ADDIS ABABA UNIVERSITY

ADDIS ABABA INSTITUTE OF TECHNOLOGY
SCHOOL OF CHEMICAL AND BIOENGINEERING

Characterization and Optimization of Gesho for Substituting as an Alternative Source to Beer Hops.

A Thesis submitted to the School of Graduate Studies of Addis Ababa University in partial fulfillment of the requirements for the Degree of Master of Science in Chemical Engineering (process Engineering Stream)

By:

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This is to certify that the thesis prepared by Hadas Kidey, entitled Characterization and Optimization of Gesho for Substituting as an Alternative Source to Beer Hops and submitted in partial fulfillment of the requirements for the Degree of Master of Science in Chemical Engineering (process Engineering Stream). It complies with the University rule and agreed criteria with regard to originality.

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Declaration

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

Hadas Kidey Gebremicael	_____	_____
Name	Signature	Submission Date

This thesis has been submitted for examination with my approval as a university advisor.

Professor. Belay weldegiorgis	_____	_____
Advisor	Signature	Date

Abstract

Beer is made of from malt, hop, distilled water and yeast. The study was conducted with the aim of Characterization and Optimization of Gesho for Substituting as an Alternative Source to Beer Hops. In this study substitute of hops by effective use of Gesho were investigated using optimizing the process variable gesho (*Rhamnus prinoides*) to hop mixing ratio (25 up to 100 %), wort boiling temperature (75 up to 95 ^{o}C), wort boiling time (45 up to 90 min) and wort gravity (15 up to 20 ^{o}P). However, it is not always economically feasible to brew with 100% imported hops, and at present time breweries are forced to minimize their costs without changing the quality of their beer. All the experiments were conducted at Raya beer production S.c. 25 series of experiments involving (25%, 62.7% and 100% hops substitute by Ethiopian gesho (*Rhamnus prinoides*) with full barley malt serving as a control was conducted. Based on ANOVA analysis, process parameters have significant positive effect ($P<0.05$) on standard beer bitterness. The best levels of hoped wort boiling parameters for higher bitterness value 24.3 BU were gesho to hop mixing ratio of 25% having wort gravity 15.12^{o}P for 79.86 min at 94.16℃. The major characteristics of the beer (bitterness, flavor and alcohol content) were evaluated for optimized one with compared to the control beer. The results showed that 25% substitution of hops b *Rhamnus prinoides* (gesho) is promising in the beer production. The results of the study showed that the average bitterness finished beer determined was 17.20 BU.

Key words: *Rhamnus prinoides,* beer, hop and bitterness

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Acronyms and abbreviations

ANOVA	Analysis of Variance
AOAC	Association of official analytical chemistry
BBD	Box-Behnken Design
Bu	bitterness unit
CCD	Central Composite Design
DOF	Design of Experiment
DPPH	1, 1-Diphenyl-1-picrylhydrazyl
EDTA	Ethylenediamine triacetic Acid
Min	minute
RSM	Response Surface Methodology
T^O	Temperature
TPC	Total Poly phenol Content
UV/VIS	UV/visible
^OP	Degree plato
%	percentage

1. Introduction

1.1 Background

Beverage is formed principally from malt, hop, distilled water and yeast. Beer production worldwide may be a viable industry. Additionally, among business refreshments in 2006, brew positions fourth in per capita utilization behind carbonated sodas, drinking water and intermittent followed by milk and organic product drinks inside the utilization ofAmerica (Vn, Uw, and Ri 2020).

Brew is that the third most sizzling beverage the planet close to tea and water. Lager can be an elegant combination; very 400 unique mixtures are described in lager and, additionally, contains macromolecules like proteins, nucleic acids, sugars and lipids. Assortment of the constituents of lager are gotten from the crude materials and endure the blending measure unaltered. Others are the aftereffects of substance and biochemical change of the crude materials during malting, squashing, bubbling, aging and molding (Science 1982b).

The brew organization is a critical worldwide endeavor, with yearly deals of $294.5 billion. per annum , different countries spend tons on liquor and among these; brew is that the most customarily devoured drinks around the world. It's additionally the third most blazing among every single thought about thing, after water and tea (Chakraborty 2018).

Nigeria is in tropical district and since lager creation in Nigeria has never declined with prepared market as utilization rate keeps on expanding, the importation of bounces gets inescapable. Subsequently, the need to investigate some Nigerian plants that would substitute jumps in lager preparing (Okafor et al. 2016).

Ethiopia is pulling in additional speculation as a business and world serious for liquor creation subsequently; drink venture has become the worthwhile commercial center for the wine and brew Industry and has to a largest extent added to measure. This may be credited to the counters populous and subterranean insect and growing class. This, close by a developing, to a large degree youth working populace with expanded expendable wages is that the consistent drive that expanded brew utilization in Ethiopia. Also, the bottling works organizations are changing Ethiopia's business scene with intoxicant ventures having a lie in the speculation and market

inclusion. By the tip of the year 2015. The country's export revenue from the inebriant industry would reach 17 million USD (Ethiopian brewery industry, www, 2017).

Traditionally Tella is that the most well liked Ethiopian beverage, which is made of varied row materials (Hostettman. K et al. 1996). In Ethiopia, the hops importation statistics where showed that in 2015 for imported net weight 1772 kg by 3,325,895.58 and in 2016 for net weight 170373 by 7,072,285.91. Hop plant (Humulus lupulus L.) is engaged within the beer production especially to feature bitterness (Hostettman. K et al. 1996).

The feminine inflorescences of the bounce plant contain mainly hop resins, polyphenol compounds, essential oils and other related compounds (Kavalier et al. 2011). Hops are developed all through the calm areas of the earth to fulfill the strain of the preparing enterprises bounce. It is vital to the brewing industry and a couple of of their unique chemicals have the potential to be utilized within the nutraceutical industry (Garcia et al. 2012). Bounce removes give brew its unpleasant taste, improve froth strength, upgrade fragrance and flavor, and go about as disinfectant towards microorganisms. The Gesho plant (R. prinoides), which is different from hop (Humulus lupulus) is widely cultivated in Ethiopia and is obtainable dried within the local market, the leaves and stems of this plant are accustomed impart the characteristic bitter flavour to domestically brewed beverages mentioned as Tella and Tej and it's estimated that run out 5 million people consume these beverages daily. The leafs and breaks of Gesho are indispensable ingredients within the making of these traditional fermented beverages (Anon 2013).

Fikadu ashine (2015) conducted an experiment to use Gesho as a substitution for imported hop for beer production in BGI Ethiopia, Addis Ababa. pH, mixing ratio, temperature, and time were optimized, and a bitterness value of 15.6 BU was obtained. During this study, the potential of Gesho as a substitute for imported hop was investigated where wort gravity, wort boiling time, temperature, and mixing ratio were optimized for the aim of getting standard quality beer.

1.2 Problem of the statement

Beer production in Ethiopia has increased recently due to ready markets and thus the importation of hops to satisfy the demand of the brewing industries becomes inevitable and continues to constitute an enormous proportion of Ethiopia's economy. Consequently, huge amounts of exchange are being spent by this sector on importation of hops. In Ethiopia, the hops importation statistics where showed that in 2015 for imported net weight 1772 kg by 3,325,895.58 and in 2016 for net weight 170373 by 7,072,285.91. Therefore, this indicated to look out local potential plant to substitute hops. Currently beer brewers are forced to import hop to satisfy their supply problem. So that, the country`s requirement of hops are met through import. Thus, the country has expends foreign currency for importation. Among the potential plants wont to substitute hop importation is gesho (Rhamnus prinoides L). Additionally to the present, this plant is employed as traditional medicine (Analyses 2000). Gesho might be a replacement potential substitute for imported hops which can be used as another 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 in most tropical countries, the hops are imported. With the expansion of the brewing industry in Africa, huge amounts of money are therefore being spent by developing countries for the importation of hops (Okoro and Aina 2007).

So on use R.priniodes (Gesho) in beer brewing as bittering agent the extraction of Rhamnus prinoides and comparative experiments with hops must be done. Therefore use of Rhamnus priniodes (gesho) for beer production is vital because it'll have advantages from both economic and environmental perspectives without the expense of violating the standards for beer properties. So this study were done the only choice to minimize the problems mentioned above and improve the resource use system as an honest and effective use of matrials is incredibly important for development of every country within the planet . This would the matter that needs an on the spot solution from experts within the sector instead of thinking import because the answer. Several researches were conducted on beer production in Ethiopia from Gesho. However, information on physio-chemical properties and usage (substitution) of Ethiopian Gesho isn't sufficient. The Ethiopian commercial beer industries use imported hops thanks to the shortage of researches on the feasibility and optimization of parameters like mixing ratio, wort gravity, temperature and time of beer production using local Gesho. During this study,

physio-chemical properties of local Gesho and parameters were optimized to research the potential of Gesho as a substitute for imported hops. So, this study could help contribute to tackle the matter or shortage of data on substituting hops by local Gesho.

1.2.1 General objective

The main objective of this study was to characterize the physicochemical composition of ges ho (Rhamnus priniodes) and optimize the parameters as an alternate source for Ethiopian brewery companies and to substitute the importation of hops without comprising of beer beverages quality.

1.2.2 Specific objective

- To investigate physio-chemical characterization of Gesho like composition analysis proximate analysis, and anti-oxidant activity determination.
- To investigate ratio of Gesho and hop like in range (25 -100%)
- To investigate process factors like wort gravity and wort boiling time and temperature to supply beer.
- To compare and characterize beer produced from Gesho and hop with Castel beer.
- To investigate optimum operating parameters using BBD.

1.3 Significance of the study

Currently there's a rapid development of commercial, commercial and native brewing within the country. Ethiopian has made variety of strategies which will help to extend growth alcoholic beverages and beer production. However, beer processing industries that are producing alcoholic beverages which are beer aren't developing to satisfy demand. Additionally the raw matrial (hop) imported from foreign countries are causing high cost. But, Ethiopian hops (Gesho) only used for tella and teji traditionally, isn't economically ok. Therefore, the aim of this project are going to be substitution of Gesho rather than the imported hop or as an alternate valuable products i.e. beer. this may be good once we consider from environmental, economic and societal point of view because the products are biodegradable, the population are going to be benefited from cultivation of Gesho (Rhumnus priniodes, and therefore the rate of consumption of hops are going to be decreased then on decrease the value to import hops. Production beers from hop plant thus have an economic and ecological advantage which may function an initiative (motive) for giant scale cultivation, biodiversity

conservation and environmental rehabilitation. Additionally, the value of beer will decrease thanks to a rise in production capacity. This study also will help the policy makers to possess awareness on alternative resource for production of beer product and to motivate people to practice effective utilization of resources.

2. Litrature review

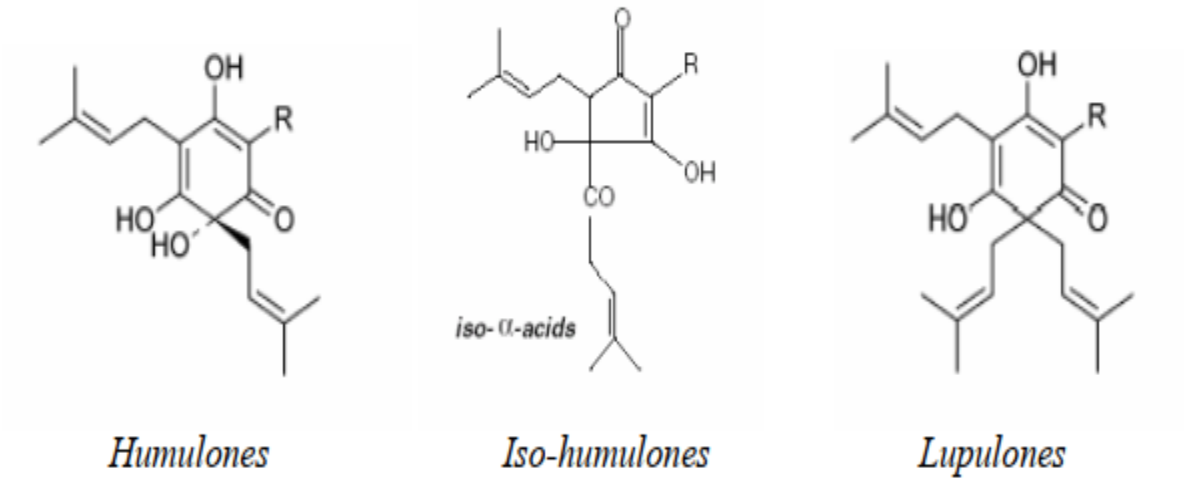
2.1 Hops

Hops are globally found crops, it is a species and family of dioecious perennial and respectively. Used to increase bitter and fragrant of the beer. The plant, which is native to Europe and western Asia, is now cultivated in North and South America, Africa, Asia and Australia and invasive is in many areas (Mongelli et al. 2015). Many literatures showed about hops categories consistent with (Schindler et al. 2019) report two category of hops those are hard resins and essential oils. Hops, the inflorescence (cone) of the common hop plant, contain both α-acids (humulones) and β-acids (lupulones) (Egts et al. 2012).

