
**Addis Ababa University**

**Addis Ababa Institute of Technology**

**School of Chemical and Bio Engineering**

# Production of barley malt beer using Gesho (Rhamnus priniodes) in Ethiopia

**(M.Sc. Thesis)**

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# Production of barley malt beer using Gesho (*Rhamnus priniodes)* in Ethiopia

**A Thesis Submitted to the School of Chemical and Bio-Engineering of Addis Ababa Institute of Technology in Partial Fulfillment of the Requirements for the Attainment of the Degree of Masters of Science in Chemical Engineering Under Process Engineering Stream.**

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# Abbreviations

Bu-bitterness unit

EDTA-Ethylenediamine triacetic acid

RPE-Rhamnus priniodes extracted

RPR-Rhamnus priniodes residual

SWY –Saccharomyces wild yeast

ARWY-Actidione wild yeast

N-Normal

O-Evolvement of gas or $CO_2$ development

DPPH- 2, 2-diphenyl-1-picrylhydrazine

CMC-Carboxymethylcellulose

## ABSTRACT

Beer is widely brewed in Ethiopia. The quality may deteriorate by oxidation. Determination of profile of antioxidant activity in Beer, and bitterness of Gesho are important. Therefore, the objective was to evaluate the suitability of *Rhamnus prinoides (Gesho)* for beer production in Ethiopia. Beer fermentation was carried out using European-brewing technology methods.

*Rhamnus prinoides* L. Herit or Gesho in Amharic is a plant which grows up to 6m which cultivated in Ethiopia and is an important commodity which is sold in almost every traditional market place. It has a potential to serve as a raw material for Brewing Industries. In this work, the malt analysis, antioxidant activity *of Gesho, Rhamnus prinoides and imported hops,* effect of process variables in beer bitterness, physico-chemical analysis of finished beer and microbiological analysis of beer were done.

Bacteria almost disappeared at the end of the fermentation and pasteurization of the finished beer. Yeasts were dominated at the middle of phases. This phenomenon may be due to the synergic effect of Gesho antibacterial substance, alcohol concentration, reduction of pH (5.4 to 4.48) and antioxidant activity. Bitter taste value of 15.6Bu of Gesho being in the range of standard i.e.15-18Bu of hops was found to be comparable with the values of common varieties of hops. This should indicate that Gesho can serve as a substitute for hops used in the breweries.



**Keywords**: Antioxidant activity, Beer, Bitter taste, Herkule and *Rhamnus prinoides.*

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## Chapter one

### 1. Introduction

### 1.1. Background

Beer is one of the world oldest prepared beverages, possibly dating back to the early Neolithic or 9000 B.C and is recorded in the written history of ancient Egypt and Mesopotamia (Michael, 2004). It is also an ancient beverage. Clay tablets also describing the beer brewing process and dating back more than 5,000 years have been found in Mesopotamia. According to these tablets, Sumerians used to prepare "beer bread" out of germinated barley seeds. By crumbling this bread into water, they obtained a liquid called "Sikarur", which was finally boiled and mixed with a few herbs, resulting in a drink free of harmful bacteria. Over time, different types of starchy plants have been used for brewing, including maize (in South America), soy (in India and Persia), millet and sorghum (in Africa) and rice (in the Far East). Nowadays, beer production using barley malt is the most common brewing process worldwide (FAO, 2009). Water, hops, malt and yeasts are the only raw materials for beer brewing accordingly to Germany purity law of 1516(Kunze, 2004).

Figure 1: Brewing raw materials

# *Production of barley malt beer using Gesho (Rhamnus priniodes) in the Ethiopia*

Beer is obtained by the alcoholic fermentation of malted cereals, usually barley malt with or without other starchy material, and to which hops have been added and has been practiced for thousands of years (http://www.tuntavern.com). In the 2011 global beer production amounted to about 1.93 billion hectoliters up from 1.3 billion hectoliters in 1998. Beer is a widely consumed beverage around the world (www.statista.com).

Beer is a product valued by it physico - chemical properties (i.e. quality) as much as by its entanglement with religious, culinary and ethnic distinctiveness (i.e. tradition). Accordingly, the history of beer brewing is not only one of scientific and technological advancement, but also the tale of people themselves: their governance, their economy, their rites and their daily life (Meussdoerffer, 2009).

It is also described as a beverage containing alcohol, extract, and carbon dioxide. Beer is prepared from barley malt, raw hops, or other hop products, brewing water, and top or bottom fermenting yeast. The alcohol must be produced exclusively from these ingredients, which are converted to fermentable products during the brewing process (Eßlinger, 2009).

Tella is widely brewed and consumed in both rural and urban part of Ethiopia. It is well known as local beer (Getahun, 1976) .Since it is malt based beverage like that of commercial beer (Kunze, 1996). Tella brewing requires largely malted barley (Hordeum vulgars), Enkuro (roasted, milled and heat treated grains of maize, wheat or barely), Gesho (*Rhamnus prinoides*) leaves and stems chopped to small pieces, kita (from different grains) and derekot (Shale and Gashe, 1991).

Hops are the dried blossoms of the female hop plant known botanically as *Humulus lupulus* (*cannabinaceace*/*moraceace*).Hops are native to many north temperate areas such as Europe ,Asia and north America .The ealiest regular use in beer making is believed to have been in Germany as early as the $12^{th}$ century. Hops gained wide popularity because beer brewed without hops deteriorated fast while use of hops suppressed growth of bacteria that spoil beer. Hops also contribute to flavors of beer and to foam retention. Hops are grown commercially in many temperate countries. Recently, successful attempts to cultivate hops have been made in India. The hop resins are the soluble portion in methanol and make up

about 17% of hop by weight (Ermias and Berhanu, 2000). *Rhamnus prinoides* (Amharic, Gesho) is in the family Rhamanceae (Dlamini and Turner, 2002).

It is a wide spread plant species in east and south African countries (Alemu et. al, 2007). The only two Rhamnus species that occur in Africa are *R. prinoides* and *R. staddo*. R. prinoides is common in many parts of eastern and central Africa (Abegaz et.al, 1999). The plant is native to Ethiopia, Botswana, Eritrea, Lesotho, Namibia, South Africa, Swaziland, Uganda and it is exotic to Kenya .It also occurs in Cameroon, Sudan, and Angola (Hayeshi et.al, 2004).

*R. prinoides* grows readily in most soils, but thrives in moist, humus-rich soils of medium to high altitudes, along water courses, in riverside forest and at the margins of evergreen forests. It grows both as a cultivated plant and a natural component of mountain and revering forest in all parts of Ethiopia from 1400 to 3200 m. Although it is quite common to find *R. prinoides* cultivations throughout the country, Tigray, North Shoa around Kara Kori and Sebeta, just west of Addis Ababa, are important centers of cultivation of *R.prinoides* (Abegaz, 1996).

It has been used for bitterness in the traditional brewing process for hundreds of years in East Africa. *R. prinoides* has potential use as a commercial hopping agent in the beer industry. An extract of this plant (a naphthalene glycoside, Geshoidin) is responsible for bitterness in local alcoholic beverages (Nindi, Kgarebe, et. al, 1999) .Geshodin is a non-toxic substance found in bountiful amounts in the stem and leaves of *R.prinoides* (Pavlik, 2007)

In Ethiopia, the leaves and stems of this plant are used to impart the characteristic bitter flavor to domestically brewed beverages such as Tella and Tej and it was estimated that well over 5 million people consume these beverages everyday( Hayeshi et al, 2004) .

**1.2. Statement of problem**

The brewing industry is becoming more competitive and constantly looking for ways to improve beer quality and reduce manufacturing costs in the worldwide. In tropical countries, Hops has been imported from other temperate countries and this involves the expenditure of scare foreign exchange.

There are two fundamental limits to any history of beer brewing. First, the unambiguous definition of its object, namely beer. A consideration of hopped beer only would be too selective, and would ignore the fundamental roots of brewing technology and beer culture. The dissemination of European/American beer culture all over the world and the recent trend towards globalization generates new variants of beer - like beverages that follow regional and traditions preferences. A second difficulty arises from the availability and reliability of resources.

In the world there are five top hop producing countries. These are Germany, USA, Ethiopia, China and Czech Republic (FAOSTAT, 2010).Ethiopia is one of top five hop growing countries in the world, but nowadays, beer is produced in Ethiopia with imported hop as bittering agent. Since it is imported it creates a pressure on its foreign currency. The total beer consumption in Ethiopia is around 5.1million hectoliter of beer per year to produce this amount overall cost for hops is about 203.69 million birr per year , from which overall hop costs about 53.96 million birr per year is due to transport costs per year.

*Rhamnus prinoides* (Amharic, name: Gesho) is a cultivated indigenous shrub which is also known to occur far west as Cameroon and as far south as South Africa, and Ethiopia. It has a potential use as a commercial hopping agent in the beer industry .The leaves and stems of this plant are essential ingredients in the traditional fermented Ethiopian beverages, local beer. In Ethiopia, the plant parts are used to impart the characteristic bitter flavor for the traditional beverage and it was estimated that over 5 million people consume these beverages every day.(Hayeshi et al 2004) . .However, the role of this plant in the fermentation process of beer or beer production is not clearly established.

Gesho is a new potential substitute for imported hops which can be used as an alternative substrate and also raise economic benefits through import substitution. With the continued increase in Ethiopia and western African population great emphasis has been placed throughout the region on increasing the production of beer, improving their nutritional qualities.

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In order to use *R.priniodes* (Gesho) in beer brewing as bittering agent the extraction of *Rhamnus prinoides* and comparative experiments with hops has to be done. Therefore, the suitability of *Rhamnus priniodes* data in the beer production would be necessary to perform and it will be useful for further brewing of the 100% Gesho-beer as hop-beer in the brewing industry of Ethiopia.

## 1.3. Objectives

### 1.3.1. General objectives

The general objective of this study is to evaluate the suitability of Gesho (*Rhamnus prinoides)* for beer production in Ethiopia.

### 1.3.2. Specific objectives

The specific objectives of this study are;

- To determine the extraction of *Rhamnus prinoides* using 97% ethanol.
- Determination of antioxidant activity of *Rhamnus priniodes*.
- Identifying the bittering effect of Gesho and hops in the beer production.
- Mixing imported hops with Gesho (local hops) at various ratios and determining the effects of the mixture on beer production.
- Select the better processing parameters of the reaction temperature, pH and time of reaction.
- Conduct an actual experiment on beer by taking the best Gesho-Hop ratios bitterness value with good sensory evaluation by chemical process to compare it with the imported hops beer.

### 1.4. Significance of the study

The significance of this study:-

- It introduces the Gesho as a local raw material for use in the beer production process.
- It helps to encourage import substitution to meet growth and transformation plan.
- It helps to satisfy the brewery industry ensuring stability and sustainability of input raw material
- It provide an economically feasible option to produce hops locally, which will play a major role to substitute the imported hops which saves foreign currency and create job opportunity for people.
- It creates opportunity to increase domestic agricultural products as a raw material in the industrialization effort.

Finally, from the result of this research, farmers, and brewing company will be benefited.

## Chapter Two

## 2. LITERATURE REVIEW

### 2.1. Introduction

Beer is the most consumed alcoholic beverage in the world, and is the most popular beverage behind water and tea (Nelson, 2005). Most often beer is produced from malted barley, hops, yeast, and water, yet simple changes in the formulation has created 25,000 to 35,000 varieties of beer worldwide. Variations of this simple formulation include beer brewed from a variety of grains such as rice, millet, barley, and corn depending on the regional staple, and fermented by wild yeasts. Brewing began at home and was followed by small village breweries that eventually led to the modern day large brewery. Early records show beer was produced in ancient Babylon about 8,000 years ago. Beer was an important aspect of both the Egyptian and Mesopotamian cultures where barley was the staple grain (Papazain, 2003).

Ancient Sumerian literature, which dates to about 1800 BC, provides a hymn to the Sumerian goddess of brewing that included an ancient recipe for beer (Katz, and Maytag, 1991). Historians have argued about the advent of beer and whether ancient cultures developed beer or bread first. Historians have held that ancient cultures abandoned hunter-gatherer practices to grow grain for beer (Braidwood, 1953). Bamforth, 2006 reasons that the adoption of grain .production, and subsequent production of beer, makes brewing the world's oldest biotechnology. Beer is a product valued by its physico - chemical properties (i.e. quality) as much as by its entanglement with religious, culinary and ethnic distinctiveness (i.e. tradition). Accordingly, the history of beer brewing is not only one of scientific and technological advancement, but also the tale of people themselves: their governance, their economy, their rites and their daily life (Arnold, 1911).

Today, the brewing industry is a global business, consisting of several dominant multinational companies and many thousands of small producers ranging from brew pubs to regional breweries. The basics of brewing beer are shared across national and cultural boundaries and are commonly categorized in to two main types—the globally popular pale lagers, and the regionally distinct ales which are further categorized into other varieties such as pale ales, stout and brown ale. The strength of beer is usually around 4% to 6% alcohol by volume (Ebneth and Theuvesn, 2005).

### 2.2. Raw materials

Beer is the oldest alcoholic drink for common human kind and water, hops, malt and yeast are the only raw materials for beer brewing accordingly to Germany purity law of 1516 (Kunze, 2004).

#### 2.2.1. Malt

Malting is the controlled germination of cereals, a natural process terminating with the application of heat. Further heat is then applied to "kiln" the grain and produce the required flavour and colour. A basic rule is that, for malt to be made, the barley must be capable of germination, so maltsters source barley with a minimum germination of 98%. Processing into malt is an essential step which allows the use of barley grains in the brewing process.

In biochemical terms:

- the envelopes of the small nucleus containing starch chains are disintegrated; and
- enzymes (diastase, which will remain inside the germinated grains) are produced.

(FAO, 2009).

#### 2.2.2. Water

Quantitatively water is the major input used in the beer production. Only a small part of the water required is used directly in the beer and by far the largest part is needed for cleaning and rinsing purposes and sometimes for the condensers of cooling equipment. Supply and preparation of water is particularly important to the brewer because the water quality affects the quality of the beer produced. Water usage in the brewery is substantial and varies between 3.7 to 10.9hl of water per hl of sales beer, on average it is 6hl/hl (Kunze, 1996).

### 2.2.3. Yeast

Yeasts are unicellular microorganism which can obtain energy they need from organic carbon mainly of biomass. There are two types of yeasts that can fulfill their requirements of food and energy:

- In the presence of oxygen(aerobic) by respiration
- In the absence of oxygen(an aerobic)by fermentation

During the beer production the sugar in the wort is fermented by yeast to alcohol with the release of carbon dioxide. For this purpose the yeast fungi of the species *Saccharomyces cerivisiae* are used. Selected strains of this yeast are systematically isolated and grown as pure culture brewers' yeasts. Other stains of this yeast are used by bakers', distillers' or wine makers' yeasts. Because the yeast does not only produce alcohol but also as a result of its metabolism has a great influence on the taste and character of beer, knowledge of structure and composition of yeasts, their metabolism and their growth is important. There are a number of characteristic differences between different types and races of culture yeasts (Kunze, 2004).Brewery yeasts belong to family of saccharomyceaceae and the genus Saccharomyces. They have the main advantage that their cells propagate by budding. For the production of bottom-fermenting beers, *Saccharomyces carlsbergensis* are used, and for top fermenting beers, *Saccharomyces cerivisiae* (Donhauser, 1997).

### 2.2.4. Hops

Hop (*Humulus lupulus* L.) is a dioecious perennial climbing plant and its female inflorescence (cone) is an essential product for the brewing industry.

Figure 2: Hop (*Humulus lupulus* L.)

Total world hop acreage is about 80 thousand hectares and the Czech Republic, with 6 thousand hectares, is one of the most important world exports. Many varieties of hops are used in the world and each hop variety has a characteristic flavour profile, which is used to distinguish them (e.g. Krajl et al., 1991; Peacock & McCarty, 1992). It is also a perennial, dioecious climbing family plant of hemp family and belongs to the order (urticales) which also includes the nettle family. In brewery it is inflorescences of the female plant which are used. These contain the bitter resins and ethereal oils which supply bittering and aroma components of the beer. As far as brewing is concerned, hops are dried hop cone of female hop plant and products made from them which contains only components from hops. Hops are grown in the special growing regions where the necessary conditions exist (*Kunze, 2004*).

For this reason the majority of the world's commercial hop production occurs between latitudes 35° and 55°, either north or south of the equator. The largest producers of hops are Germany, the United States,

China, and the Czech Republic. Other important growing regions include England, New Zealand, and increasingly Argentina. Climate and soil conditions have a major effect on hops. Varieties developed in one region will have different flavor and aroma profiles when grown in another (Kunze, 1996).


Figure 3: Minimum 13 hrs daylight essential

Source: Kristiansen, 2013

Hops picking occurs at the time of seed ripening at the end of August and completed within 14 days. Harvesting consists of unfastening the hop bines from the support wires and picking the cones with short stalk. Nowadays hops are harvested entirely by hop picking machine (Kunze, 1996).

### 2.2.8. Hops in the Brewing Process

The use of hops, a dried blossom of female plant of *Humulus lupulus*, in brewery dated back to the 12$^{th}$ century. Its use is mainly preferred beside the taste it provides to beer, it also serves as a natural preservative by suppressing bacterial growth that can spoil the produce.

In brewing, hops refer to the flower harvested from the cone of the female plant Humulus lupulus. The cone-shaped flowers are valued by brewers for their resins and oils located in the lupulin glands that

impart both bitterness and aroma to the beer. Hops were not used in most ancient beer production; instead, spices and other plants were used to flavor the beer. In1079, Saint Hildegard of Germany noted the anti spoilage properties of hops, and brewers began to take note (Bamforth, 2006). However, hops were not common in beer until the early 1800s. Hops work well as a preserving agent in the brewing process because they eliminate undesirable malt proteins, aid clarification, and stabilize beer flavors. An added benefit is their ease of cultivation, and ability to impart characteristic flavor and aroma (Papazain, 2003).

Brewers use hops primarily to get bitterness, flavor, and aroma. Hops can be added at several points in the brewing process to enhance one or the other of those things. While most hops are added in the boil kettle, they can be added at a various stages prior to and after the boil as well.

Two components of hop composition are essential to beer production, the essential oils and resins. The oil portion contributes to the aroma characteristic of the final beer (Bamforth, 2006). Hop resins contains alpha acids that contribute to bitterness. The level of alpha acid is unique to each variety of hops. Alpha acids are also referred to as humulones and indicate the bitterness imparted to the beer. Hops are added to the beer during the boiling of the wort. This is necessary to promote the isomerization reaction that renders the alpha and beta acid resins water soluble, as humulinic acid and isohexenoic acid. Once water soluble, these compounds are released into the sweet wort where bitterness is imparted to the wort (Papazain, 2003).

Increased boil time increases the bitterness imparted to the final beer. The aroma that hops provide to beer is produced by essential oils, which account for 1%-2% of the total dry weight of the cone. These essential oils are volatile and easily lost during the boil .Therefore; hops are added at scheduled intervals during the boil to produce the desired flavor and aroma in the final beer. Hops are evaluated in two ways, first, by alpha acids in Alpha Acid Units (AAUs), which is the weight of hops (in ounces), multiplied by the percentage of alpha acids, determined by chemical extraction, and second, by International Bittering Units (IBUs). The IBUs estimates how much of the alpha acid is isomerized and dissolved into the beer. The IBUs are calculated from the AAUs, the volume of the boil (V), and utilization (U). Utilization takes

into account the time and gravity of the boil to describe the efficiency of the isomerization reaction (Palmer, 2006).

There is a new breed variety developed in Hulls Germany , Herkules, with high alpha acid content of hop With a yield of about 2 300 kg/ha and a good resistance to diseases Herkules shows good agronomical attributes. The new variety has an alpha content of 12 -17 % and a high cohumulone amount of 32- 38 % of alpha acids .In the past the sensory impact of high cohumulone contents on beer taste and quality of bitterness was discussed with opposed opinions( Stettner et al, 2007).

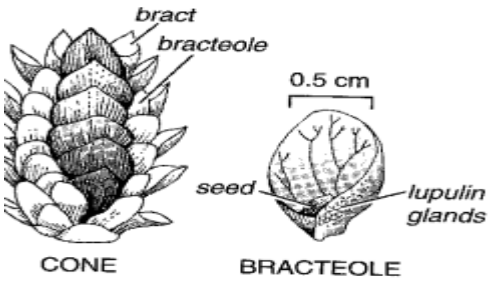



Figure 4: Diagram of hop cone and hop bracteole.

