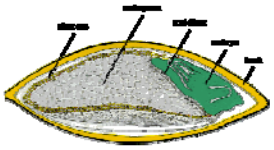

ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

FACULTY OF THECNOLOGY

DEPARTMENT OF CHEMICAL ENGINEERING

Utilization of Starch from Selected Crops as a Partial Substitute for Barley Malt in Brewing Technology

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A thesis submitted to the school of graduate studies of Addis Ababa University in partial fulfillment of the requirements for the degree of Masters of Science in Chemical Engineering (Food Engineering)

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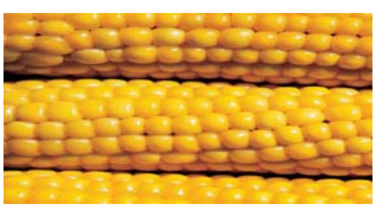

June, 2009

Addis Ababa

Acknowledgment

I thank the almighty God who gave me strength and encouragement and led me through all the rough and difficult times to complete this study.

I am very grateful to my advisors, Ato Adamu Zegeye and Dr. Amare Gessesse, for their endless help in correcting, commenting, criticizing and encouraging to accomplish this work. I also like to appreciate their patience in following up this work from the very beginning to the end. With out their dedication this work would not have been possible.

The financial assistance of the School of Graduate Studies of Addis Ababa University which made possible the implementation of this work is gratefully acknowledged.

I am greatly indebted to my family who support me all the time and to staff of Quality Assurance Department (chemists and microbiologists) in Meta Abo brewery S.C,Oljira Dinegde,Haimanot Kebede,Habtu Bazezewu,Elias Fekadu ,Mulugeta Bogale ,Woineshet Zewudie ,Mekonnen Kassa,Mekonnen Tsega,Teka Lemma ,Minda Getaneh,and Meazash Kassahun for their constructive comments, cooperation, provision of useful reference materials and encouragement during the preparation of this manuscript. I am also grateful for their valuable help in preparation of the experimental materials and their support in running the experiments. I also extend my thanks to staff of Production Division for their helpful encouragement and comments.

Finally, I am extremely grateful to the secretary of chemical engineering department Sirgut Kokeb in particular and to my friends Fentaye Lisanework,Tsige Gefeom,Yemisrach Mulugeta, and all others who directly and indirectly contributed to this manuscript in general, for their encouragement, support all the time, and voluntary participation, the assistance & help I received in using whatsoever resource they have. I should not forget also their care to me that brought me up to this success.

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List of Acronyms

AC	.	Alcohol Content
AOAC	.	Association of Official Analytical Chemists
App.	.	Apparent
ASBC	.	American Society of Brewing Chemists
BAC	.	Brewers Association of Canada
CGC	.	Canadian Grain Commission
DNSA	.	Dinitrosalisalic Acid
EBC	.	Europeans Brewing Chemists
FAN	.	Free Amino Nitrogen
Na-tartarate	.	Sodium Tartarate
OG	.	Original Gravity
SPSS	.	Statistical Package for Social Science

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ABSTRACT

Brewing process generally involves the steps of malting, mashing and fermentation. The main purpose of malting is the development of amylolytic enzymes in the grain with simultaneous degradation of high molecular substances in the cell walls enabling the achievement of a distinctive character. Barley malt is the principal ingredient in the manufacturing of beer and has traditionally been the grain of choice in the brewing industry. However, it is not always economically feasible to brew with 100% malted barley, and today's breweries are forced to minimize their costs without changing the quality or the character of their beer. Therefore, the present study was initiated to utilize Maize, Potato and Enset starch as a partial substitute for barley malt and to evaluate some physico-chemical quality attributes of the beer. All the experiments were conducted at Addis Ababa University's laboratories(Science Faculty) and Meta Abo Brewery. The quality parameters of the starch (composition and degradability) were tested. The beer underwent four series of experiments in triplicate involving the starch from the three crops (10%, 20% and 30% starch substitute from each) with full barley malt serving as a control. The major attributes of the beer (alcohol content & flavor) were evaluated for each of the 10%, 20% and 30% substitutes from the three crops with reference to the control beer. Accordingly, the collected data were subjected to statistical analysis using SPSS software with emphasis on alcohol content and sensory attributes (flavor).The results showed that 30% substitution of barley malt with Maize and Enset starch is promising in the beer production. Beer produced using these two crops showed no statistically significant difference from the control barley malt beer ($p \leq 0.05$).The present study indicated that it is possible to partially substitute full barley malt up to 30% as it is feasible in many ways. Based on the findings, a production technology involving maize starch as a partial substitute for barley-malt has been suggested.

Key words: *Barley; Beer; Partial Substitution; Starch*

Chapter 1

Introduction

1. 1 Background

The roots of beer production go back much farther to the first agrarian societies. They used a variety of grains called emmer (Triticum dicoccum), which was dehusked and baked to give flat bread. The flat breads were soaked in water and then allowed to ferment spontaneously through the action of wild yeasts. The Babylonians developed the art of brewing further, in which the emmer and barley content as well as the strength of the beer were closely regulated (Esslinger *et al.,*2005).

The Egyptians refined more the art of beer brewing and the legal requirements. They made the grains germinate and eliminated the soaked pieces of bread by sieving. From the seventh century, malting and brewing processes were researched with great experimental zeal, mainly in German monasteries. In the following centuries, brewing and dispensing rights were loaned out to several monasteries. It was also the monks who first used hops as a flavoring agent. Until the sixteenth century, only the spontaneous top fermentation, which occurs at higher temperature, was known; later, bottom fermentation also was discovered (ASBC, 1999).

Brewing technique, therefore, is based on partly on well-established scientific facts and partly on empirical knowledge gained from years of practical experience. A distinction is frequently drawn in the industry between the theoretical man who tries to explain every thing from a scientific point of view, and the practical man who relies on empirical knowledge and experience. A good brewer should be able to steer a middle course between these two extremes. On one hand, he should be in a position to make full use of scientific facts when ever these are available, and on the other, he should be guided by his practical training and feel for the process when dealing with operations which have not yet received a scientific explanation (De Clerck, 1994).

Brewing is fundamentally a natural process. The art and science of brewing lies in converting natural food materials into a pure, pleasing beverage. Although great strides have been made with the techniques for achieving high-quality production, beer today is still a beverage brewed from natural products in a traditional way (BAC, 2003).

The basic ingredients of beer are water, a fermentable starch source (such as malted barley), yeast and hops (Briggs *et al.,* 2004). Barley malt is one of the principal ingredients in the manufacturing of beer and has traditionally been the grain of choice in the brewing industry. Barley malt is preferred because, among the other reasons, it has high potential for extract development for yeast growth and fermentation. However, it is not always economically viable to brew with 100% malted barley. Therefore this study focuses to minimize the costs of brewing industries without changing the quality or the character of the beer by using mixture of fermentable starch substitutes for barley malt (Lowe *et al.,* 2004).

There are three main parts of the brewing process; malting, wort production & fermentation. During malting process, barley one of the raw materials of beer, is germinated to allow the release of amylolytic enzymes that hydrolyze the starchy endosperm of the barley grain in to soluble sugars .The malted barley is then ground and extracted with hot water, & adjuncts (Starch) are add to the grist creating a mixture of crushed barley and adjuncts. After the starches in the malt are fully hydrolyzed by the amylolytic enzymes to soluble simple sugars the wort is separated from the malt (Declerck, 1994).

Wort is an aqueous mixture of soluble and suspended substances derived from the ingredient materials .The wort is then transferred in to the kettle for boiling and hops addition, which imparts the characteristic bitter flavor and aroma of beer. After boiling the wort, is cooled and is pitched with yeast, thus starting the third step, fermentation. Following fermentation, the beer is matured, stored, filtered and packed with bottles, cans or kegs for shipping (Dougherty, 1983).

1.2 The problem statement

The production of fermented alcoholic drinks from starch-based raw materials has been practiced since long. These starch-based raw materials are different. The main starch source in brewing is malted barley. In addition, other cereals and root crops are (maize, potato, rice barley…) also used as a malt substitute. Generally starches are used in many applications throughout the food industry. The widespread use of starch is due mainly to its multifunctional nature. One segment of the food industry, which truly takes advantage of this broad versatility as a secondary starch, is the brewing industry (http://www.mapsenzymes.com, retrieved on March 3, 2008)

These secondary starch sources being often termed as adjuncts, especially used as a lower cost substitute for malted barley. In this context, obtaining part of the starch by the addition of other starch-containing raw materials (other sources of extract) of brewing adjuncts to partially substitute malt is becoming a standard procedure. Thus, the brewer needs to ensure that wort prepared from mixed grist's of malt and adjuncts does not diminish the traditionally high quality standards (Glatthar *et al.*, 2003). Starch-rich adjuncts are usually considered non-malt sources of extractable carbohydrate, which typically do not contribute to either enzyme activity or soluble nitrogen (Glatthar *et al.,* 2002).

The amount of these adjuncts used is varying widely from 10 to 30% in Europe, to 40 to 50% for some US brewers, to as high as 50 to 75% in certain African countries (Briggs *et al*., 2004). However, in certain countries, for example Germany, malt is the only permitted source of fermentable extract because of the German purity law or "Reinheitsgebot" (Esslinger *et al*., 2005).

The increased price of barley malt, low production rate of beer barley due to environmental factors and adulteration of this barley with food barley which is high in beta-glucan have negative consequences for Ethiopian brewing industries. The impact of all these changes forced brewing industries to consider barley-starch substitutes and alternative set-ups. Therefore, this study was initiated to search for possible partial substitutes for barley malt with starch from selected crops commonly available in this country.

1.3. Objectives

The general objective of this research was to utilize the starch from selected crops for partial barley malt substitute in beer production technology.

The specific objectives were to:

- Extract the starch from selected crops (Cereals and Root crops), and utilize it for partial replacement of barley malt.
- Evaluate the quality of beer produced from pure barley malt and composite malt during partial substitute.
- Evaluate cost effectiveness of the partial substitution for the beer produced from pure barley malt and composite malt..

Chapter 2
Literature Review

2.1. Overview of Ethiopian beer production

There are five beer factories in Ethiopia, Meta Abo, Harar, Bedele, Dashen and BGI Ethiopia. The first three are state owned; while the other two are owned by private investors. The factories have a capacity of producing 3.5 million hectoliters a year. Sector experts explain that the demand for beer in the country is on the rise because the existing breweries have not been able to saturate all the markets that are available in the country. The beer supply in this country does not match the current demand. Studies indicate that Ethiopia would be able to cater to three additional large scale beer factories with similar production capacities to the ones that are currently in the market before meeting the high level of demand and" if the new factory works hard on the market they might be able to get a substantial market share," an expert in the sector told Fortune (EthioBlog, 2008).

Tabl2.1 Summary of beer and malt consumption in Ethiopia

Brewing company	Production capacity(Hl)	Estimated amount of malt(Kg)
St.George brewery	1,500,000	23,710,064.090
Meta Abo brewery	750,000	11,855,032.040
Dashen brewery	750,000	11,855,032.040
Harar brewery	300,000	4,742,012.816
Bedele brewery	250,000	3,951,677.347
Total	**3,550,000**	**56,113,818.33**

2.2. Malting and beer Production

The production of beverages from malted grain, notably beer, involves many unit operations performed sequentially. The main stages include malting, wort production, fermentation and conditioning (maturation). Each of these operations is followed by a separation stage. In beer making, the wort separation stage which follows mashing is regarded as the most critical and most difficult (Declerck, 1994).The mature barley grain comprises the embryo and the endosperm, which provides a store of carbohydrates (mainly starch) and protein to support the initial growth of the germinating embryo. The endosperm comprises the starchy endosperm, which is a non-living storage tissue, surrounded by a living non-starch cell layer called aleurone. An outer husk protects the grain. (http://www.crc.dk/flab/the.htm retrieved on February 08, 200)

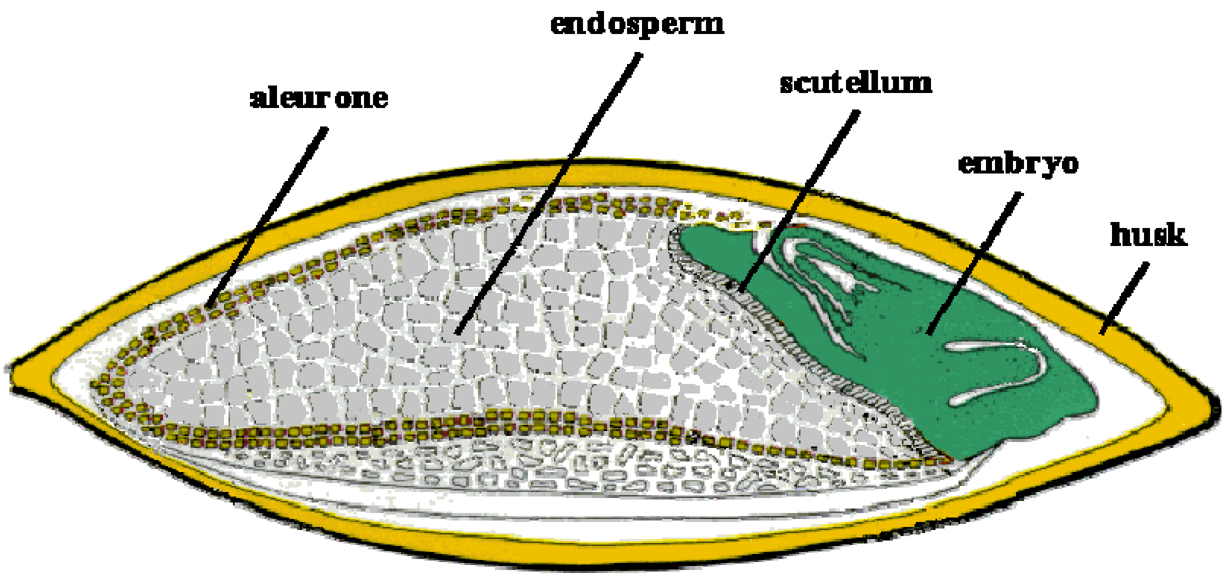


Figure 2.1.Diagramof barley grain (**Source**: http://www.crc.dk/flab/the.htm)

The use of barley for malt and brewing has empirically gradually evolved through the last few thousand years, the processes of which have been interpreted and refined by new recent advances in science and technology (Munck ,1987). Its use depends on the fact that barley has high starch content and the still adheres to the grain, even after threshing and processing to malt. Consequently it is able to form the wort filtration layer required in a latter production stage (Kunze, 1996).

Before use in the brewery the barley must first be converted in to malt (Kunze, 1996). Because barley is unsuitable material for making beer, it lacks the necessary enzymes for brewing, it lacks the friability for easily milling, it produces a highly viscous extract, which is deficient in amino acids and lacks the color and flavor required for making beer. Malting changes all these in crucial ways. These changes are collectively called modification which encompasses the sum of changes brought in the physical, chemical and biological properties of barley by the process of controlled germination called malting (Lewis and Young, 1995).

Malting is defined as allowing grain to germinate under well-controlled conditions. The main purposes of malting are the development of enzymes (amylolytic enzymes that hydrolyze the starchy endosperm of the barley grain into soluble sugars) in the grain with simultaneous degradation of high molecular substances in the cell walls (modification), the achievement of a distinctive character by color and aroma compounds, and removal of undesired aroma compounds (i. e. *S*-methylmethionine and dimethyl sulfoxide) (Esslinger *et al.*, 2005).

The malting process involves the collection of stocks of suitable barley, the storage the cereals until it is required, steeping the grain in water, germinating the grain and finally drying and curing it on the kiln. According to Briggs.*etal*,(1981), not all barleys yield suitable malts for the brewer, and selection is based on many criteria including:

i) Rapid and synchronous germination of the barley grains
ii) Even enzymic degradation of the endosperm
iii) An adequate completion of enzymes even after kilning, and
iv) Low levels of fibrous materials and total nitrogen

There are three stages of malting; steeping, germination, and kilning (Adamic, 1983; and Stefan *et al.,* 2006).

2.2.1 Steeping. Before malting, grain is screened and aspirated to remove large and small impurities and thin' corns. To initiate malting it is hydrated. This is achieved by `steeping', immersing the grain in water or `steep liquor'. Later, spraying or `sprinkling' the grain may increase the moisture content. The steep-water temperature should be controlled. At elevated temperatures water uptake is faster but microbial growth is accelerated and the grain may be damaged or killed. The best temperature for steeping immature (partly dormant) grain is low (about 12 ^{0}C, 53.6 ^{0}F). For less dormant grain a value of 16 or18 ^{0}C (60.8 or 64.4 ^{0}F) is often used (Briggs *et al.*, 2004). Cool temperatures are used to avoid the growth of microorganisms (Adamic, 1983).

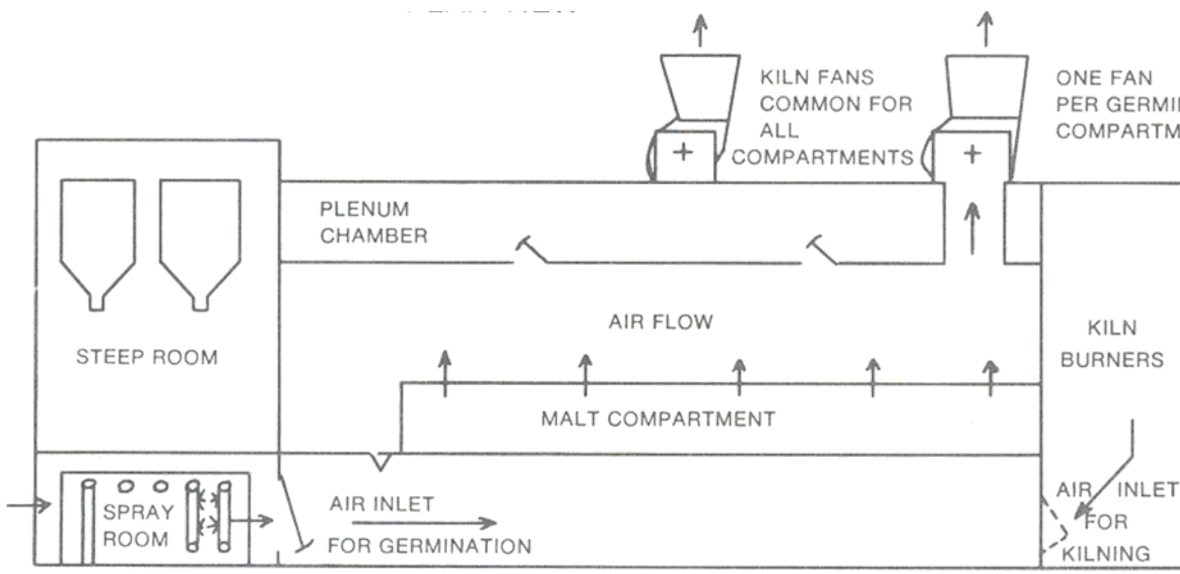


Figure2.2. Diagram of typical malt house (**Source**: Broderick, (1983) .The Practical Brewer: A Manual for the Brewing Industry, U.S.A).

As a result of water up take the barley swells by about 40 to 45% of its original volume (Kunze, 1996). This produces an increase of moisture content in the range of 44%_46%from 11-13.5% .The water uptake mechanism are purely mechanical and take place at the kernels embryo (Fix, 1989) .The water uptake of barley will vary depending on the steeping temperature, steeping time, kernel size, barley variety, and protein content of the kernel (Kunze, 1996).

When barley is steeped, the water quickly penetrates the husk and fruit-coat, and enters the corn through or near the micropyle. The embryo takes up water quickly while the endosperm hydrates more slowly, any cracks in the husk, fruit-and seed coat permit more rapid moistening of the endosperm or the embryo and, of course, easy escape of the soluble endosperm material .The loss of material is one aspect of malting loss, another loss is the loss due to respiration by embryo, consuming food reserves to yield energy, carbon dioxide and water. The respiration increases significantly when the embryo becomes active; this creates a massive oxygen demand in the steep water –hence the sparging (perfusion) of air and the air-rests during steeping (Hough *et al.***,** 1971**)**.

If the barley is not aerated, an intermolecular respiration begins (i.e. other compounds replace oxygen), which in extreme cases leads to death of the seedling (dead step) (Kunze, 1996). In the absence of oxygen the embryo has some capacity to metabolize anaerobically by converting food reserves rather wastefully in to energy, carbon dioxide and alcohol, a proportion of which enters the steep liquor. As the alcohol concentration increases it becomes progressively more toxic **(**Hough *et al.***,** 1971)**.** Under such conditions the grain will not germinate. Under aerobic conditions fermentation is repressed and germination can occur. During immersions air may be blown into the base of a steep, providing some oxygen and lifting and mixing the grain (Briggs *et al.*, 2004).

Steep water, which checks grain germination and growth if re-used, is periodically drained from the grain and replaced with fresh. The minimum acceptable number of water changes is used since both the supply of fresh water and the disposal of steep effluent are costly. Sterilants are not routinely used in steeps, but many substances, including mineral acids, potassium and sodium hydroxides, potassium permanganate, sodium metabisulphite, slaked lime water and slurried calcium carbonate and formaldehyde, have been used, as has hydrogen peroxide. `Plug rinsing' grain in the steep by washing downwards with a layer of fresh water, (with or without hydrogen peroxide or other substances), as the steep is drained is an economical possibility for removing suspended microbes, their nutrients and other substances (Briggs, 2002).

The water in the steep tank is changed every six to eight hours and the water is constantly aerated because the barley kernels respire and use up the dissolved oxygen in the water. The steeping process is stopped when the chit or rootlets are just ready to emerge. The water is drained from the tank and the moisture rich barley is transferred to the germination bed (Adamic, 1983).

2.2.2. Germination: The aim of controlled germination is to produce a green malt of a definite composition (Esslinger *et al.,* 2005), and this is where enzymatic activity starts (Fix, 1989), which allows the starch to be converted to simpler sugars in preparation for their subsequent hydrolysis during the mashing phase of beer production, but not to allow the development of a new plant. During this process, the nutrients stored in the endosperm are partly consumed. (Esslinger *et al.,* 2005).