Hard resins are lupulin glands are resinous amphiphilic molecules referred to as α-acids and β-acids, which provides a bitter taste to the beer and essential oils volatile hydrocarbons referred to as , which give beer distinct flavor and aroma profiles counting on the kinds of hops used it is an herb traditionally utilized in beer brewing to impart scent and bitterness. Iso-α-acids (IAAs) and matured hop bitter acids (MHBAs) are hop-derived bitter components that are present at concentrations of three .3-64.0 and 19.1-210 mg/L in beer, respectively (Coldea et al. 2014).

Hop bitter acids comprise α- and β-acids. Bitter α-acids are composed of three main components: nhumulone, ad-humulone and co-humulone. Likewise, bitter β-acids contains n-lupulone, ad-lupulone and colupulone. More important for brewery production are α-acids, which are only marginally soluble in water. The hop acids have pronounced bacteriostatic activity; they strongly inhibit the expansion of Gram-positive bacteria. It Contain phenyl group during this group (three within the beta-acids) are present, the stronger the bacteriostatic action is. This remarkable bioactivity is of importance for killing micro-organisms during wort boiling, which ultimately finishes up during a sterile beer. The beta-acids are delicate to oxidative disintegration and most oxidation response items have upsetting organoleptic qualities. Despite the undeniable reality that the beta-acids may secure brew against oxidation, they are, all in all, oxidation response items have terrible organoleptic attributes. Despite the undeniable reality that the beta-acids may secure brew against oxidation, they are, all in all, viewed as a negative might suspect about blending and assortment of brewers select bounce assortments that are poor in beta-acids (Keukeleire 2016).

hop varieties have traditionally been classified, based on their chemical composition, as 'bittering hops' and 'aroma hops Recent proposals suggest a more refined classification of hop varieties. On the idea of α-acid content, the bittering hops are further subdivided into 'bittering hops', 'high alpha' and 'super high alpha' varieties. Some of the foremost important traded bittering hop varieties on the planet market are Hallertauer, Magnum, Hallertauer Taurus, Herkules, Galena, Nugget, Millennium and CTZ. The representative aroma hop varieties are Hallertauer Perle, HallertauerTradition, Spalter Select, Hallertauer Mittelfrüh, Hersbruck Hersbrucker, Tettnang Tettnanger, Saaz and Cascade. At the time of harvest, the long-term average α-acid contents in the bittering and aroma varieties are in ranged from 10 to 20% and 2 to 10%, respectively(Kavalier et al. 2011).



(Elena et al. 2008).

Figure 1: The structure of α- and β-acids

R=CH2CH (CH3)2 isovaryl Humulone (lupulone)

R=CH (CH3)2 Isobutyryl cohumulone(colupulone) ; where R is an alkyl group.

2.2 Industrial hop process

Extraction of hops with supercritical CO2 was performed at temperatures and pressure in the range of 40 and 50℃ and 150 and 400 bars respectively. CO2 is thus a substitute for organic solvents of a highly nonpolar character, like hexane and pentane. The extracts contain nearly all essential oils in hops, also as a sufficiently high ratio of α-acids (humulones) and fewer bitter lupulones, besides other components like hard resins and traces of triglycerides, waxes, chlorophylls, and inorganic salts. Supercritical CO_2 purification has economic process step in assembly of brewery ingredients (Analyses 2000).

2.3 Uses of hops

During the center Ages, hops eventually became the only flavoring ingredient of beer. This unique status was codified in 1516 within the Bavarian Reinheitsgebot, which evolved into the fashionable Purity Law for beer brewed in Germany. Overall, hops became important in beer brewing by virtue of their antimicrobial properties and it used for medicinal purposes, Folk Medicine, for instance, used them against ear- and toothaches and drank hop tea as a relaxant. Hop bitters are generally known to help digestion and stimulate the appetite. Hops also are very active against inflammation or for infections (Biendl 2009). Additionally, it's utilized in pharmaceutical applications. The resin is used as potential cancer preventive activities (Farag and Porzel 2012).

2.4 Chemical composition of hops

Table 1: Average composition air dried hops.

Components	Amount (%)
α-acid	2-17
β −acid	2-10
Protein	15
Tannins(polyphenols)	3-6
Steam volatile oils	0.5-3
Moisture	8-12
Ash	10

Source: (Humulus and Bioactive 2009).

The rest consists of cellulose and other materials which are unimportant for beer production (Online, Demissew, and Mulugeta 2017). Many factors influencing α-acid formation from these the Hop variety, climate, temperature, soil moisture during sunlight, and age at harvest the sort. Additionally to the present, during drying period of the hops the greater clear effect on α-acid retention are physical conditions (bed-depth, air speed, air temperature and humidity) (Doe and Menary 1979).

2.5 Hops resins

A hop resins was the most the most trademark segments of bounces. They brew its unpleasant taste, improve froth solidness and go about as cleaning agents towards microorganisms. Upheld level of solvency, bounce saps are additionally partitioned into as hard and delicate pitch.

2.5.1 Hard resins

Hop α-acids intrinsically occur in beer in concentrations up to 4 mg/ L. They improve foam stability, suppress gushing, and contribute to the preservation of beer. However, their main contribution to beer is via isomerization during the boiling of wort with hops, thereby forming the extremely bitter iso-α-acids.

2.5.2 Soft resins

β-acids are less acidic than α-acids because the tertiary alcohol function at C-6 is replaced by an additional prenyl side chain. In contrast to α-acids that aren't bitter, these compounds have a really bitter taste and may be present in beer in quantities of a couple of mg/L. However, β-acids also as hulupones are of minor importance to the beer quality.

Iso-α-acids and Derivatives α-Acids are isomerized during the brewing process to the more water-soluble iso-α-acids, thereby yielding concentrations starting from 10 up to 100 mg/L in beers. Each iso-α-acid analogue occurs as an epimeric mixture of cis and trans-isomers and Iso-R-acids are often formed from α-acids under a spread of conditions. During the brewing process, α-acids are isomerized by boiling hops or hop extracts within the aqueous wort medium at a pH of 5.0-5.5. In practice, a final α-acid utilization yield of 25-35% is reached within the beer.

2.5.3 Hop oils

The oil of hop are volatiles during a daily 3600 second to 5400 second boil. Where bounce character is needed in lager, a little low sum (up to twenty of the whole bounce charge) of chose fragrance hops could also be inserted to a kettle 5 to fifteen minutes before the highest of the boil. Hops are typically added to Wort in 1-3 stages during the boil: Bittering, Flavor and Aroma. These stages should do with what role they're playing in your beer, and do not seem to be associated with a specific sort of hop. In other words, the identical hop variety could also be used for bittering, flavor and aroma.

Not all beers would have 3 additions; some may have only one, some may have up to 5 or 6 additions. By adding the fragrance bounces, you're adding another measurement to your brew. On the off chance that you utilized just fragrance bounces, your lager would be inadequate with regards to harshness. Insufficient alpha acids from the bounces would be isomerized in your bubble. Most plans will disclose to you when to include your jumps either on schedule from the beginning of the bubble, or time that is left inside the bubble. For instance, you'll have a brew that is envisioned to bubble for a whole of hr. The headings may advise you to include the bittering jumps half-hour into or half-hour left of the bubble. Bittering: Bittering hops are added once the wort has been collected within the kettle (or after you've added the malt extract) and a rolling boil has been achieved. They are usually boiled for hour, although some recipes involve as little as half-hour. All beers have some bittering hops. The foremost reason for this is often that without the harshness from the bounces, your brew would taste sweet. Another advantage is that bounces are a characteristic additive and may assist your brew with staying for an all-inclusive time or then again for expanded maturing periods. Enhancing: Flavoring bounces need longer than smell jumps by and large added with among 15 and half-hour remaining within the boil. They Similar with aroma hops, but the difference between them is slight it is the time that they are boiled thanks to that time.

Aroma: hoppy aroma in beer could also be a posh of sensory impressions resulting from many various volatile compounds at low concentrations, the volatiles, contained within the hop oil (0.5-3% in hops none volatile which are hop polyphenol (3-6%). due to the more volatility of their typically added near the tip of the boil, or 5 min remains to end boil they don't need longer and process called late hoping which is special method of hop adding before beer packaging.

However, the amount of hops needed is simply a fraction of the substantial quantities of malt employed within the brewery. a few grams of jumps are adequate as a quantitatively minor, yet subjectively significant fixing with pivotal effect on well-defined beer features (Keukeleire 2016).

2.6 Antioxidant

Cancer prevention agents can hinder or impede oxidation twoly: either by searching free extremists, during which case the compound is depicted as an essential cancer prevention agent, or by a component that doesn't include accurate rummaging of free revolutionaries within which case the compound could even be a secondary antioxidant. Primary antioxidants include phenolic compounds like vitamin (α-tocopherol).These components are consumed during the induction period. Secondary antioxidants operate by kind of mechanisms including binding of metal ions, scavenging oxygen, converting hydro peroxides to non-radical species, absorbing UV radiation or deactivating singlet oxygen. Normally, secondary antioxidants only show antioxidant activity is relived. This could be seen within the case of sequestering agents like acid which are effective only within the presence of metal ions, and reducing agents like antioxidant which are effective within the presence of tocopherols or other primary b (Makris et al., 2007).

2.6.1 Antioxidant capacity assay

In the first time international conference on cancer prevention agent techniques was held in Orlando, FL, in June 2004 for the express motivation behind overseeing logical issues comparative with surveying cell reinforcement limit's (AOC) in food varieties, botanicals, nutraceutials and other dietary enhancements and proposing at least one insightful strategies which might be normalized for routine appraisal of AOC (Prior et al.,). Various cell reinforcements show considerably changing antioxative adequacy in a few food frame works because of various sub-atomic design. The cell reinforcements mustn't grant any off-flavor and unseemly. It ought to be prepared to get conviently consolidated to food or food frameworks and ought to be steady at pH of the food frameworks and through food handling. Different elements which influence the effectiveness of cell reinforcements incorporate energy of initiation of cell reinforcements, redox expected security of pH and preparing and dependability (Sharma & Singh, 2013). Various measures are acquainted with live cell

reinforcement limit of food varieties and natural examples (Floegel et al. 2011).(Floegel et al. 2011)

2.7 Traditional brew of Ethiopia using Gesho

Tella is widely brewed and consumed in both rural and concrete a part of Ethiopia. It's documented as neighborhood brew since its malt based drink like that of monetary brew (Faculty 2014). Aside from the fundamental and unnecessary metals, unpredictable oils, and saponins with lower content, present phytochemical examines demonstrated that R.prinoides basically contains kinds of phenolic compounds, including flavonoids, anthraquinones, naphthols, and there for instance, the geshoidin, a naphthalene glycoside (β-sorigenin-8-O-β-D-glucoside), is one among the foremost important compounds during this plant, and partially liable for the characteristic bitterness within the popular beverages of tella. Tella Ethiopian customary brew. Its creation interaction is comparable to brew making there in the grain starch is changed over into sugars by malting. Notwithstanding, there's no yeast immunization stage for maturation yet it uses the common yeast present on the cereals (Lee, Regu, and Seleshe 2015).

2.8 Gesho (Rhamnus prinoides)

Figure 2: Gesho

Rhamnus prinoides belongs to the Rhamnaceae family. R. prinoides is also known by different names like African Dogwood, Glossy-leaf and mififi. R. prinoides is cosmopolitan in East and African country nations which incorporate Ethiopia, Botswana, Eritrea, Lesotho, Namibia, South Africa, Swaziland, Uganda and Kenya (Pillai, Santi, and Magama 2019). Gesho could

be a plant which grows up to 6 meters and for its importance within the production of domestically fermented beverages like tella and teji. In Ethiopia the leaves and steams of gesho could be a plant which grows up to 6 meters and for its significance inside the creation of locally matured drinks like tella and teji. In addition to this the leaves furthermore, steams of gesho are critical parts inside the making of standard matured beverages (Online et al. 2017).

The plant develops best in territories where the mean yearly temperature falls inside the scope of 14-22 °C, furthermore, might endure 8-32 °C. It favors a mean yearly precipitation inside the reach 600- 800mm, enduring 500-1200mm and is ordinarily found in zones with a positive season of year. By and large, moderate developing when in low precipitation territories, however it can grow 1 meter every year in wetter territory (Ferede et al. 2018).