Source: Bamforth, 2006

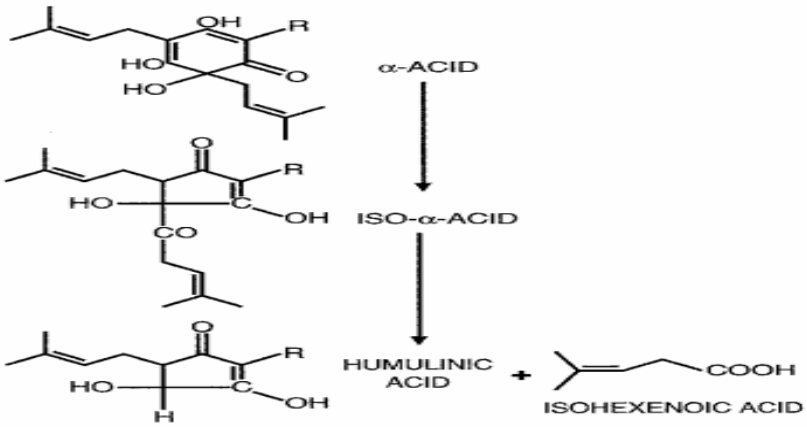



Figure 5: Schematic of the isomerization of the degradation reaction of alpha acids during the boil.

Source: Bamforth, 2006

Hops can be added at several points in the brewing process to enhance one or the other of those things. While most hops are added in the boil kettle, they can be added a various stages prior to and after the boil as well.

**Kettle Hops**: - is the name given to those hops added to the kettle during the boil. These include early addition hops for bitterness and late addition hops for flavor and aroma.

**Bittering Hops**: - Bitterness from hops comes from alpha acids found in the lupulin glands of the hop flowers. The main alpha acids are humulone and cohumulone and adhumulone. In order to become bitter these acids must be chemically altered, isomerized by boiling.

**Isomerization**: - is a chemical process in which a compound is changed into another form with the same chemical composition but a different structure. The percentage of the potential alpha acid that is isomerized is referred to as utilization. Because the length of the boil determines degree of utilization, bittering hops are usually added at the beginning of the boil or with at least 60-minutes of boiling time remaining.

**Flavor Hops**: - Hop flavor and aroma are derived from essential oils found in the lupulin glands. These oils include humulone, myrcene, geraniol, and limonene, among others. The flavors are released as these oils become dissolved into the wort during the boil. However, these oils are highly volatile and are to a large degree lost to evaporation. For this reason flavor hops are added with twenty to forty minutes remaining in the boil. This provides a compromise between isomerization of the alpha acids and loss of essential oils.

### 2.2.9. Composition of hops

The composition of the hops is extremely important for the quality of the produced from them. The average composition of dried hop weight approximately:

Table 1: Composition of hops

| | |
|---|---|
| Bitter substances (Hop resins) | 18.5% |
| Hop oils | 0.5% |
| Polyphenols (tannins) | 3.5% |
| Protein | 20.0% |
| Inorganic | 5.0% |

The rest consists of cellulose and other materials which are not importance in beer Production( Kunze, 1996).

**2.3. Traditional brew of Ethiopia, Tella and Teji**

**2.3.1. Tella**

Tella is widely brewed and consumed in both rural and urban part of Ethiopia. It is well known as local beer. Since it is malt based beverage like that of commercial beer (Getahun, 1976, Shale and Gashe, 1991; Kunze, 1996). Tella brewing requires largely malted barley (*Hordeum vulgars*), Enkuro, Gesho

(*Rhamnus prinoides*) leaves and stems chopped to small pieces, kita (from different grains) and derekot (Shale and Gashe, 1991). Generally, tella is brewing from substrates such as barley, wheat, maize, millet, sorghum, teff or other cereals. Commercial beer is mostly made from malted barley and adjunct like corn, rice or wheat provide the carbohydrate substrates for ethanol production by *Saccharomyces carlsbergensis* or *Saccharomyces cerevisiae*(Kunze, 1996). With regard to the substrate, there is no as such basic difference between tella and beer however the difference is the physical environment for fermentation process where the techniques used in brewing of Tella the standard quantity of ingredients and aseptic method are not well developed.

The quality of tella is variable from local to local, from individual to individual, even within the same individual; the quality varies from time to time. The quality of tella may be deteriorated and affected by contaminants such as fungi and different types of bacteria. To guarantee the consistency of product quality, at different stages of tella production process should be monitored for the presence of spoilage microorganisms as that of beer (Weber et al., 2008).

Tella brewing process is traditionally divided into three phases, namely tejet, tenses and difedef. At first phase, the milled bikil and the pounded Gesho (stems and leaves) are added to the water and covered with net cloth and then allowed to ferment for 1 - 2 days in big clay pot. After the first phase of fermentation (tejet), either enkuro or kita is added and ferment for about 5-7 days depending on environmental conditions, which is commonly known as tenses. At the third phase (difedef), the derekot flour is mixed with the entire content of tenses and can be kept for 10 - 12 or more days depending on the use. To prepare tella for the use of extended period of time, the amount of water and malt should be reduced while the amount of pounded Gesho should be increased but for immediate use the reverse is true. In conclusion, tella with high quality can be prepared relatively using small quantity of water. Though there are several recipes for making tella and even it appears as if every housewife has her own version of the recipe, the procedure described here is taken from one of the most known tella brewing area, Debre-Markos, Ethiopia. Hop is one of the ingredients used for bittering, flavoring and aroma imparting agent for beer brewing. In Ethiopia, Gesho (Rhamnus prinoides) is particularly used to provide a special aroma and flavor (Shale and Gashe, 1991; Berhanu et al., 1999).

**2.3.2. Teji**

Teji (indigenous honey wine) is a home-processed, but also commercially available honey wine. It is prepared from honey, water, and stems of Gesho. Sometimes, widely for commercial purposes, mixture of honey and sugar could be used for its preparation. In case where sugar is used as a part of the substrate, natural food colourant is added so that the beverage attains a yellow colour similar to that made from honey. Good quality teji is yellow, sweet, effervescent, and cloudy due to the content of yeasts. A study found that the mean alcohol content of teji was between 6.98 % V/V and 10.8 %V/V another study found that the average alcohol content of teji was 6.07%V/V (Fiseha, 2014)

### 2.3. Gesho (Rhamnus prinoides)

*Rhamnus prinoides* L. Herit, Gesho in Amharic, family Rhamnaccea, is a plant which grows up to 6 meter. It is known to occur outside Ethiopia in Cameroon, Sudan, throughout East Africa to South Africa and Angola and also in Arabian (Thulin, 1988). It is cultivated in Ethiopia and is an important commodity which is sold in almost every traditional market place in Ethiopia. Although it is quite common to find Gesho cultivations throughout the country, it is worth mentioning that regions in the Tigrai, around Kara kori in north shoa, Debre-Berhan and Sebeta, just west of Addis Ababa, are important centers of production of Gesho. It is wide spread in many parts of eastern and central Africa. In Ethiopia it is a widely cultivated multipurpose plant. The fruits are used for the treatment of ringworm infections.

The leaves and stems of Gesho are ingredients in the making of traditional fermented beverages tella and teji respectively. Although, it is generally known that Gesho imparts the characteristic bitterness of these beverages, more precise understanding of the scientific role of this plant in the brewing process is emerging only very slowly. Coady (1965) suggested that Gesho may have a role in the fermentation process. It is also indicated that the bitterness of the brew is directly related to the amount of Gesho added. Recently efforts were made to develop technologies that allow the utilization of Gesho to hop beer (Alemayehu, 1994) .Recent studies also have shown that it can serve as a commercial hopping agent in the brewery industries. Organoleptically, the most important substance is the naphthalenic glucoside,

Geshoidin, which is responsible for the characteristic bitter, favour of the beverages derived from this plant (Abegaz et al, 1999).

#### 2.3.1. Role of Gesho in tella and tej brewing process

It is not yet clear the exact role of Gesho in tella and tej production however Shale and Gashe (1991) have speculated that the role of Gesho in tella should be similar to that hops in beer. Besides its role to suppress bacteria during the fermentation process, Gesho is certainly the main agent that imparts the desirable bitter taste in to tella. It has also received positive assessment as a commercial hopping agent for beer (Alemayehu, 1994). Recently naphthalenic glycoside named Geshoidin has been identified as the substance responsible for the bitter attributes of the plant (Abegaz and Kebede, 1995).In Ethiopia,

Gesho (*Rhamnus prinoides*) is particularly used to provide a special aroma and flavor(Shale and Gashe, 1991, Abegaz, et al. 1999,).Among different chemical substances found in Rhamnus *prinoides*, naphthalenic glucoside. Geshoidin (Van Staden and Drewes, 1994) is the basic bittering agent for beverages. Although Gesho may have antibacterial effect against some groups of bacteria, its main purpose in the process is believed to impart the typical bitter taste to tella (Ashenafi, 2006).

Table 1: Composition of Gesho

| Samples | Chemical compositions (%) | |
|---|---|---|
| | Essential oils % | Total resin % |
| *Rhamnus prinoides* leaf | 1.13±0.02 | 18.46±0.03 |
| *Rhamnus prinoides* stem | 0.60±0.01 | 17.16±0.01 |
| *Humulus lupulus* control | 1.0 ±0.03 | 19.52±0.02 |

Values are means of triplicate determinations; Values within the same row followed by different superscripts are significantly different at ($p < 0.05$) (Berhanu, 2013)

Table 2: Risk of having pathogenic bacteria in local tella (Positive = +, Negative = - for tested standard bacteria).

| Code of test organisms | Filtered tella with Gesho | Filtered tella without Gesho |
|---|---|---|
| Escherichea coli(ATCC 25922) | - | + |
| Lyesria moncytogenes (ATCC 19116) | + | + |
| Staphylococcus aureus(ATCC 25923) | - | + |
| Staphylococcus sp.(clinical isolate) | - | + |
| Shigella flexneri(ATCC 12022) | - | + |
| P. vulgaris (ATCC 881) | - | + |
| Salmonella NCTC 8385 | - | + |

**2.4. Beer Production technology**

Beer is the oldest alcoholic drink for human kind and water, hops, malt and yeast are the only raw materials for beer brewing accordingly to Germany purity law of 1516(Kunze, 2004). The common processes which take place during beer brewing are malting, milling, mashing, wort boiling and fermentation.

Malting is defined as allowing grain to germinate under well-controlled conditions. The main purposes of malting are the development of enzymes in the grain with simultaneous depolymerization of high molecular carbohydrate substances in the cell wall (modification),the achievement of distinctive character by color and aroma compounds, and removal of undesired aroma compounds (i.e. S-methylmethionine and dimethysulfoxide). High yield and low malting loss are the economic goals (Michael et al, 1999).

In order to give the malt enzymes the opportunity, during mashing, to act on the malt contents and increase surface area, the malt must be broken into small fragments, this process is called Milling. The malt used for a brew is called the grist and the amount used is called the charge. Milling is a mechanical process and during milling the husks must be treated carefully because they are used as filter materials during lautering (Kunze, 1996).

Mashing is the most important process in the wort production. During mashing the grist and water are mixed together (mashed) and the contents of the malt are thereby brought into solution and the extract (filtrate) obtained .i.e. all substance which go into solution (Kunze, 2004).

During mashing the solid particles solubilized by action of brewing water and enzyme formed during malting .The optimum condition at which these enzymes act on the individual compounds are pH and temperature i.e. α-amylase ( pH= 5.6-5.8,temperature $70^{o}$C -$75^{o}$C ),β-amylase (pH=5.4-5.6, temperature $60^{o}$C-$65^{o}$C) and other enzymes also activated within their own optimum conditions. The starch breakdown is most important during mashing and the solubilization of the starch granules during mixing with water and heating proceeds in the various steps. After swelling of the starch kernels, gelatinization of the starch occurs as the enzymatic hydrolysis starts. Starch hydrolysis is allowed to continue until no more α-glucans (dextrin's), which give color with iodine, are present, and the attenuation limit is achieved (Michael et al 1999).

The aim of mashing is to form as much extract as possible. Most of extract is produced during mashing by the action of enzymes which are then allowed to act at their optimum temperatures. The α-amylase breaks down the long starch chains to smaller dextrins. It acts optimally at $72^{O}$C to $75^{O}$C and is rapidly destroyed at $80^{O}$C. The optimum $p^{H}$ is 5.4 to 5.5. Starch breakdown must be monitored because residues of undegraded starch and larger dextrins cause starch haze in the beer.

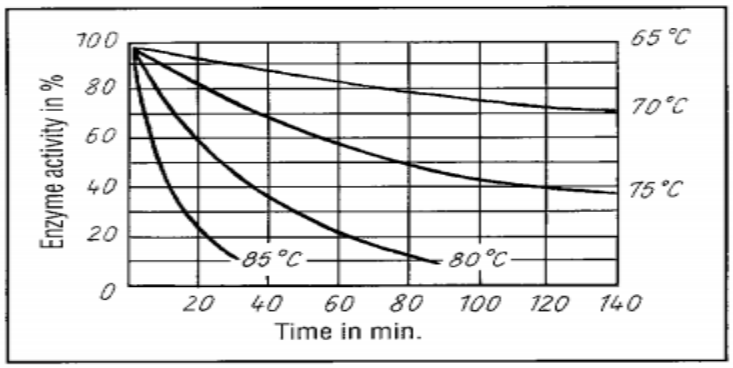



Figure 6: Dependence of enzyme activity (%) on time and temperature

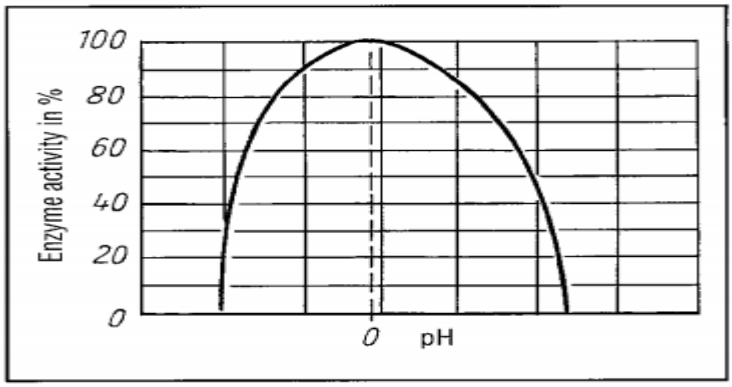



Figure 7: Dependence of enzymes on pH

The most important component of beer is alcohol formed during the fermentation from sugars. Therefore it is important to degrade starch into large extent to maltose .In the addition the intermediates products, dextrin, are produced which is not fermented. Starch degradation occurs in the three stages the sequence of which is unchangeable but which merge into one another.

Gelatinisation:-. By gelatinsation is meant the swelling and bursting of starch granules in hot aqueous solutions. The starch molecules set free into this viscous solution are more easily attacked than ungelatinised starch by amylases.

Liquefaction: -Liquefaction is meant the reduction of viscosity of the gelatinized by α –amylase.

Saccharification:-By Saccharification is meant the complete degradation of starch to maltose and dextrin's by amylases. The iodine test is used to see if it is complete (Kunze, 1996).

Wort boiling is performed in the wort kettle which is equipped with external/internal heating system; thorough boiling .The wort obtained is boiled for 1 to 2 hours during this time the hops are added. During wort boiling bitter and aromatic hop components are transferred into the wort and simultaneously proteins are precipitated. The kettles must have capacity of 9hl per 100kg of grist in order to achieve desired effect through boiling. The most important part of kettle is its heating mechanism (Michael et al, 1999).In wort boiling a number of important processes occurs:-

**A) Extraction and transformation of hop components:-**

The hop resin or bitter substances are the most important components of hops for beer productions because they give beer its bitter taste. The α-acids are completely insoluble in the cold wort. In the boiling wort the α-acids changes their structures, isomerization. The iso-compounds produced are much more soluble than the α-acids from which they are formed. On average only about one third of the α-acid added is recovered in the boiled wort in the form of isomerized compounds. Moreover, in the subsequent course of beer production substantial amounts of bitter substances are removed (FAO, 2009).

**B) Formation and precipitation of protein-polyphenol complexes**

The Polyphenols of hops and malt dissolved completely in the wort. Malt Polyphenols are more reactive than those from hops have. Compounds consisting of proteins, Polyphenols and compounds consisting of proteins and oxidized Polyphenols are insoluble in the hot wort and precipitate during wort boiling as break. By meant the flocculent particles formed during wort boiling are desirable to precipitate as completely as possible.

By break formation is encouraged by longer wort boiling:- up to 2hour boil almost all of this material is precipitated. The boiling time required for protein precipitation decreases with increasing pressure and consequently increased temperature. At temperature of $140^0$C only 3min to 5min are necessary for break formation. Vigorous movement of boiling wort and low $P^H$ 5.2before wort boiling improve the reaction between protein and polyphenol (Briggs et .al, 1997).

**C) Evaporation of water**

Boiling results in the evaporation of water and concentration of wort. Intensive boiling means vigorous movement of the kettle contents and with its extensive evaporation .the amount of water evaporated is therefore directly related to the desired protein precipitation. Consequently, for a long time an evaporation rate of 10 to 15% of the wort was a measure of good boiling.

**D) Increase in the wort colour**

Boiling results in the formation of melanoidins and also in the oxidation of Polyphenols. This causes an increase in the wort colour which on casting is slightly darker than the beer to be produced. Thus, the wort on fermentation becomes lighter in colour (Kunze, 1999)

In order to protect the produce from sour taste the pH of the wort acidity improved by boiling and adjusting to pH=5.2, where spoiling microorganisms cannot grow. At the same time in boiling the wort inhibited the action of enzymes.

The fermentation process is initiated by addition of 0.5-0.7liter of heavy yeast slurry per hectoliter of wort, corresponding to $15\text{-}20*10^6$ yeast cells per milliliter of cooled and aerated wort which is called pitching. The products resulting from the fermentation are ethanol and carbon dioxide. The other reaction products include higher aliphatic and aromatic alcohols, esters, organic acids, and polyhydric alcohols, all of which are important for the properties and quality of the resulting beer (Eßlinger et al, 1995).

**2.5. Antioxidant in the beer**

Over the last several decades, scientists have discovered that the body's formation of unstable oxygen molecules called free radicals is unavoidable and almost every cell produces tens of thousands of them each day. We are also exposed to free radicals in the environment. Thus, free radicals have endogenous and exogenous sources of origin. Our antioxidant systems are not perfect so as we age, cell constituents are constantly damaged by oxidation which may lead to diseases and therefore should be mopped up by antioxidants. It is established that body's own antioxidants are insufficient and should be restored by external sources. Food and beverages are major sources of antioxidants containing phenolic or polyphenolic nucleus with widely demonstrable activity in vitro (Halliwell and Gutteridge, 1999).Times

immemorial, beer has been known for its prophylactic, therapeutic and palliative properties (Rice Evans, 2000; Andersen et al., 2000;).

It is essentially prepared from malted Barley and Hops which contribute several constituents in it. Barley mainly contributes the sugars, amino acids, proteins, Polyphenols. Its malted form is the chief source of carbohydrates fermented by *Saccharomyces cerivisiae* converting it into ethanol and $CO_2$.

Hops added during the later stage of brewing are the chief source of AO. The AO activity of the beer is mainly due to the presence of Polyphenols, chalcones,flavonoids, catechins, proanthocyanidins and bitter compounds e.g., humulones, lupulones, etc. (Wolfhart,1993; Stevens et al., 1998). . Besides AO, hops in beer also possess sedative, chemo preventive, drug modulating and phytoestrogenic properties (Hopfenzapfen, 2000; Gerhauser et al., 2002).

Food and beverages including beer, since natural sources of AO are far more superior for better utilization than isolated preparations (Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group, 1994). Beer is an important and popular beverage liked by the majority and needs to be elucidated for its AO property and bioavailability.

**2.6. The role of pH in brewing**

$p^H$ is the measure of acidity which is the concentration of hydrogen ions $H^+$ in solution. The fall in pH is associated with the mineral composition of the brewing water and mineral treatment added to the brewing water. Thus: brewing water has pH= 7.0 (neutral), mash pH= 5.6 ±0.2, boiled wort pH =5.4 ±0.2, the fermentation process ends up with pH =4.0 ±0.2 .The low acidity during mashing comes from the precipitation of phosphates and amino acids/polypeptides derived from the malt. The optimum pH where brewing enzymes can operate within the range of the typical mash pH (5.6 ± 0.2).thus: Alpha amylase which randomly hydrolyses starch require pH= 5.2, Beta amylase which hydrolyses pairs of maltose sugar from non reducing end –optimum pH =5.5, Proteases –hydrolyses proteins to polypeptides to amino acid –optimum pH=5.5. It governs most of the physical and chemical reactions which occur and create the necessary living environment for the yeast growth, flourish and complete the fermentation process. The acidity of the beer itself contributes to the taste and character of the beer. $P^H$ has a major

effect on the rate of reaction, solubility and electrostatic charge of many molecules therefore it has an important role on beer quality and production throughout the brewing process.