The germination processes generally can be divided in to: growth process, enzyme process and metabolic changes. Germination is a physiological process during which the embryo develops rootlets and acrospire's (Kunze, 1996). Variations in acrospire's lengths indicate heterogeneity in growth. The living tissues respire and carbon dioxide and water are generated resulting in a loss of dry matter. The energy liberated supports growth and is liberated as heat (Briggs *et al.*, 2004)

Activation of enzymes and formation of new enzymes are essential process during germination and consequently in malting. Enzymes are already present in abundance in barley. They are set free or formed in ever increasing amounts during germination (Kunze, 1996). According to Fix (1989) of the many enzymes and enzyme complexes, which are contained in barley and malt, the following are the most important:

- Starch degrading enzymes above all alpha-and beta amylase
- Cytolytic enzymes above all beta glucanases and cytase

With the exception of alpha-amylase, which is not yet present in barley, all these enzymes are present in smaller amounts in barley enzymes are produced as a result of the action of hormones which are distributed with the penetration of water from the scutellum along the aleuronic layer and which causes the release and formation of enzymes. These hormones consists gibberellic acid or substances similar to gibberellic acid (Kunze, 1996).

These diffuse along the grain triggering the formation of some enzymes in the aleurone layer and the release of these and other enzymes into the starchy endosperm. Here they join the enzymes from the embryo in catalyzing modification. As germination progresses the starchy endosperm softens and becomes more easily `rubbed out' between finger and thumb. When the malt has been dried the modified material is easily crushed and `friable', and is easily roller-milled, in contrast to the tough barley (Briggs *et al.*, 2004). The physical weakening of the endosperm structure and the biochemical degradations are referred to as modification **(**Hough *et al.,* 1971).

The stages of physical modification are the progressive degradation of the cell walls of the starchy endosperm, which involves the breakdown of the troublesome _glucans and pentosans, followed by the partial degradation of the protein within the cells and the partial or locally complete breakdown of some of the starch granules, the small granules being attacked preferentially. The extent of breakdown is limited by the availability of water (Briggs *et al.*, 2004)

From the physiological point of view, there is continuity between steeping and germination. The embryonic growth is initiated in steeping (Hough *et al.,*1971) and at first supported by its own reserve substances and later by soluble materials from the modifying starchy endosperm, so there is a net migration of materials into the embryo (Briggs *et al.*, 2004), because the food reserves immediately available to it are limited, it is necessary for the abundant reserves of the endosperm to be mobilized. This is achieved by the embryo or scutellum secreting enzymes to degrade the protein, starch and cell walls of the endosperm **(**Hough *et al.,* 1971).

The balance between their rates of formation in the endosperm and their rates of utilization regulates the levels of soluble materials that accumulate by the embryo (Briggs *et al.*, 2004). Modification of starch or the enzymic break down of the endosperm there fore proceeds from the embryo end of the corn to the distal or near the scutellum (Hough *et al.,* 1971).

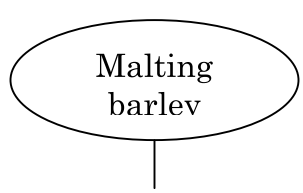
The modification of starch by the action of α- and ß-amylases occurs to only a moderate degree. The liberation, activation, and formation of the ß-amylase, as well as the de-novo formation of α-amylase during the germination process, are both important. α-Amylase can be formed only in the presence of oxygen; its production is favored by high moisture content during germination, low germination temperatures, and long germination periods (Esslinger. *et al*, 2005). The processes that have been occurred in germination are regulated by controlling the moisture content of the grain, the quality of the grain and the temperature programme of the grain during steeping and the germination period (Briggs *et al.*, 2004).

Germination takes place only under appropriate conditions in order to achieve the desired metabolic changes during the time necessary for germination. Parameters are: moisture, temperature, ratio of air to carbon dioxide, and time. Steeping and germination conditions have to be adapted to barley variety, harvest year, water sensitivity, vitality, and furthermore to the protein content and the expected structure of the endosperm matrix. The moisture content must not decrease during the entire germination period. Temperatures favorable to uniform germination ranges from 14 to 18 ^{0}C (Esslinger *et al.,* 2005).

Humidified inlet air at a temperature of 11-16^{o}C is introduced to help to maintain the temperature of the germinating grain at 16-21^{o}C. As the germination of the barley continues, the respiration of the barley generates a considerable amount of heat thus necessitating a lower inlet air temperature. As the kernels grow, the rootlet masses need to be broken up and the beds need to be aerated. This is achieved by the use of counter-rotating helical turners that are automated to move one foot per minute. Large fans in the malt house used to circulate the air cause some dehydration of the germinating kernels, so water is applied to the grain beds as a light mist. After four to six days the germinated malt is ready for kilning (Adamic, 1983).

2.2.3. Kilning: In order to stop the chemical and biological transformations that have take place during germination the green malt is dried by kilning (Esslinger *et al.,* 2005). Kilning terminates the life processes. This procedure yields a storable product. To make the malt storable, the water content must be decreased from over40%to less than 5 %(Kunze, 1996). Another function of kilning is to remove the vegetable-like flavor of the green malt and to impart to the kilned malt a specific aroma and a defined color characteristic for the type of malt required. Finally, the rootlets, which are very much valued as nutritional feed for cattle, are also removed (Esslinger *et al.,* 2005).

During kilning the germinated barley is placed in the kiln bed and hot air is passed through it (Kunze, 1996). This occurs in a stepwise fashion. In the initial stage, the moisture is easy to remove. At this stage the temperature of the kiln inlet air is maintained at 49-60^{o}C while moisture in the malt is reduced from about 48% to approximately 23%. In the second stage of kilning the temperature of the inlet air is increased to about 71^{o}C, while the moisture in the malt is reduced to 12%. The final drying stage decreases the moisture to about 3.5% by increasing the kiln inlet air to 71-88^{o}C and reducing the airflow. When kilning is complete, the dry malt is cooled to about 38^{o}C and cleaned of all roots and sprouts before it is stored. The malt is stored for at least three weeks before shipment to a brewer (Adamic, 1983).



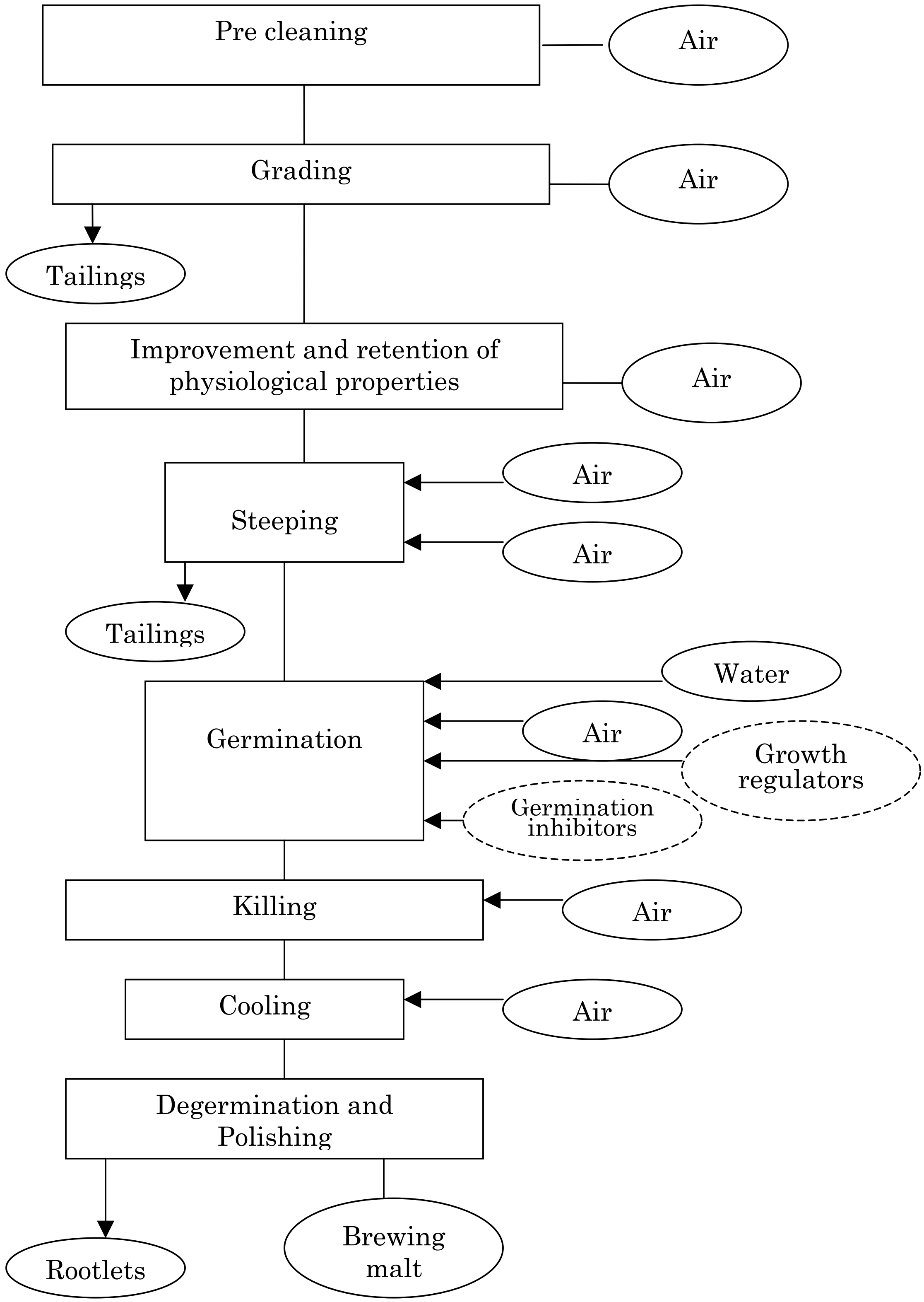


Fig2.3. Schematic diagram of malting process (Source: Esslinger, M. H., Aktiengesellschaft, B. F.and Freiberg. (2005). Beer. Ludwig Narziss, Freising, Germany)

Barley malt can be supplemented with other cereals, either malted or raw, for specific purposes (provided local legislation permits their use) called adjuncts. Adjuncts are materials, other than malt, that are sources of extract They are used because to produce a more stable beer, (as they contain less protein)**,** to produce a different flavor (maize, for example, is said to give a fuller flavor), to produce a better beer foam due to lower fat (lipid) levels and different proteins, to improve the ease of processing in the brew house, to produce beer at lower cost they yield less expensive extract than malt and/or they impart desirable characteristics to the product. For example, they may dilute the levels of soluble nitrogen and polyphenolic tannins in the wort, allowing the use of high-nitrogen (protein rich) malts and the production of beer less prone to form haze. Some adjuncts enhance head formation and retention (Graham, 2006).

2.3.1. Types of Adjuncts

Adjuncts can either be added to the cereal cooker, the mash tun or directly to the brew kettle.

Cooker mash adjuncts consist of non-gelatinized cereal products (meal, grits, flour, or dry starch) whose starches are in their native forms. A non-gelatinized adjunct needs to be heated in a separate cereal cooker to complete liquefaction since the starch gelatinization temperature of the adjunct is higher than that used for the malt saccharification (starch hydrolysis) temperature. The cooked adjunct is then added directly to the mash in the mash tun. The malt enzymes from the malt mash can be used to hydrolyze the starch from the adjunct, converting it to sugars ready for fermentation.

The adjunct can be mashed directly with the malt in the mash tun in two ways: 1) when the starch gelatinization temperature of the adjunct is lower than the malt saccharification temperature required for mashing or 2) when the adjunct has been pre-gelatinized (e.g., for flakes, torrified cereals, and refined starches).

Kettle adjuncts consist of syrups and sucrose sugar. These are sometimes called "wort extenders" because of the extract they readily contain. British brewers commonly use syrups and sugars, whereas the rest of the world is more likely to use non- and pre-gelatinized adjuncts.

2.4. Wort production

The most important process in beer production is the fermentation of sugars contained in the wort to form alcohol and carbon dioxide. To provide the necessary condition for this, the initially insoluble components in the malt must be converted in to soluble products, and in particular soluble fermentable sugars must be produced .The formation and dissolving of these compounds is the purpose of wort production. It provides the starting point for fermentation of the wort in the fermentation and storage cellar (Kunze, 1996).

Wort is an aqueous mixture of soluble and suspended substances derived from the ingredient materials such as barley and starch adjuncts/cereal adjuncts consisting of fermentable sugars (fructose, sucrose, glucose, maltose and maltotriose), dextrins, nitrogenous materials, vitamins, ions, mineral salts, trace elements, and many other constituents. In wort, the main nitrogen sources for yeast metabolism are individual amino acids, small peptides, and ammonium ions formed from the proteolysis of barley malt proteins during malting and mashing. Brewer's wort contains 19 of the 20 essential amino acids, and as with wort sugars, the uptake of amino acids is ordered (Lekkas *et al., 2007*.

The wort is then transferred into the kettle for boiling and hops addition, which imparts the characteristic bitter flavor and aroma of beer. After boiling, the wort is cooled and pitched with yeast, thus starting the third step, fermentation. Following fermentation, the beer is stored, filtered and bottled for shipping (Dougherty, 1983). There are several steps in wort production that include milling, mashing, lautering, and sparging (Dougherty, 1983).

2.4.1. Milling

Wort is produced from brew house. Malt (the ingredients) is transferred from malt storage in to, the brew house via a conveyor to a grain weighing system that assures the proper formulation of malt and adjuncts. Once the ingredients are in the brew house they are milled using a five (or six) roller mill that is often specially designed for a specific brewery based on the type of equipment being used, in which it is ground to a suitable size (Kunze, 1996). The three main objectives of milling are first and foremost to split the husk longitudinally to expose the endosperm of the kernel, to crush the entire endosperm to make the constituents accessible to enzymatic action, and to keep the amount of flour produced in the milling process to a minimum thus keeping dough formation in the mash to a minimum. The adjuncts, mainly corn grits in this case are used without any milling. Following milling, the actual mashing process can begin (Dougherty, 1983).

2.4.2. Mashing

Mashing is the process of converting starch from the milled barley malt and added adjuncts into fermentable and unfermentable sugars to produce wort of the desired composition. It involves mixing milled barley malt and adjuncts with water (brewing liquor) at a set temperature and volume to continue the biochemical changes initiated during the malting process (WO, 2005).

Most of the substances in the malt grist are insoluble. Only soluble substances can pass in to beer. It is there fore necessary to convert the insoluble (Starch, cellulose, part of high molecular weight proteins and other compounds which remain as spent grains at the end of the lautering process) materials in the grist in to soluble (such as sugars, dextrins, inorganic substances and certain proteins) materials (Kunze, 1996). All the substances, which go in to solution, are referred to as extract. The aim of mashing is there fore to form as much extract and as good extract as possible, i.e. to dissolve any soluble substances in the ingredients, to make some insoluble substances soluble through enzymatic action, and to change the chemical structure of some of the constituents through a variety of enzymatic actions (Dougherty, 1983).

The mashing process is conducted over a period of time at various temperatures in order to activate the endogenous malt enzymes responsible for the acidulation of the mash (traditionally for lagers), degradation of proteins and carbohydrates (Goldammer, 2000; Briggs *et al.*, 2004). Although there are numerous enzymes present in the mash, each with a specific role to play, according to Goldammer (2000) and De Clerk (1994) these enzymes are limited to the three principal groups and their respective processes. These are:

1) phytases: acidifying, and hydrolyses Phytin to inositol and Phosphates
2) Proteolytic enzymes: protein-degrading, i.e. hydrolyses Complex protein simpler soluble bodies.
3) Carbohydrase enzymes (Amylases) break down starch in to dextrins and maltose

The enzyme catalyzed changes that occur during mashing are more complex than those normally investigated by biochemists, who usually study each enzyme acting in isolation, with a homogeneous substrate, at one temperature, with an unchanging pH and with a large excess of substrate to maintain enzyme activity (Briggs *et. al.*, 2004)**.**The most important property of enzymes is their action in breaking chemical bonds in their substrates. This activity depends on various factors (Kunze, 1996).

2.4.2.1 Factors that Affect enzyme Activity during mashing

A. 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.*, 2004).

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 in activation occurs as a result of unfolding the three-dimensional structure of enzymes (denaturation).The inactivation and destruction of enzymes activity is greater the more the optimum temperature is exceeded(Kunze, 1996). Depending on the way in which the temperature is raised, mashing processes are divided in to two types; infusion processes and decoction processes (De Clerk, 1994)

B. pH: There is also a 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, 1996).

Beta amylase is favored by a low wort pH, about 5.4. Alpha is favored by a higher pH, about 5.7. However, Beta-optimum wort is not very fermentable wort; Alpha amylase is needed to break up the larger chains so Beta can work on them. (http://www.realbeer.com/jjpalmer,retrieved on April 22, 2008).

Phosphatases cause the hydrolytic release of phosphate ions from organic phosphates in the malt thus changing the pH to about 6 due to the mixture of K_2HPO_4 (pH = 8.4) and KH_2PO_4 (pH = 4.7). So that other enzymes can work, the pH needs to be decreased to between 5.2 and 5.7. The acidity of the mash is increased by the addition of calcium sulfate, $CaSO_4$. Calcium sulfate reacts with the alkaline potassium phosphate to create a precipitate of $Ca_3(PO_4)_2$. This adjusts the pH to the optimum range (Dougherty, 1983).

C. Mashing time: The enzymes certainly do not work uniformly through out mashing. Instead two time-dependant stages in enzyme activity can be distinguished.

1. The maximum enzyme activity is reached after 10-20min.the maximum enzymes are higher at temperatures “between” 62-68 ^{0}C.
2. After 40-60min enzymes activity at first decreases rapidly, but the reduction in activity continuously decreases.

In general with increasing mashing time the concentration of extract solution increases, but the rate increase becomes slower and slower. With increasing mashing time (especially when mashing at 62-63 ^{0}C) the maltose content increases and with it the attenuation limit. This wort should produce a vigorous main fermentation. From this it must be concluded that the effect of the mashing temperature must always be considered in relation to the duration of mashing (Kunze, 1996).

2.4.2.2 Chemical changes at mashing

A. Dough-in/Acid Rest

The acid rest is responsible for reducing the initial mash pH for traditional decoction mashing of lager beers. This temperature rest (holding period) has no longer used by any commercial brewery. It is sometimes used by home brewers for "Doughing In"- mixing the grist in with the water to allow time for the mash to liquefy and time for the enzymes to be distributed. The use of the 20 minutes rest at temperatures near 100°F (40°C) has been shown to be beneficial to improving the yield from all enzymatic malts. This step is considered to be optional but can improve the total yield by a couple of points (Goldammer, 2000). Malt is rich in phytin, an organic phosphate containing calcium and magnesium. Phytase breaks down phytin into insoluble calcium and magnesium phosphates and phytic acid. The process lowers the pH by removing the ion buffers and producing this weak acid. This stage is known as the Acid Rest but it is not used nowadays. It can take several hours for this enzyme to lower the mash pH to the desired range. Today, through knowledge of water chemistry and appropriate mineral additions, proper mash pH ranges can be achieved from the outset without needing an acid rest (http://www.realbeer.com/jjpalmer, retrieved on April 22, 2008)

B. Protein Rest

The protein rest is responsible for reducing the overall length of high-molecular-weight proteins - which cause foam instability and haze - to low-molecular-weight proteins in the mash **(**Goldammer, 2000**).** The naturally occurring proteolytic enzymes that are used in the mash degrade the proteins in the grains into forms of protein, which improve the quality and fermentation characteristic of the beer (Miller, 1992). There are three types of proteolytic activity that occurs in the mash; solubilization of insoluble proteins, partial digestion of soluble proteins, and continued breakdown of the soluble protein fractions into peptones that can ultimately be utilized by the yeast in fermentation (Dougherty, 1983).

The yeast used in brewing requires amino acid proteins as nutrients. In a temperature range of 103-122 ^{0}F (40-50 ^{0}C), proteolytic enzymes (Peptidase) become active that break down nitrogen based proteins into amino acid proteins providing these nutrients for later use by the yeast. These nutrients are very significant in developing the attenuation of the wort, which is the ability of the yeast to ferment and convert fermentable sugars into alcohol and Carbon dioxide. The temperature of the mash is then raised to between122-140 F (50-60 ^{0}C), which activates other proteolytic enzymes (proteinase) that "break down proteins into forms that improve the foam potential of the beer and aid in clarity."(Miller, 1992). This stage in the mashing process is known as the protein rest (Hough *et al.,* 1971).

C. Starch conversion

Starch is comprised of the mainly linear polymer amylose and the highly branched amylopectin. By far the most important change brought about in mashing is the conversion of starch molecules into fermentable sugars (maltose) and unfermentable dextrins. The main principal enzymes responsible for starch conversion are alpha-amylase and beta-amylase. Alpha and beta amylase hydrolyze amylose and amylopectin from starch to dextrin's and eventually to maltose. The beta amylase works to convert the starch to maltose and the alpha amylases work more effectively on the amylopectin to break it into dextrin's and more slowly into maltose (Dougherty, 1983).

Alpha-amylase very rapidly reduces insoluble and soluble starch by splitting starch molecules into many shorter chains (i.e., partially-fermentable polysaccharide fractions - dextrins and maltotriose) that can be attacked by beta-amylase. Given a long enough "rest," the alpha-amylase can dismantle all the dextrins to maltose, glucose, and small, branched "limit dextrins." However, starch conversion is more effective by the faster-acting beta-amylase. Beta-amylase is more selective than alpha-amylase since it breaks off two sugars at a time from the starch chain. The disaccharide it produces is maltose, the most common sugar in malt. Together, alpha- and beta-amylase are capable of converting only 60 to 80% of the available starch to fermentable sugars (WO, 2005; Goldammer, 2000).

When brewing from grist's low in enzymes such as high adjunct grist's, mashing may be performed in the presence of added enzyme compositions comprising the enzymes necessary for the hydrolysis of the grist starch (WO, 2005). Starch is found as granules (small or large). Particle size has an influence up on conversion on mashing. Starch carbohydrate consists of about 20% of a straight-chain component, amylase, which gives a characteristic intense blue color with iodine, and about 80% of a branch-chain component, amylopectin, which forms a thick viscous solution when boiled with water, and difficult to saccharify and does not give a blue color with iodine .On hydrolysis with a mixture of alpha and beta amylase, starch gives a maximum yield of 80% of maltose; the remaining 20% of the hydrolysate consisting of dextrins due to incomplete degradation of amylopectin(De Clerk, 1994)

Three distinct stages can be distinguished in the enzymic breakdown of starch: gelatinization, liquefaction, and saccharification. In hot aqueous solution a large amount of water is incorporated in to starch molecules. This results in an increase in volume which causes the closely packed starch granules to swell and finally to burst. Viscous or sticky solutions are formed. This process during which no chemical degradation is formed is called gelatinization. The starch molecules set free in to this viscous solution are more easily attacked than ungelatinised starch by amylase. By liquefaction is meant the reduction in viscosity of the gelatinized starch by alpha amylase and saccharification is the complete degradation of starch to maltose and dextrin's by amylases.