In Ethiopia, R. prinoides (gesho) are regularly found developing inside the wild on the whole territories, normally at heights of 1500- 2500 m. In most provinces, it's cultivated in Ethiopia similarly. It's present in many gardens near houses and it's sometimes cultivated as a field crop on a bigger scale. In markets, fresh leafy branches are often sold. In keeping with the Land Utilization Report, gesho covers approximately 1% (=5000 ha) of the full acreage under permanent cultivation in Ethiopia. The foremost important provinces for gesho are: Shoa (1800 ha), Gojam (1200 ha), Begemdir (1000 ha), Arussi (500 ha), Sidamo (400 ha) it's been speculated that the role of Gesho in tella should be almost like that of hops in beer. Thanks to the phenolic nature of Geshoidin, it's the ability of scavenging active oxygen radicals or its chemical antioxidant activity (Roma 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 African country. The plant is native to Ethiopia, Botswana, Eritrea, Lesotho, Namibia, Republic of South Africa, Swaziland, Uganda and it's exotic to Kenya. It also occurs in Cameroon, Sudan, and Angola. It's cultivated in Ethiopia. R. prinoides cultivations throughout the country, Tigray, North Shoa around Kara Kori and Sebeta, just west of capital of Ethiopia, are important centers of cultivation of R. prinoides (Hostettman K et al. 1996). In Tigray, R. prinoides is cultivated within the districts of south of Ahferom, Ganta Afeshaum and alittle a part of Werei Leke. Gesho incorporates a considerable value in Ethiopia. It's one in every of the foremost and precious crops used for industrial uses both locally for domestic use and industrially (Girmay 2015)

(Amabye 2016) anticipated that utilizing the fluid concentrate of Rhamnus prinoides and of methanol/water concentrate of Rhamnus prinoides of that Rhamnus prinoides found in the market of Mekelle includingnutritional structure: rough protein 8.5%, unrefined fiber (about 25.6%), debris (about 9.5%), carbs (about 70.5%), moisture 9.5%, and lipid (about 3.5%) by using AOAC (Association of Analytical chemists procedures). The ash content of about 9.5% indicates that the leaves are rich in mineral elements. The general and supplement examinations of Rhamnus prinoides utilizing standared phytochemical screening strategy assume a urgent part in evaluating its dietary significance (0.05). The use of Rhamnus

priniodes as substitute for hops within the Ethiopian brewing industry has been chronicled by fekadu ashine (Anon 2017). Through the method he used to do the work pH, Temperature and time were main process parameters and He recommended using 50% of Ethiopian hops for beer brewery companies. This is a continuation to our earlier reported studies (Anon 2017).Values are means of triplicate determinations; Chemical composition of Gesho.

Table 2: Determination of essential oil, total, soft and hard resins from fresh leaf and stem powder of Rhamnus prinoides

Samples	**Chemical compositions (%)**			
	Essential oils %	Hard resin (%)	Soft resin (%)	Total resin %
Rhamnus prinoides leaf	1.13±0.02a	2.73± 0.01c	15.73±0.03a	18.46±0.03b
Rhamnus prinoides stem	0.60± 0.01c	1.0 ±0.03d	12.40±0.02c	17.16± 0.01c
Humulus lupulus control	1.0 ±0.03d	6.33±0.02a	13.20± 0.01b	19.52±0.02a

Qualities inside a similar line followed by various superscripts are fundamentally extraordinary

at ($p < 0.05$) (Faculty 2014).

Physicochemical characteristics of Rhamnus prinoides leaf and stem powder were determined in comparison with reference standard commercial hops. The results obtained from Rhamnus prinoides for all out tar, delicate gum, hard gum and fundamental oil were discovered to be similar with thatof the values of different commercial varieties of hops obtained from the literature and with that of commercial hop used as control in the laboratory. Thus, Rhamnus prinoides can substitute the standard commercial hops for even beers brewed for commercial purpose.

2.8.1 Antioxidant Properties of Gesho

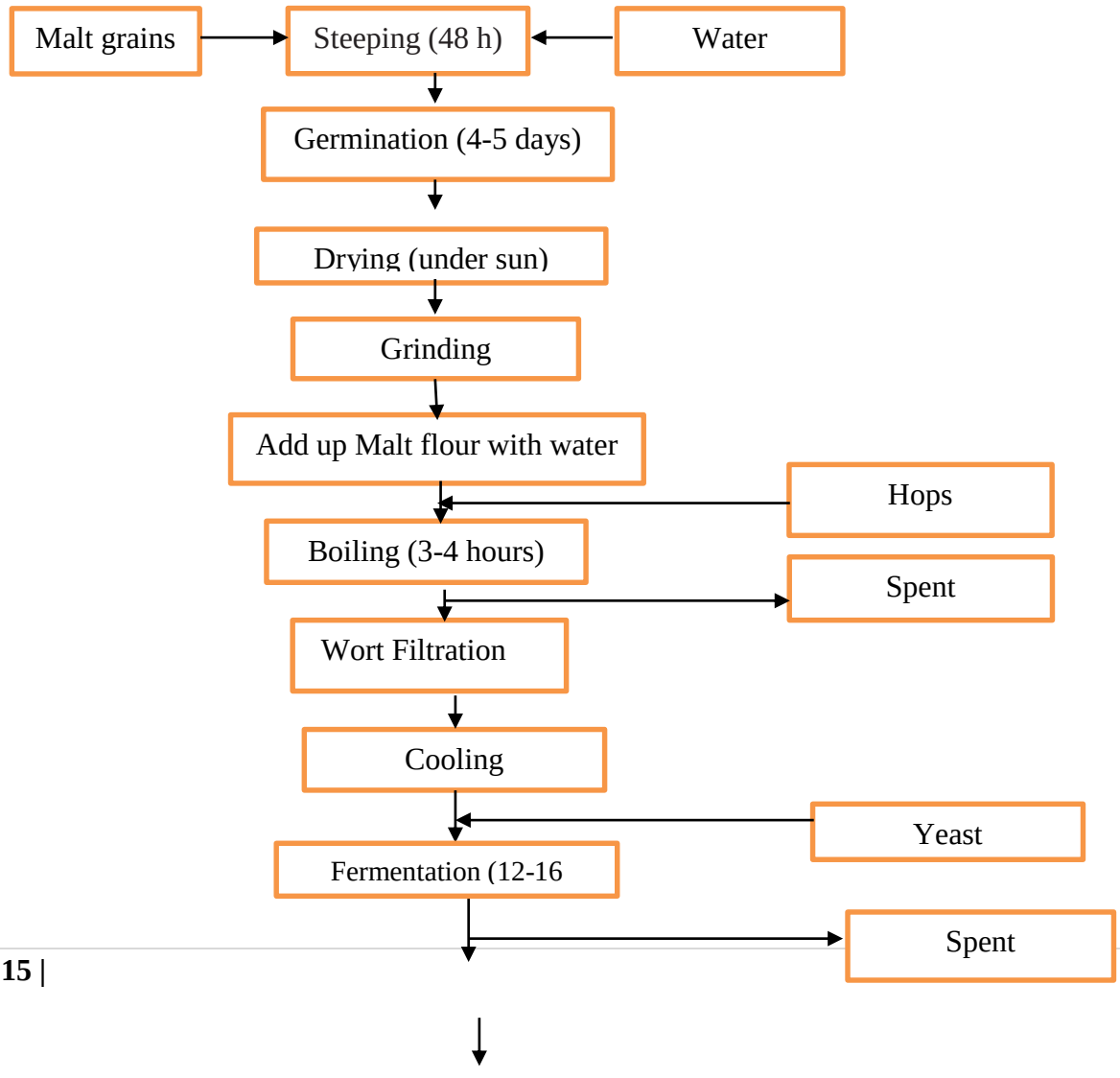
This crude extract was used for further investigation for potential antioxidant properties. The antioxidant proporties of 97% ethanolic extracts from leaves of R. prinoides has been reported previously (Anon 2017) and Methanolic and aqueous extracts from roots of R. prinoides have also evaluated for his or her her DPPH radical searching movement (Kimondo et al., 2019).

The AChE inhibitory activity of R. prinoides was found to be above that of some Portugese and Danish medicinal plants (Pillai et al. 2019).

Several literature works show that GESHO is endowed with this antioxidant characterics. And also, this antioxidant activity of Gesho was tested by researchers with their different method and different extracting agents. Consistent with the report of (Pillai et al. 2019) used hexane, chloroform, ester and methanolic as an extracting agents of leaves of R. prinoides, and (Anon, 2017), 97% ethanolic extract from leaves of R. prinoides. This 97% ethanolic extract from leaves of R. prinoides showed higher extremist searching action and lower IC50 esteem. This 97% ethanol may need more extractive power of active constituents than hexane, chloroform, ester and methansolic.

2.9 Beer brewing process

The four main ingredients used in all brewing processes are malted barley, water, yeast, and hops.



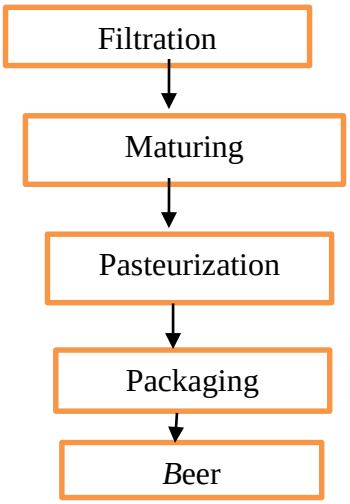


Figure 3: Beer brewing process flow diagram

2.9.1 Mashing Programs

The objectives of mashing are to make and extract into solution, fermentable sugars, amino acids, vitamins, etc., from malt (Lyumugabe et al. 2012). The mashing process are often divided into decoction and infusion way. Boths of them begin within the same way, bruised malt is poured into water at a temperature usually 37–52 °C, and this phase is named primordial mash. In subsequent phases, the temperature is being gradually increased reaching optimum for every range where ±certain groups of enzymes are the foremost active. At temperatures around 37 °C the foremost active are phosphatases, from 40–55 °C proteases, around 58–65 °C β– amylases are activated and α–amylases around 70– 72 °C for gelatinization/liquefaction and 78°C for mashing-off and malt enzyme inactivation. Within the infusion process, the mash is heated gradually according to the brewing scheme, and thus the mashing is directed in one container, which makes the tactic simpler and fewer energy consuming. With the decoction technology process, an area of the mash is heated separately from the rest, usually delivered to the boiling point. This procedure is repeated with the mash portions usually two or 3 times, then this technological process is named i.e. double or triple mash procedure. Infusion technology compared to the decoction one demands about 20 kind of energy and saves about 18 some time (Kryl and Gregor 2012).

2.9.2 Wort boiling

The functions of wort-boiling where describe by (Coldea et al. 2014). Among the most functions

- Maximum isomerization; usually occurs within 60 to 70 minutes of boiling and accounts for around 60% of the whole alpha acid present. Unfortunately, final conversion value of alpha acid into iso-alpha acid within the beer is around 40% thanks to lost throughout the fermentation and maturation process and is lost in any foam formed.
- To precipitate unwanted nitrogenous material; during the brewing process it is necessary to decrease the extent of high relative molecular mass nitrogen, which comes from the malt.
- Reducing Wort pH; it is vital to understand the required decrease in pH (generally around pH 5.0)
- To terminate enzymatic action; Enzymes believe their three dimensional structure for his or her the scope of 50-75°C) the tertiary design of the catalyst gets denatured, which they lose their movement. When the wort has arrived at edge of boiling over there's generally no remaining catalyst action. ? A large portion of the bounce oil volatiles are lost during a regular 60 to 90 minute bubble. Where late bounce character is needed in lager, alittle sum (up to twenty of the entire hop charge) of selected aroma hops are often added to the kettle5 to fifteen minutes before the maximum of the boiling process.
- To sterilize the wort; brewing raw materials like malt, hops and infrequently brewing water itself are infected by micro-organisms, and if wort boil microorganism are destroyed in the process to avoid spoilage of wort and beer. Increase in Colour; the color of wort increases during the boil.
- To evaporate excess water. Boiling water is removed as steam becomes thickening the wort. The quantity of water removed during the boil is linearly to speed of evaporation he major purpose being the evaporation of water to concentrate the wort. Additional methods wont to concentrate wort or gaining high gravity wort were: Parti-gyles, Sugar adjuncts, Weak wort recycling, Dewatering grains and High extract wort separation techniques like the Mash Filter. Traditionally conditions for wort boiling were a 90 minute boil with a minimum of 10% evaporation per hour. Wort boiling hop utilization are often suffering from several factors. Especially time, wort gravity, hop rate and wort IBU within the list below have a bigger impact than others (Justus 2018).
- Wort gravity: High gravity brewing (HGB) may be a procedure that employs wort of upper than normal (11-12°Plato) concentration, normally at a limit of 18°Plato. Consequently, a later stage dilution with water is required. This process may be a technological innovation

that has become popular thanks to product quality and economic advantages, like more efficient use of existing plant facilities, reduced energy, labor and capital costs, possible use of upper adjunct ratios, improved beer smoothness, improved flavor and haze stability and increased ethanol yields per unit of fermentable extract (Dragone et al. 2007).utilization decreases with increasing wort strength.