Table 3:DPPH Assay of undiluted 100% and 50% Light (LB4.4) and Strong (SB4.1) Beer samples at their native i.e. 4.4, 4.1 respectively and physiological i.e.7.3 pHs. PH in the native beer samples was adjusted to 7.3with 0.1 N NaOH (LB7.3 and SB7.3 respectively).

| Commercial Beer Type | Conc. (%) | pH | % Scavenging of 250µM DPPH |
|---|---|---|---|
| Light (LB) | 100 | 4.4 | 96.91 |
| | 50 | 4.4 | 85.81 |
| Light (LB) | 100 | 7.3 | Nil |
| | 50 | 7.3 | Nil |
| Strong (SB) | 100 | 4.1 | 74.85 |
| | 50 | 4.1 | 81.88 |
| Strong (SB) | 100 | 7.3 | Nil |
| | 50 | 7.3 | 2.3 |

Source: - (Singh, Sharma et al. 2007)

### 2.7. The role of temperature and time in brewing

By mashing for a long time at $62^{O}C$ to $64^{O}C$ beer with a higher attenuation limit is obtained. By exceeding these temperatures and mashing for long time at $72^{O}C$ to $75^{O}C$ a dextrin-rich beer with a lower attenuation limit is obtained. The mash temperatures have an extremely large effect and so during mashing rests at the optimum temperatures for the amylases are always used.

- Maltose production rest at 62 to 65$^{O}$C which is optimum temperature for β-amylase.
- Saccharification rest at 72 to 75$^{O}$C which is optimum temperature for α-amylase.
- Mashing-off temperature of 76 to 78$^{O}$C.

The enzymes certainly deactivated throughout mashing. Instead two time- dependent stages in enzyme activity can be distinguished:

- The maximum enzyme activity is reached after 10 to 20min.
- The maximum enzyme after 40 to 60 min. enzymatic activity at first decrease rapidly, reduction in the activity continuously decreases. From this it must concluded that the effect of mashing temperature must always be considered in the relation to the duration of mashing

In general: with increasing mashing time the concentration of the extract solution increases. But the rate of increase becomes slower and slower and with increasing mashing time (especially when mashing at 62 to 63$^{O}$C the maltose content increases and with it the attenuation limit. This wort should produce a vigorous main fermentation.

**2.8. Effects of alcohols on man**

The main active ingredient of beer is alcohol and therefore, the effects of alcohol apply to beer. The moderate consumption of alcohol, including beer is associated with a decreased risk of cardiac disease, stroke and cognitive decline (Stampfer, 2005).

. The long-term effects of alcohol abuse, however, include the risk of developing alcoholism and alcoholic liver disease. High ethanol consumption over the years leads to damage. For healthy man, the limit is about 60g per day and for women 50g per day. However, this value strongly dependent on body weight, health status and other factors. Ethanol related high levels of NADH +H and acetyl-coA in the liver leads to increase synthesis of neutral fats and cholesterol. However, since the exports of this in the form of VLDLs are reduced due to alcohol, storage of lipid occurs. This increase in the fat content of the liver (from less than 5% to more than 50% of the dry weight) is initially reversible. However, in chronic alcoholism the hepatocytes are increasingly are replaced by connective tissue. When liver, cirrhosis occurs, the damage to the liver finally reaches an irreversible stage, characterized by progressive loss of liver functions (Koolman, and Roehm, 2005).

## Chapter Three

## 3. Materials and Methods

### 3.1. Structure of the Thesis

The research was conducted following this general flow sheet which contains the major unit Operations and activities performed during the study.

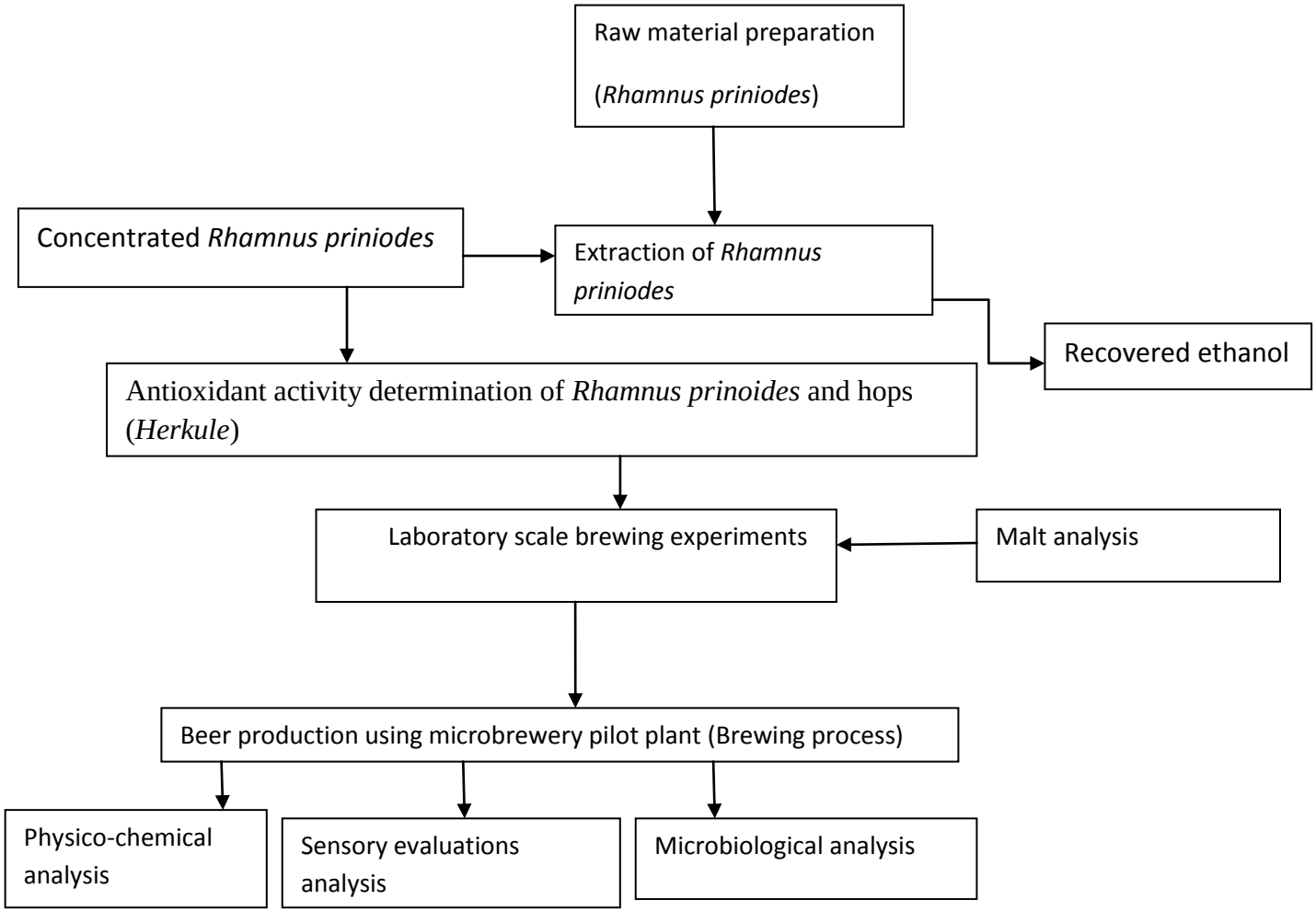



Figure 8: Structure of experimental design

## A) Materials used

### i. Sample

The sample used in this study is *Ramuns.priniodes* which was collected from central market (merkato), Addis Ababa. It was grown in Debre-Berhan, one of the important centers of *Rhamnus priniodes* (Gesho) production. The other samples like Barley malts, hops, yeasts and other chemicals used for brewing were collected from the warehouse of the BGI Ethiopia plc.

### ii. Apparatus

The equipments used to conduct this study are: - Wiley mill, Shaker (Hy-5A), rotary evaporator, pump, UV/Vis/NIR spectrophotometer, Erlenmeyer flask(2000ml),canonical flask(250ml),Hammer mill, mashtun, wort kettle, whatman.no 2. filter paper, Extractor i.e. separatory funnel for cold extraction, digital weighing balance , weight bottle, stopper, oven, tong , water bath, measuring cylinder, mash bath, prototype, beaker, glove, mask, goggles, conical flask, and glass stopper,10ml volumetric flasks, test tubes, micropipettes, vortex meter.

### iii. Reagent

Chemicals reagents used in this study are:-97% ethanol, lubricant oil and distilled Water(Ethiopia forest product utilization research institute laboratory, A.A ), methanol analytical grade and DPPH free radial(2,2-diphenyl-1-hydrazine) food science and nutrition laboratory, A.A), $CaCl_2$, $H_2SO_4$, isooctane, concentrated hydrochloric acid(3NHCl), octanol, sugars was used as adjuncts, brewing water, yeast (*Saccharomyces carlsbergensis*), hop, Gesho(Merkato, A.A) and other additives like Vitamin.C(ascorbic acid), sulphuric acid, sodium hydroxide, calcium chloride. All these and other reagents and standard are analytical Grade (BGI Ethiopia plc laboratory).

### 3.2. Raw material collection, transportation and storage

Gesho (*Rhamnus priniodes)* was brought from the central market (Merkato), Addis Ababa, transported to the laboratory of Forest Product Utilization Research Center of Ethiopian Environmental and Forest Research Institute for extraction. Raw materials like Barley malts (local plus imported), hops, yeasts and other chemicals used for brewing were collected from the warehouse of the BGI Ethiopia plc.

### 3.3. Methods

***Rhamnus prinoides L*** **.Herit**, the fresh plant leaves and stems of *Rhamnus prinoides* was bought from central market(Merkato), and the extraction would be carried out in Ethiopia Forest Product Utilization Research Center of Ethiopia Environmental and Forest Research Institute. The moisture of freshly harvested sample of Gesho leaves have moisture content of 75 -80%.

Figure 9: Gesho(*Rhamnus prinoides)*

Drying was performed in an open air protected from direct exposure to sunlight for five days to moisture content of 10.4%. The dried materials were powdered using Wiley mill (Retsch SR200) (Ethiopia forest product utilization research institute lab.)

Figure 10: Mill (Retsch SR200) Powdered *Rhamnus priniodes*

Cold extraction of dried and milled sample of Gesho was performed with 97% of ethanol by socking for 72hr (Ethiopia forest product utilization research institute lab.). The experiments done for production of barley malt beer using Rhamnus Priniodes (Gesho) and imported hops based on European brewing technology, physico-chemical analysis; sensory quality evaluation and microbiological analysis are conducted in the BGI Ethiopia Plc St. George brewery. The comparative analysis of Rhamnus prinoides with hops on the beer production is done by using Design Expert software7.0

### 3.4. Extraction process of *Rhamnus prinoides*

Extraction of well vented and dried samples of Gesho was performed by socking in 97% ethanol as an extracting agent. A weight of 750g of the dried and powdered *Rhamnus prinoides* leaves with stem put in 2000 ml Erlenmeyer flask was soaked in 1500ml of 97% ethanol for a period of about 72 hours with by shaking on a shaker Hy-5A Manoeuvre. Fresh solvent was used in every 12 hr socking extract was filtered through a medium density filter paper fitted in Buchner funnel. And the extract of *Rhamnus prinoides* i.e. ethanol extracted concentrated using rotary evaporator (Ethiopia forest product utilization research institute lab.).

Figure 11: Hy-5A may oeuvre style vibrator (shaker)

### 3.5. Antioxidant activity determination of *Rhamnus prinoides* and hops (*Herkule*)

The antioxidant activity of the *Rhamnus prinoides* ethanolic extract, residual and Herkule using ethanol as a solvent was estimated using the method of Kirby and Schmidt (2004) with slight modification. The method of Kirby and Schmidt uses DPPH (2, 2-diphenyl-1picrylhydrazine) as a free radical. For measuring free radical scavenging ability of a sample using this method, the methods are grouped in two according to the chemical reactions involved: hydrogen atom transfer reaction-based methods and single electron transfer reaction-based methods. This radical shows a strong absorption maximum at 517 nm (purple), in the presence of antioxidants, the color turns yellow.

Accordingly an amount of 0.004% DPPH and 0.3 mg/ml of standard ascorbic acid was prepared in a 98% methanol solvent and 50 mg/ml of *Rhamnus prinoides* extract, *Rhamnus prinoides* residue and Herkule extract were also prepared using ethanol (97%) as a solvent. Then 20$\mu$l, 40$\mu$l, 60$\mu$l, 80$\mu$l, 100$\mu$l, 120$\mu$l, 140$\mu$l, 160$\mu$l, 180$\mu$l , 200$\mu$l and 240 $\mu$l sample was taken from 50 mg/ml of *Rhamnus prinoides*, *Rhamnus prinoides* residual and *Humulus lupulus* stock solution , 98$\mu$l, 96$\mu$l, 94$\mu$l, 92$\mu$l, 90$\mu$l, 88$\mu$l, 86$\mu$l, 84$\mu$l, 82$\mu$l , 80$\mu$l and 76 $\mu$l of the methanol and 4ml of 0.004% of DPPH free radical solution were added to each sample in the different test tubes and mixed using a vortex mixer for 20s. All test tubes were then incubated for 30 minutes in the dark cabinet at room temperature. This makes the reaction to happen and prevents the absorption of light by DPPH that may interfere with the actual absorbance of the solution. The same procedure was repeated for standard by replacing the Rhamnus prinoides extracted,

residual and humulus lupulus sample with equal amount of 0.3 mg/ml for ascorbic acid. Control sample (without extracted, residual, humulus lupulus sample and ascorbic acid) that is blank was run in parallel. Finally, free radical scavenging activities was read spectrophotometrically by monitoring the decrease in absorbance at 517nm using high performance UV-Vis spectrophotometer and Cuvette(1-cm,quartz) inhibition of free radical DPPH in percent (I %) was calculated as follows.

$$I\% = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) X100 \ldots\ldots\ldots\ldots\ldots a$$

Where:

- A $_{control}$ is the absorbance of the control reaction (containing all reagents except the test compound)
- A $_{sample}$ is the absorbance of the test compound.

Figure 12: In the presence of antioxidant colour turns to yellow

(Food science and nutrition lab.)

### 3.6. Brewing process

Beer brewing beguns with malted grain, which was passed through a milling machine to crack the dried kernels and grind them into a coarse powder. The cracked malt was then steeped with hot water in a large, stainless steel vat called a mash tun, to produce thick, sweet liquid called wort. The wort was boiled, or brewed for two hours in a large kettle. After it was cooled, the wort was then transferred to a fermentation tank where yeasts slowly convert the grain sugar to alcohol and $CO_2$. The liquid, now

beer, may then be passed through a filter to remove the yeast residue. The wort was pumped into a large conditioning tank to age where it undergoes fermentation. During aging, the beer becomes naturally carbonated. The finished beer was then mechanically bottled, and may be pasteurized to kill any of the remaining yeast and any other micro-organisms. The longer process – generally one to two weeks, depending on the temperature at which the bottles are kept – results from a "natural fermentation" or aging process that minimizes the use of additives. The brewing process can be described in detail as follows:

### 3.6.1. Milling

Barley malt was milled using the Buhler-Miag disc attrition (mill, type DLFUW24050, serial No.20352, Germany) to a near-floury consistency as fine grinding 0.2mm and mashed with distilled water as ratio 1:4 of grist to distilled water.


Figure 13; Mill, type DLFUW24050, serial No.20352, Germany, BGI Ethiopia plc.

### 3.6.2. Malt analysis

The physico-chemical analysis of malts was performed in the BGI Ethiopia Plc. St.George brewery using the mash bath. It was done using mashing program described below and its physico-chemical analysis done using Anton paar alchololizer plus by following malt analysis format and its results were described in the result and discussion part(BGI Ethiopia plc).

Figure 14: Mash bath

Figure 15: Anton paar alcholoLizer plus, DMA4500

### 3.6.3. Mashing program

The 6kg of barley malt i.e. local plus imported malt was placed in the cleaned steeliness steel tank i.e. mashtun and mixed with 25L of hot water at $62^0$c was added to the grist while stirring to prevent clotting and lump formation. The temperature of mash was decreased to about $48^O$c on mixing .This was then raised to between 54 $^O$C and $56^O$C due to steam in the coil and held for 30min while slowly stirring in the mashtun with pH=5.4. The temperature program was performed as following: $55^o$c for 30min, $62^o$c for 30min, 72 $^o$c for 20min, $78^O$C for 30min and then 5L of hot water was added as sparging water and held at $78^o$c for 10min. At the end of the stand-on period, starch presence or absence test was done using iodine solution. This test involved taking a sample of the mash; cooling it to $20^o$c dropping it on a white tile and adding a three drops of iodine. A blue-back colouration indicates presence of starch or yellow colour indicates iodine normal i.e. Saccharification complete. Then it was filtered through polystyrene cloth, and transferred to wort kettle and then the cleared wort was analyzed for extract

### 3.6.4. Wort boiling

The 25liter of hot wort ($78^O$C) obtained from mashtun is boiled for 45min. During this time after 15min of wort transferred the 4g of imported hops and 175.5g of Gesho were added to 25L of wort in the wort kettle, then boiled at $80^O$c for 45min. During wort boiling bitter and aromatic hop or Gesho components transferred into wort and simultaneously proteins are precipitated. Then the wort was cooled to $20^O$c using ice water as a coolant. Then cold wort was transferred to fermentation tank with yeast to ferment.

### 3.6.5. Fermentation

Twenty liter of clear wort which is obtained from one mash operation and filled into 40liter fermentation tank and aerated for 10min in order to obtain 8mg/liter of dissolved oxygen. Aerated wort was acclimatized to 10 $^{O}$C and 180g per 20liter of Inoculation of Saccharomyces cerevisiae at 10$^{O}$C wort at concentration 12x10$^{6}$ cells/ml were performed and kept the Fermentation through cooling system at 9 $^{O}$C and after 6days fermentation the temperature increased to 15$^{O}$C in order to reduce vicinal diketones (VDK) which is in the high amount that is 0.15ppm would disturb beer flavour for two days. Fermentation was finished when extract content 12.5$^{O}$P or 80% of sugar converted into $CO_2$ and alcohol. The extract content determined using (Anton Paar DMA 45,000, Austria) and pH were determined after filtered wort through filter paper (what man filter paper), and the rest of wort was kept at 12 $^{O}$C. After fermentation was finished lager beer was subjected to age at 3$^{O}$C for 3 days in order to produce clear beer. During aging, the beer becomes naturally carbonated, which was done by adding 180g/20L of yeast and 200g of sugar per 180 gram of yeast and 5.37 alcohol %v/v and 4.5 ppm of $CO_2$ was obtained. Then it was filtered by Whatman .paper. The finished beer is then mechanically bottled, and pasteurized (7) to kill any of the remaining yeast and any other micro-organisms for at least of1hrs.Beer was racked in order to reduce cell concentration to less than 2x10$^{6}$ cells/ml and then they were adjusted $CO_2$ to be 4.5mg/liter and chilled at cool room at 7$^{O}$C for a week. The chilled beer was filtered through sterile filter pad supplemented with course filter aids (120g/hl) under $CO_2$ pressure. The cleared beer was filled in the bottle at $CO_2$ 4.5mg/liter at 3 $^{O}$C, and were kept at 3 $^{O}$C for further analysis.

Figure 16: Micro brewery pilot plant, BGI Ethiopia plc lab.

### 3.7. Experimental design and Statistical Analysis

Experimental design selected for this study was a $2^3$ Full-Factorial analysis with two level of treatment for comparative experiment and the main parameter was beer bitterness gained. The experiment was analyzed by the Design Expert software 7.0.0 and the study conducted to develop and evaluate *Rhamnus priniodes* in beer production by varying *Rhamnus priniodes* ratios with hops.

It was also used to identify the relationship existing between the dependent responses (beer bitterness) and independent process variables as in the brewing process. The three independent variables or factors studied were: brewing temperature ($80^o$c and 90℃), time (45min and 90 min), $P^H$ (5.2 and 5.4) for actual variable levels. For each factor, an experimental range was adjusted based on the results of literature data and on the performance of preliminary experimental trials. These three factors: temperature, $P^H$ and time

were selected as independent variables, because of their influence on brewing process. In this study, hop ratio was controlled as constant during each brewing process. Therefore, in this work, physico-chemical analysis; sensory quality evaluation and microbiological analysis are conducted in the BGI Ethiopia Plc which means comparisons are performed using one-way analysis of variance (ANOVAs) for all data for each processing stage and the influence of process variables on bitterness of beer will be analyzed a $2^3$ Full-Factorial experiment. To do these, design expert 7.0.0 software package has been employed.