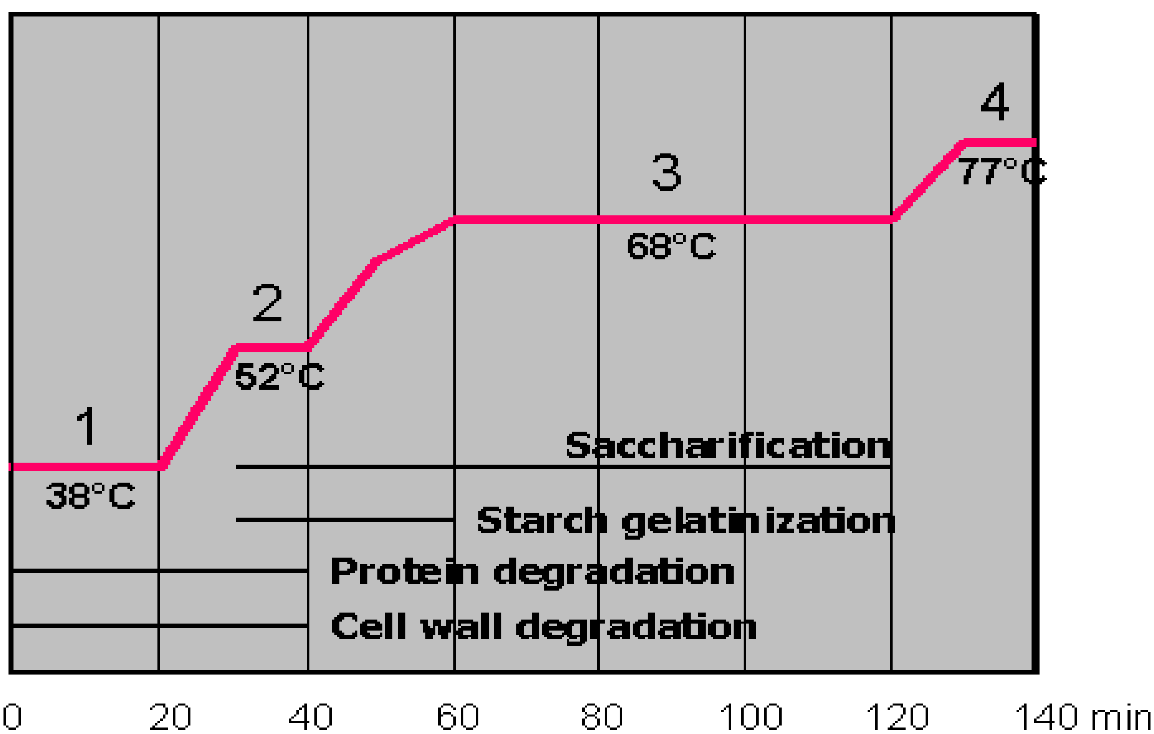


Fig2.4.Temperature programmed mashing process

(Source: http://www.crc.dk/flab/mashing.htm)

2.4.3 Lautering

At the end of mashing process, the mash consists of a watery-mixture of dissolved and undissolved substances. The liquid sweet wort, which contains the extract, is separated from the residual solids, the spent grains or draft

The aqueous solution of the extract called wort, and the undissolved part is referred to as spent grains. The spent grains consist specially of the husk, the seedling and other materials, which don't go in to the solution on mashing (Briggs *et al.*, 2004). Only the wort is used for beer production, and for this purpose it must be separated as completely as possible from the spent grains. This separation process is called lautering. When this wort has been drained from the spent grains the later still contains extract. This extract retained by the spent grains is washed out by hot water. This process is called sparging (Kunze, 1996).

When the mash is completely transferred to the lautertun and properly leveled, the wort is circulated through the grain bed that has formed on the bottom of the tank. This helps to form filter bed and clearer wort with the help of the lautering machine. As the first wort is drawn off, sparging begins. Sparging is done to dilute and reduce the viscosity of the first wort encouraging more rapid flow through the grain bed. Sparging is completed when the final volume of wort is appropriate in the kettle. Spent grains are removed from the lautertun with the help of lautering machine, which sweeps out the grains (Dougherty, 1983)

2.4.4 Wort boiling and Hops addition

The next step in brewing is to boil the wort. The wort obtained is boiled for 1-2 hours. During this time the hops are added. Wort boiling is performed in the wort kettle, which is equipped with every thing needed for vigorous thorough boiling. The end product of wort boiling is the casting wort, i.e. is the hot finished wort (Kunze, 1996)

According to Briggs *et al.*, (2004), the principal changes that occur during wort boiling are:

1. Inactivation of malt enzymes
2. Sterilization of the wort
3. Extraction and isomerization of compounds derived from hops
4. Coagulation of protein material in the wort
5. Formation of protein/polyphenol complexes

6. Formation of flavor and color complexes
7. Fall in wort pH
8. Concentration of wort gravity through evaporation of water
9. Evaporation of volatile compounds in wort derived from mashing
10. Evaporation of volatile compounds in wort derived from hops.

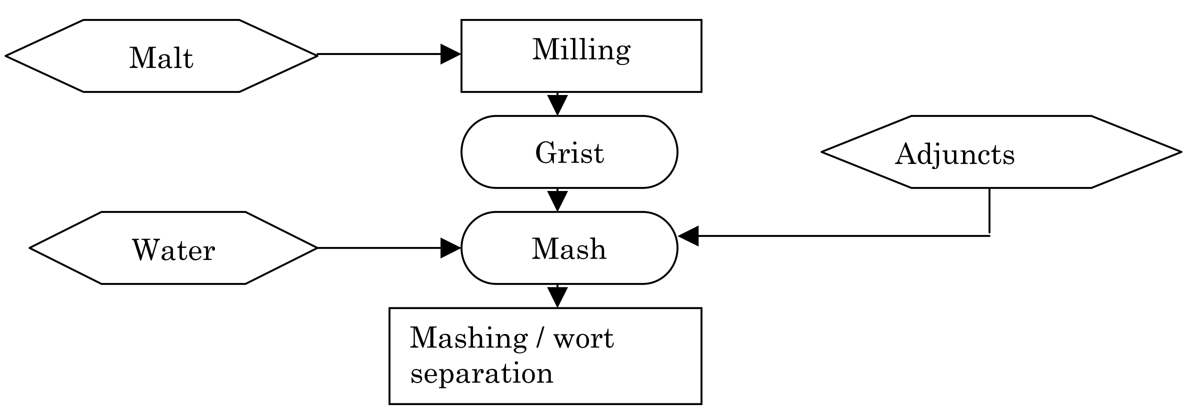
In order to remove the hop pellets from the beer, a whirlpool tank is used. In this tank suspended particles of hops migrate to the bottom and center of the whirlpool creating a trub cake as the hot wort rotates in the vessel. The wort starts to rotate as it is being pumped into the tank from the kettle. The wort stays in the whirlpool for a set amount of time before the wort is removed and cooled, but the wort temperature is not allowed to drop below 82 ^{o}C. The "clear" wort is drawn out of the whirlpool with a draw-arm positioned just above the trub cake. The wort is finally cooled via a plate type cooler that consists of a stainless steel frame that holds many stainless steel recessed plates pressed tightly together. This allows the wort and cooling medium to pass each other in a counter flow manner in shallow layers between the adjoining plates (Strauss, 1983).

2.5. Fermentation, Maturation and Filtration

Once the wort is cooled it can be fermented. Wort is a highly complex medium consisting of fermentable sugars (fructose, sucrose, glucose, maltose and maltotriose), dextrins, nitrogenous materials, vitamins, ions, mineral salts, trace elements, and many other constituents. In wort, the main nitrogen sources for yeast metabolism are individual amino acids, small peptides, and ammonium ions formed from the proteolysis of barley malt proteins during malting and mashing, collectively known and measured as Free Amino Nitrogen (FAN) 3, 19. Brewer's wort contains 19 of the 20 essential amino acids, and as with wort sugars, the uptake of amino acids is ordered (Briggs *et al.*, 2004).

The fermentation starts when the wort leaves the cooler at about 8 –10 ^{o}C and is aerated to about 8 ppm dissolved oxygen. Once the wort is aerated, the yeast is pitched (yeast added to the wort). The pitched yeast must be clean and sanitary to prevent the introduction of wild yeast or bacteria into the wort. After about ten to twenty hours the wort is covered by white foam changing to creamy-white thick foam as time passes. Fermentation is dependent on four basic parameters including the wort composition, which are the nutrients for the yeast, the yeast itself, and the processing conditions such as time, temperature, volume, pressure, agitation, and vessel shape and size. At the end of the fermentation, 8-10 days, the yeast will flocculate and settle to the bottom of the tank, leaving a "green beer" to be stored. Following the fermentation, the beer is transferred to the storage tank and stored at low temperatures, 30 ^{o}F (Knutsen, 1983).

Two different classes of beer must be distinguished here. "Bottom fermentation" or lager beers, which are fermented at low temperatures (5-10 ^{0}C) with a special strain of yeast which falls to the bottom of the fermenting vessels, and "top fermentation" beers, which are fermented at relatively high temperatures (15-10 ^{0}C) with a different strain of yeast which rises to the surface of the fermenting wort and is skimmed off. After fermentation, the beer is stored for several weeks in the case of top fermentation beers, and for several months with bottom fermentation beers. The object of this prolonged storage or conditioning process is to mature the beer and improve its flavor. Finally, the beer is racked and filtered in to casks or bottles (De Clerk, 1994).



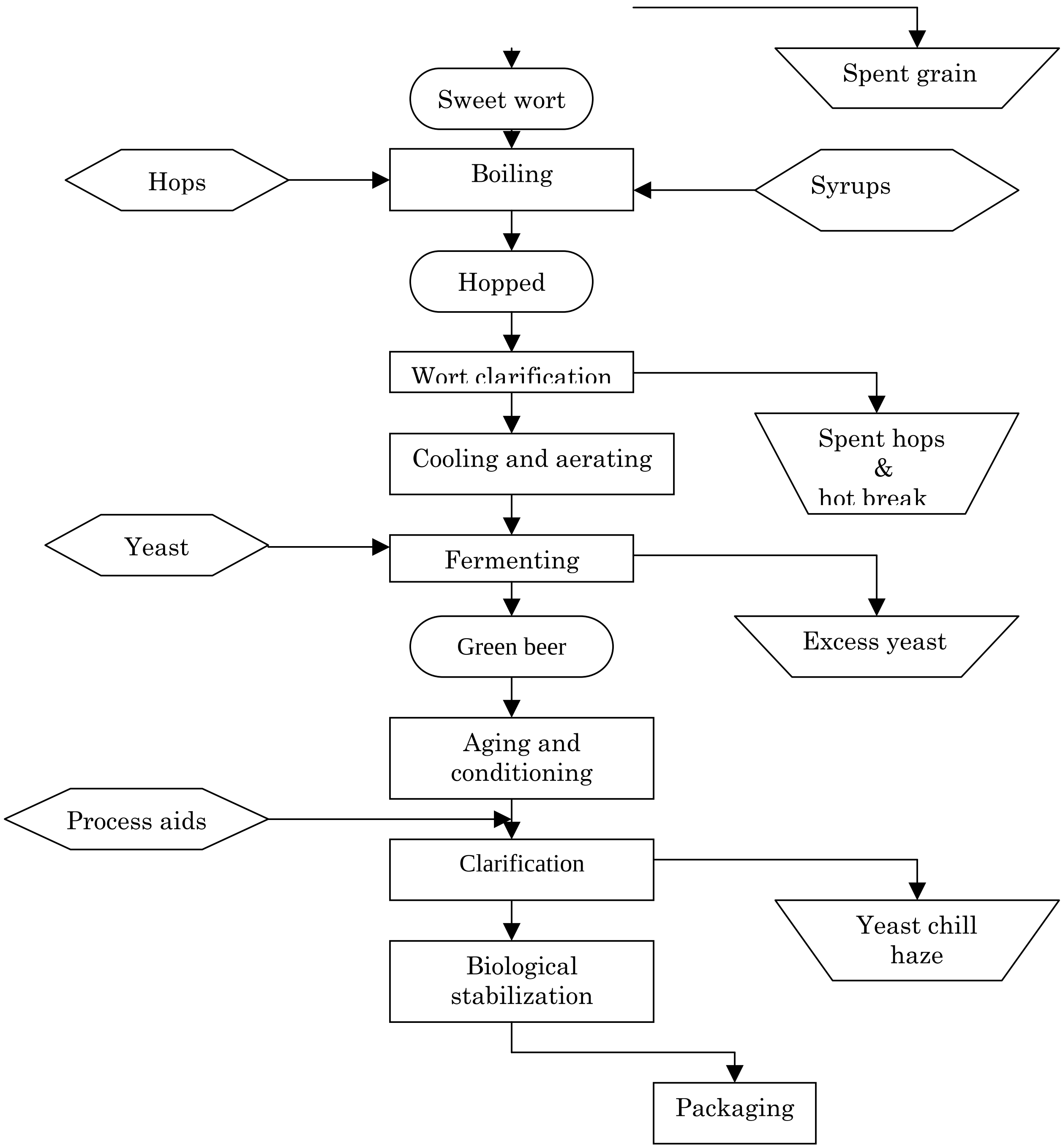


Fig2.5. General flow chart of beer production process (Lewis andYoung, 1995)

Materials and Methods

3.1. Materials

3.1.1. Beer raw materials

- Malted barley of beka variety from Assela Malt Factory

- Hops (pelleted and extracted) from Meta Abo Brewery
- Yeast of bottom fermentation (Carlsberg genesis strain) from Meta Abo Brewery and spring water (brewing liquor) was used.
- Potato and Maize was bought from market & Enset from southern Ethiopia of Gurage zone

3.1.2. Chemicals &Equipments

- **Chemicals:** All chemicals used in this study were analytical grades such as
- Sulphuric Acid (H_2SO_4) ,Calcium Chloride ($CaCl_2$), CalciumSulphate ($CaSO_4$), 3,5-dinitrosalicylic acid (DNSA), Sodium-tartarate, Sodium hydroxide (NaOH), Phenol , Sodium acetate
- **Equipments:** Equipments, which were used for experimental work, are:
- Buhler-Miag disc miller (0.2-1.0mm,DLFU-1980,Germany),
- Sieve (mesh size of 2.8-2.2mm,Sortmks-3332,PFEUFFR,Germany),
- Mash bath (Congress, temperature programmed, RS232,Czech),
- Anton Paar DMA5000digital density meter(DMA35N,Austria-Europe),
- Kjeldahl apparatus(Gerhardt, Germany)
- Color comparator (lovibond,3000 comparator with color cuvett),
- Saccharometer, measuring cylinder, oven, refractometer (RFM340,Bellingham), centrifuge, vortex,
- Spectrophotometer(T60U model, PG Instruments Ltd)

3.2. Method

3.2.1 Sample collection, transportation and Preparation

3.2.1.1 Starch from potato, maize and enset

- **Potato starch**

Fresh potatoes (10kg) were washed to remove dirt and loose soil, and then peeled by hand to remove the skin (peels). The peeled potatoes were sliced and chopped with hand - rasping or grating machine to produce a slurry or pulp. The slurry was then sieved to separate the fibrous tissue from the starch milk; considerable quantities of clean water were used at this stage in order to ensure efficient separation of starch granules from the slurry. The starch milk was collected and left in settling bath for 12 h. When the starch sinks to the bottom, the liquid was drained away. The surface layer of the starch mass usually contains impurities and was therefore scraped off, leaving a creamy-white mass below, which was stirred vigorously with water and then left to settle. This washing and settling process was repeated more until the starch was to be sufficiently pure. Thereafter, the solution was passed through muslin cloths, to separate the starch from debris left in the solution. Finally the starch cake was dried at room temperature, by spreading it out in trays in the sun and weighed, evaluated for quality (Charles, 1953)

- **Maize Starch**

Maize starch was extracted from the kernel through a process of wet milling. The process employs techniques of grinding, screening and centrifugation to separate purified starch from fiber, oil and tightly bound protein (National Starch and Chemical Company, 1996)

Dried raw maize grains were handpicked and cleaned to remove foreign material, and broken kernels before analysis. After cleaning, the maize kernels were transferred in to steeping bath and steeped in water at 50 ^{0}c for 72 h in a weak solution of sulfurous acid to prevent germination of the steeped maize, helps to loosen the starch protein matrix in the steeped maize kernel gradually, prevents fermentation process and facilitates purification (Singh, 1995). Subsequently the maize kernels were crushed and ground with water in an attrition mill to remove the germs. The germ removal step was followed by fine grinding in an impact mill. The resulting suspension was led over 1mm sieve for separation from fiber and other maize components and left for 12h for the starch to settle. For complete separation of white starch washing was done repeatedly. Finally the resulted starch was dried at 40 ^{0}c until fully dried powder was formed (Al-Hakkak, 2006)

- **Enset Starch**

The corm part of Enset (Amecho) was scuffed (chopped down) on the grater or rasping machine .The chopped pieces were mixed with water and left to settle for 1h. Settling and washing was done until the starch got white. White starch was separated with muslin cloth and left to dry at room temperature.

3.2.2. Physicochemical Analysis

3.2.2.1. Extraction and assay of malt enzymes

In this research commonly 0.2M Sodium-acetate buffer with pH = 5 was used as the extraction medium. This buffer enhances the release of more enzymes rather than another medium such as the mixture of NaCl in water, as pointed out by Osman (2002). Approximately, 0.75 g malt flour was weighed in duplicate into centrifuge tubes and 4 ml extraction medium was added with mixing. Extraction was performed for 30 min at 30 ^{0}C with regular vortexing for 5 s at 5 min intervals and was terminated by centrifugation for 5 min at10,000 rpm. The super-natants were filtered through wet muslin cloths in to eppindrof tubes (Osman, 2002, and Maryam, 2008).

Starch-degrading enzymes were determined on the basis of using 1% soluble starch as the substrate and measuring the amount of reducing sugars released with DNSA (dinitrosalisalic acid). The soluble starch solution was prepared by dissolving 1g soluble starch in 100ml_ distilled water which is boiled with stirring, and cooled to room temperature (Hossein *et al.*, 2005). To assay, 0.1ml extracted enzyme was added with mixing to pre-equilibrated (pH=5) soluble starch solution (0.9ml). The reaction was continued or incubated for 10min at 40 ^{0}c and terminated by adding 2ml freshly prepared DNSA solution, mixing and boiled for 5min.Standards were treated similarly with the exception that enzyme extract was added to the controls after DNSA. The samples & standards were measured to their relative activity with spectrophotometer read at 540nm after cooling to room temperature (Osman, 2002).In this investigation white wheat starch sample was used as the substrate. One unit of amylase activity was defined as the amount of enzyme that released 1µmol of reducing sugar equivalent to glucose per min under the assay condition (Hossein *et al.*, 2005).

3.2.2.2. Starch degrading malt enzymes optimal temperature, pH and their stability

The optimal temperature profile was evaluated by incubating the enzymes with natural starch substrate (1%) in the temperature range of 30-80 ^{0}C with 5 ^{0}C intervals. The reaction was continued or incubated for 10min at each temperature (5 ^{0}C intervals) and terminated by adding 2ml freshly prepared DNSA solution, mixing and boiled for 5min.Standards were treated similarly with the exception that enzyme extract was added to the controls after DNSA. The samples & standards were measured to their relative activity with spectrophotometer read at 540nm after cooling to room temperature (Osman, 2002).

PH optimization of enzymes was done with selected buffers (acetate, phosphate, citrate, tris and glycine) at pH range of 3-5 ,5-7 ,6.5-8 ,7.5-9 ,9-10)respectively.

The substrate (starch-natural) was adjusted to each pH as the buffer. Then the amylolytic activity was determined by incubating for 10min at 40 ^{0}c and terminated by adding 2ml freshly prepared DNSA solution, mixing and boiled for 5min.

Thermo stability of starch-degrading enzymes were determined using similar procedures as temperature profile except that incubating time was 30minfor temperatures 30-80 ^{0}Cand 60min for specifically 40 ^{0}C, 45 ^{0}C, 50 ^{0}C.

The buffer (pH) stability was determined as the enzyme was diluted to different buffers (described in pH profile for the pH range of 3-10) according to the determined dilution factor and incubated for 60min at room temperature. The substrate was prepared at pH 5 acetate in equal volume dilution .in order to minimize the effect of pH change; the incubated enzyme was taken in to 10 times dilution factor with distilled water. Then the activity was measured as usual assaying system (i.e.10min incubation period at 40 ^{0}C)

3.2.2.3 Malt quality analysis

The brewing malt (from Assela Malt Factory) was homogenized by thorough mixing to make uniform samples. It was then sampled and analyzed for basic quality parameters common to most brewing specification. Three types of malt samples were analyzed, all are from the same malting factory (Assela).

- **Sieve test**

Grading of malt was performed by using sieves of mesh size, 2.8, 2.5, 2.2, and less than 2.2mm from 100g of malt .The corns of size >2.8mm, >2.5mm, >2.2mm and <2.2mm was weighed separately. Foreign matters and half corns were separated out and mixed with corns of size <2.2mm.The results were calculated as percentage (%) (Kunze, 1996). The weight of malt at each grade in grams was converted in to percentage of malt.

- **Thousand kernel weight**

The thousand corn's weight of the malt sample was determined by counting 500 kernels from the sample of malt. The counted 500 kernels were weighed on balance. The result was calculated as percentage in dry basis (Declerk, 1994).

- **Milling**

Malted barley was milled in to fine and coarse- grind with a Buhler-Miag disc mill set to (0.2mm) and (1.00 mm) respectively. Moisture content of malt was determined on a fine-ground sample dried at 105°C for 3h in a convection oven method (ASBC, 1992).

- **Mashing**

Mashing was performed by time-temperature program mash system as described in EBC-methods (congress mashing procedure) for both fine and coarse grind. 50 g of fine and coarse ground malt was mashed in to 200ml stainless still beaker of preheated water and incubated at 45 ^{0}Cwith stirring for 30min.The temperature was increased over a 25min period at a rate of 1^{0}C per min to 70 ^{0}C.It was held for 60min at 70 ^{0}C for complete saccharification. The mash was tested for starch conversion after 10 min and throughout till the conversion was complete by using iodine starch conversion test. The pH of mash was also determined as in Dawn *et al (*2004).