- Hop rate: using more hops per barrel will decrease efficiency.
- Temperature and Time: higher temperatures facilitate isomerization. Thermometer was the equipment wont to measure temperature. Wort boiling degradation or isomerization of α-acids to iso-α-acids which maintain the bittering quality of the α-acids and are soluble in beer unlike the primary addition of α-acids. The best method used to the amount of iso-α-acid in beer is measured by spectrophotometry. Longer duration in hot wort equates to higher utilization.

Hop utilization measures the percentage of α-acids added to the wort which are actually utilized (Elena et al. 2008)

% Utilization = (Iso-α-acids in beer)/ (α -acids added to wort kettle) ×100

Wort IBU: as you approach a saturation in bitter- ness, utilization will drop

It is now commonly accepted that the vast majority of the bitterness in beer derives from the α-acids of hops, in the main, by direct isomerization but to some extent by way of oxidation products (Univer 1944). It is usual to calculate the utilization of potential bitter substances from the equation:

$$\% \text{ Utilization} = \frac{\text{Bitterness units in beer} \times 100}{\text{Concentration of } \alpha\text{−acids added to wort}}$$

- To calculate hot-side hop utilization, we'd like to first calculate the dosage of α-acid, or IBU max. This assumes that IBU is only represented as iso-α-acid, which was addressed in the introduction. The equation is as follows:

$$\text{IBU max} = \frac{(W)(AA)(3865.4)}{V} \quad \text{(Justus 2018)}$$

- **Type of hop product:** Extraction of hops provides a simple and rapid method of effecting isomerization of alpha-acids to iso-alpha-aids. Utilization of alpha-acid when

using whole hops, hop pellets, or hop powder is typically in beteween 30-40%. Both pilot-scale and commercial trials with extruded hops have given utilization in the range 50- 60% without any effects on beer quality (Jaroslava and Bafrncov 2015). Extracts will have better utilization than pellets and whole cone hops.

Isomerization of hops

The most important chemical conversion occurring during wort boiling is the thermal isomerization of the α-acids into the bitter tasting iso-α-acids via an acyloin-type ring contraction (Wiley et al. 2008).

(Elena et al. 2008) suggested that the hop varieties with low alpha (Styrian Aurora) acids contents have a better utilization in wort kettle than high alpha hops (Worrior). This yield, increase with boiling time (60-120oc), wort concentration (12-14 op) and pH (5.1-5.8). Unfortunately, the high pH value is not a good decision because a lot of unlikable reactions should be developing for other wort chemical compounds (proteins and polyphenols). The traditional brewing process, hops are boiled with wort in a copper vessel for 1-2 hours, during which the resins go into solution and are isomerized to produce the bitter principles of beer. Nowadays, beer bittering is generally controllable in brewing practice, as modern commercial breweries can adjust hopping rates based on the α-acids or iso-α-acids content obtained by appropriate analytical techniques (e.g. IBU or HPLC (Hao et al. 2014 After aging, the item is weakened, as a rule with oxygen free water, to get lager with normal ethanol content (5%) or wanted liquor content (Jane 2015).

Kettles are fitted with a heating plant that heats the wort from mash temperature (65–78°C) to boiling temperature, which is simply above 100°C (at sea level) thanks to dissolved solids. Boil length can range from 30 to 120 min. Liquid adjuncts and hops are often added at various points during boiling. Following boiling, the solid material precipitated is removed and therefore the purified wort is cooled ready for fermentation. This process stabilizes the wort, removes unpleasant flavors, and extracts hop components that give beer its distinctive flavor. Utilization is all encompassing. It is a big picture quantification of the entire process, taking into account all factors affecting final bitterness level - not only the quantity of iso-alpha acids that are actually produced by isomerization, but also the loss of these bitter compounds to

various processes and conditions such as losses to trap (the insoluble precipitate that forms during kettle boiling, resulting mainly from protein coagulation), yeast, filters, degradation products, vessel and piping walls, etc Utilization was shown to decrease with increasing wort gravity (Hellhammer 2005).

2.9.3 Fermentation

Fermentation is a wort production process from sugar used yeast as catalyst and finally into ethyl alcohol and CO_2. In Western breweries, the fermentation process is started by selected yeast strains (S.cerevisae or S.carlsbergensis) and the fermentation time ranges between 12-16 days at 10-16 °C (Lyumugabe et al. 2012). East will fill in straightforward media which contain fermentable starches to give energy and carbon 'skeletons' for biosynthesis,sufficient nitrogen for protein union, mineral salts and at least one development factors. Yeasts additionally require atomic oxygen (Science 1982a). Along these lines it is critical to control pH esteem in the scope of 4.0-5.0 (Lin et al. 2012). In traditional lager brewing, pitching rate often ranges from 5–20 million cells/mL wort, but high gravity fermentation technologies has used high pitching rate of four or five times to the traditional pitching rate. The fermentation process reduced the sugar content obtained from wort kettle i.e. (the sweet wort). (Coldea et al. 2014) has investigated that fermentation optimization by compensating alcohol content (0-15 % vol.); genuine concentrate (0-10 % wt.); evident concentrate (0-10% wt.); wort (0-20 %) and relative thickness (0.95-1.05 g/cm3) were assessed utilizing the programmed analyzer Ferment star(Funke-Gerber, Germany) in 25 days of maturation and Wort gathered from the maturation tank in the day 13 arrived at an aging evaluation of 81.35 %. Liquor is a side-effect of yeast digestion and is harmful to the yeast; common preparing yeast can't get by at liquor fixations above 12% (Lee et al. 2015). After maturation, the item is weakened, ordinarily with oxygen free water, to acquire lager with standard ethanol content (5%) or wanted liquor content (Jane 2015).

2.10 Factors that affect beer quality

A. The quality of the crude materials utilized production: hops, barley malt, yeast and water. Malted barley is a key ingredient in most beers because it provides an optimized source of nutrition for yeast and a suite of color and flavor property. The resulting malts are analyzed for quality following by American Society of Brewing Chemists (ASBC) procedures for α-

amylase, β-glucan, diastatic power, free amino nitrogen (FAN), Kolbach index, malt color, malt extract, and viscosity.

B. wort heat treatment: strongly affecting final quality and overall fermentation performance. The conventional method of wort readiness is delicate bubbling includes the consistent expulsion of froth from the bubbling wort surface. These days, wort sanitization which is not so much difficult but rather more energy proficient are getting more famous.

C. Wort preparation: is the most un-well known in enormous scope lager creation, and simply includes blending of all crude materials in proper proportions that shows how much jumps, yeas and malted grain with the right volume of water.. We had to know the correct mixing rate of ingredients used to produce beer in stage of wort preparation. The lack of heat treatment can cause many -problems during fermentation, mainly caused by naturally occurring rich micro flora with an abundance of lactic and acetic bacteria, as well as other anaerobic.

3. Materials and Methods

3.1 Experimental frame work of the thesis

The research was conducted based on the following general diagram which shows main unit operations and all activities performed during the work.

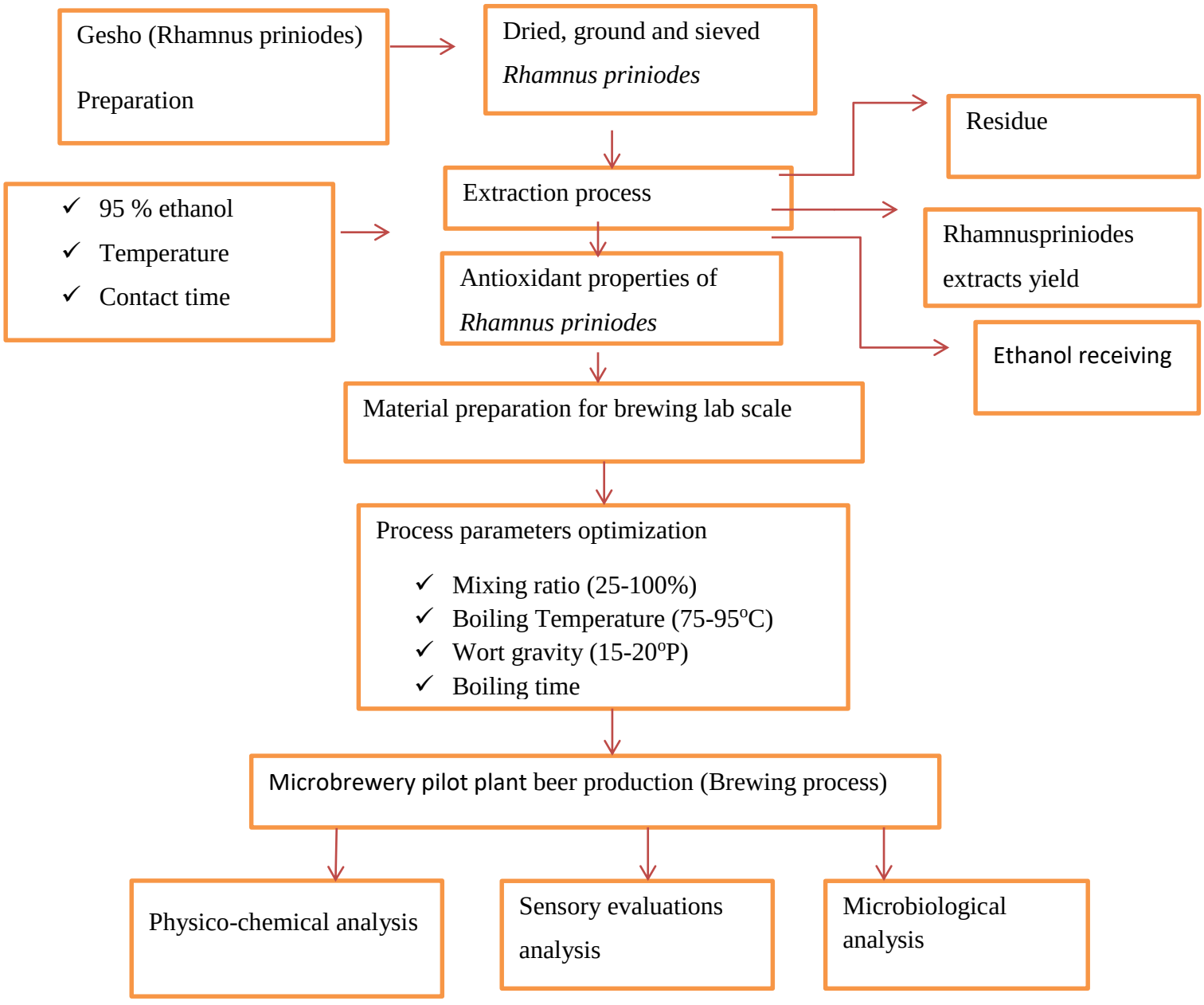


Figure 4: Conceptual frame work of the experiment

3.2 Materials and equipment

The essential crude materials that were utilized for the creation of brew are Rhamuns.priniodes (Gesho) gathered from certain spots around Addis Ababa,merkato market where as malt grain, yeast and water from raya distillery share organization plc. In addition, using the Rhamnus.prinoides leaves beer production laboratory work were performed in Michew, Ethiopia.

3.2.1 Materials used to determine the physico-chemical characteristics of Gesho

Disc mill DLFU Buhler(serial.no 20355505), Masher bath-R4, Kjedahl, Inkjel (serial no.4021467), Behr Distillation S3, Digital Thermostatic Water Bath, Oven (ser.no1401587), digital balance, condenser, sieves Plan sifter MLUA(BUHLER 10525178), hot plate Magnetic stirrer/rpl-1200, Different volume of glass beakers (100ml, 500ml, 1000ml,2000), Test tubes 25ml, Desiccators, Spectrophotometer (Genesys 10S UV-Vis), centrifuge-HERMLE 1400 (serial.no76150192), LG-Automatic Diacetyl (1.001 model) and Julabo Thermostatic Water bath (serial.no 10255353).