### 3.7.1. Experiments done in laboratory scale.

**Table 4: Experiments done for 100% local hops (*Rhamnus priniodes)***

| std | Run | Block | Factor1 A:temp [ $^0$C] | Factor2 B:PH [PH] | Factor3 C:time [min] | Response1 beer bitterness [Bu] |
|---|---|---|---|---|---|---|
| 2 | 1 | Block1 | 90 | 5.2 | 45 | |
| 3 | 2 | Block1 | 80 | 5.4 | 45 | |
| 5 | 3 | Block1 | 80 | 5.2 | 90 | |
| 6 | 4 | Block1 | 90 | 5.2 | 90 | |
| 7 | 5 | Block1 | 80 | 5.4 | 90 | |
| 4 | 6 | Block1 | 90 | 5.4 | 45 | |
| 8 | 7 | Block1 | 90 | 5.4 | 90 | |
| 1 | 8 | Block1 | 80 | 5.2 | 45 | |

Table 5: Experiments done for 100% imported hops (Herkule)

| std | Run | Block | Factor1 A:temp [$^0$C] | Factor2 B:PH [PH] | Factor3 C:time [Min] | Response1 beer bitterness [Bu] |
|---|---|---|---|---|---|---|
| 2 | 1 | Block1 | 90 | 5.2 | 45 | |
| 3 | 2 | Block1 | 80 | 5.4 | 45 | |
| 5 | 3 | Block1 | 80 | 5.2 | 90 | |
| 6 | 4 | Block1 | 90 | 5.2 | 90 | |
| 7 | 5 | Block1 | 80 | 5.4 | 90 | |
| 4 | 6 | Block1 | 90 | 5.4 | 45 | |
| 8 | 7 | Block1 | 90 | 5.4 | 90 | |
| 1 | 8 | Block1 | 80 | 5.2 | 45 | |

Table 6: Experiments done for 50:50 local hops (Rhamnus priniodes) to imported hop (Herkule)

| std | Run | Block | Factor1 A:temp [ $^0$C] | Factor2 B:PH [PH] | Factor3 C:time [Min] | Response1 beer bitterness [Bu] |
|---|---|---|---|---|---|---|
| 2 | 1 | Block1 | 90 | 5.2 | 45 | |
| 3 | 2 | Block1 | 80 | 5.4 | 45 | |
| 5 | 3 | Block1 | 80 | 5.2 | 90 | |
| 6 | 4 | Block1 | 90 | 5.2 | 90 | |
| 7 | 5 | Block1 | 80 | 5.4 | 90 | |
| 4 | 6 | Block1 | 90 | 5.4 | 45 | |
| 8 | 7 | Block1 | 90 | 5.4 | 90 | |
| 1 | 8 | Block1 | 80 | 5.2 | 45 | |

Table 7: Experiments done for 75:25 local hops (Rhamnus priniodes) to imported hop (Herkule)

| Std | Run | Block | Factor1 A:temp [ $^0$C] | Factor2 B:PH [PH] | Factor3 C:time [min] | Response1 beer bitterness [Bu] |
|---|---|---|---|---|---|---|
| 2 | 1 | Block1 | 90 | 5.2 | 45 | |
| 3 | 2 | Block1 | 80 | 5.4 | 45 | |
| 5 | 3 | Block1 | 80 | 5.2 | 90 | |
| 6 | 4 | Block1 | 90 | 5.2 | 90 | |
| 7 | 5 | Block1 | 80 | 5.4 | 90 | |
| 4 | 6 | Block1 | 90 | 5.4 | 45 | |
| 8 | 7 | Block1 | 90 | 5.4 | 90 | |
| 1 | 8 | Block1 | 80 | 5.2 | 45 | |

Table 8: Experiments done for 25:75 local hops (Rhamnus priniodes) to imported hops (Herkule)

| Std | Run | Block | Factor1 A:temp [ $^0$C | Factor2 B:PH [PH] | Factor3 C:time [Min] | Response1 beer bitterness [Bu] |
|---|---|---|---|---|---|---|
| 2 | 1 | Block1 | 90 | 5.2 | 45 | |
| 3 | 2 | Block1 | 80 | 5.4 | 45 | |
| 5 | 3 | Block1 | 80 | 5.2 | 90 | |
| 6 | 4 | Block1 | 90 | 5.2 | 90 | |
| 7 | 5 | Block1 | 80 | 5.4 | 90 | |
| 4 | 6 | Block1 | 90 | 5.4 | 45 | |
| 8 | 7 | Block1 | 90 | 5.4 | 90 | |
| 1 | 8 | Block1 | 80 | 5.2 | 45 | |

**4.2**. **Bitterness measuring method (**Questionnaire for overall acceptability ranking test attached as appendix-D)-colour, bitterness, foam stability……

**Determination of Beer bitterness (spectrophotometer method-EBC method 4.9.3)**

**A) Sampling method**

A sample the cooled wort before pitching where the composition is representative ,aspectic sampling is recommended to prevent contamination and Store at refrigerator at 5-10$^o$c until use.

**B) Sample preparation**

A sample must be degassed without loss of foam by adding 2-4 drops of octanol into the bottle and pouring 5-20 times from one beaker to another or use maginetic stirrer at least of five minutes. If Yeast contained in beer must be clarified by centrifuging 15minutes at 3000rpm.

**C) Procedure**

- 10ml of degassed test sample was pipetted into conical flask with glass stopper which was acidified with 1ml of 3N HCl and was filled with 20ml of isooctane (2,2,4-trimethylpentane) ,Stopper the flask tightly and place it in mechanical shaker ,Shake vigorously for 15minutes.at room temperature and then allow decanting for 10 minute until the supernatant organic phase will create.
- Measure the absorbance of isooctane at 275nm in a1.ocm quarz cell or cuvette using Isooctane from the batch as the blank. The optical density is expressed in terms of European brewing convention bitterness units as follows.

  Expression of result will be come;-

  Bitterness (Bu) = 50*ABS275 (EBC, 1998).

Figure 17: Bitterness shaker

### 4.3. Sensory quality evaluation

An acceptability, aroma and flavour profile (colour, Bitterness,estery,foam stability and tingly)tasting was conducted on five beer samples, that is 100% Gesho beer,100% imported hop beer, 75% Gesho beer, 50% Gesho beer and 25% Gesho beer. The sensory analysis was conducted using 8 member-trained panelits using sensory evaluation form in the appendix D. The panelists constituted 8 male who are staff at executive position and currently have been working in St.George brewery and who has experiences and familiar of beer drinking. The discussion for meaning of each attribute in score sheet and agreement that these Gesho beers were presented as new products on shelve was carried out before beer tasting.

The study was served at $7^{O}C$ so that panel members could easily pick flavour notes. Serving order was changed for every group of three and samples were served in clean and odourless drinking glasses. All assessors had one score sheet for five samples and tasted all samples within 30min. The hedonic scale (4 levels) was designed 0 to 4 scores for evaluate the colour, bitterness, tingly and estery. The score 0 was extremely dislike, 1 was dislike, 2 was normal, 3 was like, 4 was like very much. And finally attribute was overall impression, if scored as 0 was undrinkable,1 was drinkable but not prefer another glass, 2 was drinkable and prefer one more, 3 was good ,4 was very good . Panelists were introduced to rinse their mouths with water before starting and between sample evaluations. Parameters for evaluation are shown in the questionnaire in the Appendix D. The ratings of each sensory attribute were converted to numerical score and the numerical score were collected for statistical analysis

## Chapter Four

## 4. Results and Discussions

### 4.1. Antioxidant activity of *Rhamnus prinoides* extracted, residual and Herkule

Antioxidant activity of *Rhamnus prinoides* extracted was evaluated by its free radical scavenging activity on stable DPPH radical. Ascorbic acid was used as standard and a control sample was run in parallel.

Table 9: Inhibition effect of ascorbic acid (standard) on DPPH assay

| Ascorbic acid conc. (mg/ml) | Absorbance at 517 nm | Inhibition effect (%) |
|---|---|---|
| 0.000 | 0.722±0.001 | 0.00±0.00 |
| 0.020 | 0.546±0.012 | 24.38±1.66 |
| 0.040 | 0.411±0.044 | 43.07±0.01 |
| 0.060 | 0.32o±0.052 | 55.68±0.06 |
| 0.080 | 0.143±0.002 | 80.19±0.02 |
| 0.100 | 0.048±0.003 | 93.35±0.004 |
| 0.120 | 0.051±0.008 | 92.94±0.10 |
| 0.140 | 0.047±0.002 | 93.49±0.04 |
| 0.160 | 0.045±0.001 | 93.77±0.08 |
| 0.180 | 0.045±0.000 | 93.77±0.00 |
| 0.200 | 0.045±0.000 | 93.77±0.00 |
| 0.240 | 0.050±0.000 | 93.07±0.00 |

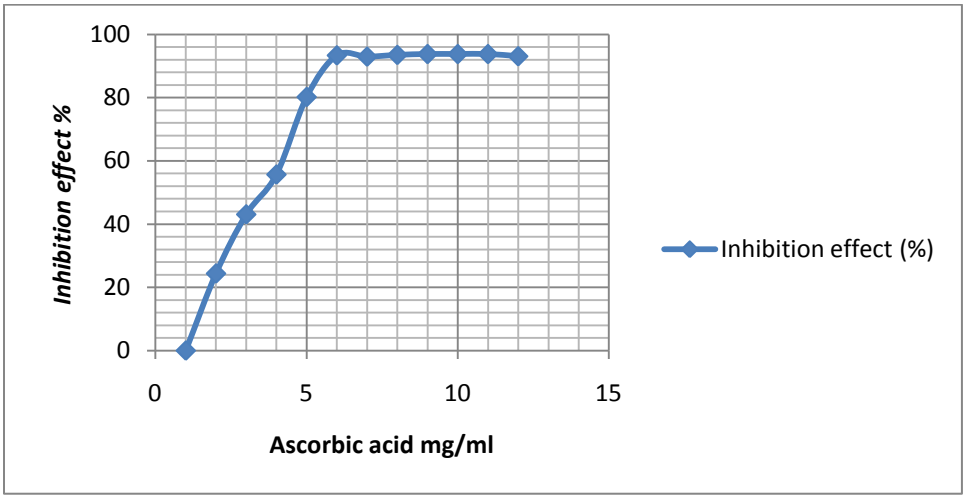



Figure 18: Inhibition effect of different concentration of ascorbic acid on DPPH free radical at 517 nm

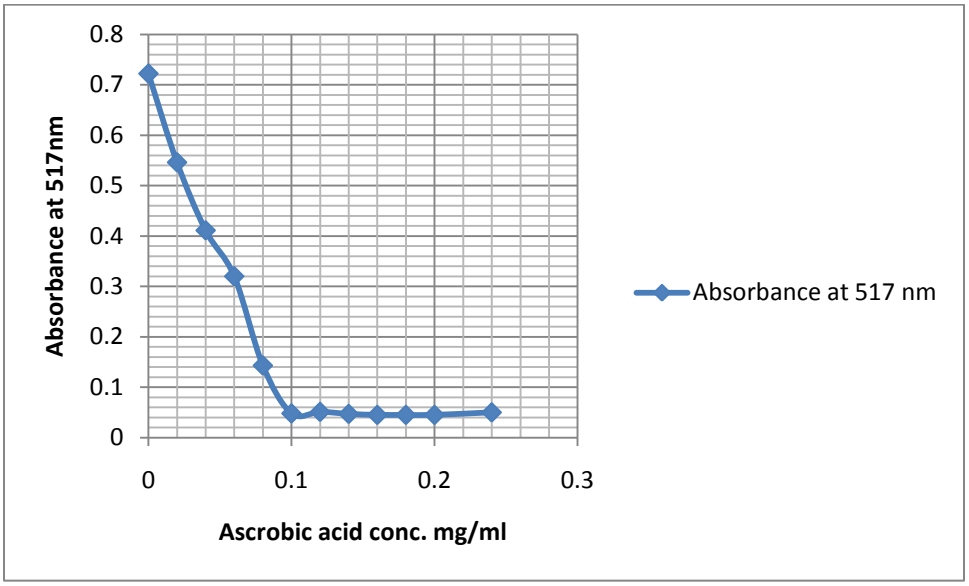



Figure 19: Effect of different concentration of ascorbic acid with DPPH free radical on absorbance at 517 nm.

The above Table(Table 11) shows that ascorbic acid is the strong antioxidant having maximum inhibition effect of 93.77 and the inhibition effect increase in the first phase until the maximum value of inhibition effect is achieved and decreased with further addition of ascorbic acid.

Table 10: Inhibition effect of hops (Herkule) on DPPH assay

| Herkule conc. (mg/ml) | Absorbance at 517nm | Inhibition effect (%) |
|---|---|---|
| 0.000 | 0.915±0.013 | 0.00±0.00 |
| 0.020 | 0.798±0.004 | 11.68±0.437 |
| 0.040 | 0.664±0.002 | 27.43±0.219 |
| 0.060 | 0.54±0.028 | 40.98±3.06 |
| 0.080 | 0.448±0.007 | 51.04±3.716 |
| 0.100 | 0.364±0.004 | 60.22±0.437 |
| 0.120 | 0.308±0.000 | 66.34±0.000 |
| 0.140 | 0.267±0.012 | 70.82±1.311 |
| 0.160 | 0.230±0.01 | 74.86±1.093 |
| 0.180 | 0.207±0.001 | 77.38±0.109 |
| 0.200 | 0.190±0.001 | 79.23±0.109 |
| 0.24 | 0.175±0.001 | 80.87±0.109 |

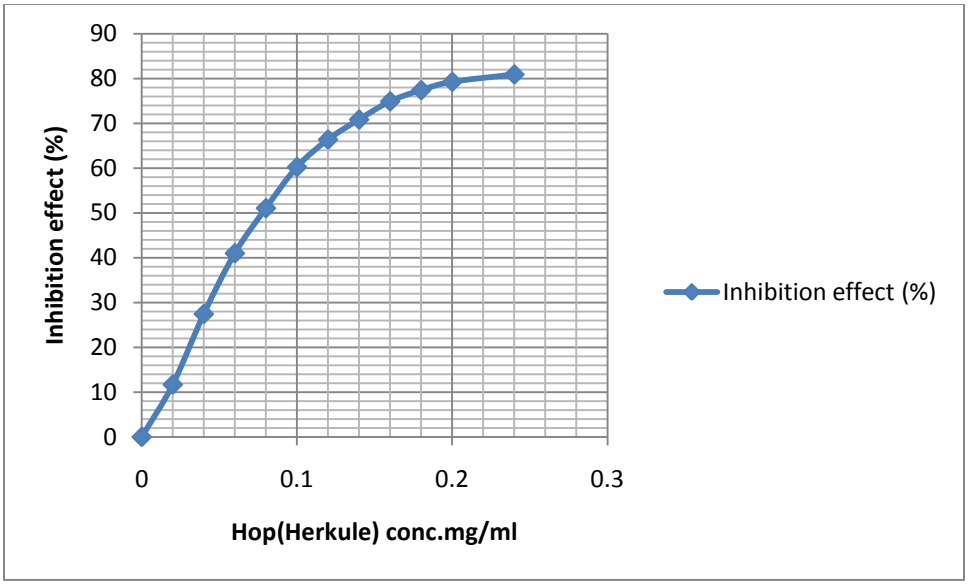



Figure 20: Scavenging effect of different concentration of hop (herkule) on DPPH free radical at 517 nm

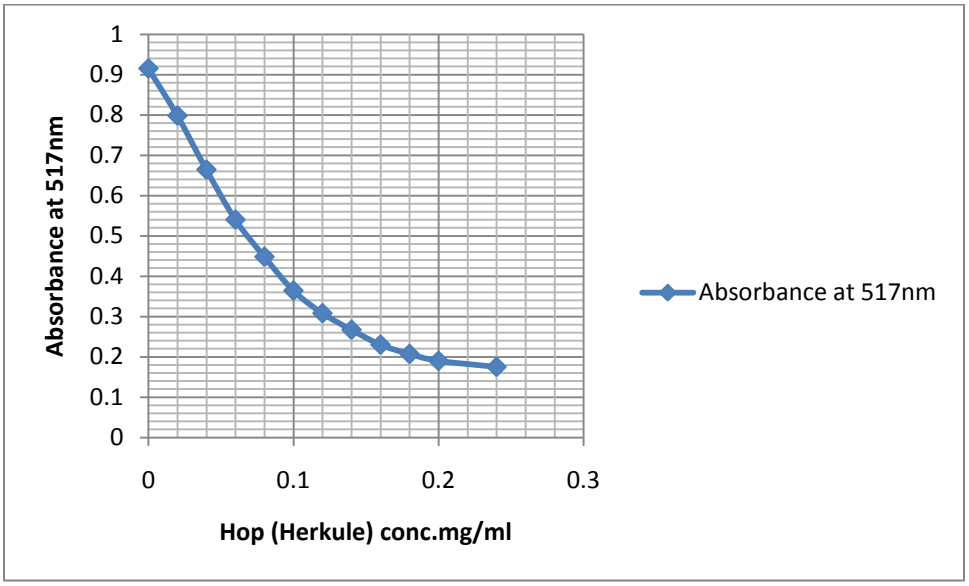



Figure 21: Effect of different concentration of hop (herkule) with DPPH free radical on absorbance at 517 nm

The above Table (Table 12) shows hop (herkule) is strong antioxidant having maximum inhibition effect of 80.87 at concentration of 24 mg/ml and the inhibition effect increase in the first phase until the maximum value of inhibition effect is achieved and decreased with further increase in concentration of herkule.

The nature of the graph (Figure21:) of inhibition effect versus the concentration of *herkule* (Rk) increases in the first phase until it reaches the maximum, then there is no change in effect with an increase in concentration of the herkule. In fig.21, the AO of the hop graph trend is similar to that of the standard sample of ascorbic acid (Figure 18 :) with varying inhibition degree. The effect of antioxidant on DPPH radical scavenging is due to their hydrogen donating ability. When a solution of DPPH is mixed with that of a substance, it can generate a hydrogen atom. This results in the reduced form of DPPH- H (non-radical) with change of the violet color to yellow when it reaches its maximum inhibition effect. DPPH scavenging activity is usually presented by $IC_{50}$ value, defined as the concentration of the antioxidant needed to scavenge 50% of DPPH present in the test solution. Therefore, extract concentrations

providing 50% inhibition ($IC_{50}$) were calculated using the data plotted in Fig 18….and…. Lower $IC_{50}$value reflects better DPPH radical-scavenging activity and has strong antioxidant potential.

This study compared the free radical scavenging activity of imported hops (Herkule) against the standard ascorbic acid. The scavenging of DPPH radical was determined by level of reduction in absorbance at 517 nm, which is dependent to concentrations (Fig18 and 20). Herkule and ascorbic acid showed a maximum radical scavenging as 80.87% and 93.77% at concentrations of 24mg/ml and 0.160mg/ml, respectively. Inhibitory concentration ($IC_{50}$) was calculated for herkule and ascorbic acid to be 5.2 and 0.2 mg/ml, respectively. This was due to the fact that Hops added during the later stage of brewing are the chief source of AO compounds such as Polyphenols, chalcones, flavonoids, humulones, and lupulones. Different reports indicate that the activity of the beer is mainly due to the presence of Polyphenols, chalcones,flavonoids, catechins, proanthocyanidins and bitter compounds e.g., humulones, lupulones, etc. (Wolfhart,1993; Stevens et al., 1998). Besides AO, hops in beer also possess sedative, chemo preventive, drug modulating and phytoestrogenic properties (Hopfenzapfen, 2000; Henderson et al., 2000; Miranda et al., 2000b).