- **Wort analysis**

After the initial mash, clear wort was collected and separated from spent grain by filtration using filter paper through filter funnel. The quality of the produced wort was evaluated for the following parameters; pH, original extract, color, filterability according to the brewing method of analysis (Dawn *et al.,* 2004*).* Color was determined with Lovibond 3000 color comparator and its filterability is below 60 min. Specific gravities of sweet wort for fine and coarse grind were determined at 20 °C with an Anton Paar DMA 5000 digital density meter. The difference between the fine and coarse extract was then calculated to determine the extract difference according to the following formula:

$$E_{f(c)}\% = \frac{(M+800)P\times 100}{(100-P)(100-M)} \qquad (1)$$

$$E_d = \%\text{ extract of fine grind} - \%\text{ extract of coarse grind}$$

Where, M-moisture content, P-Plato (saccharometer reading), $E_{f(c)}$ - extract of fine or coarse grind, Ed -extract difference, 800-constant

- **Protein analysis**

Malt Protein (total and soluble) content was measured using Kjeldahl method of protein determination (EBC, 1998) as the reference. Its Kolbach Index (KI) was calculated using the ratio of soluble protein to total malt protein (Declerk, 1994)

$$\text{Total protein content } (\%) = \frac{(T-B)\times 14\times N \text{ of acid}\times 6.25}{\text{Wt of sample}\times D\cdot M} \qquad (2)$$

$$\text{Soluble protein content } (N_v) = \frac{(T-B)\times 14\times 1000}{V} \qquad (3)$$

$$Kolbach \quad Index = \frac{\% \quad of \quad soluble \quad protein}{\% \quad of \quad Total \quad protein} \tag{4}$$

Where, Nv-soluble nitrogen content in1-liter of wort

T- Acid titration value (ml)

14 - Atomic weight of nitrogen

V - Sample volume (ml)

N-normality of acid

$$N_s = \frac{N_v \times E' \times 6.25}{100{,}000 \times E_w}$$

Where, Ns-soluble nitrogen content as percentage on dry basis.

3.2.2.4 Starch (Potato, Maize, Enset) analysis

- **Moisture content**

Starch from each source was evaluated for its moisture content by the oven drying method (AOAC, 1984). A measured weight of the sample was put in to a measuring pan and allowed to stand for 3h in a drying oven at 105 ^{0}C. Its result was expressed as percentage of the weight of the sample analyzed.

$$\% \quad of \quad moisture \quad content = \frac{W_2 - W_1 \times 100}{Wt \quad of \quad sample} \tag{5}$$

Where, W_1 = weight of empty drying pan, W_2= weight of pan + sample

- **Ash content**

Ash content was determined by the gravimetric method (AOAC, 1984) following furnace incineration and the total ash was converted to percentage.

$$\% \quad of \ ash = \frac{M_1 - M_2}{M_3} \tag{6}$$

Where, M = moisture content,

M_1 = mass of crucible,

M_2 = mass of fresh sample + Crucible

M_3 = mass of sample

- **Crude protein**

Samples of ground starch were mixed thoroughly to make a uniform mix and analyzed for its nitrogen content by Kjeldahl method (AOAC, 1984). Total nitrogen was converted to percentage protein using a standard factor of 6.25.

$$Total \quad protein \quad content \quad (\%) = \frac{(T-B)\times 14\times N \quad of \quad acid \times 6.25}{Wt \quad of \quad sample \times D\cdot M} \qquad (2)$$

Thereafter the moisture, ash and protein contents were subtracted from the crude starch of each crop to estimate the pure starch as indicated under here.

Starch content = Crude starch – (Moisture+ Ash+ Crude protein)

In doing so the fiber content of enset was considered as reported by Gebremariam and Brandt (1995).

3.2.2.5. Beer Production

- **Wort production**

For wort production, a series of experiments were performed in which starches produced from three different variety of crops (potato, enset, maize) were used as partial substitutes for barley malt in grist. Starch was added in each of the series of experiments as a substitute for barley malt: 0%, 10%, 20%, and 30% in grist (Briggs *et al.*, 2004). Experiments were carried out on laboratory scale by applying infusion-mashing procedure and brewing water was used for wort production. The starches were first gelatinized to their gelatinization temperature before adding to malt in grist formation in order to make available the starch granules to amylase (in the malt) attack. 50g of grist (malt + starch) was mashed in 200ml of preheated water and incubated at 45 ^{0}C with stirring (700rpm) for 30 min in stainless steel beaker. The temperature was increased over a 25min period at a rate of 1^{0}Cper min to 70 ^{0}C, 100ml of preheated water to70 ^{0}C was added, and held for a further 60min until the mash was completely saccharified. The mash was tested for its starch conversion using the iodine starch conversion test. The pH and odor of mash was also determined after cooling it to room temperature (Denault *et al.,* 1981). The content was made 450g with the addition of cold brewing water (sparge water) .The clear wort was collected and separated from the spent grain by filtration using filter paper through filter funnel. Finally clear wort analysis was performed using the standard of European brewery convention (EBC, 1998).

- **Hoped Wort boiling**

Upon completion of wort filtration, hop extracts and pellets were added and wort was boiled for 90min at boiling temperature. Following the boil, the hop debris and trubaceous matter were separated from the hopped wort by filter paper after cooling to 85^0C.

- **Fermentation**

Fermentation was carried out in a 2000ml 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 (12^0P-12.3^0P) and maintained at 13 ^{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 24h to calculate fermentability (attenuation limit).

Fig 3.6.Fermentation process (Picture)

- **Final product (Beer) analysis**

The final beer product was examined for color (Lovibond comparator), pH (pH-meter), apparent extract (hydrometer), refractive index (refractometer), taste, smell and odour (sensory) by standard EBC method. The alcohol content, original gravity (OG), real extract, real and apparent degree of fermentability was calculated based on refractive index and apparent extract results in relation with the density of beer(EBC,1998).

$$Real\ extract = 0.251 + 1.2980 \times S + 0.1179 \times R \quad (7)$$

$$Alcohol\ Content\ (AC) = 0.323 - (2.774 \times S + 02691 \times R) \quad (8)$$

$$Original\ Gravity = \frac{(2.0665 \times AC + Real\ extract)}{100 + (1.0665 \times AC)} \times 100 \quad (9)$$

$$App\ Degree\ of\ fermentation = \frac{Original\ extract - App\ extract}{Original\ extract} \times 100 \quad (10)$$

$$Real\ Degree\ of\ fermentation = \frac{Original\ extract - Real\ extract}{Original\ extract} \times 100 \quad (11)$$

Where, S- Specific gravity, R- Refractive index

3.2.3. Experimental design and data analysis

The three beer varieties including pure barley malt (control), namely barley-maize malt, barley-potato malt, and barley-enset malt were produced in triplicate and evaluated under completely randomized design. The collected data were subjected to analysis of variance (ANOVA) by using SPSS statistical soft ware (SPSS version 15.0, 2006). The mean Values of the quality parameters of the three beer varieties were compared using LSD test and the statistical significance was set at 5% level.

Chapter 4

Results and discussions

4.1. Assay of starch degrading enzymes

4.1.1 Extraction and assay of malt enzymes

It was established that 4ml acetate buffer was used to extract enzyme from 0.75g malt flour, yielded an average of 3.15ml enzyme;the difference being retained by the grist This result was nearly the same to the result obtained by Osman (2002) ,which was reported as an average of 3.18. This indicates that starch degrading enzymes were extracted well with acetate buffer (at pH=5) which seemed to be best extraction medium as it is in the range of the optimum pHs (4.00-5.50) of the enzymes which is suitable for mashing and starch degradation (Osman, 2002). The 3, 5-dinitrosalicylic acid (DNSA) was chosen for assay over the similar reducing agent *p*-hydroxybenzoic acid hydrazide because it was less damaging on starch and produced much less background color in the blank and controls.

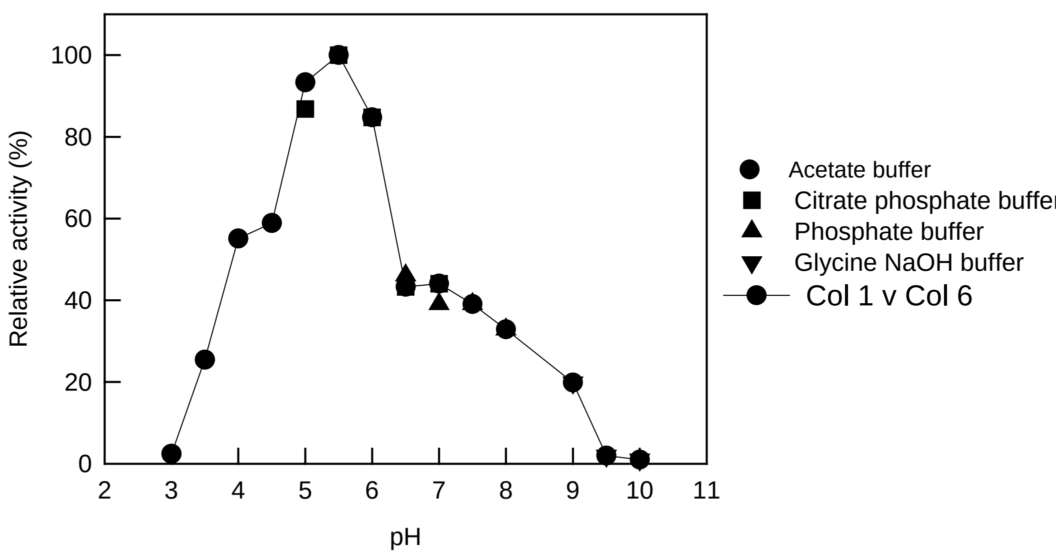


Fig 4.7.The PH profile of barley malt starch degrading enzymes assayed with natural substrate (soluble starch).

4.1.2. Starch degrading enzymes optimal temperature, pH and their stability

The pH optima for starch-degrading enzymes assayed with natural substrates using different buffer pHs are shown in Fig 4.7. The optimum pH was (pH 5.5). It also coincides with the recommended pH (5.5) of industrial mashing as described by Kunze (1996). Therefore, pH 5.5 was chosen as the pH of assay for starch-degrading enzymes to evaluate their roles in starch- degradation during mashing. The pH stability is also shown in Fig4.8.

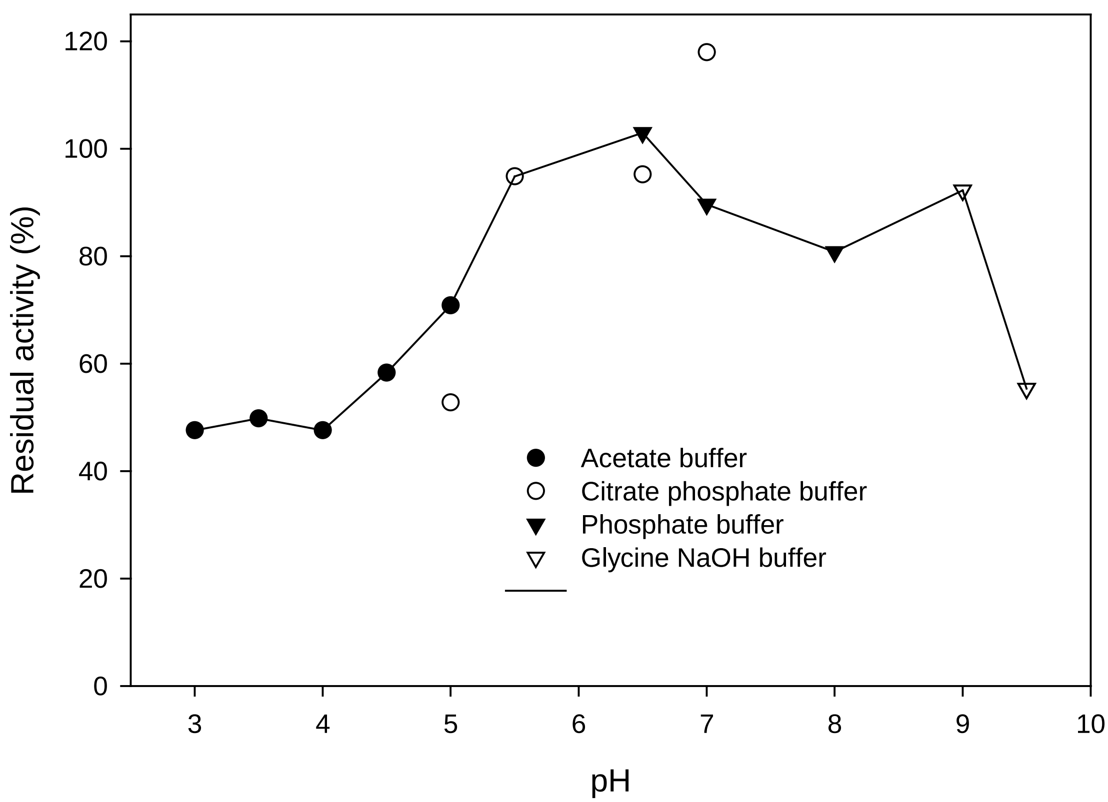


Fig4.8.The PH stability of Barley malt starch degrading enzymes.

Thermo stability is an essential requirement of starch degrading enzymes for the industrial use of barley. The results of optimal temperatures and thermo stability of starch-degrading enzymes are shown in Figs 4.9 & 4.10 (A, and B) respectively. These results indicate the optimal temperature was 55 ^{0}C. It is possible to note that there is a possibility of losing more than 30 % of their activity within 30 min of mashing, at a temperature of 50 ^{0} c (Osman, 2002).According to Annocer(2008), the lowest temperature (45 ºC) is the optimal temperature for cell wall degrading enzymes, ß-glucanases. The proteases works best at 52 ºC, the ß-amylase best at 63 ºC and the α-amylase at 72°C. The last step in the mashing is inactivation of the enzymes at 78 ºC. Therefore, this result shows that the commulative effect of enzyme activities are high at low temprature and decreases when the mashing temprature increases . Temperature is a controlling factor in enzyme activity and wort fermentability, since it affects the range and extent of sugar extraction, as well as the type and amount of sugars extracted (Reilly.*etal*, 2004)

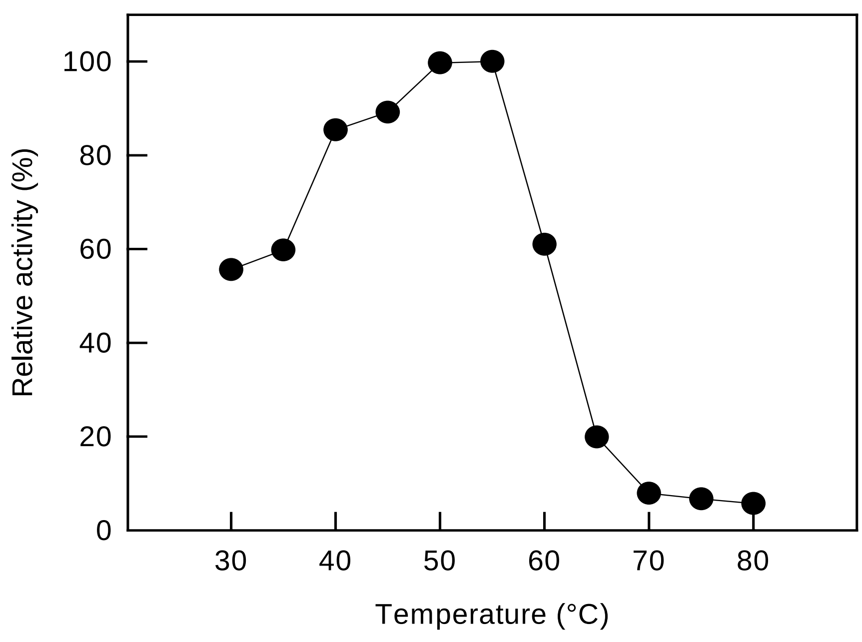
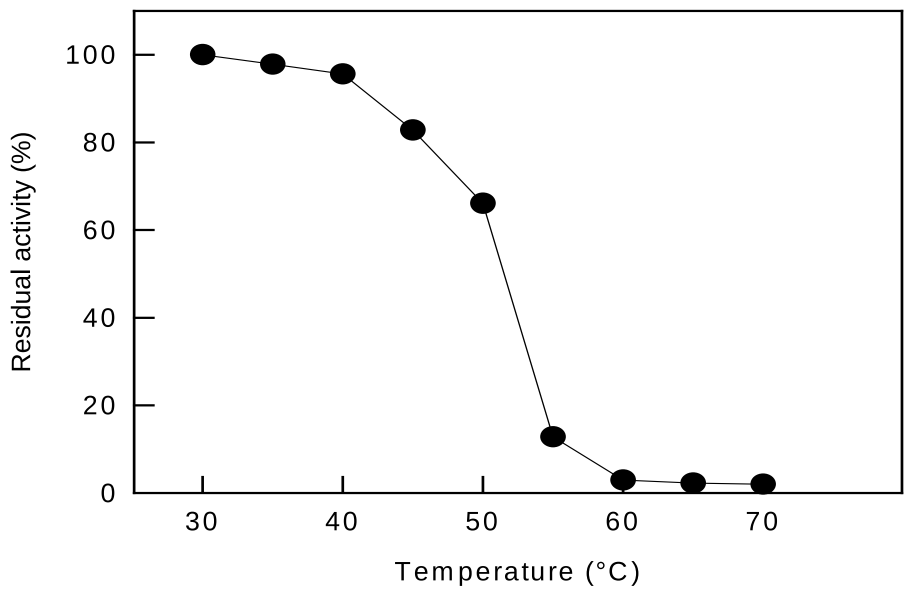


Fig4.9.Optimal temperature of starch degrading enzymes with soluble starch incubated at 10 min.



Fi
g4.10A. Temperature stability of malt enzyme for 30min of mashing time

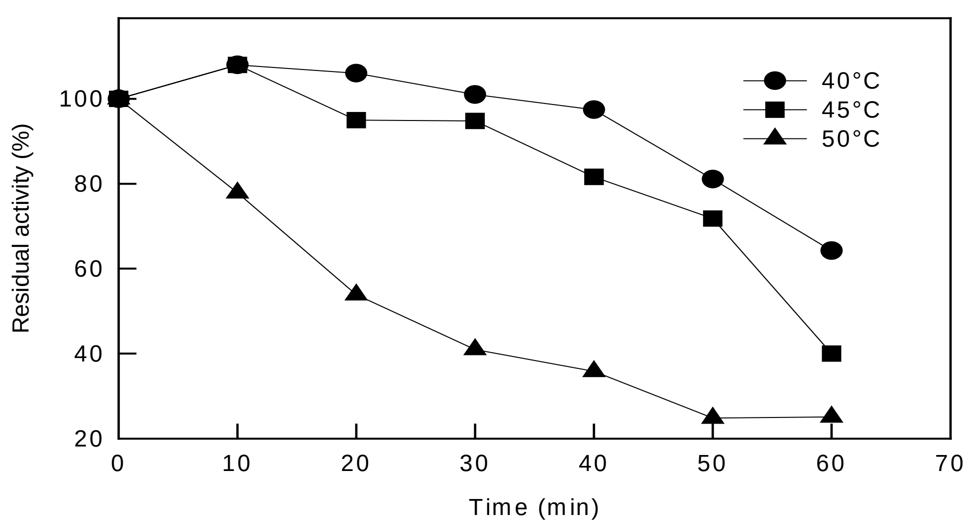


Fig4.10B.Thermo stability of starch degrading enzymes measured with soluble starch for the mashing time of 60min at 40 ^{0}C, 45^{0}C, 50^{0}C.

4.2 Starch (Potato, Maize, Enset) analysis

The starches from each source were analysed for their moisture, protein and ash content. The results are given below in Table 4.2

Table4.2**.** Proximate analysis result of starches on dry basis (Means $\pm$SD)

Source of starch	Moisture (%)	Protein (%)	Ash (%)	pH(Acidity)
Potato	13.78 $\pm$0.38	0.24 $\pm$ 0.03	0.47 $\pm$0.05	6.45
Maize	12.00 $\pm$ 0.00	5.58 $\pm$ 0.22	0.36 $\pm$0.05	4.24
Enset	19.09 $\pm$ 0.11	0.42 $\pm$ 0.02	0.58 $\pm$0.08	6.77

The moisture content of potato starch was in lines with a dextrin manufacturer's standard in America (14%) and its ash content should never exceed 0.5 %(Charles, 1953). The results obtained in maize starch was showing less variations in moisture & differ in ash content from the data (Moisture =13.5%, ash =0.15%) reported by Ningbo Shengda Kaifu International Trading Co., Ltd (China).

Fig 4.11 .Starch of Enset (picture)

The moisture and ash contents of Enset starch were slightly higher than others (Maize and Potato). The difference in moisture content may depend on the extent of drying (Chen, 2003). The starch granule structure of enset is similar to potato as reported by Gebremariam and Schmidt (1995). The protein content (on dry basis) of maize starch was higher than potato and enset and this is expected due to the gluten content. The protein and ash content of enset starch was different from the results obtained by (protein = 0.35%, ash = 0.16%) Gebremariam and Schmidt (1995)

Fig4.12. Shows Maize and its starch (picture)

Fig4.13. Shows Potato and its starch (Picture)

4.3. Malt analysis

The analysis results of the three malt types from Assela Malt Factory are shown below in Table 4.3**.** The slight difference in quality parameters is not unusual for malts in the same geographical location and same factory due to various factors (cropping, malting, and processing, instrumental and human factor). Although there was some variation in some quality parameters all the three malts were with in the specification. On the basis of results, Malt-1 was chosen for this research as control malt (full barley malt) due to its yield (extract), filtration time, Saccharification performance, and its protein content. This evaluation was carried out because the barley seeds are collected from different plots.

