, removal of microorganisms and removal of dissolved materials. As the water being used is from spring source, its quality in purity & mineral composition is fit for brewing without any complicated treatment. The suspended matters are removed using mechanical filter & pressure sand filters. After the physical treatment chlorination is done & then chorine is dechlornated using activated carbon media. Ultra violet ray is fitted at the out let of the treatment plant so as to kill any microorganism that could escape from the chemical treatment.

Chlorine and coagulant

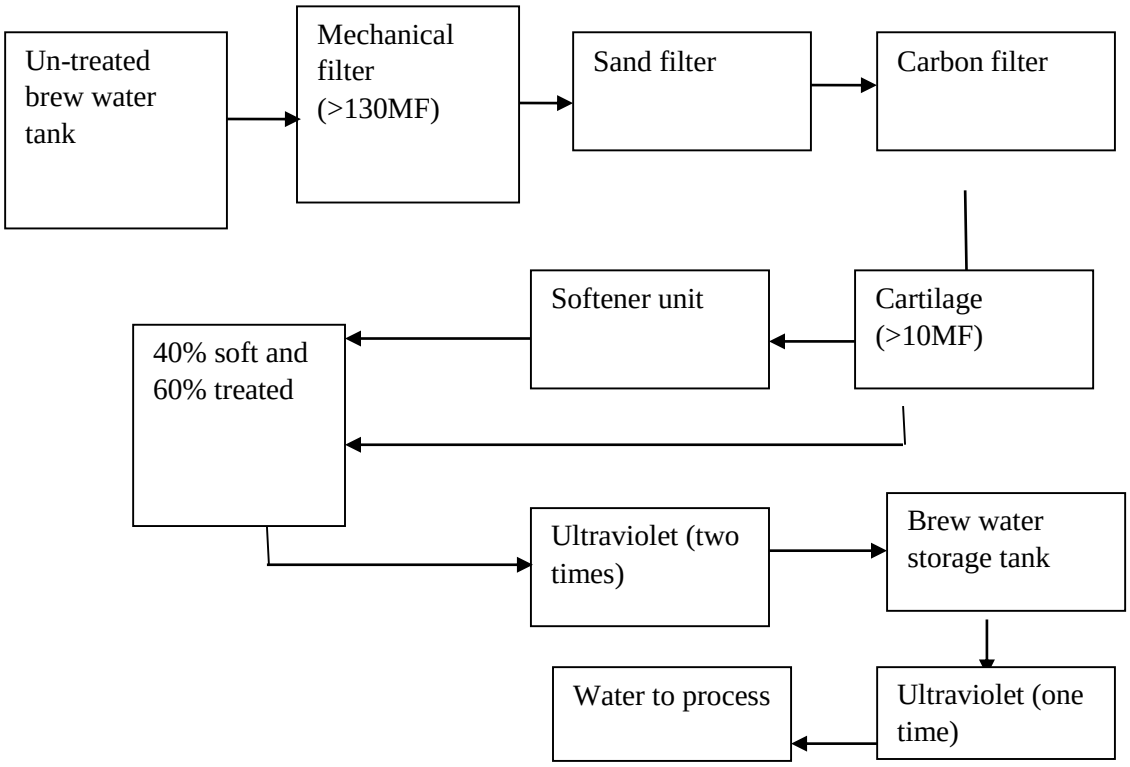


Figure 12: Brew water treatment flow sheet

5.1.3.5 Boiler Plant

This section is steam generation plant from treated water at high temperature about 150 C – 160 C and distribute into other plants that need steam. The fire tube boiler fit for the brewery uses diesel oil, butane gas, automation air and electrode to produce the required steam. Softener is fit to decrease the hardness of the water by using resin after the water is treated in water treatment plant. The condenser in the boiler plant is used to collect all condensates as reservoir to heat the soft water to save energy before it is sent to boiler.

5.2 MATERIAL & ENERGY BALANCE

5.2.1 Material Balance

Plant capacity of the Brewery is 600, 000 hl per annum. Supply of the product is limited to less number of consumers mainly around the region & the daily production is 2,000 hl out of which 20 % is served in the form of keg & 80 % in the form of bottle.

- The annual production considering 300 working days per annum**.**

  = (2,000 hl/ day) * (300 day /annum) = <u>600,000 hl / annum</u>

<u>Raw material requirement</u>

1 hl beer = 16 kg of malt

2,000 hl beer = X ?

$$X = \frac{2{,}000\ \text{hL} * 16\ \text{kg of malt}}{1\text{hL}}$$

X = <u>32,000 kg malt</u>

Hence, for 2,000 hl per day, 32,000 kg malt per day is needed.

For the sake of safety the brewery handles the malt only for six months;

32,000 kg malt per day * 150 days = <u>4,800,000 kg</u> of malt i.e. (4,800 ton)

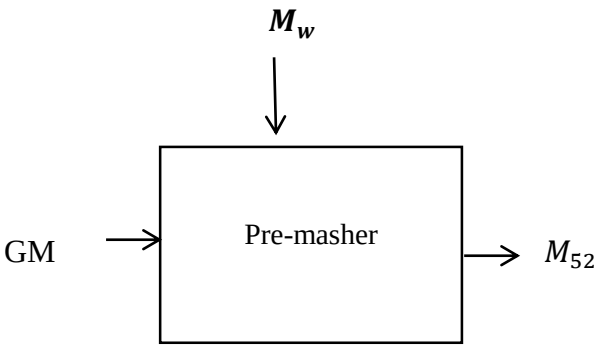
Mass balance analyses

Assumptions:

- ✓ Analysis is done for one brew
- ✓ Mass loss in mash filter as a vapor during spent grain discharge is not considered because it is to small
- ✓ Mass loss during sampling not considered because it is too small
- ✓ 3.5% mass loss is assumed in fermentation
- ✓ Four brew CCT is used for mass calculations
- ✓ Real density of our mash is 20^0 *P* and that of wort is 15^0 P
- ✓ 85% of our wort is water, and 75% of the 15% is fermentable

Pre-masher

Input	**output**
Water volume (V_w) = 140 hl	mash at 52 ℃(M_{52})
Grist malt (GM) at 20 ℃ = 5000 Kg	



Since no chemical reaction, no consumption and generation term, also not anything to accumulate in the pre-masher. So the general mass balance equation becomes