3.2.2 Materials/equipment used to optimize wort and used for beer manufacturing

Mash bath fitted with mechanical stirring, Mashing beakers of 600ml stain less steel, Porcelain spot plate, Filter funnel (165mm plastic or 150mm glass diameter), Microbrewery plant, PH-meter, Lovis2000M (Micro viscometer) and Density meter (DMA35)

3.2.3 Materials/equipment used to characterize beer

Refractive index(RI),density meter analyzer(DMA), CO2-meter(Haffmans In Pack 2000 CO2 meter), Foam stability tester (Nibem-TPH), Genesys (10S UV-Vis Spectrophotometer), Automatic shaker (RY-002 model 170615-1) and different size pipettes (2ml, 5ml, 10ml) and Haze meter (HAFFMANS Vot 90/25)

3.2.4 Chemicals & Reagents Used

95% ethanol, lubricant oil and distilled Water, methanol analytical grade and DPPH free radial(2,2-diphenyl-1-hydrazine), CaCl2, H2SO4, isooctane, concentrated hydrochloric acid(3NHCl), Octanol, brewing water, yeast (*Saccharomyces cervisa*), hop, Gesho and other additives like Vitamin C (ascorbic acid), calcium chloride, Sodium carbonate (Na_2CO_3), sulfuric acid (H_2SO_4), sodium hydroxide (NaOH), potassium hydroxide (KOH), petroleum

ether (C_6H_{14}), hydrochloric acid (HCl), and DPPH ($C_{18}H_{12}N_5O_6$). Some of them were bought from market while others were gained from BGI Raya brewery quality assurance laboratory.

3.3 Methodology

3.3.1 Raw material collection, drying and stabilizing

Gesho leaves and steams after they reach for harvest are harvested or collected by picking manually with hand or by cutting from the bottom with the shoot. After the leaves are collected, they're dried on sun for better quality. Sun dried leaves have good green yellowish color, but if the leaves aren't well dried or stored on shade, the leaves have dark brown to blackish color which indicates poor quality. Within the raya society, gesho plants are cut from the underside along with the shoot, and chopped with konchera and dried. For this study, 10 kg Gesho (Rhamnus priniodes) was collected from merkato market, addiss ababa Ethiopia 50 birr for 1 kg and removed impurities mixed with them using hands. The moisture of freshly harvested sample of Gesho leaves have moisture content of 75 -80%. They were allowed to dry on sun for 6 days (10.5% moisture content). Basedon (Geisenfeld 2013) strategy to build solidness the water substance of green jump cones is typically diminished from approx. 80 % after reap to less than 12 %. In addition to this, finally the dried gesho in this study once they were dry the sheets will then be further reduced in size by means of disk mills, After milling, the material was further dried on oven at 50 ^oC for 24hrs (to a moisture content 5%) and screened using sieves 0.2 mm in order to get the fines and to separate the core material from the surface material and packaged by clean polyethylene bag shown below in figure 6.

Figure 5: Disc mill DLFU Buhler

Figure 6: Gesho powder after milled and sieved

3.3.2 Gesho Extraction Methods

Gesho leaves and steams were milled through a 0.2mm screen using a Disc mill DLFU Buhle. 725 g of milled Gesho powder was placed in a 10 liter flask and mixed with 2.5 L 95% quality ethanol. The flask was closed by aluminum and stirred vigorously for 5hours and at 30ºC. The mixture was filtered through a Buchner Funnel and the residual Gesho where washed with 250 ml of ethanol. The filtrate was sorted for 24 hour in a refrigerator. The Gesho residue was returned to flask, and rinsed with 1.5 liter ethanol and mixed thoroughly before being left unstirred at 30^0C. After a further 24 hours the contents of the flask where filtered through WhatmanNo.1 filter paper and the residue washed with 500 ml ethanol. The two filtrates were combining then evaporated to dryness in a rotary evaporator under vacuum to remove the ethanol from the extracts by using the method of (Extraction, Hops, and Quality 1992) with little modification.

Figure 7: rotary evaporator under vacuum

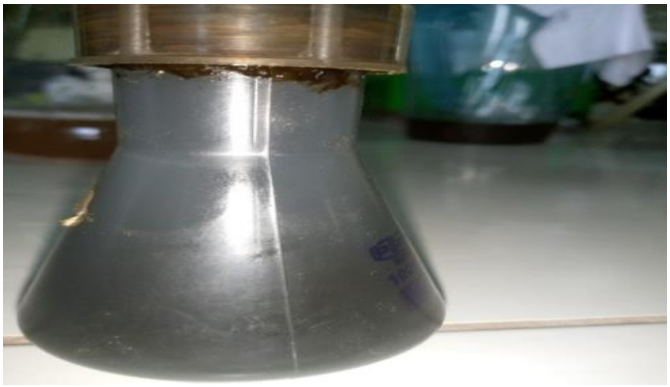


Figure 8: Extracted Gesho

3.4 Characterization of Gesho (Rhamnus prinoides**)**

The leaves and steams of the plant were dried for the estimation of moisture, ash, proteins, and fiber, fat and total carbohydrates. The samples were analyzed for composition analysis (moisture, proteins, fat, carbohydrates and ash) using the AOAC procedures and α-acids, soft resins, essential oils and antioxidant activity determination of extract were done using ASBC 1976, steam distillation and DPPH radical scavenging (IA), respectively.

3.4.1 Proximate analysis

Proximate analysis of Gesho powder and extracts such as moisture content, ash content, protein, total carbohydrate, and crude fat were done using the method of Association of Official Analytical Chemists (AOAC, 2000).

α-acid determination (hard resins)

To a 0.15 g of the samples was added 100 ml cold methanol in (Gallenkamp) flask shaker. The solution was then centrifuged at 2500 rpm for 20 min and the decanted supernatant was acidified with 0.002 N HCl and its absorbance at 355, 325 and 275 nm was determined using spectrophotometer (Pye-unicam sp6-550 uv/vis. Model).

the α-acid was determined using AOAC (2000) and ASBC 1976 methods:

$$\alpha\text{-acid (mg/L)} = 73.79\ (A_{325}) - 51.56\ (A_{355}) - 19.07\ (A_{275}) \quad (3.1)$$

Where A is absorbance reading at the specified wave length.

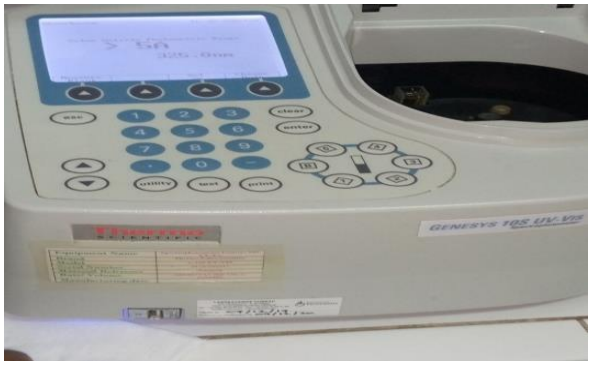


Figure 9: Spectrophotometer Genesys 10S UV-Vis

Resin Determination

Gesho (Rhamnus prinoides) resins were determined the using method described by (Okoro and Aina 2007).

Soft Resin: 20 grams of the gesho (Rhamnus prinoides) sample was dissolved in 20 ml of n-hexane thoroughly stirred and filtered using paper. Filtrate was dried to a continuing weight at 500C. The soft resins were calculated because the percentage of the first weight of sample dissolved within the n-hexane.

$$\text{Soft Resin (\%)} = \frac{\text{Weight of dried filtrate} \times 100}{\text{Weight of sample}} \qquad (3.2)$$

Essential oil determination

Gesho (Rhamnus prinoides) essential oils were determined by using the tactic of steam distillation which is that the commonest method within some modification of (Jane 2015). Sample preparation method wont to obtain gesho (Rhamnus prinoides) volatile oil. The essential oils of varied gesho samples were isolated by steam distillation employing a Clevanger apparatus. The experimental conditions were as follows: 20 g of dried and milled Gesho cones (ground during a mortar with pestle) were weighed into a 2,000-ml distillation flask. Next, the quantity of deionized water 550 ml was added, and therefore the mixture was distilled for 4 h. Obtained essential oils were collected from the condenser. The volatile oil was calculated because the percentage of the first weight of sample.

Ash Determination

At heat i.e. 5500C all organic compounds are burnt off. Minerals (ashes) are less volatile than other food compounds and aren't destroyed at this heat. The inorganic material remained is named ash (mineral)

Apparatus:

a. Muffled furnace (CARBOLITE AAF 1100)

b. Crucibles with lids

c. Desiccator

d. Analytical weighing balance

Ash content decided consistent with method (Naeem et al. 2018). Took clean crucibles at temperature and weighed them without their lids .The dry sample (free of moisture) of three g Gesho powder was incinerated at 550 °C on the crucibles and placed with lid cover transferred them to the Muffled furnace for 3 h) until light whitish grey ash was obtained then the ash content was calculated supported the subsequent formula:

$$\text{Ash } (\%\frac{W}{W}) = \frac{M1-M2}{M3} \times 100 \quad (3.3)$$

Where M1 = mass of empty dish and ash, g; M2 =mass of empty dish, g; M3 = mass of the dry sample, g.

Moisture content determination

Dampness content assurance Sample was gauged and set in hot air stove withset temperature at 130 0C for 60 minutes (an hour). The weight after drying is used to determine moisture content of the sample.

Apparatus:

a. Hot air oven

b. Moisture dishes

c. Desiccator

d. Plastic spatula

e. Analytical weighing balance

Method:

The method was supported drying a 2 g sample in an oven at 130 °C for 1hr (60 min) to a continuing weight and determining moisture content by the load difference between dry and wet material.

Figure 10: oven

The percentage of moisture content (wet basis) was then computed as follows:

$$\text{Moisture } (\%\frac{W}{W}) = \frac{W0 - Wf}{W0} \times 100 \qquad (3.4)$$

Where W0 = weight of the wet sample, g; W f = weight of dry sample, g;

Determination of total proteins:

Protein was determined by the Kjeldahl method.

The method was supported drying a 2 g sample in an oven at 130 °C for 1hr (60 min) to a continuing weight and determining moisture content by the load difference between dry and wet material. Protein decided by the Kjeldahl method. All nitrogen is converted to ammonia by digestion with a mix of concentrated vitriol and concentrated phosphoric acid containing potassium sulfate as a boiling point raising agent and selenium as a catalyst. The ammonia

released after alkalization with caustic soda is steam distilled into boric acid and titrated with vitriol. Digestion: 0.5gram of gesho samples were taken during a tecator tube and 6ml of acid mixture (5parts of concentrated orthophosphric acid with 100 parts of concentrated sulfuric acid). Was added, mixed- thoroughly and a 3.5ml of 30% oxide was added step by step. As soon on the grounds that the vicious response had halted, the cylinders were shaken for a couple minutes and put back to the rack. A 3.00g of the impetus combination (ground 0.5g of selenium metal with 100g of potassium sulfate) was added into each cylinder, and permitted to look for about 10min before assimilation. At the point when the temperature of the digester came to 370oC, the cylinders were brought down into the digester. The absorption was proceeded until a straightforward arrangement was acquired, about 1h.The cylinders inside the rack was moved into the tubes within the rack was transferred into the fume hood for cooling, a 15ml of deionizedwater was added, and shaken to avoid precipitation of sulfate within the solution.

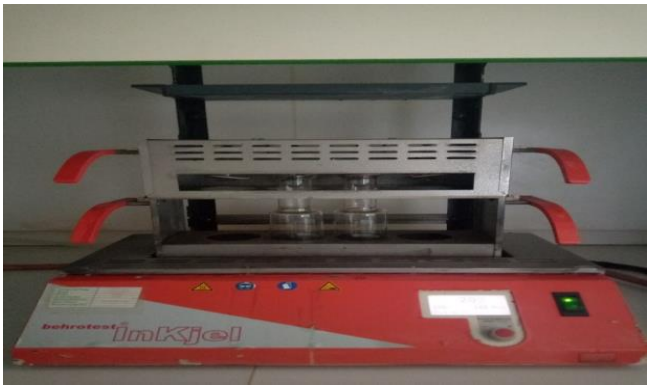


Figure 11: Digestion

Distillation: A 250ml conical flask containing 25ml of the boric acid receive. The processed and weakened arrangement was moved into the example compartment of the distiller. The cylinders were washed with two parts of about 5ml de-ionized water and furthermore the washes were added into the arrangement. A 25ml of 40% sodium hydroxide arrangement was added into the compartment and washed down with a minuscule low measure of water, stoppered and furthermore. A 100ml arrangement of the example was refined, at that point the collector was brought down so as that the tip of the condenser is over the outside of the distiller.

The refining was proceeded until a whole volume of 150ml is gathered. The tip was washed with a few milliliter of water before the recipient was eliminated.