Table 11: Inhibition effect of Rhamnus prinoides extracted on DPPH assay

| RPE conc. (mg/ml) | Absorbance at 517nm | Inhibition effect (%) |
|---|---|---|
| 0.00 | 0.891±0.011 | 000±0.00 |
| 0.02 | 0.792±0.006 | 11.110±0.673 |
| 0.04 | 0.697±0.004 | 21.770±0.449 |
| 0.06 | 0.631±0.005 | 29.181±0.561 |
| 0.08 | 0.558±0.003 | 37.374±0.337 |
| 0.10 | 0.494±0.004 | 44.557±0.449 |
| 0.12 | 0.444±0.005 | 50.168±0.561 |
| 0.14 | 0.373±0.000 | 58.137±0.000 |
| 0.16 | 0.320±0.002 | 64.085±0.224 |
| 0.18 | 0.270±0.006 | 69.697±0.673 |
| 0.20 | 0.243±0.004 | 72.727±0.449 |
| 0.24 | 0.165±0.005 | 81.148±0.561 |

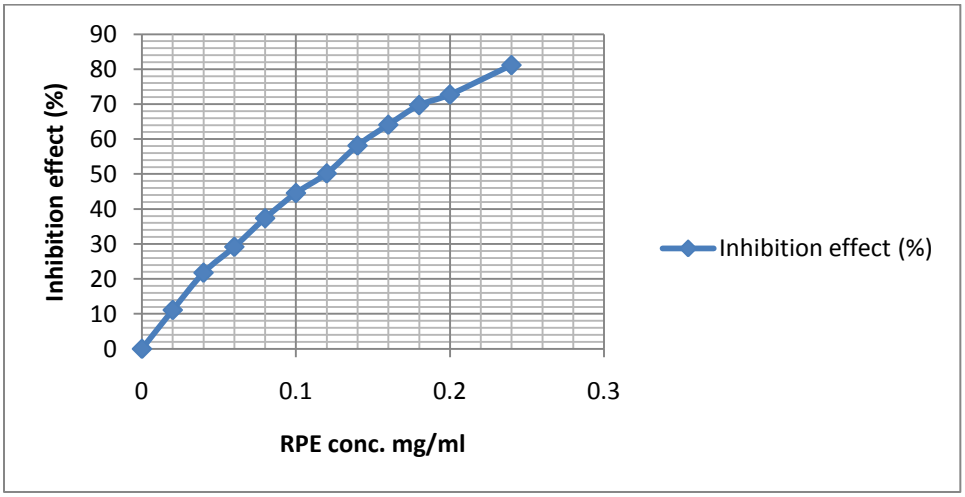



Figure 22: scavenging effect of different concentration of hop (herkule) on DPPH free radical at 517 nm

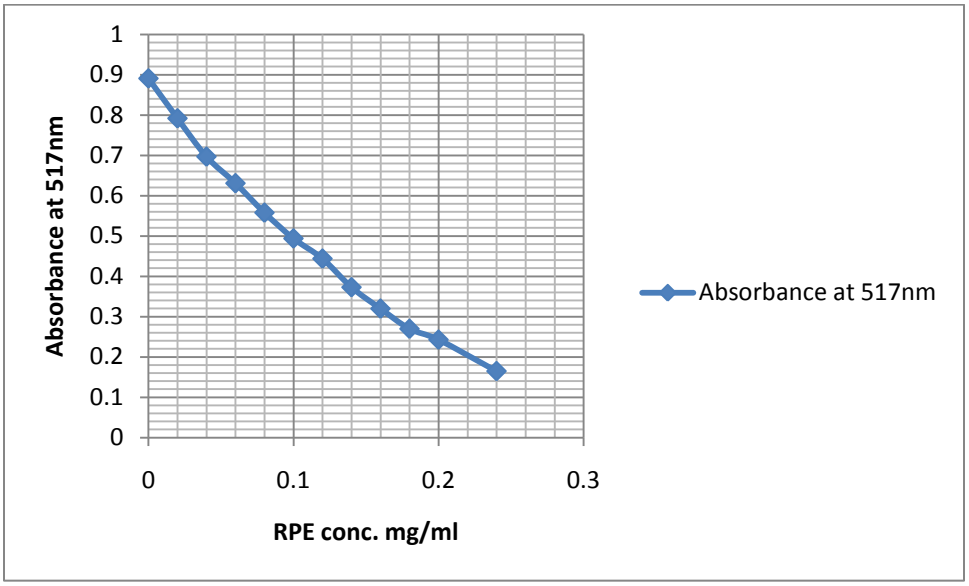



Figure 23: Effect of different concentration of *Rhamnus prinoides* extracted with DPPH free radical on absorbance at 517 nm

The above Table 23 shows *Rhamnus prinoides* extract is strong antioxidant having maximum inhibition effect of 81.148 at concentration of 24 mg/ml and the inhibition effect increase in the first phase until the maximum value of inhibition effect is achieved and decreased with further increase in concentration of *Rhamnus prinoides* extracted. The trend of the graph in fig.23 is similar to that of the standard ascorbic acid (Fig.18) with varying degree of inhibition and comparable to that of the imported hop acivity.

This study evaluated the free radical scavenging activity of *Rhamnus prinoides* extracted and ascorbic acid. The scavenging activity of DPPH radical was determined by level of reduction in absorbance at 517 nm, which is dependent to concentrations (Figure 23 and….). Rhamnus prinoides extracted and ascorbic acid showed a maximum radical scavenging as 81.148 % and 93.77 % at concentrations of 24mg/ml and 0.160 mg/ml, respectively. Inhibitory concentration ($IC_{50}$) was calculated for *Rhamnus prinoides* extract and ascorbic acid to be 5.2 and 0.24 mg/ml, respectively. This was due to the fact that *Rhamnus prinoides* extract contains antioxidant compound such as polyphenol, Geshodin, etc (Abegaz and Kebede 1995).

Ascorbic acid, Herkule and Gesho are considered to be three of the most oxdatively stable AO in beer. Considering that the antioxidant activity of the *Rhamnus prinoides* ethanolic extract is mainly due to its polyphenol, humulone, cohumulone, etc.

Table 12: Inhibition effect of *Rhamnus prinoides* residual on DPPH assay

| RPR conc. (mg/ml) | Absorbance at 517nm | Inhibition effect (%) |
|---|---|---|
| 0.00 | 0.936±0.000 | 0.000±0.00 |
| 0.02 | 0.834±0.002 | 10.897±0.214 |
| 0.04 | 0.722±0.008 | 22.86±0.855 |
| 0.06 | 0.638±0.000 | 31.838±0.000 |
| 0.08 | 0.573±0.003 | 38.782±0.321 |
| 0.10 | 0.498±0.017 | 46.795±1.816 |
| 0.12 | 0.423±0.012 | 54.808±1.282 |
| 0.14 | 0.356±0.015 | 61.966±3.788 |
| 0.16 | 0.300±0.004 | 67.949±0.427 |
| 0.18 | 0.245±0.001 | 73.825±0.106 |
| 0.20 | 0.199±0.003 | 78.739±0.321 |
| 0.24 | 0.136±0.006 | 85.470±0.641 |

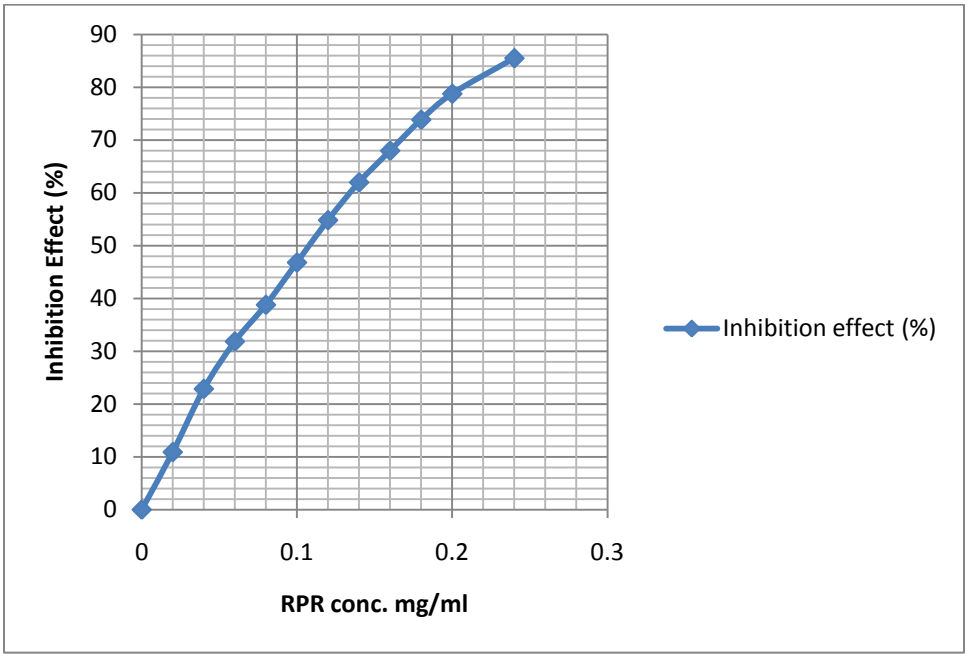



Figure 24: Scavenging effect of different concentrations of RPR on DPPH free radical at 517 nm

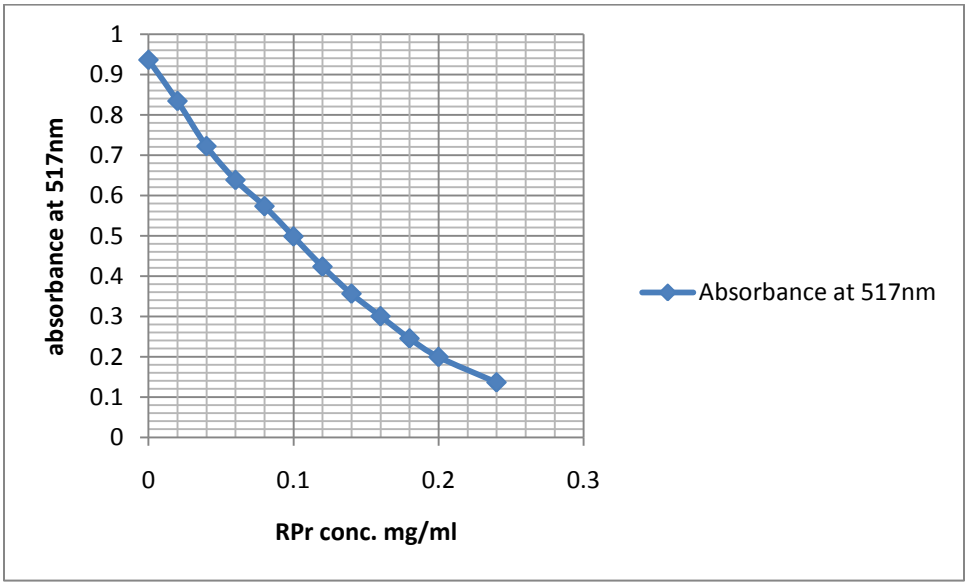



Figure 25: Effect of different concentration of RPR with DPPH free radical on absorbance at 517 nm

The above Table 13 shows *Rhamnus priniodes* residual has a strong antioxidant activity having maximum inhibition effect of 83.05 at concentration of 0.18 mg/ml and the inhibition effect increase with further increase in concentration of *Rhamnus priniodes* residual.

The nature of the graph (Figure 24) of inhibition effect versus the concentration of Rhamnus *priniodes* residual (RPR) increases with increase in concentration of the RPR having a linear functionality. The graph trend in fig.24 is unlike that of the standard ascorbic acid graph trend in fig18 where the ascorbic acid has two regions: an increasing in inhibition till it reaches 93.77% and an asymptotic region to the maximum inhibition with further increase in concentration. .

This study evaluated the free radical scavenging activity of Rhamnus priniodes residual and ascorbic acid. The scavenging of DPPH radical was determined by level of reduction in absorbance at 517 nm, which is dependent to concentrations (Fig. 23and.24) Rhamnus priniodes residual and ascorbic acid showed a maximum radical scavenging as 85.47 and 93.77 % at concentrations of 0.24 and 0.16 mg/ml, respectively. Inhibitory concentration ($IC_{50}$) was calculated for Rhamnus priniodes residual and ascorbic acid to be 5.2 and 0.24 mg/ml, respectively.

**4.2. Raw materials (malt) analysis**

Table 13: Malt analysis of local and imported (BGI Ethiopia plc lab.)

| | **Local malt assela analysis** | | | | **Imported malt analysis** | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Properties | Fine extract: EBC 4.5.1 | | Course extract: EBC 4.5.2 | | Fine extract: EBC 4.5.1 | | Course extract: EBC 4.5.2 | | standard |
| Density(g/cm) | 1.03320 | 1.03318 | 1.03109 | 1.03307 | 1.03351 | 1.03354 | 1.03112 | 1.0310 | - |
| Specific gravity | 1.0351 | 1.0350 | 1.0330 | 1.0349 | 1.03540 | 1.03543 | 1.0331 | 1.0333 | - |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Extract %humid | 78.3 | 77.4 | 73.6 | 77.3 | 78.4 | 78.9 | 78.3 | 77.4 | 77-83 |
| Extract %dry | 82.0 | 81.8 | 77.10 | 81.0 | 82.3 | 81.8 | 82.0 | 81.8 | 77-83 |
| Time of Filtration (hr) | 1.14 | 1.17 | 1.00 | 1.08 | 1.05 | 1.12 | 1.03 | 1.04 | - |
| Moisture content(%):EBC 4.2 method | 4.4 | 4.5 | 4.2 | 4.2 | 4.5 | 4.2 | 4.4 | 4.1 | 4-5.5 |
| Colour:EBC4.5 7 method | 4.0 | 4.0 | 3.9 | 4.1 | 4.2 | 4.0 | 3.8 | 3.9 | 2.5-4.0 |
| PH:EBC 8.17 method | 5.8 | 5.8 | 6.02 | 6.04 | 5.74 | 5.72 | 5.84 | 5.88 | 5.8-6.2 |
| Wort viscosity (mpas):EBC 4.8 method | 1.248 | 1.257 | 1.306 | 1.285 | 1.251 | 1.255 | 1.294 | 1.330 | 1.45-1.60 |
| Protein | 10.2 | 10.2 | 10.2 | 10.2 | 10.6 | 10.6 | 10.6 | 10.6 | |

Table 14 indicates that the malt analysis of local and imported were nearly the same results or properties but the filtration rate of course extract is more better than that of fine extract which implies that course powdered malt is more advisable than fine powder for beer production due that filtration is very important in the beer production.

### 4.3. Effects of boiling conditions on *Rhamnus priniodes* and imported hop for *beer bitterness*

The effect of boiling time, boiling temperature and PH on beer bitter taste value was studied and evaluated for good value of beer bitterness for production of beer in the pilot plant scale. The experimental values of beer bitterness at different boiling conditions are presented in table 16. These results were input into the Design Expert® software version 7.0.0 for further analysis and the statistical analysis of the boiling conditions is discussed in the following section.

Table 14: Experimental results for 100% local hops (*Rhamnus priniodes)*

| Std | Run | Block | Factor1 A:temp [ $^0$C] | Factor2 B:PH [PH] | Factor3 C:time [min] | Response1 beer bitterness [Bu] |
|---|---|---|---|---|---|---|
| 2 | 1 | Block1 | 90 | 5.2 | 45 | 18.997±0.026 |
| 3 | 2 | Block1 | 80 | 5.4 | 45 | 16.85±0.130 |
| 5 | 3 | Block1 | 80 | 5.2 | 90 | 17.73± 0.095 |
| 6 | 4 | Block1 | 90 | 5.2 | 90 | 18.36±0.060 |
| 7 | 5 | Block1 | 80 | 5.4 | 90 | 16.85±0.130 |
| 4 | 6 | Block1 | 90 | 5.4 | 45 | 18.91±030 |
| 8 | 7 | Block1 | 90 | 5.4 | 90 | 17.93±1.60 |
| 1 | 8 | Block1 | 80 | 5.2 | 45 | 18.45±0.453 |

The resulting data(Table 15) were analyzed using Design expert® 7 software to determine the effects of boiling PH, boiling temperature and boiling time. All experiments were carried out in a randomized order to minimize the effect of unexpected variability in the observed response due to extraneous factors.

Analysis of variance (ANOVA) is employed to test the siginificance of the developed models. Appendice-$A_1$ shows the summary of the analysis of variance (ANOVA) of the one response i.e beer bitterness. The detail ANOVA for the one responses is given at Appendice-$A_1$.

Table 15: Experimental results for 100% imported hops (Herkule)

| Std | Run | Block | Factor1 A:temp [$^0$C] | Factor2 B:PH [PH] | Factor3 C:time [Min] | Response1 beer bitterness [Bu] |
|---|---|---|---|---|---|---|
| 2 | 1 | Block1 | 90 | 5.2 | 45 | 18.073±0.015 |
| 3 | 2 | Block1 | 80 | 5.4 | 45 | 18.31±0.040 |
| 5 | 3 | Block1 | 80 | 5.2 | 90 | 21.64±0.320 |
| 6 | 4 | Block1 | 90 | 5.2 | 90 | 20.89±0.315 |
| 7 | 5 | Block1 | 80 | 5.4 | 90 | 19.19±0.120 |
| 4 | 6 | Block1 | 90 | 5.4 | 45 | 19.82±0.210 |
| 8 | 7 | Block1 | 90 | 5.4 | 90 | 21.32±0.435 |
| 1 | 8 | Block1 | 80 | 5.2 | 45 | 21.62±0.180 |

The resulting data( Table 16); were analyzed using Design expert® 7 software to determine the effects of boiling PH, boiling temperature and boiling time. All experiments were carried out in a randomized order to minimize the effect of unexpected variability in the observed response due to extraneous factors.

Analysis of variance (ANOVA) is employed to test the siginificance of the developed models. Appendices-$A_2$ shows the summary of the analysis of variance (ANOVA) of the one response i.e Beer bitterness. The detail ANOVA for the one responses is given at Appendices-$A_2$.

Table 16: Experimental results of 50% *Rhamnus Priniodes*

| Std | Run | Block | Factor1 A:temp [ $^0$C] | Factor2 B:PH [PH] | Factor3 C:time [Min] | Response1 beer bitterness [Bu] |
|---|---|---|---|---|---|---|
| 2 | 1 | Block1 | 90 | 5.2 | 45 | 22.57±0.105 |
| 3 | 2 | Block1 | 80 | 5.4 | 45 | 24.02±0.295 |
| 5 | 3 | Block1 | 80 | 5.2 | 90 | 22.08±0.325 |
| 6 | 4 | Block1 | 90 | 5.2 | 90 | 21.82±0.550 |
| 7 | 5 | Block1 | 80 | 5.4 | 90 | 22.55±0.175 |
| 4 | 6 | Block1 | 90 | 5.4 | 45 | 23.61±1.410 |
| 8 | 7 | Block1 | 90 | 5.4 | 90 | 22.01±0.415 |
| 1 | 8 | Block1 | 80 | 5.2 | 45 | 23.42±0.393 |

The resulting data( Table 17) were analyzed using Design expert® 7 software to determine the effects of boiling PH, boiling temperature and boiling time. All experiments were carried out in a randomized order to minimize the effect of unexpected variability in the observed response due to extraneous factors.

Table 17: Design summary

| | |
|---|---|
| Study Type | Factorial |
| Initial Design | Full Factorial |
| Center Points | 0 |
| Design Model | 2FI |
| Runs | 8 |
| Blocks | No Blocks |

| **Factor** | **Name** | **Units** | **Type** | **Low Actual** | **High Actual** | |
|---|---|---|---|---|---|---|
| A | Temp | Oc | Categoric | 80 | 90 | Levels: 2 |
| B | Time | min | Categoric | 45 | 90 | Levels: 2 |
| C | PH | PH | Categoric | 5.2 | 5.4 | Levels: 2 |

| **Response** | **Name** | **Units** | **Obs** | **Analysis** | **Minimum** | **Maximum** | **Mean** | **Std. Dev.** | **Ratio** |
|---|---|---|---|---|---|---|---|---|---|
| Y1 | Beer bitterness | Bu | 8 | Factorial | 21.82 | 24.02 | 22.76 | 0.77 | 1.10 |

### 4.3.1. Model adequacy check

The adequacy of the model was checked by analysis of variance (ANOVA) and some diagnostic plots. The model was found to be highly significant with the correlation coefficients of determination of R-squared, adjusted R-squared and predicted R-squared having a value of 0.9547, 0.9207and 0.8188 respectively. The quality of the model developed could be evaluated from their coefficients of correlation. The value of R-squared for the developed correlation is 0.9547. It implies that 95.47% of the total variation in the degree of bitterness is attributed to experimental variables studied. The adequacy of the model was further checked with analysis of variance (ANOVA) as shown in table5.4. Based on the 95% confidence level, f-value is a test for comparing model variance with residual (error) variance. If the variance close to the same, the reaction will be close to one and It is likely that any of the factors have the significant effect on the response with the p-value less than 0.05. It is calculated by model mean square divided by residual mean square; here the model f-value of 4.52 implies the model is significant. There is only 0.01% chance that a 'model F-value' this large occur due to personal error or disturbance.

Analysis of variance (ANOVA) is employed to test the siginificance of the developed models. Table 19 shows the summary of the analysis of variance (ANOVA) of the one response i.e Beer bitterness.

Table 18: Analysis of variance (ANOVA)

| Source | Sum of Squares | Df | Mean Squares | F-value | P-value | |
|---|---|---|---|---|---|---|
| Model | 4.52 | 3 | 1.51 | 28.10 | 0.0038 | Significant |
| A | 0.53 | 1 | 0.53 | 9.89 | 0.0347 | |
| B | 3.33 | 1 | 3.33 | 60.62 | 0.0014 | |
| C | o.66 | 1 | 0.66 | 12.33 | 0.0246 | |
| Residual | 0.21 | 4 | 0.054 | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Cor Total | 4.73 | 7 | | | | |

The Model F-value of 28.10 implies the model is significant. There is only a 0.38% chance that a "Model F-Value" this large could occur due to noise.