Input = Output

Mass of water(M_w) calculation

Water density (ρ_w) at 52 ℃, 987 Kg/m^3

Correlation

1m^3= 10 hl

$M_w = \rho_w \times V_w$

$= 987 Kg/m^3 \times 140\ hl \times \frac{1\ m^3}{10\ hl}$

= 13,818 Kg

Now mass balance

Input = output

GM+M_w= M_{52}

M_{52}= 18,818Kg

Mash kettle

Input

M_{52} = 18,818 Kg

mass of additives (M_{ad}) = 18.5 Kg

NB. Additives are $CaCl_2(4Kg)$, $Caso_4(2Kg)$, ceramix (0.5 Kg), H_3PO_4(optional) 6-8 L of 85 % H_3PO_4 solution having a density 1.5 Kg/m^3 taking 8 L the calculated mass is 12 Kg

Output

Mash at 76 ℃ (V_{76}), 165 hl having an original gravity of 20 ^oP and this is corresponding to 1.083 specific gravity (SG). Therefore it is possible to calculate its density and mass as follows

Take density of reference (ρ_{reff}) density of water at room temperature 1000 Kg/m^3

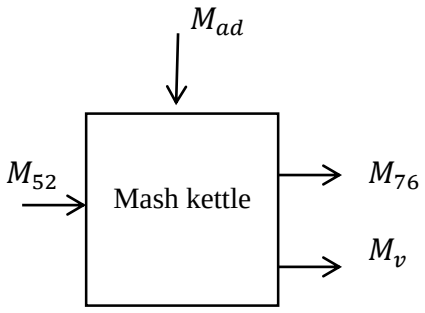
SG = $\frac{\rho_{mash\ 76}}{\rho_{reff}}$

$\rho_{mash\ 76}$= SG× ρ_{reff}= 1.083×1000 Kg/m^3= 1,083 Kg/m^3

Now calculate mass of mash at 76℃ (M_{76})

M_{76}=$\rho_{mash\ 76} \times V_{76}$), = 1,083 Kg/m^3 ×165 hl× $\frac{1m^3}{10\ hl}$ = 17,869.5 Kg

Mass of vapor (M_v) =?



Now mass balance is done in Masher

Input = Output

$M_{52} + M_{ad}$= $M_{76} + M_v$

$18{,}818Kg + 18.5\ Kg = 17{,}869.5\ Kg + M_v$

M_v= 967 Kg i.e $\frac{967}{967+17{,}869.5} \times 100$ = **5.13** %

Mash filter

Input

M_{76} = 17,869.5 Kg

Sparging water (V_s), 130 hl which is hot water at 78 ℃ and its density (ρ_s) at this temperature is

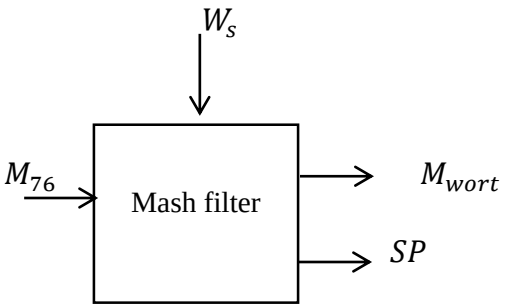
$972.85 Kg/m^3$

Output

Wort volume at 76 ℃ (V_{wort}), 271 hl having an original gravity of 15 ^oP which is corresponding to a specific gravity value of 1.061

Spent grain (SP) =?

During spent grain discharging, there is vapor loss but since too little, it is not considered in the calculation.



First calculate mass of sparging water and wort

Mass of sparging water

W_s= $V_s \times \rho_s$ = 130 hl× 972.85 Kg/m^3 $\times \frac{1m^3}{10\ hl}$ = 12,647.05Kg

Mass of wort

Density of wort (ρ_{wort}) = SG× ρ_{reff} = 1.061 × 1,000 Kg/m^3= 1,061 Kg/m^3

M_{wort}= $\rho_{wort} \times V_{wort}$= 1,061 $Kg/m^3 \times 271\ hl \times \frac{1m^3}{10\ hl}$ = 28,753.1 Kg

Now mass balance

Input = output

$$\boldsymbol{M_{76} + W_s = M_{wort} + Sp}$$

17869.5 Kg + 12647.05Kg = 28,753.1 Kg + $\boldsymbol{Sp}$

$\boldsymbol{Sp}$ = **1,763.45**Kg

Wort pan

Input

$\boldsymbol{M_{wort}}$ = 28,753.1 Kg

Mass of additives (M_{add}) = 14.025 Kg

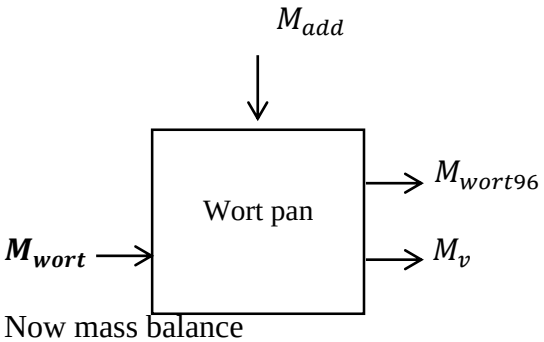
NB the additives in wort pan are hop extract (4Kg), hop pellet (10Kg) and $ZnSo_4$ (0.025Kg)

Output

Wort volume at 96 ℃ (V_{wort96}), 255 hl no change in original gravity occur so its mass becomes

M_{wort96} = $\rho_{wort} \times V_{wort}$= 1,061 $Kg/m^3 \times 255\ hl \times \frac{1m^3}{10\ hl}$ = 27,055.5 Kg

Mass of vapor (M_v) =?



Now mass balance

Input = output $\boldsymbol{M_{wort}} + M_{add} = M_{wort96} + M_v$

28,753.1 Kg + 14.025 Kg = 27,055.5 $Kg + M_v$

M_v = 1,711.625Kg

Whirlpool

Input

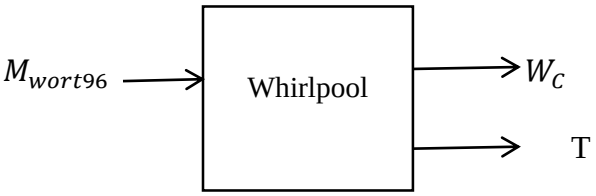
M_{wurt96}= 27,055.5 Kg

Output

Clear wort volume (V_c), 250 hl no change on density again also, so its mass (W_C)

Clear wort mass (W_C) = $\rho_{wort} \times V_{wort}$= 1,061 $Kg/m^3 \times 250\ hl \times \frac{1m^3}{10\ hl}$ = 26,525 Kg

Trub (T) =?