Figure 12: Distillation

Titration: The distilled solution was titrated with 6ml 0.1N HCl using indicator solution (mixture of bromocresol & methyl red) to a reddish colour.

Mg nitrogen in the sample = V×N×14

g nitrogen sample/100g sample = mg nitrogin×100/mg sample

The percentage of Protein was calculated as:

$$\%\text{Nitrogen} = \frac{(V-vb)\times N\times 14}{W}\times 100 \qquad (3.5)$$

Crude protein (%) = Total nitrogen (%) * F

Where, V=Volume of hydrochloric acid consumed to neutralize the test material (ml) Vb=Volume of the acid consumed to neutralize the blank

F=Conversion factor of total nitrogen to crude protein (6.25)

14=Equivalent weight nitrogen N= Normality of standard hydrochloric acid.

Determination of fat content:

The fat/ oil content of the sample decided by treating about 10 grams of Rhamnus prinoides sample was weighed and extracted with Petroleum Ether in an extraction apparatus for 16 hours. The extract was dried, cooled in desiccators, weighed and therefore the mass was recorded. The half of fat decided using an equation.

$$\% \text{ of fat} = \frac{(wt.of\ Soxl\ heat\ flask\ with\ extracted\ fat) - (Wt.of\ empty\ Soxl\ heat\ flask) \times 100}{Weight\ of\ sample\ (g)} \quad (3.6)$$

Determination of fiber content:

5 grams of Rhamnus prinoides sample was extracted using Petroleum ether. The fat free material was transferred during a beaker and 200 ml of dilute vitriol was added and boiled. Whole boiling acid during a flask is connected to condenser and boiled for half-hour. The flask was removed, filtered and washed thoroughly with boiling water followed by washing in boiling caustic soda and again refluxed for half-hour. The contents were separated and washed with bubbling water and in the long run washed in ethanol. The buildups were dried and burned in mute heaters at 660°C and hence the cauldron close by debris was gauged and level of fiber was determined. % of crude fiber

$$\% \text{ of crude fiber} = \frac{(Wt\ .of\ crucible\ with\ before\ washing - Wt.of\ crucible\ after\ washing) \times 100}{Weight\ of\ sample} \quad (3.7)$$

Determination of total carbohydrates

The percentage of total carbohydrates was calculated by the difference method according to this equation (100 - Total moisture + Total ash + Total protein + Total fat) the percentage of carbohydrates was calculate (3.8)

3.4.2 Gesho (Rhamnus prinoides) extracts characterization
PH value of the extract

The pH value of Gesho extract was directly measured by pH meter at room temperature.

Figure 13: PH meter

Specific gravity of the extract

The sample was filled into graduate (50 ml) and its temperature was recorded. Hydrometer was accustomed measure the specific gravity of the gesho extract at the recorded temperature. Hence, the density of the gesho extract is set using specific gravity. Alternatively the subsequent method was used.

Weight of beaker measured in (g)

Weight of Gesho extract with beaker in (g) Thus, Weight of extracts = Weight of Gesho extract with beaker in (g) - Weight of beaker measured in (g), Equal volume of water was measured.

Weight of water with beaker in (g)

Weight of water= Weight of water with beaker in (g) – weight of beaker (g)

Then, the particular gravity of the Gesho extricate was acquired by taking the proportion of green tea concentrate to mass of water.

Determination of Antioxidant Activity of Gesho

The estimation of the DPPH revolutionary searching action was performed by philosophy depicted by Brand-Williams et al (Garcia et al. 2012). To assess the subterranean insect oxidative action of explicit mixtures or concentrates, the last mentioned are permitted to respond with a steady extremist, 2,2-Diphenyi-l-picrylhydrazyl (DPPH) during a methanol arrangement (Garcia et al. 2012).

To evaluate the ant-oxidative activity of specific compounds or extracts, the latter are allowed to react with a stable radical, 2,2-Diphenyi- l-picrylhydrazyl (DPPH) during a methanol

solution (Cuvelier and Berset 1995). The decrease ability of DPPH revolutionary is chosen by the reduction in its absorbance at 5l7 nm, instigated by cancer prevention agents. The abatement in absorbance of DPPH extremist is caused by cell reinforcements, because of the response between cancer prevention agent particles, and radicals, which ends up within the scavenging of the novel by hydrogen donation. It's visually noticeable as a change in color from purple to yellow. Hence, DPPH is typically used as a substrate to guage the antioxidative activity. The extremist rummaging movement of the concentrate was estimated in terms of hydrogen giving or extremist searching capacity utilizing the steady revolutionary DPPH. At the point when the DPPH revolutionary is searched, the shade of the response combination changes from purple to yellow with diminishing of absorbance at frequency 517 nm. Determination of DPPH radicals scavenging activity was estimated with the tactic used by (Cuvelier and Berset 1995). Percentage inhibition DPPH radical scavenging was calculated using the equation:

DPPH radical scavenging (IA) % = $\frac{A_{control} - A_{sample}}{A_{control}} \times 100$ (Erasto et al. 2008) (3.9)

Where, a sample is the absorbance in the presence of the sample of the extracts and a control is the absorbance of the control samples out of extract

Determination of Antioxidant Activity of the Extract with the DPPH Radical Scavenging Method using a UV/Visible Spectrophotometer

A) Preparation of reagent and blank solution

DPPH (0.004%) was prepared by dissolving 0.01 g of DPPH in 250 mL of methanol. Then, 4 mL of this solution was added to the test tube containing 1 mL of methanol and mixed well.

B) Preparation of standard solution

The standards used for this study, were ascorbic acid solutions without extract concentration. Two (2.0) g of this acid was dissolved in to 40 mL of methanol to prepare a stock solution (50 mg/mL) of ascorbic acid. Successive dilutions were made from this stock solution to obtain solutions with concentrations 1.0, 2.0, 4.0, 6.0, 8.0, 10.0 and 12.0 mg/mL by mixing 20, 40, 80, 120, 160, 200 and 240 µL of standard ascorbic acid with 980, 960, 920, 880, 840, 800 and 760 µL of methanol solvent respectively in different test tubes and 4 mL of 0.004% DPPH was added to each sample concentrations in the test tubes and the mixtures of the standards were incubated for in the dark for 30 mins at room temperature. The absorbance was measured after 30 min incubation, against the blank at 517 nm reading using a UV/Visible Spectrophotometer.

C) Preparation of sample solution

50 mg/mL sample stock solution of the extract was prepared by 2.0 g of extract into 40 mL of ethanol. From each stock solution, 20, 40, 80, 120, 160, 200 and 240 µL of sample solution were withdrawn and mixed with 980, 960, 920, 880, 840, 800 and 760 µL of methanol Serial dilutions were made from this stock solution to obtain solutions with concentrations of (1.0, 2.0, 4.0, 6.0, 8.0, 10.0 and 12.0 mg/mL) respectively in isolated test tubes and4 mL of 0.004% DPPH was added to each sample concentration in the test tubes and mixed well. After that the mixtures samples were incubated also 30 min in the dark at room temperature. The absorbance was measured After 30 min incubation against the blank (the same mixture without the ascorbic acid) at 517 nm reading using a UV/Visible Spectrophotometer.

Determination of Antioxidant Activity

Scavenging capacity of the 1, 1-Diphenyl2picrylhydrazyl (DPPH) free radical was used to determine the antioxidant activity of samples as detailed by (Cuvelier and Berset 1995). Results were expressed as the inhibition dose for 50% of reduction in the initial levels of DPPH free radical. IC50 value of an antioxidant is the concentration of the antioxidant required to give 50% inhibition of the DPPH assay. It was named because the ‘‘Inhibition Concentration” (IC50) and determined by sequential sample dilutions versus the antioxidant activity scavenger percentage as described by (Cuvelier and Berset 1995).

Experiments were carried out according to (Proestos and Komaitis 2008) with a slight modification. The antioxidant capacity of an unknown sample was determined and the DPPH solution without sample solution was used as a control. The inhibitory concentration 50 (IC50 or EC50) values of extracts (the concentration that causes a decrease in the initial DPPH concentration by 50%) was calculated by using the calibration %DPPH radical scavenging concentration (g/ml) and expressed in (g/ml). Results were compared with ascorbic acid which used as standards. A lower value of IC50 represents higher antioxidant activity. The IC50 values were calculated from graphs by plotting extract concentrations vs. percentage inhibition of DPPH radical using Microsoft Excel. Each experiment was administered in triplicate and therefore the averages of the three values were wont to calculate IC50 values.

Standard deviation was calculated for each concentration from the three replicate values of the experiment. The free DPPH radical percentage of standard ascorbic acid and ethanol extract of

Gesho were calculated using equation (3.9) list above. The IC50 values of the standard ascorbic acid Table 4.2 and that of the sample Table4.3 was determined from the plots as indicated in Figure 4.2 and Figure 4.3. Comparing this IC50 values we can see that the extract manifested the strongest capacity for neutralization of DPPH radicals. According (Krofta, Mikyška, and Hašková 2008) the highest antioxidant activities of 70 to 80% .IC50 value (concentration of substrate that causes 50% loss of the DPPH activity) of hop ethanol extract (49.8) was found to be similar to ascorbic acid. On the contrary, vitamin C IC50 value was less than of hop extracts. The higher the antioxidant capacity, the lower is that the value of EC50.

3.5 Mixing ratio of Gesho to Hop

Raya brewery uses 13.5 kg of CO_2 extract hop per 1640 hectoliter of sweet wort. The alpha acid value of CO_2 extract hop is 40 % while Ethiopia's Gesho alpha acid value is 11.5 %. 500 ml of sweet wort sample was taken, and the amount of CO_2 extract hop and Gesho to produce 500 ml of sample were calculated to be 0.04115 and 1.5064 grams respectively. The amount of hop and Gesho and hop used were calculated accordingly.

Table 3: Mixing ratio of Rhamnus prinoides (gasho) to hops

Gesho to hop mixing ratio (%)	Gesho used (g)	Hop used(g)
100:0	0.9415	0
62.5:37.5	0.0154	0.077
25:75	0.377	0.03086

3.6 Experimental procedure

3.6.1 Design of experiments (DOE) and optimization

Experimental runs were done through Design-Expert software version 11. (Box- Behnken). The first task before conducting the experiments was selection of potential parameters to be varied. The four main factors selected in this study were wort boiling temperature, wort gravity, wort boiling time and Gesho to hop mixing ratio with their three levels. The levels of the selected factors are determined from the literature research.

Table 4: Factors and Levels of variables used for General Factorial Design

Factors	Levels
Temperature(℃)	75-95
Wort boiling Time (min)	45-95
Wort gravity ($°plato$)	15-20
Gesho/hop Mixing ratio (%)	25-100

The experimental result was analyzed using Box- Behnken (BBD) of Design-Expert software to develop, 25 experimental runs were generated (Table 3.3). Each of these four factors was evaluated for their effect at the specified levels and based on the result of the beer analysis, the optimum parameters were used to rework the experiment for the validation of the optimum yield. Furthermore, this design of the experiment helps us to optimize of process parameters using Response Surface Methodology (RSM). Significance of the result was set from analysis of variance (ANOVA).

Table 5: Randomized Experimental Runs

		Factor 1	Factor 2	Factor 3	Factor 4
Std	Run	A:mixing ratio	B:temperature	C:time	D:wort gravity
		%	℃	Min	^{o}p
19	1	25	85	90	17.5
1	2	25	75	67.5	17.5
17	3	25	85	45	17.5
7	4	62.5	85	45	20
13	5	62.5	75	45	17.5
3	6	25	95	67.5	17.5
6	7	62.5	85	90	15
11	8	25	85	67.5	20
9	9	25	85	67.5	15
4	10	100	95	67.5	17.5

18	11	100	85	45	17.5
15	12	62.5	75	90	17.5
16	13	62.5	95	90	17.5
2	14	100	75	67.5	17.5
14	15	62.5	95	45	17.5
20	16	100	85	90	17.5
12	17	100	85	67.5	20
10	18	100	85	67.5	15
5	19	62.5	85	45	15
24	20	62.5	95	67.5	20
22	21	62.5	95	67.5	15
8	22	62.5	85	90	20
23	23	62.5	75	67.5	20
21	24	62.5	75	67.5	15
25	25	62.5	85	67.5	17.5

3.7 Beer production process and controlling process factors

3.7.1 Malt analysis

Finished malt is analyzed by making a laboratory mash which provides a sign of brewing performance. The physio-chemical analysis of malts was performed in B.G.I raya brewery Share Company Using the mash bath for the analyses of original extract, colour, wort viscosity, pH .etc. Also moisture and humidity were determined by the moisture analyzer equipment .it was done using mashing program described below and its physio-chemical analysis done by index of refraction plus by following malt analysis format and its results were described within the result and discussion part.