The values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, C are significant model terms. The Values greater than 0.1000 indicate the model terms are not significant.If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

| | | | |
|---|---|---|---|
| Std. Dev. | 0.23 | R-Squared | 0.9547 |
| Mean | 22.76 | Adj R-Squared | 0.9207 |
| C.V. % | 1.02 | Pred R-Squared | 0.8188 |
| PRESS | 0.86 | Adeq Precision | 14.535 |

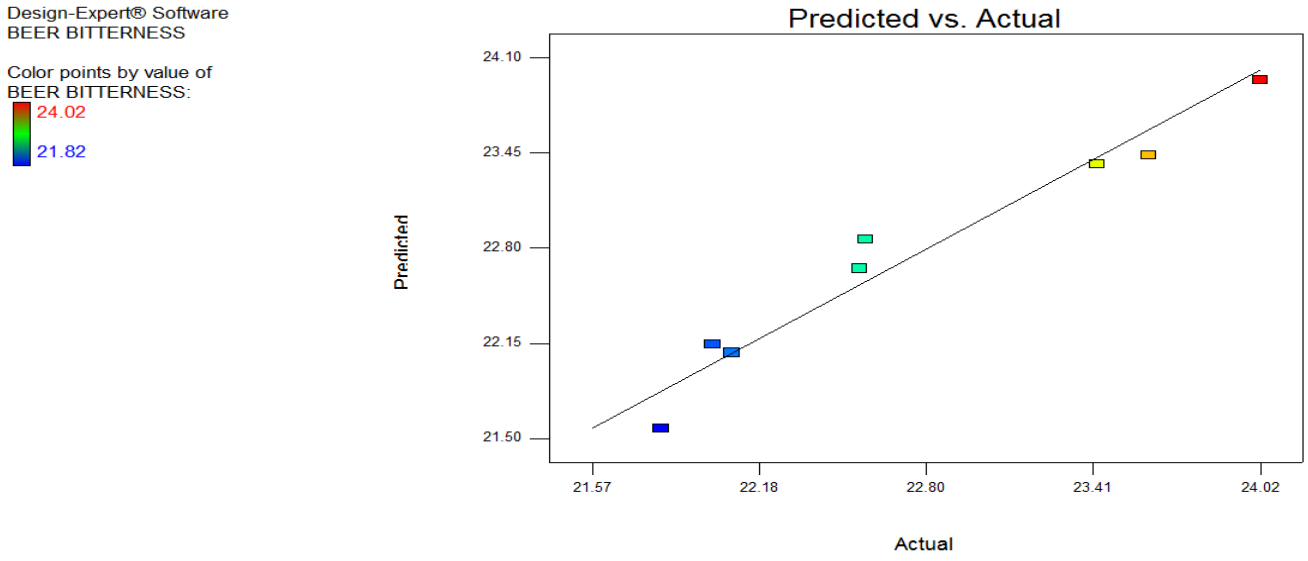
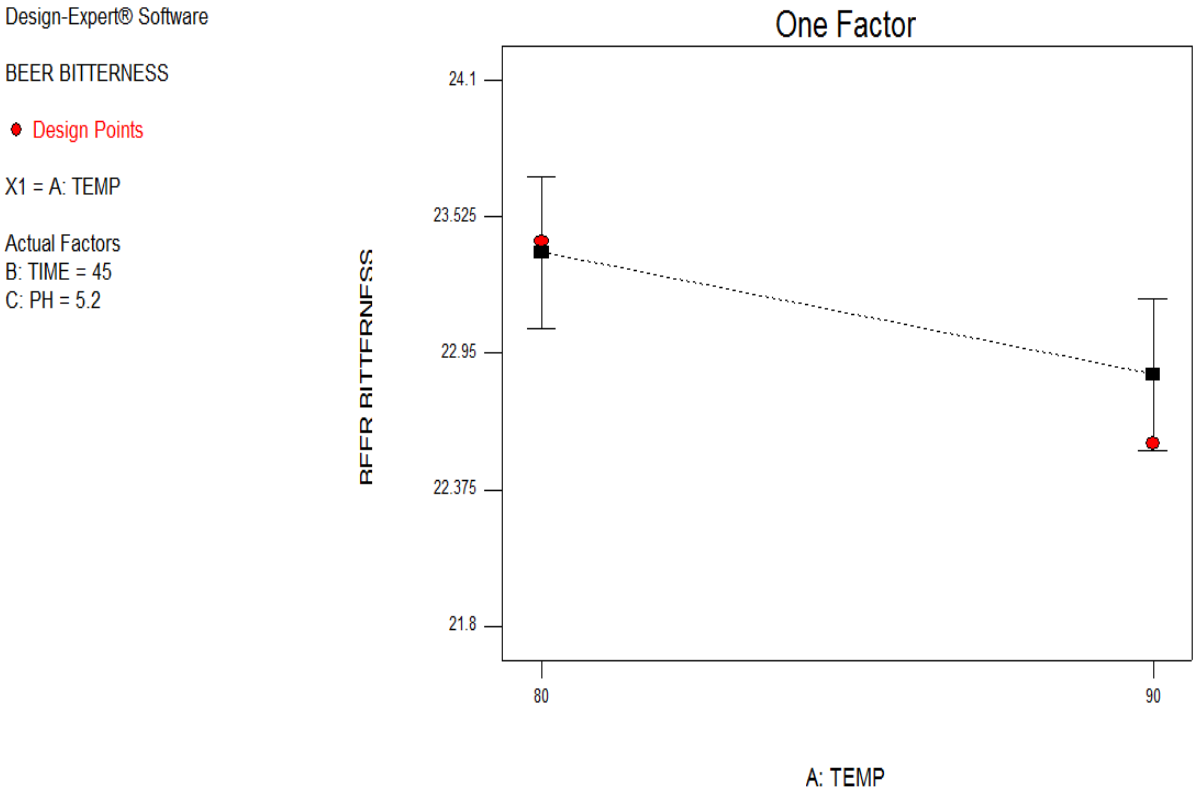



Figure 26: predicted versus actual degree of beer bitterness

The graph of predicted value obtained using the developed correlation versus actual values is shown in the figure (26). The results in the figure4.2.1 demonstrated that the regression model equation provided a very accurate description of the experimental data, in which all the points are very close to the line of perfect fit. This result indicates that it was successful in the capturing the correlation between the three wort boiling process variables to the design of Beer bitterness.



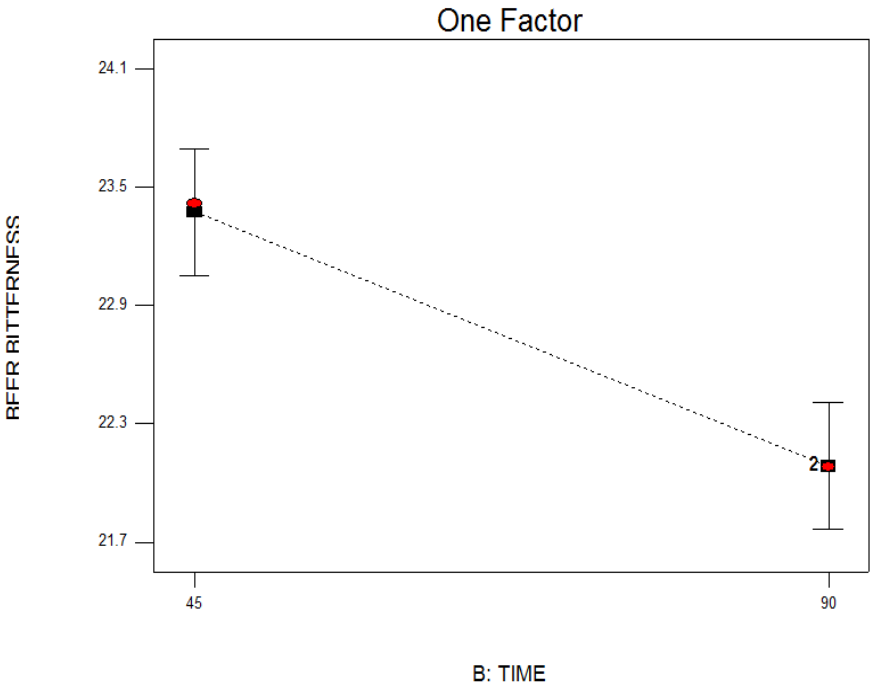
Design-Expert® Software

BEER BITTERNESS

Design Points

X1 = B: TIME

Actual Factors
A: TEMP = 80
C: PH = 5.2



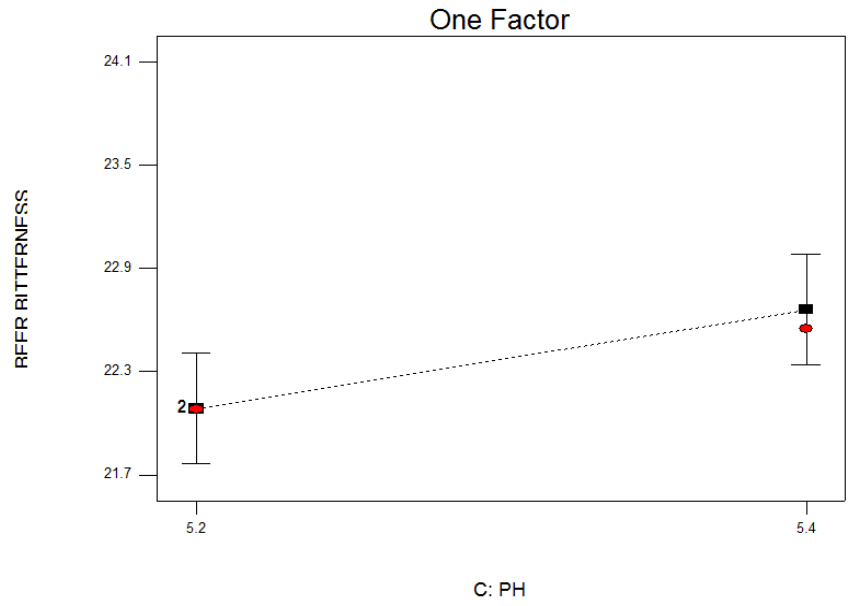

Design-Expert® Software

BEER BITTERNESS

Design Points

X1 = C: PH

Actual Factors
A: TEMP = 80
B: TIME = 90



Design-Expert® Software

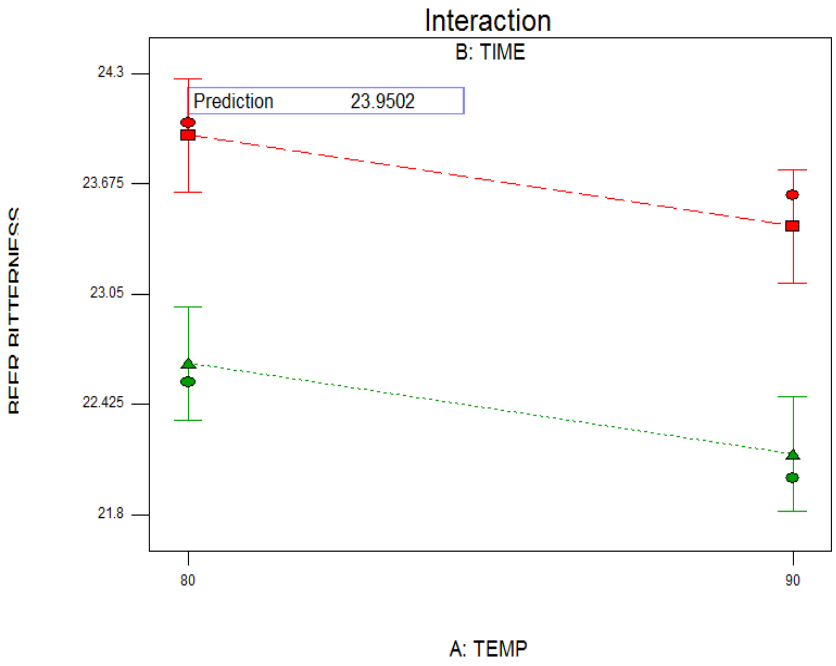
BEER BITTERNESS

Design Points

B1 45
B2 90

X1 = A: TEMP
X2 = B: TIME

Actual Factor
C: PH = 5.4



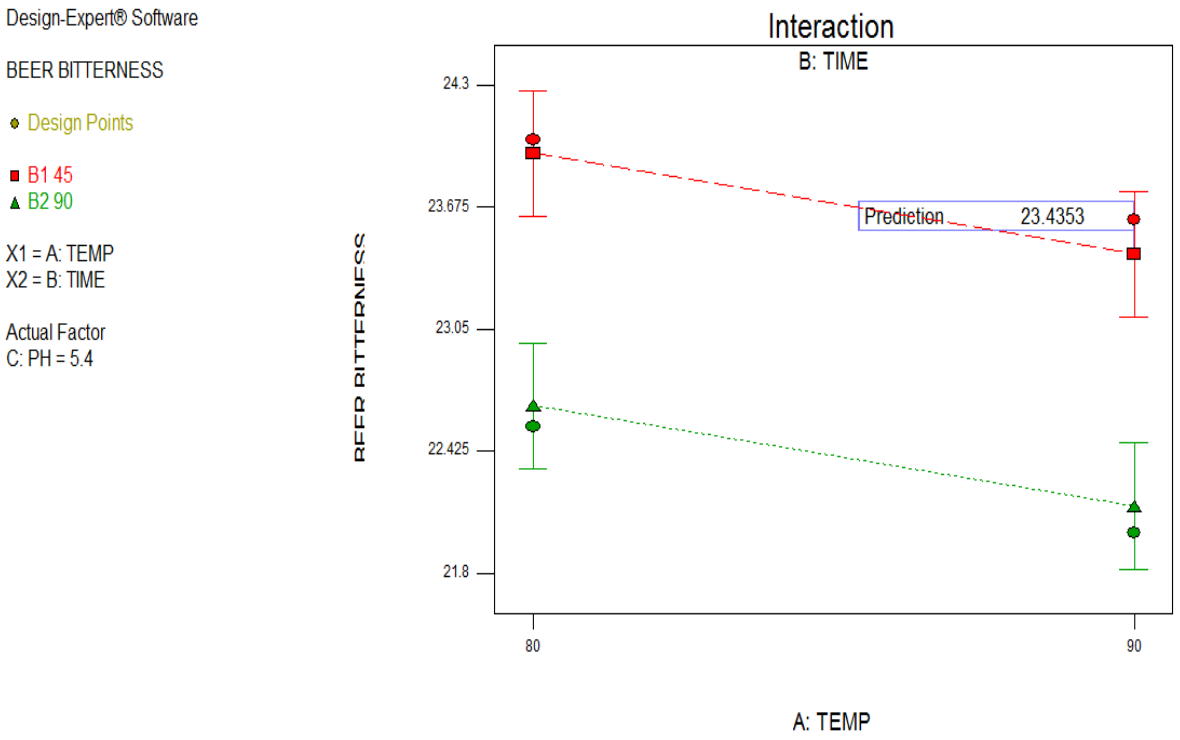



Table 19: Experimental results for75:25 local hops (Rhamnus priniodes) to imported hop (Herkule).

| Std | Run | Block | Factor1<br>A:temp<br>[ $^0$C] | Factor2<br>B:PH<br>[PH] | Factor3<br>C:time<br>[min] | Response1<br>beer bitterness<br>[Bu] |
|---|---|---|---|---|---|---|
| 2 | 1 | Block1 | 90 | 5.2 | 45 | 22.07±0.075 |
| 3 | 2 | Block1 | 80 | 5.4 | 45 | 21.68±0.315 |
| 5 | 3 | Block1 | 80 | 5.2 | 90 | 21.80±0.115 |
| 6 | 4 | Block1 | 90 | 5.2 | 90 | 21.58±0.250 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 7 | 5 | Block1 | 80 | 5.4 | 90 | 22.39±0.670 |
| 4 | 6 | Block1 | 90 | 5.4 | 45 | 21.69±0.165 |
| 8 | 7 | Block1 | 90 | 5.4 | 90 | 21.56±0.235 |
| 1 | 8 | Block1 | 80 | 5.2 | 45 | 21.87±0.090 |

The resulting data(Table 21) were analyzed using Design expert® 7 software to determine the effects of boiling PH, boiling temperature and boiling time. All experiments were carried out in a randomized order to minimize the effect of unexpected variability in the observed response due to extraneous factors.

Analysis of variance (ANOVA) is employed to test the siginificance of the developed models. Appendix-$A_3$ shows the summary of the analysis of variance (ANOVA) of the one response i.e Beer bitterness. The detail ANOVA for the one responses is given at Appendix $A_3$.

Table 20: Experimental results for 25:75 local hops *(Rhamnus priniodes*) to imported hops (*Herkule*)

| Std | Run | Block | Factor1 A:temp [ $^0$C | Factor2 B:PH [PH] | Factor3 C:time [Min] | Response1 beer bitterness [Bu] |
|---|---|---|---|---|---|---|
| 2 | 1 | Block1 | 90 | 5.2 | 45 | 22.48±0.485 |
| 3 | 2 | Block1 | 80 | 5.4 | 45 | 24.47±0.515 |
| 5 | 3 | Block1 | 80 | 5.2 | 90 | 24.42±0.895 |
| 6 | 4 | Block1 | 90 | 5.2 | 90 | 21.83±0.715 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 7 | 5 | Block1 | 80 | 5.4 | 90 | 23.85±0.235 |
| 4 | 6 | Block1 | 90 | 5.4 | 45 | 23.46±0.265 |
| 8 | 7 | Block1 | 90 | 5.4 | 90 | 23.85±0.250 |
| 1 | 8 | Block1 | 80 | 5.2 | 45 | 24.19±0.504 |

The resulting data( Table 22) were analyzed using Design expert® 7 software to determine the effects of boiling pH, boiling temperature and boiling time. All experiments were carried out in a randomized order to minimize the effect of unexpected variability in the observed response due to extraneous factors.

Analysis of variance (ANOVA) is employed to test the siginificance of the developed models. Appendices-$A_4$ shows the summary of the analysis of variance (ANOVA) of the one response i.e Beer bitterness. The detail ANOVA for the one responses is given at Appendices-$A_4$.

**4.4. Sensory quality evalution results**

Table: Acceptability score on colour, bitter taste, estery aroma and foam stability

| sample code | colour | Bitter taste | Estery aroma | Foam stability |
|---|---|---|---|---|
| 150 | 1.2 | 3.2 | 2.4 | 3.4 |
| 200 | 4.0 | 4.0 | 3.8 | 3.8 |
| 250 | 2.6 | 3.2 | 2.8 | 3.6 |
| 300 | 3.4 | 3.3 | 3.0 | 3.6 |
| 235 | 3.8 | 3.6 | 3.6 | 3.8 |

The overall acceptability tasting gave the results shown in the figure below:

Table 23: Overall acceptability taste score

| sample code | acceptability taste |
|---|---|
| 150 | 2.55 |
| 200 | 3.9 |
| 250 | 3.05 |
| 300 | 3.33 |
| 235 | 3.7 |

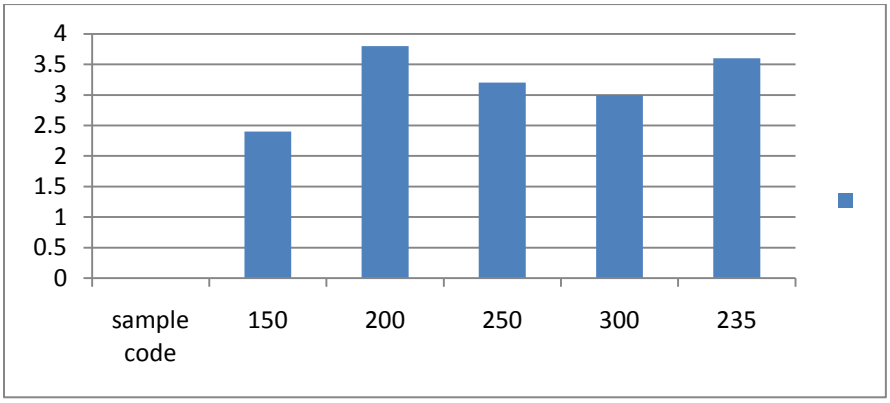



Fig:Acceptability taste score

KEY:- Sample code

150 -100% Gesho beer

200 -100% imported hop beer (herkule)

250 -75% Gesho beer

300-50% Gesho beer

235-25% Gesho beer

The above results show that the preferred samples, ranging from the most preferred to the least prepared were in the order: 200, 235,300,250 and 100. This judgment may be arrived at based on the combine flavour attributes of colour ,bitterness, foam stability, and estery exhibited by each sample from table below buttressed with ANOVA results, the foam stability and bitter taste flavour attributes were not significantly different ($p>0.05$) between samples.