Now mass balance

Input = output

$M_{wort96} = W_c + T$

T= 27,055.5 Kg −26,525 Kg = 530.5Kg

When we calculate this trub in percentage $\frac{530.5}{27,055.5} \times 100$ = <u>1.96</u> %

Mass balance for 4 brews CCT (fermenter)

Input

Input mass of wort (W_{in}), 4× W_c= 106,100 Kg

Input mass of yeast (M_y), 500 Kg

Input mass of air (M_{air}), using rule of thumb for 2^0 P wort, 1mg/l air now for our case for 15^0 P wort 7.5 mg/l of air

Then mass of air = 7.5 $mg/l \times$ volume of wort = 7.5 $mg/l \times$ 100,000 l= 750,000 mg

M_{air}= 750,000 $mg \times \frac{1\ g}{1000\ mg} \times \frac{1\ Kg}{1000\ g}$= 0.75 Kg

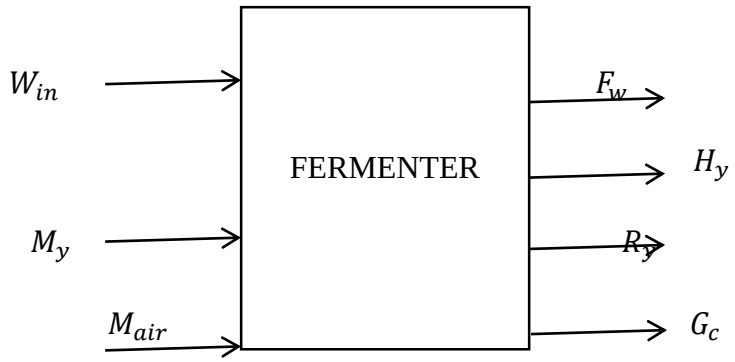
Output

Fermented wort (F_w),

Harvested yeast (H_y),

Residual yeast (R_y),

Generated $CO_2(G_c)$,



Total fermented sugar

From one brew, 85 % is water and 15 % extract and from the 15 % the 75 % is fermentable. So the amount of fermentable sugar is calculated as:

F_w= 4× 26,525 × 0.15 × 0.75 = 11,936.25 Kg this is the mass of total fermented sugar for 4 brew CCT.

Total harvested yeast

Theoretically, three times of the input yeast is to be harvest but currently 3,000 kg from 4 brew CCT and 4,000 Kg from 6 brews CCT is harvested in current situation.

So, H_y= 3,000 kg

Total carbon dioxide generated

From one hl, 2 Kg of carbon dioxide are generated which can be calculated from the equimolar equation of the chemical reactions.

So $G_c = \frac{1000\ hl \times 2\ Kg\ CO_2}{1\ hl}$= 2,000$Kg$

Now mass balance,

$W_{in} + M_{air} + M_y = F_w + H_y + R_y + G_c$ substituting numerical values

106,100 Kg + 0.75 Kg + 500 Kg = 11,936.25 Kg + 3,000Kg +2,000Kg + R_y

R_y= 89,664.5 Kg this is the total recovered and loss (Residual yeast). In fermentation during sampling, harvesting and pitching 3.5 % mass loss is expected. The recovered yeast becomes 85,933.47 Kg and the lost is 3,731 Kg

5.2.2 Energy Balance on a Selected Unit Operation

More than 50 % of the total heat consumed is in the brew house (Scheer, 2014).

Assumptions during calculation:

- ✓ Since viscosity source are $\beta - glucans$, simple sugar and pentose but not spent grains and assuming little amount of these loss with spent grains dynamic viscosity of wort is equal to that of mash
- ✓ In the course of fermentation, there is density and specific heat capacity difference but since small difference are not considered, taken as constant
- ✓ Environmental effect on the fermenter is not considered since vessels are insulated
- ✓ Fouling in plate heat exchangers is negligible due to frequent CIP
- ✓ Heat transfer analysis for conical and cylindrical is taken the same
- ✓ Equations used for actual heat loss are empirical correlations but their error is not considered
- ✓ Steam properties are evaluated using its temperature by applying interpolation
- ✓ Throughout the calculation properties such as stainless steel thermal conductivity, mineral wool thermal conductivity, mash specific heat capacity, wort specific heat capacity, steam temperatures are considered as constants
- ✓ One dimensional steady state heat transfer is considered
- ✓ Radiation heat transfer is not considered
- ✓ Vacuum formation is negligible since there is air discharge valves in mash kettle and wort pan

Hammer mill

The energy required by hammer mill is going to be calculated using bond`s equation.

First to calculate work index

Input

Classic milling two is the milling chamber

The circuit feed diameter, 2.88mm

The circuit product diameter, 2.25 mm

Input malt size (d_{in}), 88 % is 2.88 mm

Input power (W), 55 KW

Milling capacity ($C_{milling}$), 8 $tone/hr$

Grinding capacity, $tone/hr$

Input mass of malt (M_{malt}), 5000 Kg

Output

Grist size (d_{out}), 2.25 mm

Now first to calculate the input work

Work input = $\frac{W}{C_{milling}} = \frac{55\ KW}{8 tone/hr}$ = 6.875 $KWh/tone$

Then the Operating Work Index (W_{in}) of this circuit, is calculated as follows:

$W_{in} = \frac{6.875\ KWh/tone}{\left(\frac{10}{\sqrt{d_{out}}} - \frac{10}{\sqrt{d_{in}}}\right)} = \frac{6.875\ KWh/tone}{\left(\frac{10}{\sqrt{2.25\ mm}} - \frac{10}{\sqrt{2.88\ mm}}\right)}$ = 8.88 $KWh/tone$

Now to calculate the electrical energy needed to reduce from 2.88 mm to 2.25 mm

E = $M_{malt} \times W_{in} = \frac{1\ tone}{1{,}000\ Kg} \times 5{,}000\ kg \times 8.88 KWh/tone$

E = 44.4 KWh = 159,840 KJ

Mash kettle

Input

Input mash temperature ($T_{mash\ in}$) = 52 ℃

Input mash mass ($M_{mash\ in}$) = 18,818 Kg

Output

Output mash temperature ($T_{mash\ out}$), 76 ℃

Specific heat capacity of mash (Cp_{mash}) (20 °P), 3.6 $KJ/Kg.K$

In Raya Brewery the steam produced is at 145 ℃, *so* properties are to be evaluated at this temperature.

h_s Enthalpy of steam (from steam table), 2741 KJ/Kg

h_c Enthalpy of condensate (from table), 612.9 KJ/Kg

Heat of vaporization (h_{fg}) $= h_s - h_c$ = 2,128.1 KJ/Kg,

The question here is how much mass of steam (M_S) is required to heat mash from 52 ℃ to 76 ℃?