Table4.3**.** Analysis results of barley malts (Means ± SD)

Parameters	Standard (EBC)	Analysis results		
		Malt-1	Malt-2	Malt-3
Thousand kernel weight(g)	25-35	30.70 ± 0.27	31.88±3.08	28.00±1.24
Moisture (%)	3-5.8	4.65 ± 0.60	4.83 ±0.31	4.49 ± 0.15
Extract fine, (%)	77,min	79.59 ± 0.46	78.92±0.38	77.83±0.27
Extract Coarse (%)	75,min	77.67 ± 0.14	77.58±0.73	76.49±0.19
Fine/Coarse extract Difference	2-2.6	1.94 ± 0.60	1.35 ± 0.59	1.34±0.29
Saccharification time	10-20min	11.67 ± 2.89	13.33 ±2.89	15.67±1.15
Wort clarity	Clear	Clear	Clear	Clear
Filtration time	≤1h	34.00 ± 1.73	36.33±1.55	40.00 ±0.00
Color, EBC Unit	2.5-4,EBC	3.50 ± 0.00	3.00 ±0.00	3.00 ± 0.00
Total protein (%),Dray basis	9-11.5	11.11 ± 0.24	10.28±0.21	10.45 ±0.87

4.4. Beer Production

4.4.1 Brewing using full barley malt (Control brew-Malt-1)

4.4.1.1 Mash and sweet wort analysis

The selected malt (M-1) was used to brew lager beer using an infusion-congress mashing (as described in the method of mashing protocol). The brewing data is shown in Table 4.4. According to the values of analytical parameters of mash and sweet wort analysis presented in this table; the saccharification time, lautering, its color, yield or extract content was all in good range. Its soluble protein, important for yeast fermentation, was in required standards according to EBC (1998)

Table4.4.The analysis results of Mash &Sweet Wort for full barley malt (Means ±SD)

Parameters	Values	Std(EBC)
Mash pH	5.74 $\pm$ 0.05	5.50-5.80
Sweet Wort Extract /Yield, ^{0}P	8.43 $\pm$ 0.03	-
Sweet Wort color, EBC	3.50 $\pm$ 0.00	2.5-4,EBC
Soluble Protein (%)	3.13 $\pm$ 0.04	2.5-3.5
Kolbach Index (Sprotein/Tprotein)	28.17 $\pm$0.51	-
Sweet wort clarity	Clear	Clear
Mash Lautering/Filtration time	34.00 $\pm$1.73	≤60 min
Saccharification time, in min	11.67 $\pm$2.89	10-20min
Iodine Test (mash)	Negative	Negative

Kolbach Index= (Soluble protein/Total protein)

4.4.1.2 Quality of wort

Quality wort is important for producing quality beer. Beer wort was analyzed for quality parameters .As it is shown in Table 4.5 the analysis results of beer wort quality parameters such as color ,extract, pH , bitterness and its saccharification were fulfilling the standards(Declerk,1996).

Table 4.5.Analysis results of beer wort (Means ± SD)

Parameters	Values	Std(EBC)
Wort pH	5.25 $\pm$ 0.06	5.00-5.30
Wort color, EBC units	8.50 $\pm$ 0.00	7.00-11.00
Original Gravity, ^{0}P(as-is)	11.99 $\pm$0.02	11.80,min
Bitterness, BU unit	32.67 $\pm$0.58	20.00-40.00
Wort Iodine test	Negative	Negative
Wort boiling time, min	90.00 $\pm$0.00	-

4.4.1.3 Fermentation

The fermenting worts were monitored daily for its extract or sugar drop. 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 asses the fermentability and quality of the wort .The wort produced was found to have an attenuation limit of 2.60 and subsequent fermentation studies showed complete attenuation after 120 h. The details are given in Table 4.6 below.

Table 4.6 Fermentation of full barley Malt brews.

Time (hr)	Original gravity,^{0}P	pH
0	11.99	5.25
24	8.00	4.90
48	4.50	4.63
72	3.00	4.55
96	2.75	4.50
120	2.60	4.50

4.4.1.4 Beer analysis results

Final beers were analysed for a range of key quality parameters and the results are shown below in Table4.7. The alcohol content, original gravity, real and apparent degree of fermentation and those measured directly by instruments are reported. For a normal beer, the parameters were conforming to the quality standards of beer. The values are also in line with the values of the ASBC standard (ASBC, 1999., EBC, 1998).

Table4.7. Full barley malt beer analysis (Means ± SD)

Quality parameters	Value	Std(EBC)	QSAE
Original extract, (%) by mass	12.48 ± 0.57	10-14	11.5min
Beer color, EBC unit	7.50 ± 0.00	5-10	-
Beer pH	4.51 ± 0.07	4.35-4.65	3.9-4.5
Apparent Extract, (%) by mass	2.57 ± 0.06	1.70-2.70	1.5min
Refractive index	39.92 ± 0.49	-	-
Alcohol content, (%) by mass	4.16 ± 0.23	3.50-4.50	3.5min
Apparent degree of fermentation (%)	79.42 ± 0.48	75-85	-
Real degree of Fermentation (%)	65.3 ± 1.23	60-70	-
Real extract, (%) by mass	4.45 ± 0.16	3.5-4.5	-
Bitterness ,BU units	24.00 ±1.00	20 (min)	20-40

4.4.2 Results of brewing trials with starch as a partial substitute for malt

All the starch substitutes were gelatinized with malt and malt amylolytic activity were determined on the composite to know whether additional microbial enzyme is needed other than malt amylase. For all the three starches (potato, enset, maize) in the three starch-malt compositions (10%, 20%, and 30%), the saccharification time was normal (10 to 20min).This showed that malt usually contains an excess of amylolytic enzymes, so up to 30% of the malt can be replaced with a starch fraction at the mashing step without the need to add enzymes as described by Ahvenainen and Kuhanen(1993).

4.4.2.1. Brewing with Potato starch composite

4.4.2.1.1. Results of mash and sweet wort analysis

As with the all-malt brew, at 10% starch substitutes the yield of extract was the same. However as the percentage of starch increases the yield of extract was a bit higher than the control brew. Color of sweet wort at 20% and 30% substitute was less than the control brew but color of sweet wort in 10% substitute was similar to the control. . Lautering/Filtration time at 30%grist was higher than the control brew and in 10% and 20% grist. Saccharification time was good for all worts produced from potato starch-malt composite (<20 minutes). The details are given in Table 4.8

Table 4.8.Analysis results of potato starch-malt composite mashing and sweet wort (Means ± SD)

Parameters	Substitute in percent			Full barely malt
	10	20	30	
Mash pH	5.64±0.02	5.60 ±0.01	5.65 ±0.02	5.74 ± 0.05
Sweet wort extract, ^{0}P	8.43 ± 0.06	8.47 ±0.06	8.57 ±0.06	8.43 ±0.03
Sweet wort color, EBC unit	3.50 ± 0.00	3.33 ±0.10	3.25 ±0.00	3.50 ±0.00
Sweet wort clarity	Clear	Clear	Clear	Clear
Lautering/filtration time (min)	35.00±0.00	41.47±2.89	48.33±2.89	34.00±1.73
Saccharification time	15.0 ± 0.00	16.33±1.15	19.33±1.15	11.67±2.89
Mash Iodine test	Negative	Negative	Negative	Negative

4.4.2.1.2 Beer wort quality

As indicated in Table 4.9**,** the beer wort produced with 30% of potato starch substitute yielded the highest extract content (12.32). Wort produced with 20% had a bit higher extract content than control wort (12.05); where as the wort produced with 10% substitute was equal as the control brew in yield (11.99).

Generally original gravity/extract content was increasing as the amount of substitute increases, it is clear that on advantage of adjuncts are used mainly because they provide extract at a lower cost (a cheaper form of carbohydrate) than is available from malted barley (Beer Adjuncts from http://pubs.acs.org/cen/whatstuff/84/841...Retrieved on 23/05/09), while the color of the wort decreases. It is not unusual that color source of wort is from the kilning of barley in the malting process as explained by Olgica and Jalena (2007).

Table.4.9**.** Wort quality analysis results of brews with potato starch (Means ± SD)

Parameters	Substitute in percent			Full barley malt
	10	20	30	
Wort pH	5.29 ± 0.09	5.23 ±0.03	5.23 ± 0.23	5.25 ± 0.06
Wort color, EBC unit	8.50 ± 0.00	8.42 ±0.14	8.00 ± 0.00	8.50 ± 0.00
Original gravity, ^{0}P	11.99 ± 0.01	12.05±0.05	12.32 ±0.06	11.99 ±0.02
Bitterness, BU	32.67 ± 1.53	32.67±2.31	31.67 ±1.53	32.67 ±0.58
Wort boiling time,min	90.00 ± 0.00	90.00±0.00	90.00 ±0.00	90.00 ±0.00
Wort iodine test	Negative	Negative	Negative	Negative

4.4.2.1.3 Fermentation

As the control brew, the fermenting worts were monitored daily for gravity and the results are shown below in Table4.10**.** There was fermentability difference between the proportions, as the percentage of the substitute increases, fermentability decreases. Real and apparent wort attenuation decreased with an increase in composite malt share. The gravity drop was slower than the control brew in all the proportions. There were indications for potential fermentation problems. Occasionally the beers were stop fermenting a few degrees above the required final gravity. This may be caused by little oxygen added to the wort, flocculation of the yeast. Thickness of the mash may also be the factor because the amylase activity is highly dependant on it, which in turn limits the amount of fermentable worts. The pH drop during fermentation is a normal drop (ASBC, 1999).

Table 4.10**.**Fermenting process of composite brewing

Time (h)	Drop of gravity and its pH

	10%	pH	20%	pH	30%	pH
0	11.99	5.30	12.05	5.23	12.32	5.23
24	9.80	4.92	9.84	4.91	10.97	4.90
48	5.54	4.83	5.60	4.88	5.67	4.91
72	5.03	4.82	5.14	4.80	5.21	4.67
96	3.51	4.73	3.63	4.69	4.17	4.66
120	3.32	4.70	3.58	4.69	4.01	4.59
144	3.28	4.65	3.45	4.65	3.91	4.62
168	3.28	4.66	3.42	4.63	3.67	4.59
192	3.26	4.66	3.40	4.65	3.65	4.57
216	3.25	4.66	3.40	4.65	3.65	4.57

4.4.2.1.4. Beer quality

The finished beers were analysed for quality parameters. Results are given in Table4.11**.**There was a difference between the control beer and composite beer for most parameters, as would be expected from the fermentation results. Alcohol concentrations were in general lower than that of the all-malt beer suggesting that the extracts were not fermentable. This was indicated by the less attenuation limits for the composite beers and by their high original gravity which probably is the reason described in the fermentation process (Table 4.11). Fermentation performance was significantly slower than the all-malt brewing which is reflected in alcohol contents (Annex-2). It is below the average in all the three compositions, though it is with in the acceptable range. The real degree of fermentation was near the lower limit standard as described in EBC (2005) due to higher real extract and the apparent degree of fermentation was lower than the control brew but in the range of standards.

Table4.11. Analysis results of finished beers from potato starch-malt composite brews (Means ± SD).

Parameters	Substitute in percent			Full barley malt
	10	20	30	
Apparent extract (%)	3.25±0.05	3.40 ± 0.19	3.62 ±0.27	2.57 ± 0.06
Beer pH	4.66±0.00	4.65 ±0.01	4.57±0.05	4.51 ± 0.07
Original gravity (%)	13.77±0.23	13.62±0.34	14.91±0.11	12.48 ±0.57
Color	7.50±0.00	7.50±0.00	7.25±0.00	7.50 ± 0.00
Alcohol content (%)	3.80±0.08	3.72±0.07	3.63±0.06	4.16 ± 0.23
Real extract (%)	5.24±0.09	5.34±0.22	5.78±0.24	4.45 ± 0.16
App degree of fermentation (%)	76.29±0.03	75.08±0.84	75.71±1.62	79.42 ±0.48
Real degree of fermentation (%)	61.86± .15	60.60±0.68	61.31±1.38	65.3 ± 1.23

4.4.2.2. Brewing with Enset starch

4.4.2.2.1. Mash and Sweet wort of enset starch as composite malt

The brewing result of the Enset starch as the partial substitute of malt is given in Table 4.12**.** Enset starch gave a good yield of extract content in both sweet wort and beer wort when compared with the full malt brew in all the three proportions (10%, 20%, and 30%) Olgica and Jalena (2007). For the first two proportions(10%, 20%), its yield of beer wort was better than potato starch brew .However its lautering/filtration was slower than the control brew and potato brew, but was still adequate, whereas its saccharification time was a little higher than the control brew and nearly the same with potato starch brew. The substitute of enset starch from 10% up to 30% resulted in increased lautering and saccharification time with in the acceptable range.

4.4.2.2.2. Beer Wort quality

Results of the beer wort analysis for these brews are indicated in Table 4.13. The sets of brews did not exhibit any significant variations other than the wort extract content as already noted in sweet wort. Substitute of starch in 10-30% caused the wort color to decrease compared to control wort. Wort pH value is good for beer production according to Kunze (1996) and wort clarity was not influenced by increased starch share.

Table4.12**.** Analysis result of mash and sweet wort brewing partly with Enset starch and comparison with full barley malt (Means ± SD)

Quality descriptors	Substitutes in percent(Enset)			Full barley malt
	10	20	30	
Mash pH	5.67 ± 0.02	5.65 ± 0.09	5.63 ± 0.03	5.74 ± 0.05
Sweet wort extract/ yield	8.50 ± 0.00	8.58 ± 0.03	8.68 ± 0.03	8.43 ± 0.03
Sweet wort color	3.33 ± 0.14	3.25 ± 0.00	3.08 ± 0.14	3.50 ± 0.00
Sweet wort clarity	Clear	Clear	Clear	Clear
Lautering time, min	40.00 ±0.00	45.00 ±0.00	52.67 ±2.52	34.00 ±1.73
Saccharification time(min)	15.00 ±0.00	15.00 ±0.00	18.67 ±1.15	11.67 ±2.89
Iodine test	Negative	Negative	Negative	Negative

Table4.13**.** Wort quality analysis results of Enset brewing and comparison with full barley malt wort (Means ± SD)

Parameters	Substitute in percent(Enset)			Full barley malt
	10	20	30	
Wort pH	5.31 ± 0.15	5.29 ±0.19	5.30 ±0.18	5.25 ± 0.06
Wort color, EBCunits	8.50 ± 0.00	8.17 ±0.14	7.75 ±0.00	8.50 ± 0.00
Original Gravity, ^{0}p(as-is)	12.25 ±0.05	12.27±0.06	12.33±0.21	11.99 ±0.02
Bitterness, BU unit	32.33 ± 1.53	32.33±0.58	32.33±1.53	32.67 ±0.58
Wort Iodine test	Negative	Negative	Negative	Negative
Wort boiling time, min	90.00 ±0.00	90.00±0.00	90.00±0.00	90.00 ±0.00

4.4.2.2.3. Beer analysis results

As with the earlier brews, all the parameters were assessed & results are given below in Table4.14. All brews in the composition (10%, 20%, and 30%) were fermentable as indicated by the values of apparent extract, which is with in the EBC standards (EBC, 2005). They provided an above average alcohol concentration, which is little less alcohol concentration than the control brew and better than potatoes starch beer. The potatoes starch was found to be below average. Beers produced with 10-30% of substitute yielded equivalent real and apparent attenuation with the control beer and lower in original gravity than the control beer. There was insignificant variation in color and bitterness. The refractive index of the control brew was higher than enset brew indicating that it is better in original gravity and alcohol content. Generally the enset brews were good in overall performance except the alcohol content in the 10% substitute which became below the average value. It is however with in the acceptable range.

Table4.14. Results of enset starch composite beer analysis compared with the control beer (Means ± SD).

Quality Parameters	Substitute in percent (Enset)			Full barley malt
	10	20	30	
Original extract (%),by mass	11.92±0.04	12.11±0.25	12.14±0.06	12.48 ± 0.57
Beer color, EBC	7.50 ±0.00	7.50 ±0.00	7.33 ±0.14	7.50 ± 0.00
Beer pH	4.36 ±0.00	4.34 ± 0.03	4.38 ±0.01	4.51 ± 0.07
Apparent Extract (%),by mass	2.43 ±0.04	2.29 ±0.29	2.44 ±0.05	2.57 ± 0.06
Refractive index	38.35±0.04	38.5 ±0.77	38.63±0.22	39.92 ± 0.49
Alcohol content (%),by mass	3.97±0.03	4.09 ±0.17	4.03 ±0.02	4.16 ± 0.23
App degree of fermentation (%)	79.59±0.34	81.15±2.29	79.89±0.40	79.42 ± 0.48
Real degree of Fermentation (%)	64.54±0.22	65.61±1.82	64.42±0.41	65.3 ± 1.23
Real extract (%),by mass	4.23 ±0.02	4.21 ±0.24	4.28 ±0.05	4.45 ± 0.16
Bitterness, BU	21.57±0.67	21.33±0.32	21.55±0.95	21.73 ±2.02

4.4.2.3. Brewing with Maize starch as a partial substitute for malt

4.4.2.3.1. Mash and sweet wort analysis results

Table4.15 Mash and sweet wort analysis results in maize starch brew

(Means± SD)

Quality attributes	Substitute in percent (Maize starch)			Full barley malt
	10	20	30	
Mash pH	5.59 ±0.01	5.59 ± 0.08	5.57 ±0.15	5.74 ±0.05
Sweet wort extract/ yield (^{0}P)	8.40 ±0.00	8.57 ± 0.06	8.77 ±0.06	8.43 ±0.03
Sweet wort color, EBC	3.50 ±0.00	3.50 ± 0.00	3.42±0.144	3.50 ±0.00
Sweet wort clarity	Clear	Clear	Clear	Clear
Lautering /Filtration time, min	38.67±1.15	40.00 ±0.00	40.00±0.00	34.00±1.73
Saccharification time, min	13.33±1.53	14.67 ±0.58	17.00±0.00	11.67±2.89
Iodine Test	Negative	Negative	Negative	Negative

The sweet wort yield or extract content of the three composite brews for maize starch were better than of all brews (Potato, Enset, Control).The color of the sweet wort was similar to the control brew for 10% ,and 20% proportions , but for the 30% composite brew it was little less than the control, as would be expected from the proportions of maize ,which imparts less color than malt(Baxter,2002).The lautering time of the brews was less in the 10%substitute(38.67 min) and it was equal for the 20% and 30% composite(40.00 min).The lautering/filtration time was better when compared to the two starch composite brew(Potato and Enset starch) but higher than the full malt(34.00) . Starch conversion /Saccharification time was also better than potato and enset starch, and higher from the all-malt brew. There was no difference in the wort clarity. Beer wort color and pH did not show large variation. However, there was considerable difference between beer wort extracts in maize brew grist's in increasing order of starch portion (12.00±0.00, 12.22±0.03, 12.47±0.06) respectively and control brew (11.99±0.02).

The extract content in 10% starch substitutes of the potato starch brew, maize brew and control brew (11.99±0.01 to 12.00 +0.00) did not show great variation except in enset starch which is12.25 ± 0.05. As the general trend in all the three starch shares an increase in substitution percentage showed an increase in original gravity or extract content (Table4.16).

4.4.2.3.2. Beer wort quality analysis

Table 4.16 .Results of wort analysis of maize brewing (Means ± SD)

Quality attributes	Composite in percent (Maize starch)			Full barley malt
	10	20	30	
Wort pH	5.32 ± 0.02	5.24 ± 0.07	5.26 ±0.02	5.25 ±0.06
Wort color, EBC units	8.58 ±0.14	8.50 ± 0.00	8.42 ±0.14	8.50 ±0.00
Original Gravity, ^{0}P(as-is)	12.00 ±0.00	12.22 ±0.03	12.47±0.06	11.99±0.02
Bitterness, BU unit	32.2 ±0.80	32.50 ±0.90	31.8 ±0.85	32.67±0.58
Wort Iodine test	Negative	Negative	Negative	Negative
Wort boiling time, min	90.00 ±0.00	90.00 ±0.00	90.00±0.00	90.00±0.00

4.4.2.3.3. Beer analysis results

The maize starch beers found to have mean values of 7.50, EBC color Unit in the three composites (10%, 20% and 30%). This color value was not showing a difference on the other brews too except at 30% enset and potato starch substitute, which is a little less .The pH values of enset and maize substitutes showed insignificant variation, but the variation was noticeable between pH of maize substitute beers(Table4.17),and control beer as well as potato substitute beers(Table11).The apparent extracts of the maize substitutes were indicated as 2.38 in 10%, 2.50 in 20% & 2.47 in 30% substitution which were less than the control brew. The apparent extract of maize and enset brew indicated that the fermentablity was better than the control. The alcohol content, which is part of the major component of beer, in maize brews was found to be 4.09% 4.15% & 4.04% in increasing order of substitute. The results of the entire physicochemical parameters of maize brew are in the normal ranges (Table4.17).