However, ANOVA results shown a significant difference in color and estery flavor attributes between the samples ($P<0.05$). These two flavor attributes invariably may the determining factors for the panel's rating in the acceptability taste. The 25% and 50% Gesho beer starch substituted beer was the best rated sample. The foam stability and bitter taste value profile of 25% and 50% Gesho beer substituted sample even suggested but in order to pronounce the right bitter taste of 50% Gesho beer was selected for final pilot plant scale beer production which could give beer of good quality from sensory perspective. From table…. below color tends to be the only flavor that puts sample table and a head on the others. The panelists' therefore judged and rated the sample with their colors as the most influencing flavor attribute. First impression counts. Most consumers drink with their eyes and the appearance is often more important than taste (O'Rourke, 2002)

### 4.5. Microbrewery pilot plant brewing process results

#### 4.5.1 Microbiological analysis of 50% *R.priniodes* beer

Table 24: Extent of detection in the number of colony (N/O**)**

| Micro organisms | Unmatured beer(48hrs) | Unpasteurized beer | Pasteurized beer | Standard |
|---|---|---|---|---|
| micro organisms | day2 | day4 | day6 | standard |
| wort bacteria | +++++++ | +++++ | - | 1/0.2ml |
| lactic bacteria | - | - | - | 1/0.2ml |
| Swy | - | - | - | 1/0.2ml |
| Arwy | - | - | - | 1/0.2ml |

Determination of dynamics of microorganisms in beer fermentation, the amount of bittering agents in comparison with commercial hops and antimicrobial activity of Gesho against bacteria are important to design aseptic condition for beer brewing process. In consideration with these significant points, this study was attributed using profound scientific methods. As the result, profile of microorganisms, quantity of bittering agents and antimicrobial activates of Rhamnus prinoides were determined in this section.

### 4.5.2. Finished beer analysis

**Table 25: Finished beer analysis results**

| **parameters** | **50%Gesho beer(*Rhamnus priniodes*)** | **Imported hop(*Herkule*)** | **St.George beer standard** |
|---|---|---|---|
| Alcohol(%v/v) | 4.87 | 4.55 | 4.71-4.78 |
| Alcohol(%w/w) | 3.86 | 3.56 | 3.69-3.75 |
| Extract content($^{o}$p) | 11.40 | 11.02 | 10.95-11.15 |
| Real extract(%w/w) | 3.98 | 4.08 | 3.75-3.85 |
| Apparent extract(%w/w) | 2.21 | 2.42 | 2.05-2.13 |
| ADOF (%) | 80.63 | 77.98 | 81.27-80.89 |
| Specific gravity | 1.0086 | 1.0095 | - |
| Bitterness(Bu) | 15.6 | 16.55 | 15-18 |
| Colour(EBC) | 7.95 | 8.26 | 8-10 |
| VDK(ppm) | 0.44 | 0.07 | <0.15 |
| Polyphenol(ppm) | 244.6 | 232.4 | <120 |
| Foam stability | 232 | 224 | >200 |

| | | | |
|---|---|---|---|
| CO2(ppm) | 4.5 | 5.65 | 5.60-6.0 |
| F.G(ppm) | 1.38 | 1.77 | <1.0 |
| PH | 4.48 | 4.73 | 4.05-4.35 |

Ethanol is major end product of beer. It forms part of end by products of glycolytic pathway of wort fermentation. Ethanol level is generally very high, as result of high gravity brewing. Analysis of result in the Table 25 shown that there was significant difference ($p<0.05$) in the ethanol levels between samples

pH values as shown in the Table 25 the general drop of pH as wort ferments. However, the pH trends changed as fermentation as progressed to the end yielding the final pH of 4.48 for Gesho beer and 4.73 for imported hop beer due to formation of organic in the fermentation process. During wort sugar metabolism, several organic acids, which do not only impart flavour but also give rise to pH drop in the beer. Lewis and young stated that the fall in the pH is the result of ammonium ion ions, potassium ion, and the amino acids by yeast and consequent release of hydrogen ions and secretion of organic acids**.**

Bitterness levels dropped during and at the end of fermentation. O'Rourke, 2000 mentions the following factors as culminating in the loss of bitterness during fermentation: excessive fobbing resulting in the beer loose, and hence, bitterness, CO2 evolution with the volatile hops components. Based on the analysis above finished beer mechanically bottled and packed as shown below:

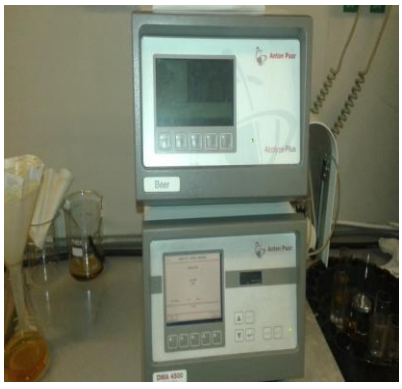


Figure 27: Anto paar alchololizer

Figure 28: Bottled beer

# Chapter Five

## 5. Conclusions and Recommendations

### 5.1. Conclusion

From the study realized that utilization of bitter effects derived from Rhamnus priniodes were tested. Furthermore; bitter taste and antioxidant activities are suitable for application in brewing. From the study the investigation have proved that antioxidant activity value, bitter taste, foam stability and antibacterial properties of Rhamnus priniodes fall behind that of regular imported hop, then Rhamnus priniodes and imported hops to increase bitterness content of the beer, so that the 25% and 50% Rhamnus priniodes was screened for their suitability for beer production based on their bitter taste but in order to pronounce the bitter taste of Rhamnus priniodes in the beer production 50% of Rhamnus priniodes was selected. With selected processes variables of temperature= $80^{O}$C, time=45min and pH=5.4 due to its high bitter taste effect, beers were produced on the pilots scale. The beer was found to have a pH of 4.48, an alcohol content of 4.87%, colour of 7.95EBC and bitterness of the beer were 15.6Bu. Similarly, the commercial brand of beer (imported hop beer) was also tested for the above mentioned parameters which did not differ much with that of 50% Rhamnus priniodes beer. From the sensory quality evaluation, it was found that Rhamnus priniodes beer was comparable with that of commercial beer except differing slightly in colour which can be further improved.

From the study also realized that ethanolic extract of Gesho has a good antioxidant activity and its application in beer brewery with 1:1 proportion with imported bittering agent hop resulted a beer of good quality. Therefore, incorporating Gesho as a bittering agent gives an advantage to the breweries and the country as well an economic advantage by:

- Increasing profit margin of the brewery
- Reducing import of hop with
- Increasing agricultural inputs to industries
- Creating job opportunities

Thus, Rhamnus priniodes can be used for commercial beer production as an alternative to imported hop beer.

### 5.2 Recommendation

Ethiopia can benefit from the beer products produced by partial replacement of imported hops by Rhamnus priniodes (Gesho) which is cheaper plant source that allow more consumers with reduced cost but still valuable nutritional content, valuable beer quality like good bitter taste and aroma, colour which also provide expected flavours. From the result of the work carried out, I recommend the following:

- ✓ The 50% of *Rhamnus priniodes* having good bitter taste, antioxidant activity and foam stability content should be screened for good quality beer production so that it is very advisable for country like Ethiopia which do have availability of *Rhamnus priniodes* resources which was used for traditional brewing like Tella and Teji, and now I recommend it to use commercial beer production**.**
- ✓ The 50% Rhamnus priniodes beer should be used for brewing industries as bittering agent and pasteurized in order to discourage spoilage by microbes and to extend its shelf-life even better.

**Reference**


- Abegaz, B. and Kebede, T. 1995. Geshoidin: A bitter principle of Rhamnus prinoides and other constituents of the leaves. Bulletin of Chemical Society of Ethiopia 9: 107-114.
- Abegaz, B.M, Ngadjui, B.T, Bezabih, M. And Modee, L.K, 1999: Novel natural products from marketed plants of eastern and Southern Africa, pure applied chemistry 71(6):919-926.
- Abegaz, B.M., 1996: Bull. Chem. Soc. Ethiopia., 10, 57.
- Alemu, H.; Abegaz, B.M.; Bezabih, M., 2007: Bull. Chem. Soc. Ethiopia., 21, 189.
- Andersen, M.L., Outtrup, H. and Skibsted, L.H, 2000: Potential antioxidants in beer assessed by ESR spin trapping. J Agric Food Chem483, 106–11.
- Anonymous, 1996: Good news for beer drinkers. Science274, 1309–10.
- Arnold, J.P, 1911: Origin and History of Beer and Brewing, Alumni Association of the Wahl - Henius Institute of Fermentology, Chicago, IL.
- Ashenafi, M. 2006: A review on the microbiology of indigenous fermented foods and beverages of Ethiopia. Ethiopian Journal Biological Sciences 5(2): 189-245.
- B.E.Briggs, 1997: malts and malting, Chapman and Hall, Andover.
- Bamforth, C.W, 2006: Scientific principles of malting and brewing. American Society of Brewing Chemists. St. Paul, MN.
- Berhanu, A, 2013: Microbial profile of Tella and the role of Gesho (Rhamnus prinoides) as bittering and antimicrobial agent in traditional Tella(Beer) production, journal.
- Braidwood, R.J., Suaer, J.D, Helback, H., Mangelsdorf, P.C., Cuter, H.C., Coon, C.S., Linton, R., Steward, J., Oppenheim, A.L, 1953. Did man once live by beer alone? American Anthropologist. 55(4):515-526
- Dlamini, M.D.; Turner, S, 2002: Rhamnus prinoides L'Hérit, Witwatersrand National Botanical Garden: South African National Biodiversity Institute, South Africa.
- Ebneth. O.J. and Theuvesn, L, 2005: Globalization of the Brewing Industry: 5th International PENSA Conference Journal, Ribeirao Preto.
- Ermias D. and Berhanu, A, 2000: Modern and tradition brewing in Ethiopia; Addis Ababa; Ethiopia, CSE.

- Eßlinger, H.M 1995: Fermenters and storage tanks",Brauwelt international ,50-154
- Eßlinger, H. M. 2009: Handbook of Brewing: Processes, Technology, Markets; WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim ISBN: 978-3-527-31674-8
- FAO, 2009: Agribusiness handbook, Barley malt Beer; Viale delle Terme di Caracalla, 00153 Rome, Italy.
- Meussdoerffer, F. G. 2009: A Comprehensive History of Beer Brewing; WILEY-VCH Verlag GmbH & Co. KGaA, WeinheimISBN: 978-3-527-31674-8.
- Gerhauser, C., Alt, A., Heiss, E., Gamal-Eldeen, A., Klimo,K.,Kn
- Getahun, A. 1976: Some common medicinal and poisonous Plants used in Ethiopian folk medicine. Faculty of Science, Addis Ababa University, Addis Ababa, Ethiopia.
- H.Michael E., F.B.Aktiengesellschaft, Freiberg/Sachsen, 1999: Beer concepts, Federal Republic of Germany, Vol.4, p659-690.
- Halliwell, B. and Gutteridge, J.M.C. 1999: Free radicals in biology and medicine, Clarendon Press.
- Hayeshi, R.; Mukanganyama, S.; Hazra, B.; Abegaz, B.; Hasler, J. Phytother. Res. 2004, 18, 877.
- Henderson, M.C., Miranda, C.L., Stevens, J.F., Deinzer, M.L. and Buhler, D.R. 2000: In vitro inhibition of human P450 enzymes by prenylated flavonoids from hop, Humulus lupulus.Xenobiotica30, 235–51.
- Hopfenzapfen, Lupuli flos. 2000: In Europä isches Arzneibuch, Nachtrag 2000, Deutsche Ausgabe, Deutscher ApothekerVerlag, Stuttgart: Govi-Verlag - Pharmazeutischer VerlagGmbH Eschborn.
- http://www.tuntavern.com/pages/hisbeer.htm accessed on 2010/08/21
- Katz, S.H., and Maytag, F. 1991. Brewing an ancient beer. Archeology. 44(4):24- Kayodé, A.P.P., Hounhouigan, J.D., Nout, M.J.R. 2007. Impact of brewing process operations on phytate, phenolic compounds, and in vitro solubility of iron and zinc in opaque sorghumbeer. LWT. 40:834-841.
- Kimura, S., Hayatsu, H. and Arimoto-Kobayashi, S. 1999: Glycine betaine in beer as an antimutagenic substance against 2-chloro-4-methylthiobutanoic acid, the sanma-fish mutagen. Mutat Res439, 267–76.

- Kool, J. and, Roehm, K. 2005: Color atlas of Biochemistry. (2nd edition). Newyork: Thieme publishers.
- Krajl, D., J. Zupanec, D. Vasilj, S. Kralj & J. Pšeničnik, 1991: Variability of essential oils of hops, Humulus lupulusL. J Inst Br 97: 197–206
- Kristiansen, A. G, 2013: The Scandinavian School of Brewing.
- Kunze, W. 1996: Technology Brewing and Malting. 7th Edn. VLB Berlin, Germany.
- Kunze, W., 1999: Technology Brewing and Malting, 2nd ed, Verlag der Berlin.
- Kunze, W., 2004: Technology brewing and malting, 3rd completely updated edition, VLB Berlin, Germany.
- Kusche, M., G. Stettner, A. Stephan, W. Mitter and D. Kaltner, 2007: Influence of the new high alpha hop variety Herkules on beer quality. Proceedings of the European Brewery Convention Congress, Venice.
- Michael, M., 2004: Beer and Its Drinkers. An ancient near eastern Love Story. Near eastern archaeology. 67(2):84-95
- Miranda, C.L., Aponso, G.L., Stevens, J.F., Deinzer, M.L. and Buhler, D.R. 2000b: Prenylated chalcones and flavanones as inducers of quinine reductase in mouse Hepa 1c1c7 cells, Cancer Lett149, 21–29.
- Nindi, M.M., Kgarebe, B.V., Wolfender, J.L, Abegaz, B.M., 1999 Phytochem. Anal., 10, 69.
- O'Rourke, T., 2002:The role of pH in brewing." Brewer International 2(8): 21-23.
- Palmer, J.J., 2006: How to brew. Everything you need to know to brew beer right the first time. Brewers Publications. Boulder, CO.
- Papazain, C. 2003: The complete joy of home brewing. 3rd ed. Harper Collins. New York, New York.
- Pavlik, J.W. 2007: ARKIVOC, 9, 1
- Peacock, V.E. & P. McCarty, 1992: Varietal identification of hops and hop pellets. MBAA Techn Quart 29: 81–85
- Rice-Evans, C.A. ed. 2000: Wake up to flavonoids, Royal Society of Medicine International Congress and Symposium Series.RSM Press.
- S.donhauser, 1997: Characterization of yeast species and strains",Brauwelt international 52-58

- Shale, S. and Gashe, B.A., 1991: The microbiology of Tella fermentation. Sinet Ethiopia Journal of Sciences 14: 81-92.
- Singh, N., R. Sharma and A. K. Balapure, 2007: pH regulated scavenging activity of beer antioxidants through modified DPPH assay." Toxicology and industrial health 23(2): 75-81.
- Stampfer, Meir.J, 2005: Consumption of Trans fatty acids is related to plasma biomarkers of inflammation and endothelial dysfunction; Journal of nutrition.
- Stevens, J.F., Miranda, C.L., Buhler, D.R. and Deinzer, M.L.Chemistry and biology of hop flavonoids. J Am Soc BrewChem56, 136–45.
- Tessema, Alemayehu Demissie, 1994: PhD dissertation, Moscow state Academy of Food products; Moscow.
- Thulin, M., 1988: In the flora of Ethiopia, vol.3 Hedberg,I;Edwards s..eds; Addis ababa and Asmara,Ethiopia,and Uppsala, Sweden.
- Van Staden, L.F. and Drewes, S.E., 1994: Knipholone from Bulbine latifolia and Bulbine frutescens. Phytochemistry 35: 685–686
- Weber, D.G., Sahm, K., Polen, T., Wendisch, V.F. and Antranikian, G., 2008: Oligonucleotide microarrays for the detection and identification of viable beer spoilage bacteria. Journal of Applied Microbiology 105: 951–962
- Wohlfart, R. Humulus. In Hänsel, R. Keller, K. Rimpler, H. and Schneider, G. eds. Hagers Handbuch der Pharmazeutischen Praxis (volume 5). Springer, 47–458.
- www.statista.com/statistics /270275/worldwide beer production/

**Appendices-A**

**Experimental results**

**Appendices 1**: Experimental results and ANOVA for 100% local hops (*Rhamnus priniodes)*

| std | Run | Block | Factor1 A:temp [ $^0$C] | Factor2 B:PH [PH] | Factor3 C:time [min] | Response1 beer bitterness [Bu] |
|---|---|---|---|---|---|---|
| 2 | 1 | Block1 | 90 | 5.2 | 45 | 18.997±0.026 |
| 3 | 2 | Block1 | 80 | 5.4 | 45 | 16.85±0.130 |
| 5 | 3 | Block1 | 80 | 5.2 | 90 | 17.73± 0.095 |
| 6 | 4 | Block1 | 90 | 5.2 | 90 | 18.36±0.060 |
| 7 | 5 | Block1 | 80 | 5.4 | 90 | 16.85±0.130 |
| 4 | 6 | Block1 | 90 | 5.4 | 45 | 18.91±030 |
| 8 | 7 | Block1 | 90 | 5.4 | 90 | 17.93±1.60 |
| 1 | 8 | Block1 | 80 | 5.2 | 45 | 18.45±0.453 |

**Response 1**: **Beer bitterness**

**ANOVA for selected factorial model**

**Analysis of variance table [Partial sum of squares - Type III]**

| Source | Sum of Squares | df | Mean Square | F Value | p-value Prob > F | |
|---|---|---|---|---|---|---|
| Model | 4.14 | 3 | 1.38 | 7.46 | 0.0408 | significant |
| *A-Tem* | *2.33* | *1* | *2.33* | *12.61* | *0.0238* | |
| *B-Time* | *0.68* | *1* | *0.68* | *3.70* | *0.1268* | |
| *C-PH* | *1.12* | *1* | *1.12* | *6.08* | *0.0692* | |
| Residual | 0.74 | 4 | 0.18 | | | |
| Cor Total | 4.88 | 7 | | | | |

The Model F-value of 7.46 implies the model is significant. There is only a 4.08% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

| | | | |
|---|---|---|---|
| Std. Dev. | 0.43 | R-Squared | 0.8484 |
| Mean | 18.01 | Adj R-Squared | 0.7348 |
| C.V. % | 2.39 | Pred R-Squared | 0.3938 |
| PRESS | 2.96 | Adeq Precision | 7.941 |

The "Pred R-Squared" of 0.3938 is not as close to the "Adj R-Squared" of 0.7348 as one might normally expect. This may indicate a large block effect or a possible problem with your model and/or data. Things to consider are model reduction, response tranformation, outliers, etc. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 7.941 indicates an adequate signal. This model can be used to navigate the design space.

| Factor | Coefficient Estimate | df | Standard Error | 95% CI Low | 95% CI High | VIF |
|---|---|---|---|---|---|---|
| Intercept | 18.01 | 1 | 0.15 | 17.59 | 18.43 | |
| A-Temp | 0.54 | 1 | 0.15 | 0.12 | 0.96 | 1.00 |
| B-Time | -0.29 | 1 | 0.15 | -0.71 | 0.13 | 1.00 |
| C-PH | -0.37 | 1 | 0.15 | -0.80 | 0.047 | 1.00 |

**Final Equation in Terms of Coded Factors:**

Beer bitterness =
+18.01
+0.54 * A
-0.29 * B
-0.37 * C

**Final Equation in Terms of Actual Factors:**

Temp 80
Time 45
PH 5.2
Beer bitterness =
+18.13750

Temp 90
Time 45
PH 5.2
Beer bitterness =
+19.21750

Temp 80
Time 90
PH 5.2
Beer bitterness =
+17.55250

Temp 90
Time 90
PH 5.2
Beer bitterness =
+18.63250

Temp 80
Time 45
PH 5.4
Beer bitterness =
+17.38750

Temp 90
Time 45
PH 5.4

Beer bitterness =
+18.46750

Temp 80
Time 90
PH 5.4
Beer bitterness =
+16.80250

Temp 90
Time 90
PH 5.4
Beer bitterness =
+17.88250

**Appendices-2**: Experimental results and ANOVA for 100% imported hops (Herkule)

| std | Run | Block | Factor1 A:temp [$^0$C] | Factor2 B:PH [PH] | Factor3 C:time [Min] | Response1 beer bitterness [Bu] |
|---|---|---|---|---|---|---|
| 2 | 1 | Block1 | 90 | 5.2 | 45 | 18.073±0.015 |
| 3 | 2 | Block1 | 80 | 5.4 | 45 | 18.31±0.040 |
| 5 | 3 | Block1 | 80 | 5.2 | 90 | 21.64±0.320 |
| 6 | 4 | Block1 | 90 | 5.2 | 90 | 20.89±0.315 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 7 | 5 | Block1 | 80 | 5.4 | 90 | 19.19±0.120 |
| 4 | 6 | Block1 | 90 | 5.4 | 45 | 19.82±0.210 |
| 8 | 7 | Block1 | 90 | 5.4 | 90 | 21.32±0.435 |
| 1 | 8 | Block1 | 80 | 5.2 | 45 | 21.62±0.180 |

**Response 1**: **Beer bitterness**

**ANOVA for selected factorial model**

**Analysis of variance table [Partial sum of squares - Type III]**

| Source | Sum of Squares | df | Mean Square | F Value | p-value Prob > F | |
|---|---|---|---|---|---|---|
| Model | 5.06 | 3 | 1.69 | 0.67 | 0.6123 | not significant |
| *A-Temp* | *0.058* | *1* | *0.058* | *0.023* | *0.8867* | |
| *B-Time* | *3.38* | *1* | *3.38* | *1.35* | *0.3102* | |
| *C-PH* | *1.62* | *1* | *1.62* | *0.65* | *0.4665* | |
| Residual | 10.03 | 4 | 2.51 | | | |
| Cor Total | 15.09 | 7 | | | | |

The "Model F-value" of 0.67 implies the model is not significant relative to the noise. There is a 61.23 % chance that a "Model F-value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case there are no significant model terms. Values greater than 0.1000 indicate the model terms are not significant.