3.7.2 Wort production

The method of wort production was done according method described (Healy et al. 2017) with slight modifications. The malt utilized in these trials was produced commercially and contained 1.5-2% of nitrogen. Fine grist was prepared during a Buhler disc mill at 0.7 mm, this mashed malt where mixed. initiative is preparing the three wort samples from 100% malt wort and

water (1:4 ratio), using an infusion mashing regime and therefore the wort concentrations were set (15 -20 °plato). 50 g malt in 200 mL hot liquor (Liquefaction). Place in automatic mashing apparatus at 45°C with constant stirring for 30 min (gelatinization stage). Then Add 100 ml water and therefore the temperature was increased 70 0C with(1°C)⁄min, (scarification stage) and keep at 70oC for 1 hour. Finally, mashing-off temperature (78°C) achieved and therefore the whole portion of mash was cooled to temperature in 10 – 15 minute. And made up to 450 g before filtration. The mash was filtered through Whatman No 597 ½ paper using 100ml of water during filtration for dilution. Sweet wort samples were taken for analysis after the primary 100 ml dilution by water. Original extract and relative density was measured by automatic density meter.

Figure 14: Mash bath

Figure 15: Density meter analysis (DMA 35)

3.7.3 Wort boiling

500ml Wort was obtained from mashing of 78oc affect the various parameters: temperature, wort gravity, time and Rhamuns.priniodes (gesho) to hop ratio to urge hopped boiled wort was

administered during a 2000 ml Erlenmeyer flas . At this stage 1.55 g (extracted Gesho and hop) which suggests Gesho 0.942g and hop 1.506g containing (hopped with Magnum hop variety (40% α-acids from BGI Raya Brewery Factory) ratios at the ranges (25-100%) was administered in 400 mL bottles for (45-90 min) and therefore the wort concentrations (15-20°plato) at temperature ranges (75-95℃). hops were obtained from Czech Republic and Gesho from Merkato, adissababa, Ethiopia. it had been also cooled in order that proteins which are sensitive to lower temperatures would be separated out of solution and will be far away from the wort. Then the sample was immediately chill right down to 10oC to stop microbiological contamination. From theobtain final hopped wort sample was taken for analysis. Cooled wort was stored at 20°C for further analysis. . Hot wort was cooled to 12-16oC where yeast are often safely added without being destroyed by heat and therefore the cold wort goes to next step fermentation process.

3.7.4 Fermentation

Fermentation of 500 mL of every wort was administered during a 2000 ml Erlenmeyer flask inoculated yeast from Microorganism collection of Microbiology Lab, Department of microbiology BGI raya brewery sc. was performed at 12-16 °C in 500 mL glass measuring cylinders stoppered with foam and covered with cotton. For 500 ml each wort was add 80 gram of yeast (12x 106 cells/mL) at 10°C and transferred to a static 16°C incubator. the entire fermentation process was performed at temperature . Fermentation was followed by measuring mass loss thanks to CO2 volatilization over time and fermentation was deemed complete when mass stabilized. Following fermentation, beers were clarified by centrifugation to get rid of yeast and haze, cold-stored for twenty-four h to stabilize, then re-centrifuged to yield bright beer. Samples were taken for analysis and ethanol analysis. Agar brewing strain of baker's yeast utilized in this study originated from Microorganism collection of Microbiology Lab, Department of microbiology.

$C_6H_{12}0_6 + O_2 + \text{Yeast} \longrightarrow C_2H_5OH + CO_2$

Glucose + oxygen + yeast ⟶ Ethanol +carbon dioxide

Media Preparation

The stock culture was maintained on malt – agar slants at 4°C. Yeast propagation was performed within the 12oBx all – malt wort in an incubator at 28oC. the specified inoculum size was prepared by centrifuging the culture above at 4000 rpm at °C for 10 min. All fermentations were administered during a bioreactor containing 500 ml of sterile 24oBx wort. the first fermentation was conducted at 17oC and completed when 85% of the reducing sugars had been consumed. The entire fermentation process (5 days) was monitored (temperature, pH, alcohol content, apparent and total wort extract). Alcohol content, wort extract (real and apparent wort extracts), and density were evaluated using the automated analyzer Fermentostar (Funke-Gerber, Germany). This device was wont to monitorize the beer fermentation. Fermentostar can register values for alcohol content (0.00-15.00 % vol.); real extract 0.00-10.00 % wt.; apparent extract 0.00-10.00 % wt.; wort 0.00-20.00 attempt to density 0.95-1.05 g/cm3 (Coldea et al. 2014). During the anaerobic phase, yeast cells convert sugars to ethanol and CO2 . 'Bottom fermentation' was conducted: the temperature during primary fermentation process was 8-10°C, for five days.

Figure 16: Beer Fermentation Process

3.8 Characterizations of produced beer

Product characterization is that the comparing of the gained product with the quality and similar product of various brands. The obtained final composition of beer specially correlated with the initial extract content of wort. Extract may be a weight percent of dissolved material within the wort solution. Characterization of beer (Original gravity, apparent and real extract, apparent degree of fermentation and ethanol) were performed by index of refraction beer analyzer. Total polyphenols, Calcium, pH, bitterness, vicinal dactyl ketone (VDK) and colour

were all measured consistent with the present European Brewery Convention (Analytical EBC 1987). (Analytical EBC 1987).


Figure 17: Finished beer using Refractive index beer analyzer (RI)

3.8.1 Parameters used for Characterization of beer Quality

Determination of bitterness in a beer

10ml of test sample (degassed without a loss of foam) was pipetted into conical flask with glass stopper which was acidified with 1ml of 3N HCl then add 20ml of isooctane (2, 2, 4-trimethylpentane), Stopper the flask tightly and place it in mechanical shaker, Shake vigorously at 600 rpm for 15minutes at temperature then allow decanting for 10 minutes until the supernatant organic phase will create. Measure the absorbance of isooctane at 275nm in a1.0 cm quartz cell or cuvette using Isooctane as blank. The optical density is expressed in terms of European brewing convention bitterness units as follows.

Bitterness (BU) = $50*Abs_{275}$ (EBC) 3.10

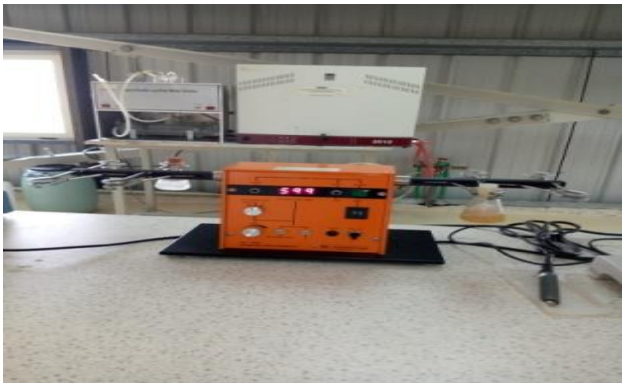


Figure 18: Mechanical shaker

Determination of specific gravity using Pycnometer or Density bottle

Principle: ratio of weight of beer to same volume of water

Weigh empty bottle, Weigh bottle with 50ml beer and Weigh bottle with 50ml water.

N.B: All measurement at 20 ^{o}C

$$S.G. = \frac{(Weight bottle + beer) - (Weight bottle)}{(Weight\ bottle + water) - (Weight bottle)} \quad 3.11$$

Figure 19: density measuring pycnometer

Color determination

Colour of the finished beer was measured at 430 nm by a UV–vis spectrophotometer (Juri et al. 2015) according to the EBC method . Colour was expressed in EBC units.

Colour was calculated using equation (3.12):

$$C = A_{.430} \times f \times 25 \quad (3.12)$$

Where C indicates the colour (EBC), f indicates the dilution factor and A indicates the absorbance at 430 nm.

pH measurement

The pH estimations were performed by a pH meter (PB-11, Sartorius, Germany). Prior to pH estimation, the CO_2 was taken out from the brew tests by shaking at room temperature (18 – 20°) for 5 seconds

Determination of Ca^{2+}

Pipette 10 ml of degassed sample in to 250 ml conical flask. Add 90 ml distilled water and add 3ml of 8N potassium hydroxide solution mix well. Add Calcon-red indicator and titrate with 0.01M EDTA until the colour changes from pink to grey blue. Result could be calculated according the formula:

$$Ca^{2+}\ (ppm) = V_{0.01\ M\ EDTA} X 40.08 \qquad (13)$$

N.B: V=Volume

Figure 20: Conical flask 250 ml

Determination of CO2 in beer

The CO2 content was communicated in grams per liter. All estimations were completed in tests of packaged brews, in three-fold. Results were communicated as normal qualities CO2 Content (g/l).

Figure 21: Haffmans InPack 2000 CO2 meter

Determination of polyphenol in a beer

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 add 0.5 ml of 0.5% ferric reagent and 8ml of CMC/EDTA was added except 0.5% ferric reagent wasn't added to the blank. Response time allowed for 10min then Measure the absorbance of the sample at 600nm in a1.0 cm glass cuvette against the blank. The Expression of result could.be;-

Polyphenol (pph) = $820*Abs_{600}$ (EBC).

Figure 22: Volumetric flask (25 ml)

Determination of VDK

Sample preparation:

Add 100 ml of beer into the distillation flask and begin distillation. Control the heating rate carefully to stop over foaming. The available period under gentle heating should be a minimum of 6min until the primary drop is collected into the receiving cylinder. Collect 25 ml of the distillate within 8-10 minutes and blend thoroughly .Pipet 10 ml of the distillate into 50 ml volumetric flask with glass stopper. Add 0.5 ml of 1% O-phenaylenediamine mix and place the flask 20-30 minutes in dark place .add 2ml of 4N HCl and blend the sample thoroughly then measure the sample at 335nm within 20 minutes against the blank.

Blank preparation: Add 10 ml of water into 50 ml of volumetric flask with glass stopper. Add 0.5 ml of 1% O-phenaylenendiamine mix and place the flask 20-30 minutes in dark place .add 2ml of 4 N HCl mix the sample thoroughly and measure at 335nm within 20 minutes

Result: VDK (ppm) =Abs $_{335}$ x 2.7(Factor)

Haze Determination

In an especially problematic sort of haze, β-Glucans has been shown to be the divisor. These are the so-called "Invisible" or "Pseudo" hazes that during a 90 ° (permanent haze) scatter haze meter usually offer high readings, while the beer is bright to the attention for all intents and purposes.

Figure 23: Haze meter

Head

Head is one among the standard parameter of beer. Different brews of Rhamnus prinoides (gesho) beer will vary in product quality counting on the ratio and quality of raw materials used and initial wort gravity. The necessity arises to use a taste panel to work out the standard and acceptability of the beer. Therefore, my beer is within the expected range.

Figure 24: beer foam

Sensory analysis

The sensory research was performed on a population of 15 individuals. A sensory study of clarity, colour, flavor, taste and foam was conducted on a population of 15 people. The findings (Table 6) and therefore the summary table were expressed as a percentage below. Clarity: 2/3 agree;

A. Color: 3/3 extremely agree;
B. Flavor: 4/4 excellent;
C. Taste: 4/5 agree;
D. Foam: 8/10 agrees.

They all enjoyed our gesho (Rhamnus prinoides) beer, with the exception of the taste criterion at the mouse feeling, which wasn't excellent. The findings are alluded to in Table 4 above. Our Gesho beer was fine, with a mark of 21/25. My gesho (Rhamnus prinoides) beer was enjoyed by all 15 people. Nevertheless, they found that the froth which was very strong at the opening. The results are mentioned within the table above. The standard control meets the standards. that's why we would like to match the chemical characteristics with those of traditional beer,

Microbiological analysis

Microbiological research, there was no microbiological analysis for the subsequent reasons. Therefore, all hazards of physical, chemical and biological origin were reduced to the limit. we've endeavoured to enforce and obey the principles of private hygiene, clothing, climate,

premises, equipment, etc. in the least stages of production from raw materials until the finished product. On the opposite side, we will examine the subsequent germs if microbiological research is to be performed: Lactobacillus, Pediococcus, Acetobacter, Zymomonas mobilis (a) Gram-positive bacteria (Science 1982b)

1. Catalase-negative rods, e.g. Lactobacillus

2. Catalase-negative cocci or cells in cubical packets, e.g. Pediococcus

(b) Gram-negative bacteria

1. Capable of oxidizing ethanol; grows well on glucose but not lactate; if motile, then polar flagellation, e.g. Acetomonas

2. Capable of oxidizing ethanol grows well on glucose and lactate; if motile, then peritrichous flagellation, e.g. Acetobacter Which isn't harmful but contaminates the beer and may influence the taste and smell of the beer. Additionally, the pH of 5.2 isn't conducive to the assembly of some pathogenic microorganisms, like yeasts, which protect our beer against other potential contaminating microorganisms. Finally, there's bitterness (21.2 BUs) that forestalls the assembly of harmful microorganisms there. However, as a precautionary measure, microbiological research should are administered, and therefore the efficacy of the preventive measures could are checked.