$Q= M_{mash\ in} Cp_{mash}(T_{mash\ out} - T_{mash\ in})$ = 18,818 Kg ×3.6 $KJ/Kg.K \times (349 - 325)K$

Q= 1,625,875.2 KJ

Actual energy loss calculation

Considering only conduction and convection heat transfer

$\dot{Q}= UA\Delta T$ where U⟶ overall heat transfer coefficient. A⟶ heat transfer area,

ΔT ⟶ temperature difference of system

Inside diameter (D_1) = 3,550 mm, height can be calculated as follows

Volume (V) = $\frac{\pi \times D^2 \times h}{4}$, h = $\frac{4V}{\pi \times D^2}$= $\frac{4 \times 27\ m^3}{3.14 \times (3.55\ m)^2}$= 2.73 m

Thickness (t) of the vessel can be calculated as V= $\pi ht(D - t)$ calculating using quadratic equation. Thickness of the vessel becomes, t = 0.733 m

$D_2 = D_1 + 2t_{ss}$= 3,550 mm + 2 ×733 mm = 5,016 mm

$D_3 = D_2 + 2t_{ins}$ = 5,016 mm + 2 × 100 = 5,216 mm

$D_4 = D_3 + 2t_{sscn}$ = 5,216 mm +2 × 0.8 mm = 5,217.6 mm

$$U = \frac{1}{\frac{1}{h_{mash}} + \frac{D_1 ln\left[{}^{D_2}/_{D_1}\right]}{K_{ss}} + \frac{1}{h_{steam}} + \frac{D_1 ln\left[{}^{D_3}/_{D_2}\right]}{K_{ins}} + \frac{D_1 ln\left[{}^{D_4}/_{D_3}\right]}{K_{ss}}}$$

h_{mash}, can be calculated as follows:

$h_{mash} = \left[\frac{K}{D}\right] \times 0.023Re^{0.8} \times Pr^{0.4}$ for forced convection where

K ⟶ mash thermal conductivity ($0.23W/M.K$)

D ⟶ Agitator diameter (propeller agitator here in raya brewery mash kettle)

Re ⟶ Reynolds number and can be calculated as $Re = \frac{\rho ND^2}{\mu}$, where

ρ ⟶ Mash density

N ⟶ Agitator speed ($0.433/sec$)

μ ⟶ Dynamic viscosity of mash (1.85 mpa.s)

thermal conductivity of mineral wool insulation (K_{ins}), 0.04 W/m. K

Thermal conductivity of stainless steel (K_{ss}), 150 W/m. K

Pr ⟶ Prandtl number and can be calculated as Pr = $\frac{Cp \times \mu}{K}$= where, Cp= specific heat capacity of mash

$Re = \frac{\rho ND^2}{\mu} = \frac{1083Kg/m^3 \times 0.433/sec \times (3m)^2}{0.00185\ Pa,sec}$ = 2,281,324.86

Pr = $\frac{Cp \times \mu}{K} = \frac{3.6\ KJ/Kg.K \times 0.00185\ Pa.sec}{0.23W/m.K}$ = 28.956

$h_{mash} = \left[\frac{0.23W/m.K}{3.55\ m}\right] \times 0.023(2281324.86)^{0.8} \times (28.956)^{0.4}$ = $698.99W/m^2.K$

h_{steam}, can be calculated as follows: the first task here is calculating Grashof number (Gr) since free convection

$Gr = \frac{g\beta(T_{steam} - T_{ss})L^3}{v^2}$ where, g⟶ gravity ($9.81m/sec^2$)

β ⟶ Volumetric thermal expansion of steam ($\beta = \frac{1}{T_{steam}} = 0.0024/K$)

T_{steam} = steam temperature (145 ℃)

T_{ss}= surface temperature (0 ℃)

v = kinematic viscosity of steam

L= height of mash vessel (2.73 m)

Properties evaluate at film temperature (T_M) = $\frac{T_{steam}+T_{ss}}{2} = 345.5\ K$

Prandtl number of steam (Pr_{steam}), 1.0945

Thermal conductivity of steam (K_{steam}), 22.01× $10^{-3}\ W/m.K$

Dynamic viscosity of steam (μ_{steam}), 114.5× $10^{-7}\ N.sec/m^2$

Specific heat capacity of steam (Cp_{steam}), $2.124KJ/Kg.K$

Density of steam (ρ_{steam}), $0.6426Kg/m^3$

Kinematic viscosity of steam (v_{steam}), 17.247× $10^{-6}\ m^2/sec$

$Gr = \frac{g\beta(T_{steam}-T_{ss})L^3}{v^2} = \frac{9.8m/sec^2)\times 0.0024/K(418\ K-273\ K)\times(2.7\ m)^3}{(17.247\times10^{-6}m^2/sec)^2}$ = 2.2567× 10^{11}

Now to calculate the multiple of Prandtl number and Grashof number

$Gr \times Pr_{steam}$= 2.2567× 10^{11} × 1.0945 = 2.4699 × 10^{11} this is on the range of 10^9 < ($Pr_{steam} \times Gr$) < 10^{12} so use the following empirical correlation to determine the Nusselt number

N_u= 0.12× $(Gr \times Pr_{steam})^{0.33}$ = 689.867 now to calculate steam convection coefficient

$N_u = \frac{h_{steam}\times D_{mash\ vessel}}{K_{steam}}$ rearranging for h_{steam}

$h_{steam} = \frac{N_u\times K_{steam}}{D_{mash\ vessel}} = \frac{689.867\times 22.01\times10^{-3}W/m.K}{3.55m}$ = $4.277W/m^2.K$