If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

| | | | |
|---|---|---|---|
| Std. Dev. | 1.58 | R-Squared | 0.3353 |
| Mean | 20.11 | Adj R-Squared | -0.1633 |
| C.V. % | 7.87 | Pred R-Squared | -1.6590 |
| PRESS | 40.11 | Adeq Precision | 2.117 |

A negative "Pred R-Squared" implies that the overall mean is a better predictor of your response than the current model. "Adeq Precision" measures the signal to noise ratio. A ratio of 2.12 indicates an inadequate signal and we should not use this model to navigate the design space.

| Factor | Coefficient Estimate | df | Standard Error | 95% CI Low | 95% CI High | VIF |
|---|---|---|---|---|---|---|
| Intercept | 20.11 | 1 | 0.56 | 18.56 | 21.66 | |
| A-Temp | -0.085 | 1 | 0.56 | -1.64 | 1.47 | 1.00 |
| B-Time | 0.65 | 1 | 0.56 | -0.90 | 2.20 | 1.00 |
| C-PH | -0.45 | 1 | 0.56 | -2.00 | 1.10 | 1.00 |

**Final Equation in Terms of Coded Factors:**

Beer bitterness =
+20.11
-0.085 * A
+0.65 * B
-0.45 * C

**Final Equation in Terms of Actual Factors:**

Temp 80
Time 45
PH 5.2
Beer bitterness =
+19.99500

Temp 90
Time 45
PH 5.2
Beer bitterness =
+19.82500

Temp 80
Time 90
PH 5.2
Beer bitterness =
+21.29500

Temp 90
Time 90
PH 5.2
Beer bitterness =
+21.12500

Temp 80
Time 45

PH 5.4

Beer bitterness =

+19.09500

Temp 90

Time 45

PH 5.4

Beer bitterness =

+18.92500

Temp 80

Time 90

PH 5.4

Beer bitterness =

+20.39500

Temp 90

Time 90

PH 5.4

Beer bitterness =

+20.22500

**Appendices 3:** Experimental results and ANOVA for 50:50 local hops (Rhamnus priniodes) and imported hop (Herkule).

| std | Run | Block | Factor1 A:temp [ $^0$C] | Factor2 B:PH [PH] | Factor3 C:time [Min] | Response1 beer bitterness [Bu] |
|---|---|---|---|---|---|---|
| 2 | 1 | Block1 | 90 | 5.2 | 45 | 22.57±0.105 |
| 3 | 2 | Block1 | 80 | 5.4 | 45 | 24.02±0.295 |
| 5 | 3 | Block1 | 80 | 5.2 | 90 | 22.08±0.325 |
| 6 | 4 | Block1 | 90 | 5.2 | 90 | 21.82±0.550 |
| 7 | 5 | Block1 | 80 | 5.4 | 90 | 22.55±0.175 |
| 4 | 6 | Block1 | 90 | 5.4 | 45 | 23.61±1.410 |
| 8 | 7 | Block1 | 90 | 5.4 | 90 | 22.01±0.415 |
| 1 | 8 | Block1 | 80 | 5.2 | 45 | 23.42±0.393 |

**Response 1: Beer bitterness**

**ANOVA for selected factorial model**

**Analysis of variance table [Partial sum of squares - Type III]**

| **Source** | **Sum of Squares** | **df** | **Mean Square** | **F Value** | **p-value Prob > F** | |
|---|---|---|---|---|---|---|
| Model | 4.52 | 3 | 1.51 | 28.10 | 0.0038 | significant |
| *A-Temp* | *0.53* | *1* | *0.53* | *9.89* | *0.0347* | |
| *B-Time* | *3.33* | *1* | *3.33* | *62.06* | *0.0014* | |

| | | | | | |
|---|---|---|---|---|---|
| *C-PH* | *0.66* | *1* | *0.66* | *12.33* | *0.0246* |
| Residual | 0.21 | 4 | 0.054 | | |
| Cor Total | 4.73 | 7 | | | |

The Model F-value of 28.10 implies the model is significant. There is onlya 0.38% chance that a "Model F-Value" this large could occur due to noise.Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, C are significant model terms.

Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

| | | | |
|---|---|---|---|
| Std. Dev. | 0.23 | R-Squared | 0.9547 |
| Mean | 22.76 | Adj R-Squared | 0.9207 |
| C.V. % | 1.02 | Pred R-Squared | 0.8188 |
| PRESS | 0.86 | Adeq Precision | 14.535 |

The "Pred R-Squared" of 0.8188 is in reasonable agreement with the "Adj R-Squared" of 0.9207.

"Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 14.535 indicates an adequate signal. This model can be used to navigate the design space.

| **Factor** | **Coefficient Estimate** | **df** | **Standard Error** | **95% CI Low** | **95% CI High** | **VIF** |
|---|---|---|---|---|---|---|
| Intercept | 22.76 | 1 | 0.082 | 22.53 | 22.99 | |
| A-Temp | -0.26 | 1 | 0.082 | -0.48 | -0.030 | 1.00 |
| B-Time | -0.65 | 1 | 0.082 | -0.87 | -0.42 | 1.00 |

| | | | | | |
|---|---|---|---|---|---|
| C-PH | 0.29 | 1 | 0.082 | 0.060 | 0.51 |
| 1.00 | | | | | |

**Final Equation in Terms of Coded Factors:**

Beer bitterness =
+22.76
-0.26 * A
-0.65 * B
+0.29 * C

**Final Equation in Terms of Actual Factors:**

Temp 80
Time 45
PH 5.2
Beer bitterness =
+23.37500

Temp 90
Time 45
PH 5.2
Beer bitterness =
+22.86000

Temp 80
Time 90
PH 5.2
Beer bitterness =

+22.08500

Temp 90
Time 90
PH 5.2
Beer bitterness =
+21.57000

Temp 80
Time 45
PH 5.4
Beer bitterness =
+23.95000

Temp 90
Time 45
PH 5.4
Beer bitterness =
+23.43500

Temp 80
Time 90
PH 5.4
Beer bitterness =
+22.66000

Temp 90
Time 90
PH 5.4

Beer bitterness =
+22.14500

**Solutions for 50% R. prinoides :50% Imported hop (Herkule)**

| Number | Temp | Time | PH | Beer bitterness | Desirability | |
|---|---|---|---|---|---|---|
| 1 | 80 | 45 | 5.4 | 23.95 | 0.968 | Selected |
| 2 | 90 | 45 | 5.4 | 23.435 | 0.734 | |
| 3 | 80 | 45 | 5.2 | 23.375 | 0.707 | |
| 4 | 90 | 45 | 5.2 | 22.86 | 0.473 | |
| 5 | 80 | 90 | 5.4 | 22.66 | 0.382 | |
| 6 | 90 | 90 | 5.4 | 22.145 | 0.148 | |
| 7 | 80 | 90 | 5.2 | 22.085 | 0.120 | |

**Appendice 4:** Experimental results and ANOVA for75:25 local hops (Rhamnus priniodes) to imported hop (Herkule).

| std | Run | Block | Factor1 A:temp [ $^0$C] | Factor2 B:PH [PH] | Factor3 C:time [min] | Response1 beer bitterness [Bu] |
|---|---|---|---|---|---|---|
| 2 | 1 | Block1 | 90 | 5.2 | 45 | 22.07±0.075 |
| 3 | 2 | Block1 | 80 | 5.4 | 45 | 21.68±0.315 |
| 5 | 3 | Block1 | 80 | 5.2 | 90 | 21.80±0.115 |
| 6 | 4 | Block1 | 90 | 5.2 | 90 | 21.58±0.250 |
| 7 | 5 | Block1 | 80 | 5.4 | 90 | 22.39±0.670 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 4 | 6 | Block1 | 90 | 5.4 | 45 | 21.69±0.165 |
| 8 | 7 | Block1 | 90 | 5.4 | 90 | 21.56±0.235 |
| 1 | 8 | Block1 | 80 | 5.2 | 45 | 21.87±0.090 |

**Response1**: **Beer Bitterness**

**ANOVA for selected factorial model**

**Analysis of variance table [Partial sum of squares - Type III]**

| **Source** | **Sum of Squares** | **df** | **Mean Square** | **F Value** | **p-value Prob > F** | |
|---|---|---|---|---|---|---|
| Model | 4.50 | 3 | 1.50 | 3.32 | 0.1384 | not significant |
| *A-Temp* | *3.52* | *1* | *3.52* | *7.81* | *0.0491* | |
| *B-Time* | *0.053* | *1* | *0.053* | *0.12* | *0.7496* | |
| *C-PH* | *0.92* | *1* | *0.92* | *2.03* | *0.2271* | |
| Residual | 1.81 | 4 | 0.45 | | | |
| Cor Total | 6.30 | 7 | | | | |

The "Model F-value" of 3.32 implies the model is not significant relative to the noise. There is a 13.84 % chance that a "Model F-value" this large could occur due to noise.Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

| | | | |
|---|---|---|---|
| Std. Dev. | 0.67 | R-Squared | 0.7134 |
| Mean | 23.57 | Adj R-Squared | 0.4984 |
| C.V. % | 2.85 | Pred R-Squared | -0.1465 |
| PRESS | 7.22 | Adeq Precision | 4.562 |

A negative "Pred R-Squared" implies that the overall mean is a better predictor of your response than the current model. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 4.562 indicates an adequate signal. This model can be used to navigate the design space.

| Factor | Coefficient Estimate | df | Standard Error | 95% CI Low | 95% CI High | VIF |
|---|---|---|---|---|---|---|
| Intercept | 23.57 | 1 | 0.24 | 22.91 | 24.23 | |
| A-Temp | -0.66 | 1 | 0.24 | -1.32 | -4.134E-003 | 1.00 |
| B-Time | -0.081 | 1 | 0.24 | -0.74 | 0.58 | 1.00 |
| C-PH | 0.34 | 1 | 0.24 | -0.32 | 1.00 | 1.00 |

**Final Equation in Terms of Coded Factors:**

Beer Bitterness = +23.57 -0.66 * A -0.081 * B +0.34 * C

**Final Equation in Terms of Actual Factors:**

Temp 80
Time 45
PH 5.2

Beer Bitterness = +23.97500

Temp 90
Time 45
PH 5.2
Beer Bitterness =
+22.64750

Temp 80
Time 90
PH 5.2
Beer Bitterness =
+23.81250

Temp 90
Time 90
PH 5.2
Beer Bitterness =
+22.48500

Temp 80
Time 45
PH 5.4
Beer Bitterness =
+24.65250

Temp 90
Time 45
PH 5.4
Beer Bitterness =

+23.32500

Temp 80
Time 90
PH 5.4
Beer Bitterness =
+24.49000

Temp 90
Time 90
PH 5.4
Beer Bitterness =
+23.16250

**Appendice 5:** Experimental results and ANOVA for 25:75 local hops (Rhamnus priniodes) to imported hops (Herkule).

| std | Run | Block | Factor1 A:temp [ $^0$C | Factor2 B:PH [PH] | Factor3 C:time [Min] | Response1 beer bitterness [Bu] |
|---|---|---|---|---|---|---|
| 2 | 1 | Block1 | 90 | 5.2 | 45 | 22.48±0.485 |
| 3 | 2 | Block1 | 80 | 5.4 | 45 | 24.47±0.515 |
| 5 | 3 | Block1 | 80 | 5.2 | 90 | 24.42±0.895 |
| 6 | 4 | Block1 | 90 | 5.2 | 90 | 21.83±0.715 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 7 | 5 | Block1 | 80 | 5.4 | 90 | 23.85±0.235 |
| 4 | 6 | Block1 | 90 | 5.4 | 45 | 23.46±0.265 |
| 8 | 7 | Block1 | 90 | 5.4 | 90 | 23.85±0.250 |
| 1 | 8 | Block1 | 80 | 5.2 | 45 | 24.19±0.504 |

**ANOVA for selected factorial model**

**Analysis of variance table [Partial sum of squares - Type III]**

| **Source** | **Sum of Squares** | **df** | **Mean Square** | **F Value** | **p-value Prob > F** | |
|---|---|---|---|---|---|---|
| Model | 0.088 | 3 | 0.029 | 0.25 | 0.8552 | not significant |
| *A-Temp* | *0.088* | *1* | *0.088* | *0.76* | *0.4320* | |
| *B-Time* | *5.000E-005* | *1* | *5.000E-005* | *4.320E-004* | *0.9844* | |
| *C-PH* | *0.000* | *1* | *0.000* | *0.000* | *1.0000* | |
| Residual | 0.46 | 4 | 0.12 | | | |
| Cor Total | 0.55 | 7 | | | | |

The "Model F-value" of 0.25 implies the model is not significant relative to the noise. There is a 85.52 % chance that a "Model F-value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case there are no significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

| | | | |
|---|---|---|---|
| Std. Dev. | 0.34 | R-Squared | 0.1601 |
| Mean | 21.83 | Adj R-Squared | -0.4698 |
| C.V. % | 1.56 | Pred R-Squared | -2.3596 |

| | | | |
|---|---|---|---|
| PRESS | 1.85 | Adeq Precision | 0.894 |

A negative "Pred R-Squared" implies that the overall mean is a better predictor of your response than the current model. "Adeq Precision" measures the signal to noise ratio. A ratio of 0.89 indicates an inadequate signal and we should not use this model to navigate the design space.

| Factor | Coefficient Estimate | df | Standard Error | 95% CI Low | 95% CI High | VIF |
|---|---|---|---|---|---|---|
| Intercept | 21.83 | 1 | 0.12 | 21.50 | 22.16 | |
| A-Temp | -0.11 | 1 | 0.12 | -0.44 | 0.23 | 1.00 |
| B-Time | 2.500E-003 | 1 | 0.12 | -0.33 | 0.34 | 1.00 |
| C-PH | 0.000 | 1 | 0.12 | -0.33 | 0.33 | 1.00 |

**Final Equation in Terms of Coded Factors:**

Beer bitterness =
+21.83
-0.11 * A
+2.500E-003 * B
+0.000 * C

**Final Equation in Terms of Actual Factors:**

Temp 80
Time 45
PH 5.2

Beer bitterness = +21.93250

Temp 90
Time 45
PH 5.2
Beer bitterness = +21.72250

Temp 80
Time 90
PH 5.2
Beer bitterness = +21.93750

Temp 90
Time 90
PH 5.2
Beer bitterness = +21.72750

Temp 80
Time 45
PH 5.4
Beer bitterness = +21.93250

Temp 90
Time 45

| | |
|---|---|
| PH | 5.4 |
| Beer bitterness | = |
| +21.72250 | |
| | |
| Temp | 80 |
| Time | 90 |
| PH | 5.4 |
| Beer bitterness | = |
| +21.93750 | |
| | |
| Temp | 90 |
| Time | 90 |
| PH | 5.4 |
| Beer bitterness | = |
| +21.72750 | |

**Appendice B: Laboratory procedures**

- **Determination of Beer bitterness(spectrophotometer method-EBC method 4.9.3)**

**i) Sampling method**

A sample the cooled Wort before pitching where the composition is representative ,aspectic sampling is recommended to prevent contamination and Store at refrigerator at 5-10$^{o}$c until use.

**ii) Sample preparation**

A sample must be degassed without loss of foam by adding 2-4 drops of octanol into the bottle and pouring 5-20 times from one beaker to another or use maginetic stirrer at least of five minutes. If Yeast contained in beer must be clarified by centrifuging 15minutes at 3000rpm.

**iii) Procedure**

- 10ml of degassed test sample was pipetted into conical flask with glass stopper which was acidified with 1ml of 3N HCl and was filled with 20ml of isooctane (2,2,4-trimethylpentane) ,Stopper the flask tightly and place it in mechanical shaker ,Shake vigorously for 15minutes.at room temperature and then allow decanting for 10 minute until the supernatant organic phase will create.
- Measure the absorbance of isooctane at 275nm in a1.ocm quarz cell or cuvette using Isooctane from the batch as the blank. The optical density is expressed in terms of European brewing convention bitterness units as follows.

  Expression of result will be come;-

  Bitterness (BU) = 50*ABS275 (EBC, 1998).

❖ **Determination of polyphenol in a beer**

**Procedure**

- A sample degassed for at least of 5times.
- 10ml of degassed test sample was pipetted into volumetric flask (25ml) with glass stopper which was basified with 0.5ml of 25% ammonium solution and 0.5% ferric reagent and 8ml of CMC/EDTA were added to the solution the blank was run in the parallel except 0.5% ferric reagent to determine polyphenol amount in the beer .
- The reaction allowed for 10min.
- Measure the absorbance of the blank at 600nm in a1.ocm quarz cell or cuvette using sample except ferric as a blank. The optical density is expressed in terms of European brewing convention bitterness units as follows.
- Expression of result will be come;-
  Polyphenol (pphl) = 120*ABS600 (EBC, 1998).

❖ **Determination of VDK (spectrophotometer method-EBC method 4.8.4)**

- Add 100ml beer into distillation flask and start distillation.
- Control the heating rate carefully to prevent over foaming.

- The available period under gentle heating should be at least 6min until the first drop is collected into the receiving cylinder.
- And collect 25ml of the distillate within 8-10min and mix thoroughly, pipette 10ml of the distillate into 50ml flask with glass stopper, then adding 0.5ml % O-phenylediamine (reagent).mix and place the flask 20-30minutes in the darkness. After adding 2ml 4N HCl (reagent) mix the sample thoroughly and measure within 20min against the blank.
- Measure the absorbance of the blank at 275nm in a1.ocm quarz cell or cuvette using isooctane as a blank. The optical density is expressed in terms of European brewing convention ppm.

VDK, ppm= 2.7*ABS335.

**Appendices-C**

Equation 1.1 Calculation of International Bittering Units

IBU = AAU × U × 10 ÷ V

AAU = Alpha Acid Units

Utilization (U) = f (G) × f (T)

F (G) = $1.65 \times 0.000125^{(Gb-1)}$ Gb = boil gravity

F (T) = [1 - e (-0.04 × T)] ÷ 4.15

10 is constant for the metric units (grams and liters), IBU is calculated in mg/L. V = Volume of the boil

Source: Papazain, 2003

**APPENDICES-D**: Questionnaire for overall acceptability ranking test

| |
|---|
| Name… |
| Product: 100% Gesho beer<br>: 100% import hop beer<br>:50% Gesho beer<br>:25% Gesho beer |
| Instructions…….. |

- Using the numbers 1, 2, 3, 4(as shown below) please indicate the intensity of the various characteristics of the coded products below.

| |
|---|
| 1.None<br>2. slightly<br>3.Moderate<br>4.Strong<br>Code colour, Bitterness, tingly, and foam stability overall acceptability<br>150……..<br>200…….<br>250……<br>300…….<br>235…….<br>Comments(optional)<br>…….<br>……..<br>THANK YOU FOR ALL |

**Declaration**

I hereby declare that this thesis is my authentic work and that all sources of materials with references to specific authors used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for M.Sc. Degree in processes engineering at Addis Ababa University. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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