Table 4.17 Values obtained from maize beer analyses (Means ± SD)

Quality Attributes	Composite in percent (Maize starch)			Full barley Malt
	10	20	30	
Original extract (%)	12.17±0.06	12.39 ±0.07	12.13	12.48±0.57
Beer color ,EBC	7.50 ±0.00	7.50 ± 0.00	7.50 ±0.00	7.50 ±0.00
Beer pH	4.36 ±0.01	4.37 ±0.00	4.36 ±0.01	4.51 ±0.07
Apparent Extract (%)	2.38 ±0.09	2.50 ±0.00	2.47 ±0.04	2.57 ± 0.06
Refractive index	38.61±0.28	39.28 ±0.11	38.74±0.04	39.92±0.49
Alcohol content (%)	4.09 ±0.03	4.15 ±0.03	4.04 ±0.04	4.16 ± 0.23
App degree of fermentation (%)	80.42±0.64	79.87 ±0.13	79.77±0.41	79.42±0.48
Real degree of Fermentation (%)	65.11±0.59	64.68 ±0.10	64.56±0.35	65.3 ± 1.23
Real extract (%)	4.24 ±0.08	4.38 ±0.01	4.30 ±0.03	4.45 ±0.16
Bitterness ,BU	21.50±0.46	21.64 ±0.18	21.67±0.85	21.73±2.02

Table4.18. Alcohol content of Potato Maize and Enset beers (30%) (Means ± SD)

Source for substitute	Alcohol Content (%)
Potato	3.63±0.06 [b]
Maize	4.04 ±0.04 [a]
Enset	4.03 ±0.02 [a]
Control	4.16 ± 0.23 [a]

Note: - Alcohol Means bearing the same superscript do not statistically differ from each other at p= 0.5 for Maize starch (30%)

4.5. Sensory Analysis

Table 4.19 Sensory evaluation results of beers (Means ± SD)

Sensory Descriptors	Product type									
	Full barley Malt	Potato substitute (%)			Enset substitute (%)			Maize substitute (%)		
	100	10	20	30	10	20	30	10	20	30
Bitterness	4.13 ± 0.12	3.33±0.12	3.33±0.31	3.13±0.12	4.13±0.31	4.13±0.12	4.13±0.23	4.00 ±0.2	4.07±0.31	4.13±0.46
Color	4.53 ± 0.12	4.20±0.35	4.20±0.20	4.00±0.20	4.53±0.12	4.00±0.20	3.87±0.12	4.53±0.12	4.13±0.50	4.20±0.35
Aroma	4.20 ± 0.20	3.20±0.20	2.80±0.00	2.93±0.12	4.20±0.20	3.60±0.40	4.20±0.20	4.00±0.00	4.13±0.23	4.27±0.12
Mouth fullness	4.33 ± 0.12	3.13±0.12	3.07±0.12	3.07±0.31	4.33±0.23	3.80±0.20	4.00±0.20	4.33±0.12	4.27±0.23	4.20±0.12
Overall acceptability (Flavor)	4.13 ± 0.12[a]	3.20±0.35	2.87±0.12	2.87±0.1[b]	4.27±0.20	3.87±0.23	4.13±0.12[a]	4.20±0.35	4.07±0.23	4.07±0.12[a]

Scores: 5 = Excellent, 4 = Very good, 3 = Good, 2 = Fair, 1 =Poor

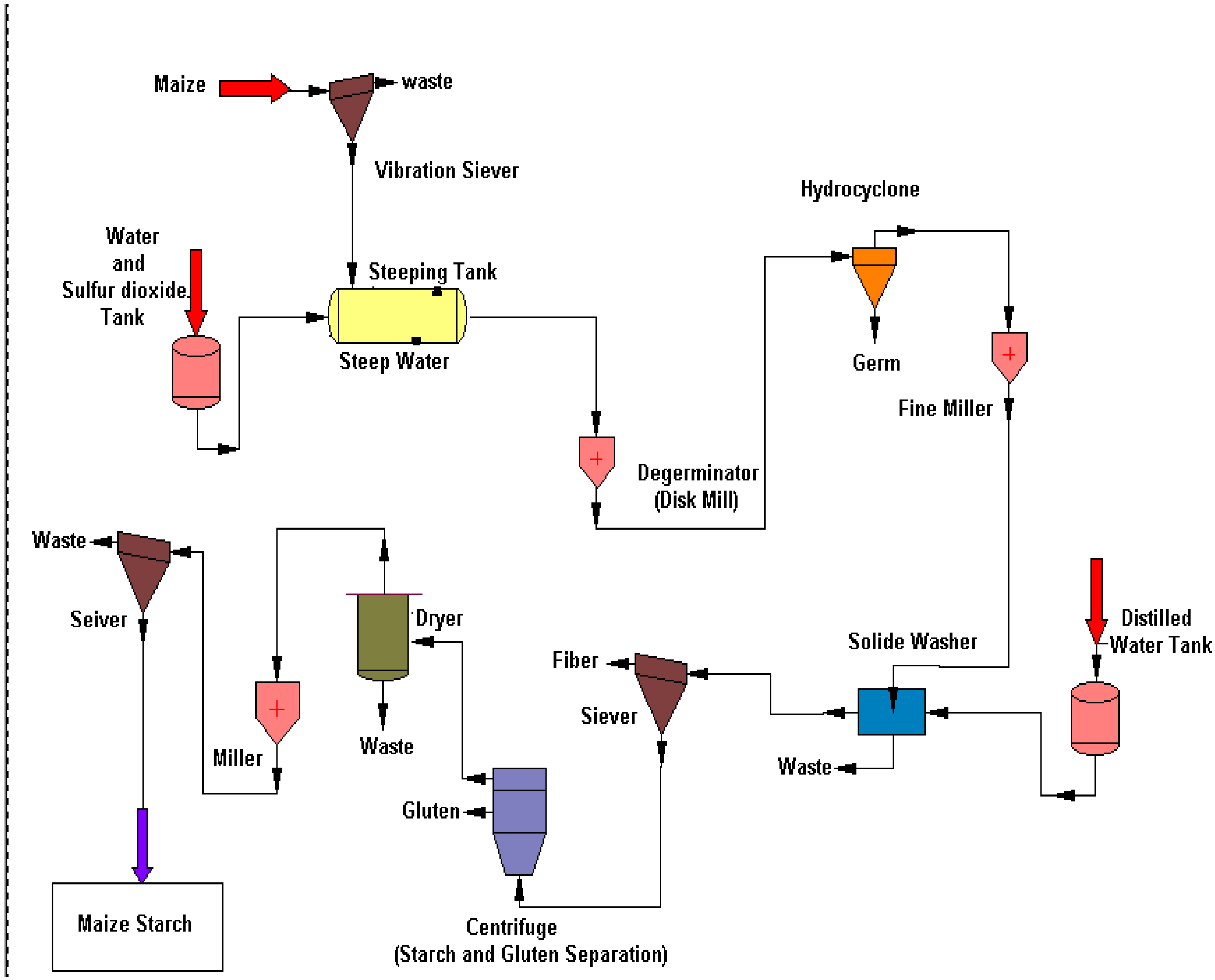
Note: - Flavour Means bearing the same superscript do not statistically differ from each other at p = 0.5 for Maize starch (30%

According to the comparison analysis of alcohol content of beers; potato substitute was found to show significant mean difference at LSD 5% with both the control and other beers. As it is indicated in the fermentation process of potato beer, (Table 4.10) the apparent extract (unfermentable dextrins) was larger than other brews .Enset and maize beers were showing good alcohol content (Ac) except that enset composite beer at 10% substitute (Ac = 3.97%) showed a significant variation when compared with the control (Ac = 4.16%) and with maize beer at composite (20%). Maize beers were comparable with in the groups, between the groups and with the control beer (see annex 2, Table 25).

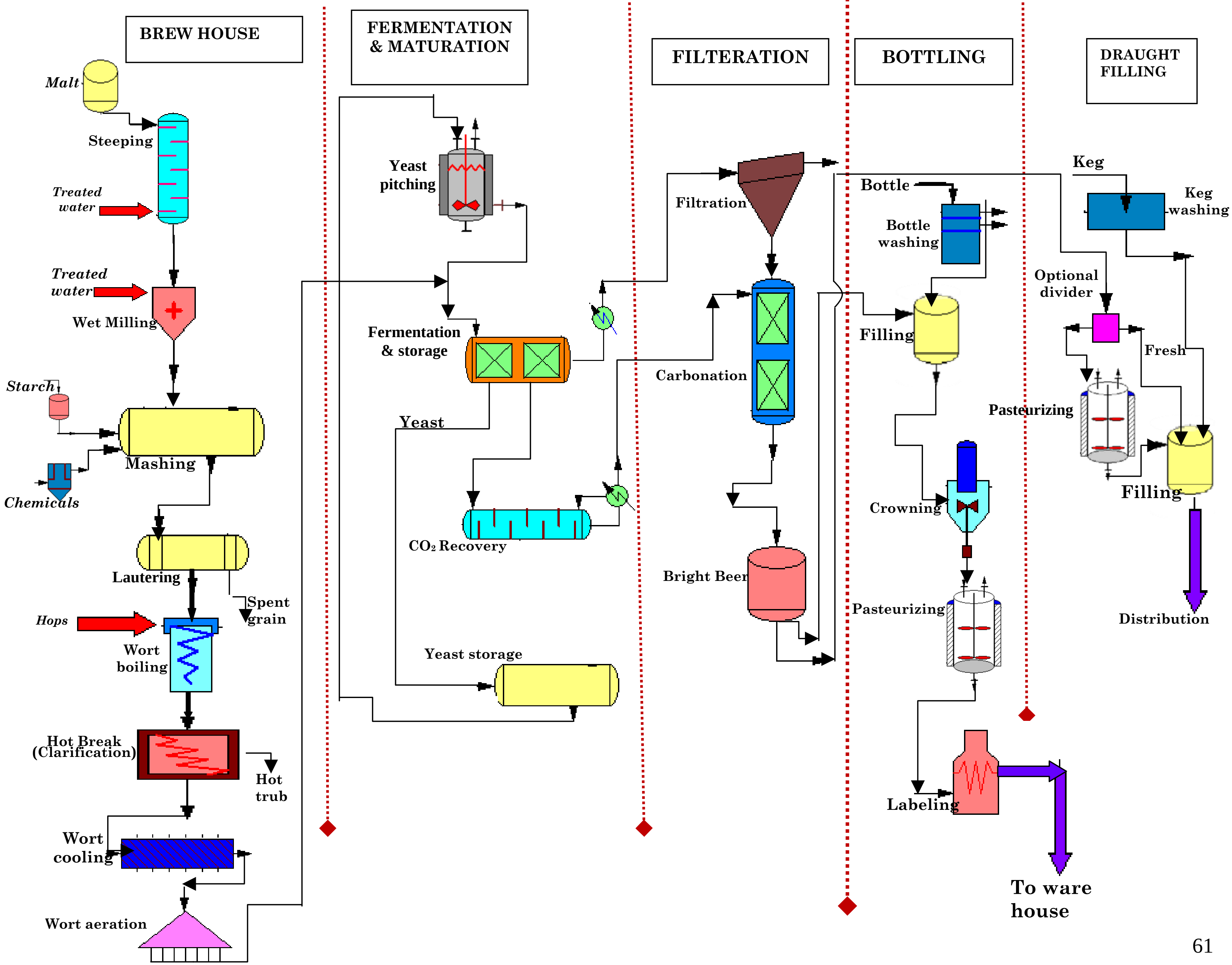
In the sensory analysis result, all the sensory parameters of the control beer were scoring above very good. The color of the beer is the first eye appeal of the consumer. This parameter was scoring above very good (4.00 to 4.53 EBC) in all beers except the beer of enset at 30% brewing which is scoring 3.87.There was no significant difference in bitterness of enset, maize and control, but the potato beer was inferior in all qualities compared to the others. Enset beer was found to have sensory values of above very good in 10% and 30% substitution, whereas the 20% for the parameters aroma, mouth fullness and flavor (3.60 ,3.80 & 3.87) respectively which is above good.

The sensory analysis results showed that the over all acceptability tests (flavor) showed significant mean difference for the potato beer in all its three composites with in the group and between the groups (Maize, Enset & Control). Enset beer at composite 20% gave a significant mean difference of with in the group and between the groups, whereas maize beers gave better results in all its composites with in & between the groups (See Annex 3, and table 26).

4.6.1 Process flow sheet of starch production from selected crop (Maize)



4.6.2 Process flow sheet of beer production



Chapter 5

Beer production plant with Adjuncts

5.1 Process Description

The steeped malt is ground in a roller milling machine and transferred to mash kettle for mashing. The process of mashing involves combining crushed malted barley with water, chemicals and starchy adjuncts (additives). The malted barley is then ground and extracted with hot water, & adjuncts (starch) are added to the grist creating a mixture of crushed barley and adjuncts. The process is continued over a period of time at different temperatures that activate and denature specific enzymes in order to break down proteins and starches. After the starches in the malt and adjuncts are fully hydrolyzed by the amylolytic enzymes to soluble simple sugars the wort is separated from the mashed grist through Lautertun (Declerck, 1994). The next step in brewing is to boil the wort. Wort boiling is performed in the wort kettle. During this time the hops are added. Then the wort stays in the whirlpool for a set amount of time before the wort is removed and cooled in order to remove suspended particles of hops (hot break). Once the wort is cooled it can be transferred to fermentation (Fermenting tank) and stored in a storage tank (Cellar beer). Finally, the beer is filtered through filter machine or candle filter and filled in to bottles or kegs (See sect…4.6).

5.2. Material and Energy Balance

Material and energy balances are fundamental in the "engineering approach" to designing new processes or analyzing the results of in-plant tests ,play an important role in process streamlining, economic analysis of alternative technologies to deliver safe and nutritious food products meeting all quality specifications, waste reduction, and selecting process modifications to increase yield(Brian and Daniel ,1997)**.**

5.2.1. Material Balance

Brewing is a process of balancing, additions of water, malt, and several soluble and insoluble components to finish a product with exact concentrations of each component. Balanced process rewards: Better control of a process, energy efficiency, water conservation, optimization of throughput Yield maximization, quality improvement .Therefore the materials required for producing beer would be obtained as follows:

Capacity of the plant = 500,000hl cast wort/year with 12% extract (12 ^{0}P)

The amount of extract in kilogram can be calculated as

- ❖ Degree Plato x S.G (specific gravity)

- ❖ Specific gravity of Wort at 12% (degree Plato) is 1.04838 (from the standard table)
- ❖ 12 x 1.04838 = 12.58056 kg of extract in 1 hl wort (Kunze, 1996)

The total extract of the cast wort for the capacity = 12.58056kg/hl x 500,000 hl

= **6,290,280kg extract/year**

But from the research results the substitute beer from maize starch was better than other substitutes up to 30%. Therefore 30% of the extract is derived from starch.

Amount of starch is (6,290,280kg extract/year x 0.3) = 1,887.084tone/year

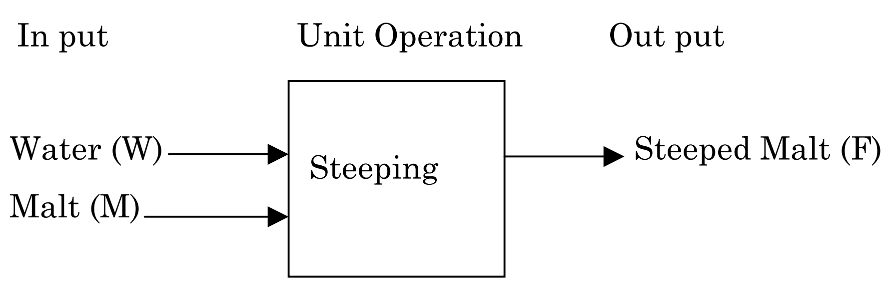
The extract derived from malt (6,290.280 -1,887.084) = 4,403.196tone/year.

In Laboratory malt analysis result 100kg malt was producing79.59kg average extract.

- ❖ Total malt required per year = $\frac{4,403,196kg}{79.59kg}$ x 100kg = **5,532.348 tone/year**

1. Brew house material balancing (Input=output)

A. Steeping



$W + M = F$. ……………………………………. (1)

The average moisture content of malt was 4.50% (from laboratory analysis)

In steeping process the moisture content of malt increases from 4.50% to 30%

- ❖ The amount of water needed is (W)

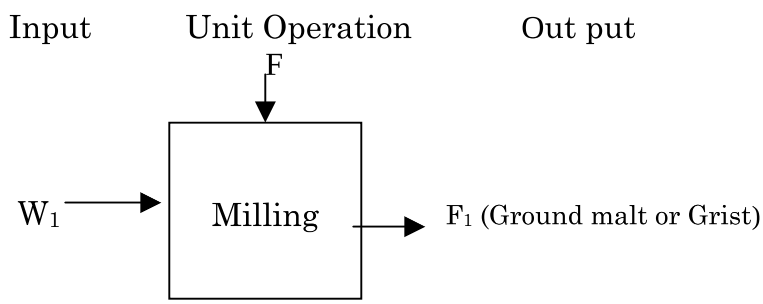
  $W = M (0.3-0.045)$

  $W = 5{,}532.348$ tone/year (0.3 - 0.045)

  $W = 1{,}410.749$ tone/year

  $F = W+M = 6{,}943.097$ tone/year…………… …… ……… ………. (1.1)

B. Milling



Where

F = Out put of (1)

Input of (2)

W_1 = Water for milling

F_1 = Output of (2)

Input of (3)

$W_1 + F = F_1$ -- ---------------- (2)

$W_1 = F_1 - F$-- ---------------- (2.1)

F (30% Moisture)

F_1 (78.25% Moisture, practical)

$W_1 = 6{,}943.097 (0.7825 - 0.30)$

$W_1 = 3{,}350.044$ tone/year

- ❖ The amount of water required for steeping & milling is

  $W + W_1 = 1{,}410.749 + 3{,}350.044$ tone/year = 4760.793 tone/year

- ❖ The amount of Grist $F_1 = W_1 + F = 10{,}293.141$ tone per year

C. Mashing The starch would be added in the mashing process (in mashtun).

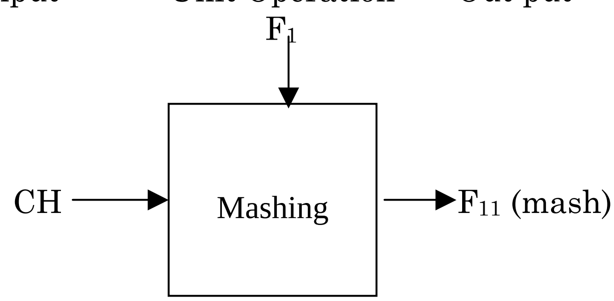
Therefore it requires water for pasting. The amount of water required for starch pasting can be calculated from the total extract (6290.280 tone /year).

Total water required for the extract = $\frac{6290.280 \times 4760.793}{4403.196}$ =6,801.133 tone/year

The water needed for starch pasting (W_{11}) =6801.133-4760.793 =2040.340tone/year

Starch paste = 1887.084+2040.340 =3927.424tone/year

Input Unit Operation Out put



Where
CH = chemicals
F_1 = Output (2)
F_{11} = output (3)

- Evaporation rate is negligible

CH = $CaSO_4$ (10 tone/year)

$Cacl_2$/kcl (7.50tone/year)

Hexamine(2.50tone/year)

H_2SO_4 (5.98tone/year

Total =25.98tone/year

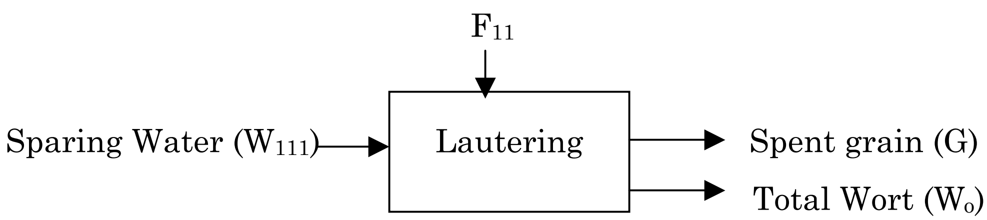
CH + F_1+ starch paste = F_{11}

F_{11} = (25.98 + 10,293.141+3,927.424) tone per year

F_{11} = **14246.545 tone per year**

D. Lautering

Input Unit operation Out put



$W_{111} + F_{11} = G + Wo$ --- (4)

From theoretical point of view 100 kg malt will give 100 - 150 kg moist spent grain.

- The average spent grain removed is 125 kg from 100kg malt and from the total malt, 6915.435 tones of spent grain removed.

To determine the total wort obtained from the lautertun some relation ships should be considered. Theoretically 10% kettle/whirl pool evaporation is expected during wort boiling. But practically (laboratory result) showed that 11% evaporation.

The total wort volume should be

X (1-0.11) = 500,000 HL cast wort

$X \text{ volume} = \frac{500{,}000 \text{ HL}}{0.89} = 561{,}797.75\text{HL} = 56179.775M^3$

The total wort before kettle boiling would have a specific gravity of (S = 1.03814gm/ml) ~10 ^oP

$W_o = 56179.775m^3 \times 1.03814$

$W_o = 58{,}322.472$ tones/year

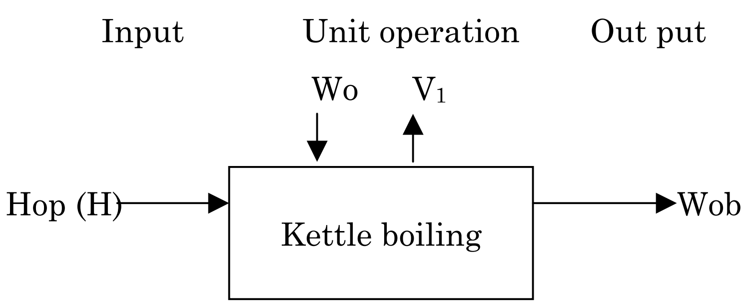
$W_{111} + F^{11} = W_o + G$

$W_{111} = (58322.472 + 6915.435) - F_{11}$

$W_{111} = 50{,}991.362$ tone/year (amount of sparging water)

Total water required for steeping, milling, sparging and starch pasting is ($W+W_1+W_2+ W_3 =$ 1410.749 + 3350.044 +2040.340 +50,991.362 = 57,792.4958 tone/year).

F. Kettle boiling



Where

H =Hop (15kg/brew as EBC standards)

WO = Total wort

Wob = boiled wort

E = Evaporation

Wc = Cold wort

$W_o + H = E + W_{ob}$ (hopped wort) --- (5)

- 4% of the wort to be clarified is lost during the rest time in the whirle- pool (hot sedimentation)

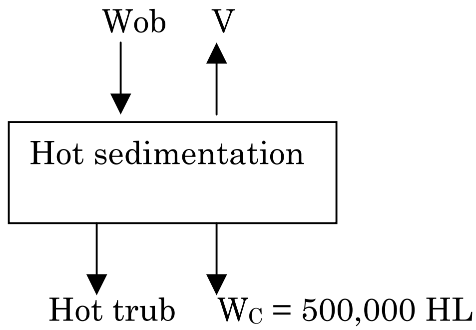
(58,322.472 + 37.50) tone/year = V_1 + 54881.446tone/year

58359.972 tone/year = V_1+ 54,449.854tone/year

Wob = 54,449.854 tone/year

V_1 = 3910.118 tone/year

G. Hot Clarification (Whirl Pool Rest)



(Cast wort) = 52413.5 tone/year (500,000 HL, S.G, = 1.04827)

Wob (Boiled wort) = V (Vapor) + Hot trub + Wc (Cast wort) -------------------------- (6)

54,449.854 = V + Hot trub + 52,413.5

V + Hot trub = 2036.354 tone/year

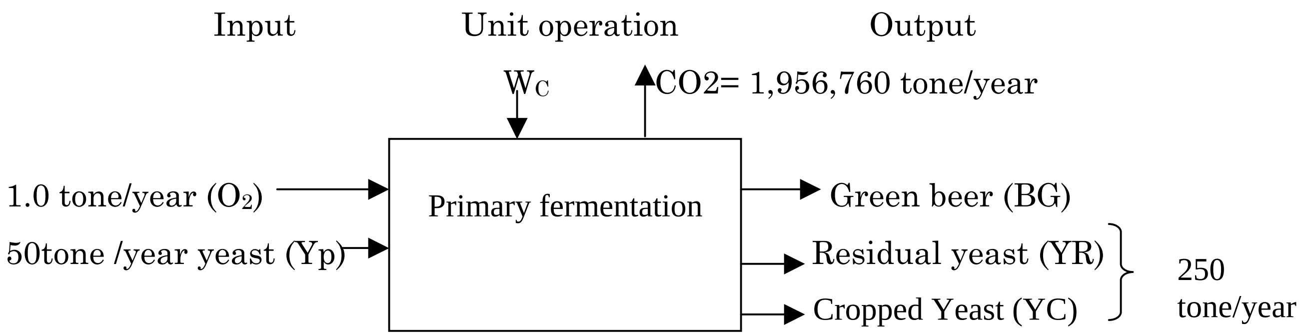
Theoretically hot trub is expected to be (0.2 - 0.4) % of boiled wort (Averagely 0.3%) (i.e. 163.350 tone/year)

V_2 = 1873.004 tone/year

Summary of brew house Material balance

Input	Amount, tone/year	Output	Amount tone/year
Malt	5532.348	Spent Grain	6915.435
Starch	1887.084	Vapor (V_1)	3910.118
Water	57792.500	Vapor(V_2)	1872.004
Chemicals	25.98	Hot trub	163.350
Hops	37.5	Cold wort	52,413.5
Total	**65275.407**	**Total**	**65275.407**

2. Fermentation (Primary fermentation)



$B_G + Y_C = Wc + Yp + Vo + O$-------------------------------------- (7)

Where B_G, green beer racked to storage

Y_C, yeast cropped

Wc, Cast wort received from brewing

O_2, Oxygen added to cold wort to stimulate yeast growth. Theoretically ranged b/n 15 - 25mg/l of wort

Yp, Yeast pitched in to the wort. Approximately 1gm/lt of wort is added. 50 tone/year is needed.