U = $\frac{1}{\frac{1}{698.99\ W/m^2.K}+\frac{1.227m}{150W/m.K}+\frac{1}{4.277W/m^2.K}+\frac{0.139m}{0.04W/m.K}+\frac{0.00109\ m}{150W/m.K}} = 0.269W/m^2.K$

Actual rate of energy loss in the cylindrical part (heat transfer area 15.6 m^2)

$\dot{q}_{cylinderical}$ = UAΔT = 0.265 $W/m^2.K \times 15.6\ m^2 \times (418K - 273K)$ = 599.43 W

Actual rate of energy loss in the bottom (conical) part (9.9 m^2)

$\dot{q}_{bottom}$ = UAΔT= 0.265 $W/m^2.K \times 9.9\ m^2 \times (418K - 273K)$= 380.41W

Now total actual rate of energy loss in mash kettle

$q = q_{cylinderical} + q_{bottom}$ = 979.84 W this is the rate only to determine the amount you should record the time the mash takes in the mash kettle. Currently in the brewery, mashing takes 155 minute. So now to calculate the amount of energy

Q= q× $time$ = 979.84 $J/sec \times 155\ minute \times \frac{60\ sec}{1\ minute}$ = 9,112.512 Kg this is the total amount of loss during mashing which is 0.56 %. Therefore the total amount of heat to be transferred to the mash kettle is

Q = 1,625,875.2 Kg

Now to calculate mass of steam required

$Q = M_s h_{fg}$ rearranging for M_s

$M_s = \frac{Q}{h_{fg}} = \frac{1{,}625{,}875.2\ \text{KJ}}{2{,}126.1\ \text{KJ/Kg}}$ = 764.758 Kg this is the total amount of steam required for one mashing

Wort pan

Input

Since sparging water at higher temperature 78 ℃ in mash filter is used and not for long time wort stay in wort collector input wort temperature ($T_{\text{wort in}}$) is, 76 ℃

Input mass of wort(M_{wort}) = 28,753.1 Kg

Specific heat capacity of wort (Cp_{wort}) = 4.1 KJ/Kg. K

Output

Output temperature of wort ($T_{\text{wort in}}$) = 96 ℃

The same question as in mash kettle is applied

How much mass of steam (M_s) is required to heat the wort from 76 ℃ to 96 ℃

$Q = M_{\text{wort}} Cp_{\text{wort}} (T_{\text{wort out}} - T_{\text{wort in}})$= 28,753.1 Kg × 4.1 KJ/Kg. K × $(369 - 349)$

Q = 2,357,754.2 KJ

Actual energy loss calculation

The same procedure to calculate height and thickness of vessel

t = 0.83 mm and h = 3.2 m

Inside diameter (D_1) = 4 000 mm

$D_2 = D_1 + 2 \times t_{ss} =$ 4000 mm + 2× 830 mm = 5660 mm

$D_3 = D_2 + 2 \times t_{ins}$ = 5660 mm + 2 × 100 mm = 5860 mm

$D_4 = D_3 + 2 \times t_{ssc}$ = 5860 mm + 2 × 0.8 mm = 5861.6 mm

Heat transfer area (A) = 34 m^2

System temperature difference (ΔT) = 145 ℃

Now to calculate the overall heat transfer coefficient:

$$U = \frac{1}{\frac{1}{h_{steam\ side}} + \frac{1}{h_{wort}} + \frac{D_1 ln[D_2/D_1]}{K_{SS}} + \frac{D_1 ln[D_3/D_2]}{K_{ins}} + \frac{D_1 ln[D_4/D_3]}{K_{ss}}}$$

h_{wort}, can be calculated as follows: for determining this first volumetric flow rate of wort ($\dot{V}_{wort}$) during recirculation must be determined because the causes of turbulence in wort pan are this circulation with the help of internal boiler spreader. It is believed that rate of evaporation is equal to rate of boiling. So

$$\dot{Q}_{boiling} = \dot{Q}_{evaporation}$$

$$\dot{V}_{wort} \times \rho_{wort} \times Cp_{wort} \times \Delta T = V_{cast\ out} \times E \times \rho_{water} \times h_{water}$$

Where:

h_{water}, heat of vaporization of water (2,261.1 KJ/Kg)

ρ_{water}, Water density at 96 ℃ (960 Kg/m^3)

E, Evaporation rate (5.6% per hour from mass balance above)

$V_{cast\ out}$ Volume of wort transferred to whirlpool (255 hl)

ΔT, Temperature difference between inlet and outlet wort (20 K for first and second brew only)

ΔT, Temperature difference between inlet and outlet wort (7 K above second brew)

n, number of recycle or circulation ($\frac{\dot{V}_{wort}}{V_{cast\ out}}$)

Now rearranging for $\frac{\dot{V}_{wort}}{V_{cast\ out}}$

$\frac{\dot{V}_{wort}}{V_{cast\ out}}$= n = $\frac{E \times \rho_{water} \times h_{water}}{\rho_{wort} \times Cp_{wort} \times \Delta T} = \frac{0.08/hour \times 960\ Kg/m^3 \times 2{,}275\ KJ/Kg}{1{,}061 Kg/m^3 \times 4.1 KJ/Kg.K \times 20\ K} = 2.1\ recycle/hr =$
$3\ recycle/hr$

Now to calculate volumetric flow rate

$\dot{V}_{wort}$= n× $V_{cast\ out}$= $3\ recycle/hr\ \times 255\ hl$ = $765\ hl/hr$ = $0.02\ m^3/sec$

So the number of brew above second brew the number of recycle is $6\ recycle/hr$ and the volumetric flow rate is $0.0425\ m^3/sec$

h_{wort}= $\left[\frac{K}{D}\right] \times 0.023 Re^{0.8} \times Pr^{0.4}$ for forced convection where

K ⟶ wort thermal conductivity ($0.23 W/M.K$)

D ⟶ diameter of circulation pipe (191.5mm)

Re ⟶ Reynolds number and can be calculated as $Re = \frac{4\rho_{wort}\dot{V}_{wort}}{\pi D \mu_{wort}}$, where

μ_{wort} ⟶ Dynamic viscosity of wort (1.85mpa.s)

$Re = \frac{4\rho_{wort}\dot{V}_{wort}}{\pi D \mu_{wort}} = \frac{4 \times 1061 Kg/m^3 \times 0.02 m^3/sec}{\pi \times 0.1915 m \times 0.00185\ Pa.sec}$ = 76,301.87

Pr = $\frac{Cp \times \mu}{K} = \frac{4.1\ KJ/Kg.K \times 0.00185\ Pa.sec}{0.23 W/m.K}$ = 32.98

h_{wort}= $\left[\frac{0.23 W/m.K}{0.1915 m}\right] \times 0.023 (53411)^{0.8} \times (32.98)^{0.4}$ = $677.16\ W/m^2.K$

h_{steam}, can be calculated as done in mash kettle since free convection

$Gr = \frac{g\beta(T_{steam} - T_{ss})L^3}{v^2}$

$\beta \longrightarrow$ Volumetric thermal expansion of steam ($\beta = \frac{1}{T_{steam}} = 0.0024/K$)

T_{steam} = steam temperature (145 ℃)

T_{ss}= surface temperature (0 ℃)

L= height of mash vessel (3.2 m)

Properties evaluate at film temperature (T_M) = $\frac{T_{steam}+T_{ss}}{2} = 345.5\ K$

Prandtl number of steam (Pr_{steam}), 1.0945

Thermal conductivity of steam (K_{steam}), 22.01× $10^{-3}\ W/m.K$

Dynamic viscosity of steam (μ_{steam}), 114.5× $10^{-7}\ N.sec/m^2$

Specific heat capacity of steam (Cp_{steam}), 2.124 $KJ/Kg.K$

Density of steam (ρ_{steam}), 0.6426 Kg/m^3

Kinematic viscosity of steam (v_{steam}), 17.247× $10^{-6}\ m^2/sec$

$Gr = \frac{g\beta(T_{steam}-T_{ss})L^3}{v} = \frac{9.8m/sec^2)\times 0.0024/K(418\ K-273\ K)\times(3.2\ m)^3}{(17.247\times10^{-6}m^2/sec)^2}$ = 3.7569× 10^{11}

Now to calculate the multiple of Prandtl number and Grashof number

$Gr \times Pr_{steam}$= 3.7569× 10^{11} × 1.0945 = 4.112 × 10^{11} this is on the range of 10^9 < (Pr_{steam} × Gr) < 10^{12} so use the following empirical correlation to determine the Nusselt number

N_u= 0.12× $(Gr \times Pr_{steam})^{0.33}$ = 816.24 now to calculate steam convection coefficient

$N_u = \frac{h_{steam}\times D_{mash\ vessel}}{K_{steam}}$ rearranging for h_{steam}

$h_{steam} = \frac{N_u\times K_{steam}}{D_{mash\ vessel}} = \frac{816.24\times 22.01\times10^{-3}W/m.K}{4\ m}$ = 4. 49 $W/m^2.K$

Now to calculate overall heat transfer coefficient

$$U = \frac{1}{\frac{1}{h_{steam\ side}} + \frac{1}{h_{wort}} + \frac{D_1 ln\left[{}^{D_2}/_{D_1}\right]}{K_{SS}} + \frac{D_1 ln\left[{}^{D_3}/_{D_2}\right]}{K_{ins}} + \frac{D_1 ln\left[{}^{D_4}/_{D_3}\right]}{K_{ss}}}$$

$$U = \frac{1}{\frac{1}{4.49\ W/m^2.K} + \frac{1}{677.16\ W/m^2.K} + \frac{1.388\ m}{150\ W/m.K} + \frac{0.1389\ m}{0.04\ W/m.K} + \frac{0.00109m}{150\ W/m.K}} = 0.27\ W/m^2.K$$

Now the actual heat loss

q= UAΔT= $0.27 W/m^2.K \times 34 m^2 \times (418\ K - 273\ K)$= 1,331.1W this is rate of actual loss. Wort boiling takes 165 minutes so to calculate amount of energy

Q = q× $t = 1{,}331.1\ J/sec \times 165\ minute \times \frac{60\ sec}{1\ minute}$ = 13,177.890 KJ this is the total amount of heat loss for first brew. Therefore, the total amount of heat required to heat the wort is 2,370,932.09 KJ

Now to calculate mass of steam required

Q = $M_s h_{fg}$ rearranging for M_s

$M_s = \frac{Q}{h_{fg}} = \frac{2{,}370{,}932.09\ KJ}{2{,}128.1 KJ/Kg}$ = 1,114 Kg

This amount of steam is only required in first brew from experience because from third brew energy water is going to be used and in second brew the surface is already heated. During this time input wort temperature to wort pan is 88 ℃. Now to calculate the amount of steam required to reach this 96 ℃

Q = $M_{wort} Cp_{wort}(T_{wort\ out} - T_{wort\ in})$= 28,753.1 Kg ×4.1KJ/Kg. K × $(369 - 361)$

Q = 943,101.68 KJ

Calculating the actual loss in the same manner

7 ℃ temperature difference between inlet and outlet of wort pan; the actual energy loss becomes 2,968.812 KJ

The total amount of heat required to heat the wort becomes, 946,070.492 KJ

Now to calculate mass of steam required

Q = $M_s h_{fg}$ rearranging for M_s

$M_s = \frac{Q}{h_{fg}} = \frac{946{,}070.492\ KJ}{2{,}128.1 KJ/Kg}$ = 444.56 Kg

How much energy do we save due to energy water comparing first brew and third brew?

Energy saved = energy used in 1^{st} brew − energy used in 3^{rd} brew

Energy saved = 2,370,932.09 KJ − 946,070.492 KJ= 1,424,861.598 KJ

In mass of steam

Mass of steam saved = M_s in 1^{st} brew − M_s in 3^{rd} brew

Mass of steam saved = 1114 Kg −444.56 Kg = 669.44 Kg

From this one it is concluded that even though there is to high mass loss in wort pan there is also very high energy saving by recovering the lost mass and making energy water.

Energy required for preparing hot water to start brew

Normally in the brewery this is done by plate heat exchanger using steam as a heating fluid.