Vo, C02 released during fermentation. From the equation equimolar amount of CO_2 and C_2H_5OH would be produced.

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

- 2/3 of the extract in the pitched wort is fermented at primary fermentation
- The extract of the cold wort was 6,290,280kg

The fermented amount of this extract at primary fermentation would be 6,290,280kg x 2/3 = 4,193,520kg of sugar/extract converted in to equimolar ethanol & CO_2 (i.e. 2,096,760kg of CO_2 & 2,096760kg ethanol). In this amount of CO_2 produced, which remains dissolved in beer is 0.3kg per hectoliter of beer. The cast wort expected is 500,00hl. The amount of CO2 dissolved in beer is 150,000kg.The CO2 escaped to the atmosphere is (2096760-150,000) 1,956760kg. The final extract to be expected after fermentation is averagely 2%.

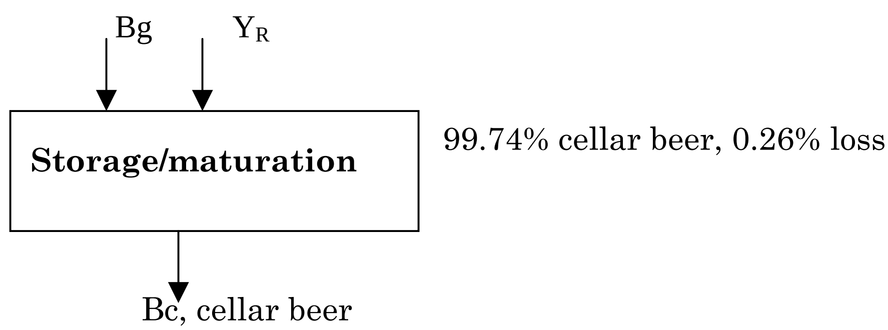
The total conversion from the extract in to CO2 would be 46.8 % (6,290,280 x 0.468 =2,943,851.04 kg extract converted to CO2). 2,096,760 kg CO2 is produced during primary fermentation.

- 2,943,851.04 - 2,096,760 = 843091.64 kg CO2 is produced in secondary cellars or during maturation and storage.
- The yeast amount will increase with five fold and 1/10 of the total will be allowed passing to storage cellar beer.
- Total yeast (50tone/year* 5) =250 tone/year.
- Residual yeast =1/10* 250 = 25 tone/year
- Cropped yeast = 250 - 25 = 225 tone/year
- $O_2 + Y_p + W_c = V_o + B_g + Y_R + Y\ crop$

(1 +50 + 52413.5) tone /year = (1,956.760 + 25 + 225 + Bg) tone/year

52464.5 tone /year = 2206.76+Bg tone /year

Bg = 50,257.74 tone/year



$B_g + Y_R = B_c$

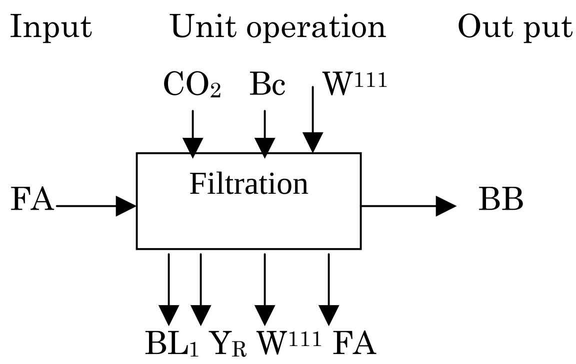
50,257.74 + 25 = Bc

Bc = 50,282.74 tone/year

Summary

Input	Amount tone/year	Out put	Amount tone/year
Cold wort	52,413.50	CO2	1956.760
Yeast	250.00	Cropped yeast	225.00
O_2	1.00	Cellar Beer	50,282.74
Total	52,464.50	Total	52,464.50

3. Filtration



Where
BB, Bright Beer
W^{111}, Water
BL_1, Beer loss
F_A, Filter aid
Y_R = residual yeast

Bottle or keg filled beer should contain about 0.50% CO2, there fore it should be set so that the beer contains more than 0.50% because some carbondioxid is lost on the way to filling

Set CO_2 averagely = 0.60 %(i.e. 0.6tone/100tone of beer)

⇨ Total CO_2 = $\frac{0.6 \times 50,282.74}{100}$ tone of cellar beer

= 301.69644 tone CO_2/year

$Bc + CO_2 + FA + W^{111} = BB + BL^1 + Y_R + FA + W^{11}$------------------------------------ (8)

50, 282.74 tone/year + 301.69644 tone/year = BB + BL^1 + 25tone

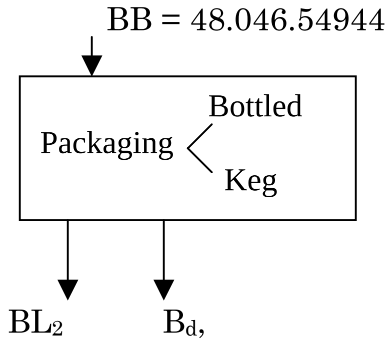
BB + BL^1 = 50,559.43644 ton/year, But 5% loss is expected

- 2,527.97188 tone/year losses
- Then, BB = 48,031.46462 ton/year

Summary

Input	Amount tone/year	Out put	Amount tone/year
Cellar Beer	50282.74	Filter aid	-
Filter aid	-	Residual yeast	25
Carbon dioxide	301.69644	Bright Beer	48,031.462
Total	50584.436	Beer loss	2527.97182
		Total	50584.436

4. Packaging



BL_2 = 2.75% Beer loss is expected due to bottle breakage, line rinsing, foaming, under fill, over fill, filter trub and for quality analysis (i.e. 1320.865277 tone/year)

Bd = distributed beer for market in bottles and kegs

$BB=BL_2+Bd$ -- (9)

48.046.54944_tone/year = (1321.28011+Bd) tone/year

Bd = 46725.26933 tone/year

Summary

Input	**Amount tone/year**	**Out put**	**Amount tone/year**
Bright Beer	48,046.54944	Packed product	46725.26933
		Beer loss	1321.28011
Total	48,046.54944	**Total**	48,046.54944

N.B .46725.26933 tone/year = 463631.7295HL/year

5.2.2. Energy Balance

5.2.2.1. Brew house

Steam consumption of the unit operations

- Heat of brewing water , Mashing , Wort boiling , Bottle and keg washing, Pasteurization

Definitions of symbols in brew house

CP= Specific heat capacity, hs= Enthalpy of steam (from steam table)

h_l= Enthalpy of condensate (table) , Hfg= Heat of vaporization (hs - hl) =2086.1kj/kg ,

Q= Heat , Ms= Mass of steam , Mw= Mass of water

Mww= mass of ice water, Mc= mass of condensate

N.B. the operation is for decoction mashing

a) Brewing water preparation

Inlet conditions

Mw= 295,893kg/day

Cp= 4179 kj/kg.k

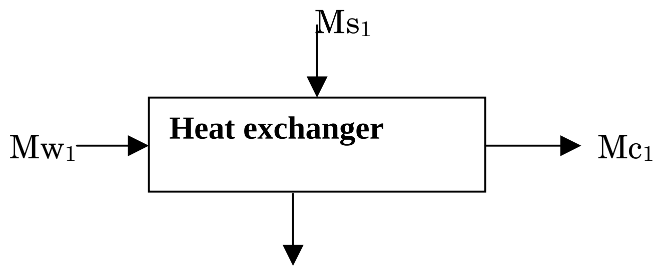
T1= 45^{O}C

T2= 70^{O}C

Out let conditions

Mw= 295,893kg/day

T_2= 70^{O}C



$$Q = Mw_1 \; Cp \; \Delta T = Ms_1 \; (hs - he)$$

$$Ms_1 = \frac{Mw_1 \; Cp \; \Delta T}{hs - hl} = \frac{295{,}893 kg/day \times 4.179 \; kj/kg.k}{2086.13 \; kj/kg} * 25k$$

Ms_1 = 14818.55kg/day x352days = 5216.1296 tone/year

b) 1st decoction

Inlet conditions

From the material balance

1/3 F^{11}, kg/year = 4748.848 tone/year

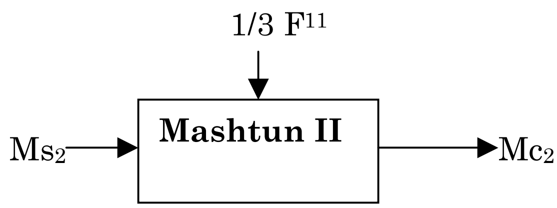
CP = 3.807

T = 58^{O}C

Out let conditions

1/3 F^{11} = 4748.848 tone/year

T = 94^{O}C



$$Q = 1/3 \; F^1 \; CP \; \Delta T = Ms_2 \; hfg$$

$$Ms_2 = \frac{1/3 \; F^1 \; Cp\Delta \; T}{hfg} = 311.984 \text{ tone/year}$$

c) Mash heating (total mash)

Inlet conditions

F^{11} = 14246.545 tone/year

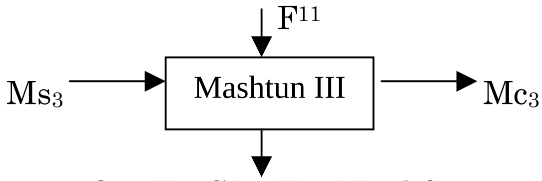
CP = 3.812

T^{i} = 63.3^{O}C

Out let conditions

F^{11} = 14246.545 tone/year

To = 67^{O}C



$$Q = F^{11}\ CP\ \Delta T = Ms_3\ hfg$$

$$Ms_3 = \frac{F^{11}\ CP\ \Delta T}{hfg} = 96.321 \text{ tone/year}$$

d) 2nd decoction

Inlet

1/3 F^{11} = 4748.848 tone/year

CP = 3.834

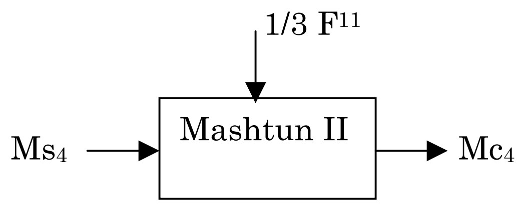
T^{o} = 67^{O}C

Out let

1/3 F^{11} = 4748.848 tone/year

Cp = 3.834

T^{o} = 94 ^{O}C



$$Q = 1/3\ F^{11}\ CP\ \Delta T = Ms_4\ hfg$$

$$Ms_4 = \frac{1/3\ F^{11}\ CP\ \Delta T}{hfg} = 235.632 \text{ ton/year}$$

e) Enzyme deactivation

Inlet condition

F^{1} = 14246.545 tone/year

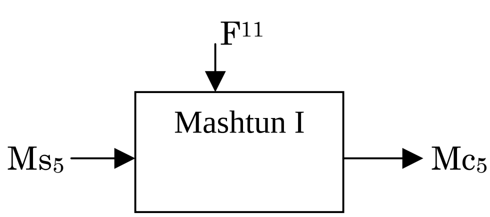
CP = 3.840

T^{o} = 72.5^{O}C

Out let condition

F^{1} = 14246.545 tone/year

T^{o} = 78 ^{O}C

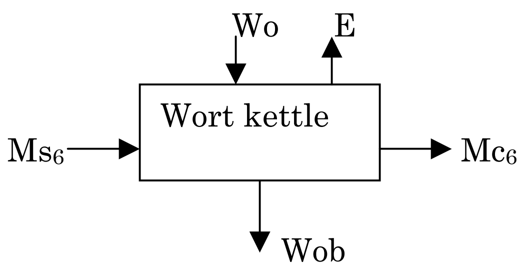


$$Q = F^{11}\, CP\, \Delta T = Ms_5\, hfg$$

$$Ms_5 = \frac{F^{11}\, CP\, \Delta T}{Hfg} = 144.23 \text{tone/year}$$

f) Wort boiling

Inlet condition	**Out let condition**
$W_o = 58322.472$ tone/year	Wob = 54449.854 tone/year
CP = 3.838 kj/kg.k	$T^o = 93\,^OC$
$T^o = 86^OC$	$hfg^1 = 2278$ kj/kg
	V = 3910.118 tone/year



$$Q = Ms_6\, hfg = Wob\, Cp\, \Delta T = Ehfg^1$$

$$Ms_6 = \frac{Wob\, Cp\, \Delta T + Ehfg1}{hfg}$$

$$= \frac{54449.854 \times 3.838 \times 7 + 3910.118 \times 2278}{2086.13}$$

$$= 4970.978 \text{ tone/year}$$

Summary of steam consumption in Brew House

Operation	***Steam consumption, tone/year***
Hot water preparation…………………………	5216.1296 ton/year
1st decoction……………………………………	311.984 "
Mash heating……………………………………	96.321 "
2nd decoction………………………………. …	235.632 "
Enzyme-deactivation……………………….. …	144.230 "
Wort boiling………………………………………	4970.978 "
Total……………………………….. ……………	**10975.290** "

5.2.2.2. Bottling Hall

A. Definitions of symbols in bottle washing (assuming which has three Caustic soda and two water zones)

B_D = Dirty bottles

Bc = Clean bottles

n = number of bottles

Ms = Mass of steam

C = Condensate

S_1 = mass of caustic soda in zone Z-I

S_2 = " " " " " " " Z- II

S_3 = " " " " " " Z- III

W1 = mass of H_2O in water Z - I

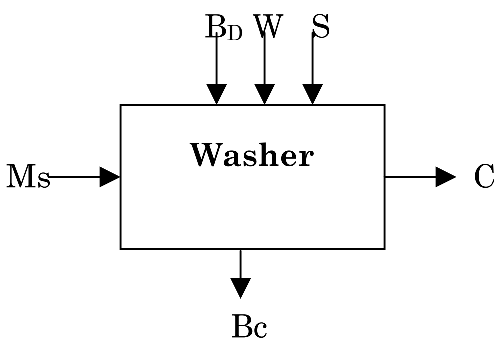
W2 = mass of H_2O in water Z - II

W3 = mass of H_2O in water Z - III

W4 = mass of H_2O in water Z - IV

W5 = mass of H_2O in water Z - V

hfg = 2086.13kj/kg

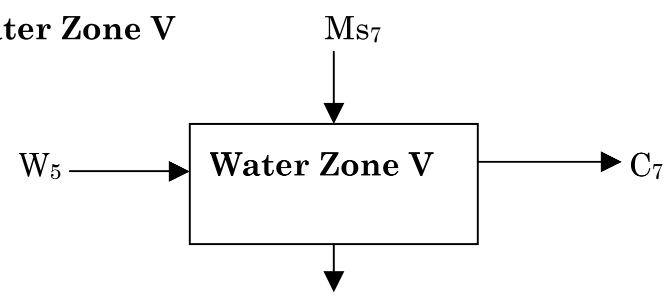


<u>Assumption</u>

$Cp_{S1} = C_{PS2} = Cp_{s3} = Cpw$

B. Heat gained by caustic soda and water

a) Water Zone V



W5 = 716 kg

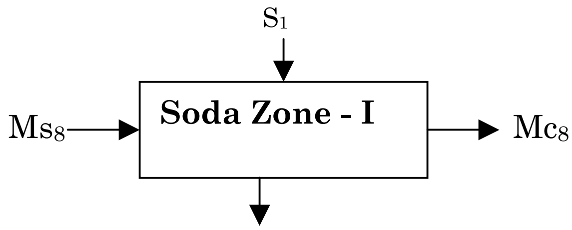
Cp = 4.18

$T^o = 28^OC, 54^OC$

$W_5\ CP\ \Delta T = Ms_7\ hfg$

$Ms_7 = \frac{WsC_P\Delta T}{hfg}$ = 37.319 kg/day×276days = 10.300 tone/year

b) Soda Zone - I



$S_1 = 16498$

$CP = 4.18$

$hfg = 2086.13$

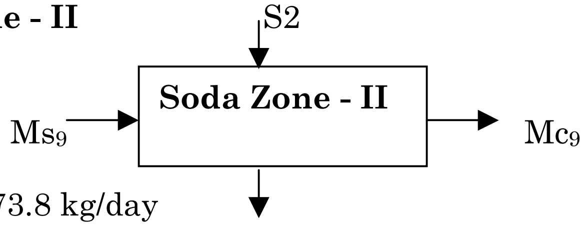
$T^o = 32^OC, 85^OC$

$Q = Ms_8\ hfg = \frac{S_1\ Cp\ \Delta\ T}{hfg}$

$Ms_8 = 1752.03$ kg/day = 1.75203 tone/day χ 276days/year

483.560 tone/year

C) Soda Zone - II



$S_2 = 16473.8$ kg/day

$T^o = 80^OC$

$hfg = 2086.13$

$S_2 = 16473.8$ kg/day

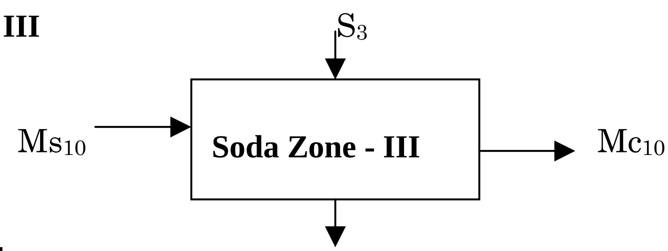
$CP = 4.18$

$T^o = 38^OC$

$Ms_9 = \frac{S_2\ \Delta TCP}{Hfg} = 1386.3663$ kg/day

1.3863663 tone/day χ 276days/year= **382.637 tone/year**

d) Soda Zone – III



$S_3 = 9212$ kg

$T^o = 70^OC$

$hfg = 2086.13$

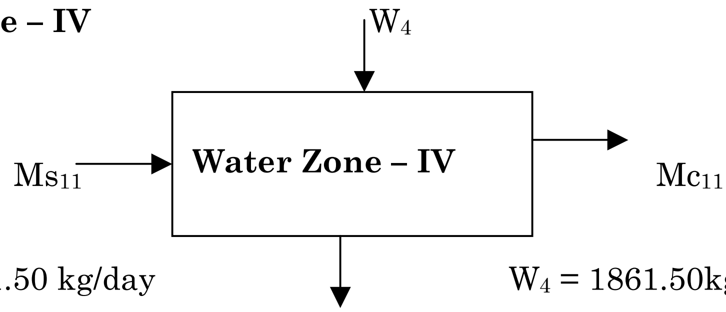
$S_3 = 9212$ kg

$CP = 4.18$

$T^o = 39^OC$

$Ms_{10} = \frac{S_3\ \Delta TCP}{hfg}$ = 572.20353 kg/day *276days/year = **157.928tone/year**

e) Water Zone – IV



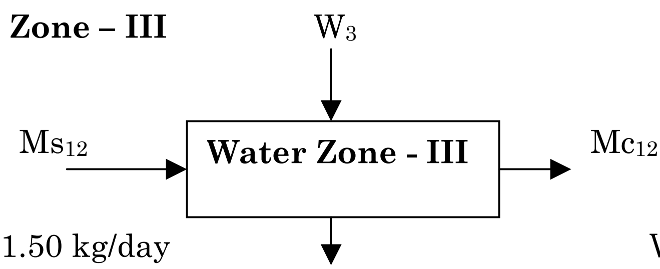
W_4 = 1861.50 kg/day
CP = 4.18 kj/kg.k
$T^{o}C$ = $25^{O}C$

W_4 = 1861.50kg/day
$T^{O}C$ = $50^{O}C$
hfg = 2086.13 kj/kg

$$Q = Ms_{11}\ hfg = W_4\ Cp\Delta T$$

$$Ms_{11} = \frac{W_4\ CP\Delta T}{hfg} = 93.252 \text{ kg/day} = 25.737 \text{ tone/year}$$

f) Water Zone – III



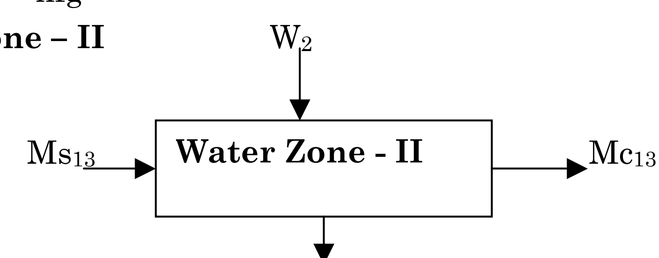
W_3 = 3971.50 kg/day
CP = 4.18 kj/kg.k
$T^{o}C$ = $28^{O}C$

W_3 = 3971.50kg/day
T^{O} = $45^{O}C$

$$Q = Ms_{12}\ hfg = W_3\ Cp\Delta T$$

$$Ms_{12} = \frac{W_3\ CP\Delta T}{hfg} = 135.2815 \text{ kg/day} = 37.338 \text{ tone/year}$$

g) Water Zone – II



W_2 = 3971.50 kg/day
CP = 4.18
$T^{o}C$ = $25^{O}C$

W_2 = 3971.50kg/day
T^{O} = $38^{O}C$

$$Q = Ms_{13}\ hfg = W_2\ Cp\Delta T$$

$$Ms_{13} = \frac{W_2\ CP\Delta T}{hfg} = 103.45056 \text{ kg/day} * 276 \text{days/year} = 28.552 \text{ tone/year}$$

- Water Zone one is the final rinsing water with out heating.