Input data

Minimum water volume to be heated, 450 hl at room temperature ($T_{water\ in}$) 20 ℃ water density at 20℃, 998.29 Kg/m^3so mass of water (M_{water}), 44,923.05 Kg

Desired water temperature ($T_{water\ mash}$), 78 ℃

Specific heat capacity of water (Cp_{water}), 4.18 KJ/Kg. K

Assume: no heat loss, neglect fouling effect on the plate heat exchanger

Heat of vaporization of steam (h_{fg}) evaluated at its temperature (145℃), 2,126.33 KJ/Kg

Q = $M_{water} Cp_{water}(T_{water\ mash} - T_{water\ in})$ = 44,923.05 Kg ×4.18 KJ/Kg. K (78 ℃ − 20 ℃)

Q = 10,891,144.242 Kg, now to calculate steam required

Q = $M_s h_{fg}$ rearranging for M_s

$M_s = \frac{Q}{h_{fg}} = \frac{10{,}891{,}144.242\ KJ}{2{,}126.33\ KJ/Kg}$ = 5,122 Kg this is the amount of steam required for starting batch process brew.

Energy required to cool wort using the provided single stage plate heat exchanger

Input data

Wort mass (M_{wort}), 26,525 Kg

assuming that no heat loss in whirlpool due to fast settling of trub because of gratings in the whirlpool; 20 minute rest in whirlpool the least recommended by whirlpool operation. Inlet wort temperature ($T_{wort\ in}$), 96 ℃

Specific heat capacity of wort (Cp_{wort}), 4.1 KJ/Kg. K

Outlet wort temperature ($T_{wort\ out}$), 8 ℃

Specific heat capacity of water (Cp_{water}), 4.18 KJ/Kg. K

Inlet water temperature ($T_{water\ in}$), 2 ℃

Outlet water temperature ($T_{water\ out}$), 78 ℃

Water mass (M_{water}),

Assuming that no energy loss means all the energy released from wort is accepted by the ice water and since CIP of the wort line is done frequently again assume no fouling in the heat exchanger even though water is highly fouling.

Now, $Q_{water} = Q_{wort}$

$$M_{water}Cp_{water}(T_{water\ out} - T_{water\ in}) = M_{wort}Cp_{wort}(T_{wort\ in} - T_{wort\ out})$$

Substituting numerical values,

$M_{water} \times 4.18\ \text{KJ/Kg. K} \times (78\ ℃ - 2\ ℃) = 26{,}525\ Kg \times 4.1\text{KJ/Kg. K}\ (96\ ℃ - 8\ ℃)$

$M_{water} = \frac{9570220\ KJ}{317.68 KJ/Kg} = 30125.35\ Kg$ this is minimal amount of hot water recovered from wort cooling, evaluating density of this hot water at 78 ℃ from table, 972.85 Kg/m^3

Now to calculate volume of the recovered hot water, V= $\frac{mass}{density} = \frac{30{,}123.35\ Kg}{972.85\ Kg/m^3} = 30.966\ m^3$

Or 309.66 hl

The energy recovered is then calculated as follow

$Q_{water} = M_{water}Cp_{water}(T_{water\ out} - T_{water\ in})$

$= 30{,}125.35\ Kg \times 4.18\text{KJ/Kg. K} \times (78\ ℃ - 2\ ℃)$

$Q_{water} = 9{,}570{,}221.2\ KJ$

Heat generated by fermentation in 4 brew CCT

Input data

Real density of the wort in the beginning = 15 °P (15%)

Real density of the wort after fermentation = 3.5 °P (3.5%)

Difference in sugar content (ΔE) = 11.5%

Energy generated by yeast (e) 586.6 $KJ/Kg\ of\ fermented\ sugar$

Volume of wort (V) = 1,000 hl

Density of wort = 1,061 Kg/m^3 now calculating the mass,

M_{wort}, 106100Kg

Specific heat capacity of wort (Cp_{wort}) = 4.1 KJ/Kg. K

Temperature of wort before fermentation (T_{before}) = 8 ℃

Temperature after fermentation (T_{after}) = 12.5 ℃

Energy gained while heating up to primary fermentation temperature:

$Q_1 = M_{wort}Cp_{wort}(T_{before} - T_{after})$ = 106,100 Kg ×4.1 KJ/Kg. K × (8 ℃ − 12.5 ℃)

Q = -1,957,545 KJ

The negative sign indicates no energy is delivered to the system and the quantity is the Energy gained while heating up to primary fermentation temperature:

Energy, that has to be discharged during primary fermentation:

$\boldsymbol{Q_2 = M_{wort} \times e \times \Delta E}$ = 106,100 Kg ×586.6 KJ/Kg × 0.115 = 7,157,399.9 KJ

Energy required to cool down to maturation temperature (0 ℃ in Raya brewery):

$Q_3 = M_{wort}Cp_{wort}(T_{after} - T_{maturation})$= 106,100 Kg ×4.1 KJ/Kg. K × (12.5 ℃ − 0 ℃)

Q = 5,437,625 KJ; total amount of energy to be drawn from the system using propylene glycol

Data of propylene glycol

30% glycol so for this glycol specific heat capacity (Cp_{glycol}), 3.91 $KJ/Kg.K$

Inlet temperature ($T_{glycol\ in}$) = - 4 ℃

Outlet temperature ($T_{glycol\ out}$) = 2 ℃

Now to calculate the amount of glycol (M_{glycol}); assuming no loss due to good quality insulation by polyurethane foam with covering by trapezoidal aluminum sheet.

Q = $M_{glycol} Cp_{glycol} \left(T_{glycol\ out} - T_{glycol\ in}\right)$ rearranging for M_{glycol}

$M_{glycol} = \frac{Q}{Cp_{glycol}\left(T_{glycol\ out} - T_{glycol\ in}\right)} = \frac{5{,}437{,}625\ KJ}{3.91\ KJ/Kg.K \times (2℃ - (-4))} = 231{,}782.82\ Kg$

Therefore, this is the amount of glycol required for fermentation of 4 brew CCT.

5.3 COST ANALYSIS

Table 11: Malted barley Requirement per annum (16 **Kg/HL)**

Amount (Kg)	Unit	Unit Price	Total Price (Birr)
9,600,000	Kg	20.00	**192,000,000.00**

Table 12: 60 % Raw Barley & 40 % Malted barley Requirement per annum

Type	Amount	Unit	Unit Price	Total Price (Birr)	Gross Total
Raw barley	3,456,000	Kg	12	73,958,400.00	
Malted barley	1,536,000	Kg	20	76,800,000.00	
Ceremix Plus	864	Kg	585.60	902,292.48	**153,719,509.44**
Ondea Pro	2,592	Kg	445.40	2,058,816.96	

Note:

i. The market price of raw barley is 10 birr for feed barley but 12 birr is considered for malt barley considering different costs like motivating farmers, agricultural experts & additional manpower of agronomist expert for the Brewery.

ii. The average moisture content of the current malt is 5 % whereas the average moisture content of the raw barley is 12 % viz moisture difference of 7 %. Therefore, additional 7 % of raw barley is considered to compensate the final extract due to the moisture difference.

Table 13: Comparison between the two scenarios

1st Scenario (using 100 % Malt)	2nd Scenario (using 60 % Raw Barley)	Difference (Birr)
192,000,000.00	153,719,509.44	**38,280,490.56**

Therefore, using 60 % of Raw Barley can make the company profitable by ***38,280,490.56*** Birr annually.

CHAPTER SIX

6 CONCLUSSIONS & RECOMMENDATIONS

6.1 CONCLUSSIONS

The use of the above-mentioned enzymes can help to use high proportions of raw barley as substituent to malted barley to produce high quality beer. This offers the brewer considerable savings & quality improvement. These exogenous enzymes can also solve the problems that arise when under modified malts are used.

Barley as adjunct containing starch with low gelatinization temperatures can be mashed together with the malt in the mashtun, where the temperatures applied are high enough to bring about the gelatinization of the raw barley starches. The malt proportion may be so small that there will not be enough diastatic power to bring about sufficient attenuation of the wort with small malt proportions. There is a high risk that insufficient nitrogenous compounds will be dissolved in the wort to provide good yeast nutrition, as normally only 30 – 40 % of the malt nitrogen is dissolved during mashing.

As shown in this paper, wort production from grists containing high levels of raw barley offers many challenges to the brewer. However, optimal levels of filterability, β-glucan breakdown, fermentable sugars, and α-amino nitrogen can be achieved with careful optimization of enzyme types, dosage levels, and mashing conditions with a large proportions of unmalted grain as adjunct.

6.2 RECOMMENDATIONS

Studies are recommended on the areas of:

- ✍ Willingness of breweries in our country to use malt substitutes,
- ✍ Awareness creation on the society for cereal crops consumption culture, and
- ✍ Awareness creation on the society as the most important part of grain is the internal content for using raw material substitution like maize, sorghum, rice, millets …

Potentially targeted beneficiaries from the outcome of this research are mainly:

1st Producers: to increase productivity & profitability

2nd Breweries: to have a choice of raw material substitution

3rd The country: to save hard currency

Further recommended studies:

Ethiopia is endowed with lot of agricultural resources among which crop farming is the most favored. Over 85 % of our populations is engaged in farming but farmers engaged in the production of cereal crops like barley, sorghum, maize, Finger millet, Pearl millet …are not motivated as the yield of these crops per a given area & the market demand are limited. These locally produced crops are insignificant quantity (underutilized) in the brewing technology. Therefore, it needs further study on possibility for enhancement and prospects for utilization of these crops for value-added products (beer) for economic, technological development, optimization of agronomical benefits of the crops and fermentability capacities.

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Appendix A

Table A1 ANOVA table for Yield of Extract of wort

Source	Sum of squares	DF	Mean square	F value	Prob > F	
Model	66.81	7	9.54	152.72	< 0.0001	Significant
A	17.68	1	17.68	282.91	< 0.0001	
B	13.80	1	13.80	220.83	< 0.0001	
C	27.73	1	27.73	443.76	< 0.0001	
AB	0.43	1	0.43	6.83	0.0188	
AC	0.06	1	0.06	0.96	0.3418	
BC	6.83	1	6.83	109.23	< 0.0001	
ABC	0.28	1	0.28	4.51	0.0497	
Pure Error	1.00	16	0.062			
Cor Total	67.81	23				

Std. Dev.	0.25	R-Squared	0.9853
Mean	77.67	Adj R-Squared	0.9788
C.V. %	0.32	Pred R-Squared	0.9668
PRESS	2.25	Adeq Precision	35.796

Table A2 ANOVA table for FAN of wort

Source	Sum of squares	DF	Mean square	F value	Prob > F	
Model	18124.42	4	4531.11	671.30	< 0.0001	Significant
A	517.08	1	517.08	76.61	< 0.0001	
B	11713.00	1	11713.00	1735.33	< 0.0001	
C	5051.80	1	5051.80	748.44	< 0.0001	
BC	842.53	1	842.53	124.82	< 0.0001	
Residual	128.24	19	6.75			
Lack of fit	15.44	3	5.15	0.73		
Pure Error	112.80	16	7.05			
Cor Total	18252.67	23				

Std. Dev.	2.6	R-Squared	0.9930
Mean	117.83	Adj R-Squared	0.9915
C.V. %	2.20	Pred R-Squared	0.9888
PRESS	204.62	Adeq Precision	69.557

Table A3 ANOVA table for Degree of Fermentability

Source	Sum of squares	DF	Mean square	F value	Prob > F	
Model	171.54	7	24.51	891.12	< 0.0001	Significant
A	56.43	1	56.43	2051.88	< 0.0001	
B	62.73	1	62.73	2280.97	< 0.0001	
C	44.83	1	44.83	1630.06	< 0.0001	
AB	5.23	1	5.23	190.06	< 0.0001	
AC	1.13	1	1.13	40.97	< 0.0001	
BC	0.67	1	0.67	24.24	0.0002	
ABC	0.54	1	0.54	19.64	0.0004	
Pure Error	0.44	16	0.028			
Cor Total	171.98	23				

Std. Dev.	0.17	R-Squared	0.9974
Mean	76.25	Adj R-Squared	0.9963
C.V. %	0.22	Pred R-Squared	0.9942
PRESS	0.99	Adeq Precision	91.217

Appendix B

Questionnaire for Overall Acceptability Ranking Test

Date	Sample ID	Appearance	Smell	Taste	Mouth-fullness	Drinkability

Notes:

i. **Definitions**

- **Appearance: Color, Clarity, Foam…**
- **Smell: Aroma of malt, hop, yeast…**
- **Taste: sweet, bitter, sour, alkaline….**
- **Mouth-fullness: Ea, CO_2**

ii. **Taste Ranking**

- **-1 Not good**
- **0 Normal**
- **+1 Excellent**