C. Heat gained by the bottles in the washer

- From the material balance the final Beer to be packaged in bottles and kegs was 46725.26933tone/year (463631.7295Hl).
- Draft filling keg amounted 30 lts and averagely 35% of the beer will be filled in kegs per year. The amount of Beer required for keg filling is 16353.84427 tone/year (162,271.1054 hl) and (46725.26933 - 16353.84427) = 30,371.42506 tone/year would be filled in bottles
- Bottle capacity = 330 cc = 0.33lt. = 3.325773×10^{-4} tone
- Beer to be filled = 30,371.42506 tone/year
- CP (bottles) = 0.6694 & Wt of single bottle = 0.272 kg (measured)
- Beer Density = 1.00781 gm/ml
- $\frac{30{,}371.42506 \text{ ton/year}}{3.325773 \times 10^{-4} \text{ tone/bottle}} = 91{,}321{,}401.25$ bottles/year

Therefore number of bottles required = 91,321,401.25 bottles/year

Total Wt of bottles = 24,839,421.14Kg

$$MS_{14}\, h_{fg} = M_B C_P \Delta T$$

$$MS_{14} = \frac{M_B C_P \Delta T}{h_{fg}} = \frac{24{,}839{,}421.14 \text{ Kg} \times 0.6694 \times 60}{2086.13} = 478.230 \text{ tone/year}$$

The amount of steam consumed by the bottles during bottle washing is equal to 478.230 tone/year

Summary of steam consumption during bottle washing.

Steam usage	**Consumption, tone/year**
For water and soda heating	1126.052
Heat gained by bottles	478.230
Total	**1,604.282**

C. Beer pasteurization

1. Definition of symbols

T = Temperature (^{O}C) , M = Mass of water, P_{beer} = Density of beer (1.00781)

M_{Be} = mass of beer, M_{B} = mass of bottles, MB_{BT} = mass of beer and bottle

T_{BB} = Outlet temperature of bottled beer from the filler, hfg = 2086.13kj/kg

2. Heat consumed by water

Pasteurizer Zones = 8, Temperature for each zone T_1 = 28, T_2 = 38.8, T_3 = 49, T_4 = 64.40, T_5 = 62, T_6 = 49, T_7 = 38.80, T_8 = 28

Water capacity of each zone

$M_1 = 4.6m^3 = 4600kg$	$M_4 = 5.1m^3 = 5100kg$	$M_7 = 2.2m^3 = 2200kg$
$M_2 = 2.2m^3 = 2200kg$	$M_5 = 22.9m^3 = 2900kg$	$M_8 = 4.6m^3 = 4600kg$
$M_3 = 1.75m^3 = 1750kg$	$M_6 = 1.75m^3 = 17500kg$	For all Zone C_P = 4.18

$T_{(initial)}\ ^{O}C = 25$

a) Zone 1 & 8

$Ms_{15}\,hfg = Ms_{16}\,hfg = M_1\,Cp\,\Delta T = M_8 Cp\,\Delta T$

$Ms_{15} = Ms_{16} = \frac{M_1\,CP\,\Delta T}{hfg} = \frac{M_8 cp \Delta T}{hfg}$ = 27.65kg/day*276= 7.6317 tone/year

b) Zones 2 & 7

$Ms_{17}\,hfg = Ms_{18}\,hfg = M_2\,Cp\,\Delta T = M_7 Cp\,\Delta T$

$Ms_{17} = Ms_{18} = \frac{2200 \times 4.18 \times 13.80}{2086.13}$ = 60.862 kg/day*276= 16.7979 tone/year

c) Zone 3 & 6

$Ms_{19}\,hfg = Ms_{20}\,hfg = M_3\,Cp\,\Delta T = M_6 Cp\,\Delta T$

$Ms_{19} = Ms_{20} = \frac{1750kg \times 4.18kj/kg.k \times 24^{O}C}{2086.13kj/kg}$ = 84.1558kg/day*276 = 23.2270tone/year

d) Zone 4

$Ms_{21}\,hfg = M_4\,Cp\,\Delta T$

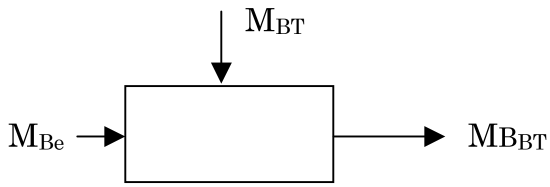
$Ms_{21} = \frac{M_4\,Cp\,\Delta T}{hfg} = \frac{5100kg \times 4.18 \times 39.40}{2086.13}$ = 402.62553kg/day*276 = 111.1246tone/year

e) Zones 5

$Ms_{22}\, hfg = M_5\, Cp\, \Delta T$

$Ms_{22} = \frac{M_5\, Cp\, \Delta T}{hfg} = \frac{2900 \times 4.18 \times 37}{2086.13} = 214.99811 kg/day*276 = 59.3395 tone/tear$

3. Heat gained by bottles and Beer in the Pasteurizer



$M_{Be}\, Cp_{Be}\, (T_{BB} - T_{Be}) = M_{BT}\, Cp_{BT}\, (T_{BT} - T_{BB})$

2.6638197 19.33618

$T_{BB} = \frac{MBe\, Cp_{Be}\, T_{Be} + M_{BT}\, Cp_{BT}\, T_{BT}}{M_{Be}\, Cp_{Be} + M_{BT}\, Cp_{BT}}$

From steam consumption of bottles in the washer

M_{BT} = 81172.106 kg/day, 24676320 kg/year

M_{Be} = 30172000 kg/year, Cp_{Be} = 3.974, CpBT = 0.6694, T_{BT} = 25OC, T_{Be} = ~3^{O}C

T_{BB} = 5.6638197^{O}C

There fore the amount of steam consumed by bottles and beer during pasteurization can be calculated as

$Ms_{23}\, hfg = M_{Be}\, Cp_{Be}\, \Delta T_{Be} + M_{BT}\, Cp_{BT}\, \Delta T$

$Ms_{23} = \frac{M_{Be} Cp_{Be}\, \Delta T + M_{BT}\, Cp_{BT}\, \Delta T_{BT}}{hfg} = 331.90358$ tone/year

5.4 Cost Estimation

5.4.1. Plant parameters

- Capacity, hectoliter per year 500,000 Cast Wort
- Number of shifts per day Two
- Working Days per year

Brew house 352

Draught filling 365

Bottle Filling 276

5.4.2. Man power requirement

Table5.20. Man power requirement

Human Resource	Number	Monthly average Salary	Total Monthly Salary	Total Yearly Salary
Managerial	2	6000.00	12,000.00	144,000.00
Professional	51	3804.00	194,004.00	2328048.00
Semi-Professional	72	2150.00	154,800.00	1,857,600.00
Non-Professional	655	1348.00	882,940.00	10,595,280.00
Total	**780**	**13,302.00**	**1,243,744.00**	**14,924,928.00**

5.4.3. Fixed asset costs

Plant Machinery	268,412,822.60
Building	49,486,505.47
Motor Vehicle	20,875,622.94
Office furniture, Fixture, Equipment	5,454,251.47
Total	**344,229,202.50**
Working capital = 0.15 x 344,229,202.5	**51,634,380.38**
Total capital investment	**395,863,582.90**

5.4.4. Cost of utilities

Table5.21.Cost of utilities

Item	**Total Qty (year)**	**Unit Price (birr)**	**Total Price (year)**
Power(KWH)	543,809.03	8.9231	4,852,462.427
Fuel(l)	4,012,030.1	5.49180	21,489,458.23
Water(M^3)	229,824	1.75	402,192.00
Total			**26,744,112.66**

5.4.5. Cost of raw materials

Table 5.22.Cost of raw materials

Item	Qty (Kg/Hl)	Total Qty (Kg/year)	Unit price (birr/Kg)	Total Price (birr)
Malt	11.58735789	5,532,348.000	15.81	87,466,421.880
Starch	3.774168	1,887,084.000	10.00	18,870,840.00
Hops	0.075	37,500.000	1408.46	52,817,250.00
Other direct materials	-	-	-	1,533,860.196
Indirect materials	-	-	-	13,961,947.06
Total				**174,650,391.100**

5.4.6. Estimation of total product cost

Table5.23. Estimation of total product cost

Item		Total Cost, birr/year
I Manufacturing Costs	**A. Material cost**	**174,650,391.100**
	B .Labor cost	
	a. Direct Labor	5,747,220.354
	b. Indirect Labor	9,932,421.534
	Total of B	**15,679,641.890**
	C Utilities	
	a. Electricity	4,852,462.427
	b. Furnace Oil	21,489,458.230
	c. Water	402,192.00
	Total of C	**26,744,112.66**
	D. Production over heads	
	a. Employees benefit	3,311,714.213
	b. Maintenance & repairing	1,630,245.908
	c .Insurance	776,197.65
	d .Depreciation	22,537,421.780
	e. Production breakages and others	1,852,612.417
	Total of D	**30,108,191.97**
	Cost of Goods Produced(A+B+C+D)	**247,182,337.600**
	Excise Tax (50%) of production cost (Off Dep.)	112,322,457.900
	Total cost of Goods produced	**359,504,795.500**
II General Expenses	a. Sales and distribution Expense	26905169.640
	b. Administrative expense	30794648.45
	C. Miscellaneous Expenses	462,969.03
	Total of General expense	**58,162,787.12**
Total product Cost (I +II)		**417,667,582.600**

5.4.7. Comparative analysis of sales

Table5.24. Sales analysis

Product	Mix ratio **(%)**	Qty(Hl)	Selling Price(l)	Total Price (birr)
Draught beer	35	162,271.1054	9.50	154,157,550.10
Bottled beer	65	301,360.6241	12.82	386,344,320.10
Total	**100**	**463,631.7295**		**540,501,870.2**

5.4.8 Gross earnings

Expecting all produced beer will be sold

Net sales = 540,501,870.2

Cost of Goods sold = 359,504,795.500

Gross profit = Net sales – Cost of Goods sold = **180,997,074.700**

General expenses = 58,162,787.12

Net profit before Tax = Gross profit - General expenses =**122,834,287.600**

Profit tax (35%) = 429, 92,000.650

Net profit After Tax **=** Net profit before Tax - Profit tax (35%) = **79,842,286.95**

Rate of return

ROI = $\dfrac{\textit{net profit}}{\textit{total capital investment}}$ x 1000 = $\dfrac{79,842,286.95}{395,\ 863,582.90}$ x 100 = **20.17%**

Pay out period = $\dfrac{FCI}{NP + depreciation}$ **=** $\dfrac{344,229,202.50}{102,379,708.70}$ = **3.4 years**

Beer production using starch substitutes is profitable as it is clearly observed from the above cost estimation. It provides a cost efficient alternative for brewing technology in Ethiopia. Utilization of starch crops for value-added products (beer) will increase the agronomical benefits of these crops and initiates the producers.

Chapter 6

Conclusion and Recommendation

6.1. Conclusion

This study indicated that the use of starches from selected crops (Maize, Enset, and Potato) as partial substitutes for barley malt was found to be promising to develop beer product, although with some differences in quality indices. The production of beer from the three types of starch sources gave good yield of extract content. Maize was found to produce the highest yield of extract from the three crop types. It is also better in alcohol content, flavor, lautering & saccharification time than others. Enset has been found to be the second important starch source that has a potential application in breweries. It has generally good yield of extract, alcohol content and flavor, however performed poor in lautering time at 30% substitute relative to others.

In this study incompatible results of the parameters were found in potato substitutes (Table 24& 25) when tested for the most important parameters of the beer (Alcohol content & over all acceptability–flavor). The extract yield was good, comparable to the control, but this did not translate through a good yield of ethanol, suggesting that the extract was not completely fermentable. In all investigated starch substitutes, in increasing the percentage of starch share, lautering and saccharification time increases, however, saccharification was under 20minutes for all substitute types, which indicates that good activity of malt amylolytic enzymes. The obtained results indicated that the use of starch substitutes (Enset and Maize) produce beers that have good analytical parameters, which make them for use in brewing technology.

6.2. Recommendation

Further studies are recommended on:

- Ethiopia is endowed with lot of agricultural resources among which crop farming is the most favoured. Over 85% of our populations engaged in farming but starch crops like enset, are consumed locally with insignificant quantity (underutilized) in the brewing technology .There fore needs further study on possibility for enhancement and prospects for utilization of starch crops for value-added products (beer) for economic, technological development, optimization of agronomical benefits of the crops and fermentability capacities.

- Potential fermentation problems in potato starch brewing. Occasionally the beers were stop fermenting a few degrees above the required final gravity.
- Modifying the existing brewing processes (in Ethiopia) as convenient for starch or cereal adjuncts.
- Detailed feasibility study for the willingness of brewing companies in our country in use of malt substitutes.

Potential targeted beneficiaries from the outcome of this research are:

- Primary Producers (farmers)
- Starch manufacturing industries
- Brewing companies
- Researchers

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Annexes

Annex1- Score Sheet for Sensory evaluation of beers

Name: ------------------------------------- Date: --------------------

Code: -------------------------------------- Signature---------------

Quality attributes	Hedonic scales				
	5	4	3	2	1
Foam					
Bitterness					
Aroma					
Flavor					
Mouth fullness					
Over all acceptability					

Key: 5 = Excellent, 4 = Very Good, 3 = Good, 2 = Fair, 1 = Poor

Comments: ---

Annex 2- Multiple Comparison of alcohols of beers

Table 25 multiple comparison of alcohols of the beers (Control, potato, Maize, Enset,)

ANOVA

Alcohol

	Sum of Squares	df	Mean Square	F	Sig.

Between Groups	.962	8	.120	13.367	.000
Within Groups	.189	21	.009		
Total	1.151	29			

(I) Mean	(J) Mean	Mean D/f	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
3.63(P_{30})	3.72	-0.09000	0.07745	0.258	-0.2511	0.0711
	3.80	-0.17000*	0.07745	0.040	-0.3311	-0.0089
	3.97	-0.34333*	0.07745	0.000	-0.5044	-0.1823
	4.03	-0.40333*	0.07745	0.000	-0.5644	-0.2423
	4.04	-0.42667*	0.07745	0.000	-0.5877	-0.2656
	4.09	-0.46500*	0.06707	0.000	-0.6045	-0.3255
	4.15	-0.52333*	0.07745	0.000	-0.6844	-0.3623
	4.16	-0.53333*	0.07745	0.000	-0.6944	-0.3723
3.72(P_{20})	3.63	0.09000	0.07745	0.258	-0.0711	0.2511
	3.80	-0.08000	0.07745	0.313	-0.2411	0.0811
	3.97	-0.25333*	0.07745	0.004	-0.4144	-0.0923
	4.03	-0.31333*	0.07745	0.001	-0.4744	-0.1523
	4.04	-0.33667*	0.07745	0.000	-0.4977	-0.1756
	4.09	-0.37500*	0.06707	0.000	-0.5145	-0.2355
	4.15	-0.43333*	0.07745	0.000	-0.5944	-0.2723
	4.16	-0.44333*	0.07745	0.000	-0.6044	-0.2823
3.80 (P_{10})	3.63	0.17000*	0.07745	0.040	0.0089	0.3311
	3.72	0.08000	0.07745	0.313	-0.0811	0.2411
	3.97	-0.17333*	0.07745	0.036	-0.3344	-0.0123
	4.03	-0.23333*	0.07745	0.007	-0.3944	-0.0723
	4.04	-0.25667*	0.07745	0.003	-0.4177	-0.0956
	4.09	-0.29500*	0.06707	0.000	-0.4345	-0.1555
	4.15	-0.35333*	0.07745	0.000	-0.5144	-0.1923
	4.16	-0.36333*	0.07745	0.000	-0.5244	-0.2023
3.97 (E_{10})	3.63	0.34333*	0.07745	0.000	0.1823	0.5044
	3.72	0.25333*	0.07745	0.004	0.0923	0.4144
	3.80	0.17333*	0.07745	0.036	0.0123	0.3344
	4.03	-0.06000	0.07745	0.447	-0.2211	0.1011
	4.04	-0.08333	0.07745	0.294	-0.2444	0.0777
	4.09	-0.12167	0.06707	0.084	-0.2612	0.0178
	4.15	-0.18000*	0.07745	0.030	-0.3411	-0.0189
	4.16	-0.19000*	0.07745	0.023	-0.3511	-0.0289
4.03 (E_{30})	3.63	0.40333*	0.07745	0.000	0.2423	0.5644
	3.72	0.31333*	0.07745	0.001	0.1523	0.4744
	3.80	0.23333*	0.07745	0.007	0.0723	0.3944
	3.97	0.06000	0.07745	0.447	-0.1011	0.2211
	4.04	-0.02333	0.07745	0.766	-0.1844	0.1377
	4.09	-0.06167	0.06707	0.368	-0.2012	0.0778

	4.15	-0.12000	0.07745	0.136	-0.2811	0.0411
	4.16	-0.13000	0.07745	0.108	-0.2911	0.0311
4.04 (M_{30})	3.63	0.42667*	0.07745	0.000	0.2656	0.5877
	3.72	0.33667*	0.07745	0.000	0.1756	0.4977
	3.80	0.25667*	0.07745	0.003	0.0956	0.4177
	3.97	0.08333	0.07745	0.294	-0.0777	0.2444
	4.03	0.02333	0.07745	0.766	-0.1377	0.1844
	4.09	-0.03833	0.06707	0.574	-0.1778	0.1012
	4.15	-0.09667	0.07745	0.226	-0.2577	0.0644
	4.16	-0.10667	0.07745	0.183	-0.2677	0.0544
4.09(E_{20}+M_{10})	3.63	0.46500*	0.06707	0.000	0.3255	0.6045
	3.72	0.37500*	0.06707	0.000	0.2355	0.5145
	3.80	0.29500*	0.06707	0.000	0.1555	0.4345
	3.97	0.12167	0.06707	0.084	-0.0178	0.2612
	4.03	0.06167	0.06707	0.368	-0.0778	0.2012
	4.04	0.03833	0.06707	0.574	-0.1012	0.1778
	4.15	-0.05833	0.06707	0.394	-0.1978	0.0812
	4.16	-0.06833	0.06707	0.320	-0.2078	0.0712
4.15(M_{20})	3.63	0.52333*	0.07745	0.000	0.3623	0.6844
	3.72	0.43333*	0.07745	0.000	0.2723	0.5944
	3.80	0.35333*	0.07745	0.000	0.1923	0.5144
	3.97	0.18000*	0.07745	0.030	0.0189	0.3411
	4.03	0.12000	0.07745	0.136	-0.0411	0.2811
	4.04	0.09667	0.07745	0.226	-0.0644	0.2577
	4.09	0.05833	0.06707	0.394	-0.0812	0.1978
	4.16	-0.01000	0.07745	0.898	-0.1711	0.1511
4.16(Control)	3.63	0.53333*	0.07745	0.000	0.3723	0.6944
	3.72	0.44333*	0.07745	0.000	0.2823	0.6044
	3.80	0.36333*	0.07745	0.000	0.2023	0.5244
	3.97	0.19000*	0.07745	0.023	0.0289	0.3511
	4.03	0.13000	0.07745	0.108	-0.0311	0.2911
	4.04	0.10667	0.07745	0.183	-0.0544	0.2677
	4.09	0.06833	0.06707	0.320	-0.0712	0.2078
	4.15	0.01000	0.07745	0.898	-0.1511	0.1711

*The mean difference is significant at the .05 level.

E =Enset, M = Maize, P = Potato, 10 =10%, 20 = 20%, 30 = 30%

Annex 3- Multiple comparison of Flavors of beers

Table 26 Multiple comparison of Overall acceptability's (Flavors) of beers (Control, potato, Maize, Enset) ANOVA

Overall acceptability (Flavor)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9.001	6	1.500	37.779	.000
Within Groups	.913	23	.040		
Total	9.915	29			

(I) Mean	(J)Mean	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
2.87 (P_{20}&P_{30})	3.20	-0.33333*	0.14091	0.027	-0.6248	-0.0418
	3.87	-1.00000*	0.14091	0.000	-1.2915	-0.7085
	4.07	-1.20000*	0.11505	0.000	-1.4380	-0.9620
	4.13	-1.36667*	0.11505	0.000	-1.6047	-1.1287
	4.20	-1.33333*	0.14091	0.000	-1.6248	-1.0418
	4.27	-1.40000*	0.14091	0.000	-1.6915	-1.1085
3.20(P_{10})	2.87	0.33333*	0.14091	0.027	0.0418	0.6248
	3.87	-0.66667*	0.16271	0.000	-1.0033	-0.3301
	4.07	-0.86667*	0.14091	0.000	-1.1582	-0.5752
	4.13	-1.03333*	0.14091	0.000	-1.3248	-0.7418
	4.20	-1.00000*	0.16271	0.000	-1.3366	-0.6634
	4.27	-1.06667*	0.16271	0.000	-1.4033	-0.7301
3.87(E_{20})	2.87	1.00000*	0.14091	0.000	0.7085	1.2915
	3.20	0.66667*	0.16271	0.000	0.3301	1.0033
	4.07	-0.20000	0.14091	0.169	-0.4915	0.0915
	4.13	-0.36667*	0.14091	0.016	-0.6582	-0.0752
	4.20	-0.33333	0.16271	0.052	-0.6699	0.0033
	4.27	-0.40000*	0.16271	0.022	-0.7366	-0.0634
4.07(M20&30)	2.87	1.20000*	0.11505	0.000	0.9620	1.4380
	3.20	0.86667*	0.14091	0.000	0.5752	1.1582
	3.87	0.20000	0.14091	0.169	-0.0915	0.4915
	4.13	-0.16667	0.11505	0.161	-0.4047	0.0713
	4.20	-0.13333	0.14091	0.354	-0.4248	0.1582
	4.27	-0.20000	0.14091	0.169	-0.4915	0.0915
4.13(Control &E30)	2.87	1.36667*	0.11505	0.000	1.1287	1.6047
	3.20	1.03333*	0.14091	0.000	0.7418	1.3248

	3.87	0.36667*	0.14091	0.016	0.0752	0.6582
	4.07	0.16667	0.11505	0.161	-0.0713	0.4047
	4.20	0.03333	0.14091	0.815	-0.2582	0.3248
	4.27	-0.03333	0.14091	0.815	-0.3248	0.2582
4.20 (M10)	2.87	1.33333*	0.14091	0.000	1.0418	1.6248
	3.20	1.00000*	0.16271	0.000	0.6634	1.3366
	3.87	0.33333	0.16271	0.052	-0.0033	0.6699
	4.07	0.13333	0.14091	0.354	-0.1582	0.4248
	4.13	-0.03333	0.14091	0.815	-0.3248	0.2582
	4.27	-0.06667	0.16271	0.686	-0.4033	0.2699
4.27 (E10)	2.87	1.40000*	0.14091	0.000	1.1085	1.6915
	3.20	1.06667*	0.16271	0.000	0.7301	1.4033
	3.87	0.40000*	0.16271	0.022	0.0634	0.7366
	4.07	0.20000	0.14091	0.169	-0.0915	0.4915
	4.13	0.03333	0.14091	0.815	-0.2582	0.3248
	4.20	0.06667	0.16271	0.686	-0.2699	0.4033

*The mean difference is significant at the .05 level.

E =Enset, M = Maize, P = Potato, 10 =10%, 20 = 20%, 30 = 30%