Table 6: Overall optimized Gesho beer quality assessment in scores

Assessment	Total score	Score
Excellent	23-25	21
Good	20-22	
Satisfactory	14-19	
Poor	<13	

3.9 Optimizing operating parameters using BBD

The optimal conditions for analytical bitterness unit were determined using box behinken-Design. The 4-Factors and 3-Levels experimental design were wont to investigate and validate beer production parameters affecting the responses. A summary of the blending ratio, wort

gravity, wort boiling time and temperature with 25 experiments were evaluated. The software Design-Expert (Trial Version 11.) was employed for experimental design, model building data analysis, Statistical significance of the model and model variables decided at a 5% probability level ($p < 0.05$).

4. Result and discussion

4.1 Characterization of Rhamnus prinoides extracts

In this research, the characterization of Gesho extracts, like pH, in reference to relative density Water analysis and proximate analysis were administered. It had been after the Green Powder of Gesho. The pH decided by a pH meter, extracted from water and cooled at temperature and obtained the worth as 4.5. The precise gravity of the extract decided on the idea of the empty beaker and sample measurement values (extracts) also because the quantity of water adequate to that of the extracts. Therefore, the essential gravity of the extracts = water mass/ extract mass, which is 0.8199. Relative density may be a substance's heaviness relative thereto of water, and it's expressed without units.

Based on the technique, the proximate analysis of gesho (Rhamnus prinoides) such as moisture content, ash content, protein, fat and total carbohydrates including crude fibers was calculated. Specified under this manuscript's materials and methods. Below is the table showing the description of the values of the proximate analysis. The behavior of antioxidants is also determined by the methods of the characterization of Gesho extracts, such as total resins, essential oils and proximate analysis and antioxidant activity, was carried out in this report. After 95 % ethanol was extracted from the green powder of Gesho and cooled at room temperature, the total resins were measured using steam distillation in accordance with the AOAC and ASBC 1976 standard method of essential oil was determined using steam distillation and obtained the value as indicated in the table below. The proximate analysis of gesho extracts such as moisture content, ash content, protein, fat and total carbohydrate including crude fibers were determined based on the procedure which was stated under the materials and methods of this manuscript. Below is the table which shows the summary of the proximate analysis values

Table 7: the proximate analysis of gesho compared with that of hops

Parameter (%)	Present study Wet base (%)	Results found in literature

		(Amabye 2016)	(Online et al. 2017) at 55 ^{o}C, 2 hr	(Humulus and Bioactive 2009)	hops
Moisture	5	9.5	10.12		10
Ash	8.33	9.5		10	8
Protein (N× 6.25)	10.5	8.5	18.055	15	15
Fat	3.4	3.5			3
Fiber	20	25.6			3.86
Carbohydrate	72.7	70.5			64
soft resin	12.12				
Essential oil	0.5		0.965	0.5-3 ($^{v}/_{m}$)	0.5
α -acid	11.5			2-17	42.5

Source: Values are means of duplicate determinations ± standard deviation

4.1.1 Determination of Antioxidant Activity

The DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical behavior of Gesho is shown in Figure 4.3. This movement was expanded by expanding the example remove fixation. The DPPH cell reinforcement measure depends on the limit of the steady free extreme 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) to decolorize within the sight of cell reinforcements. There is a surprising electron in the DPPH extremist, which is liable for absorbance at 515 nm and furthermore for a noticeable profound purple tint. At the point when an electron gave by a cell reinforcement compound is acknowledged by DPPH, the DPPH is decolorized, which can be quantitatively estimated by absorbance shifts. The IC50 estimation of the concentrate was 43.26µg/ml, rather than that of ascorbic corrosive IC50 (55.89 µg/mL), which is a notable cell reinforcement (Of and Elengi 2008). The free extremist rummaging movement or hindrance action of free extremist DPPH level of standard ascorbic corrosive and Gesho extricate were determined utilizing the relationship written in the condition (3.9). From the diagrams, as demonstrated in Figure 4.2 or inhibition activity of free radical DPPH percentage of standard ascorbic acid and Gesho extract were calculated using the relationship written in the equation (3.9). From the graphs, as shown in Figure 4.2 and Figure 4.4, the IC50 values of the standard ascorbic acid Table 4.2 and that of the sample Table 4.3 were calculated by comparing these IC50 values, we can see that the extract exhibited the strongest capacity of DPPH radicals for neutralization.

Table 8: Average absorbance of ascorbic acid and inhibition activity on DPPH

Ascorbic acid concentration ($\frac{mg}{ml}$)	Absorbance(λmax=517) Mean±SD	%IA	IC50 ($\frac{mg}{ml}$)
control	1.001	_	_
1	0.735±0.0023	27.3	1.58
2	0.492±0.064	51.2	
4	0.367 ±0.022	70. 4	
6	0.080±0.033	90.6	
8	0.067±0.018	93.9	
10	0.056±0.042	95.7	
12	0.051±0.006	97.2	

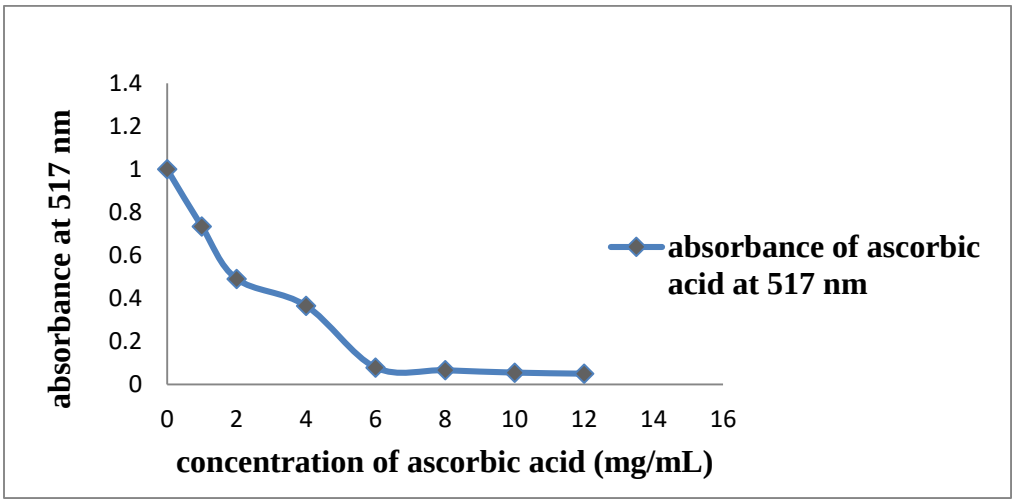


Figure 25: Absorbance of ascorbic acid at 517 nm

Table 9: absorbance of ascorbic acid at 517 nm

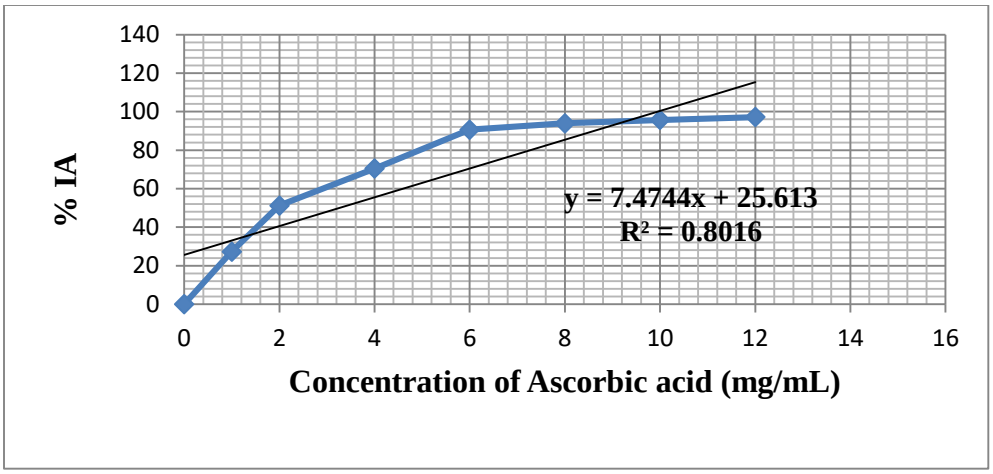


Figure 26: Inhibition activity (IA %) of ascorbic acid

Table 10: DPPH absorbance of sample and inhibition sample (Gesho extract)

Sample concentration ($\frac{mg}{ml}$)	Absorbance(λmax=517) Mean±SD	%IA	IC50 ($\frac{mg}{ml}$)
0	0.921	-	-
1	0.514	30	0.954
2	0.311	37	
4	0.21	46	
6	0.02	72.5	
8	0.014	78	
10	0.012	79	
12	0.011	78	

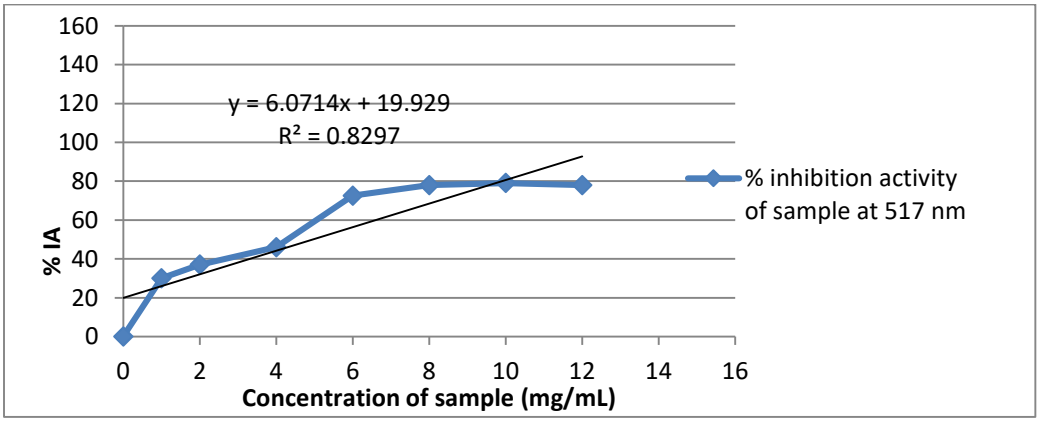


Figure 27: Inhibition activity (IA %) the sample

4.2. Malt analysis

Table 11: Malt analysis was performed in BGI Ethiopia Raya brewery share company plc.

	Local malt assela analysis				**Import 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
Specific gravity									-
Density(g/cm)									-
Extract %humid	75.03	75.90	74.07	74.26	76.07	78.36	74.63	77.2	77-83
Extract %dry	78.65	79.56	77.64	77.84	79.57	81.71	78.06	80.50	77-83
Time of Filtration (min.)	13	15			12	13			-
Moisture content(%):EBC 4.2 method	4.6	4.6			4.4	4.20			4-5.5
Colour:EBC4.5 7 method	4.6	3.4			395	3.65			2.5-4.0
PH:EBC 8.17 Method	5.98	5.95			6.12	6.00			5.8-6.2
Wort viscosity (mpas):EBC 4.8 method	-	-	-	-	-	-	-	-	1.45-1.6.0
Protein	10.44	10.06			10.33	10.13			-

4.3 Data Analysis of Response1 (beer bitterness)

The resulting data obtained during mashing was discussed using Design expert® 7 software to decide the optimum operating parameters Gesho to hop (25-100 %), wort boiling time (45 -90 min), wort boiling temperature (75-95^{o}c) and wort gravity (15-20 ^{o}p). The optimization of beer bitterness can be seen in different dimension.

Table 12: Response1 (beer bitterness)

		Factor 1	Factor 2	Factor 3	Factor 4	Response 1
Std	Run	A:mixing ratio	B:temperature	C:time	D:wort gravity	Beer bitterness
		%	^{o}c	min	^{o}p	BU
19	1	25	85	90	17.5	24.3
1	2	25	75	67.5	17.5	22.1
17	3	25	85	45	17.5	21.5
7	4	62.5	85	45	20	19.2
13	5	62.5	75	45	17.5	19.25
3	6	25	95	67.5	17.5	22.45
6	7	62.5	85	90	15	22
11	8	25	85	67.5	20	21.65
9	9	25	85	67.5	15	23.7
4	10	100	95	67.5	17.5	16.85
18	11	100	85	45	17.5	16.6
15	12	62.5	75	90	17.5	20.35
16	13	62.5	95	90	17.5	21.75
2	14	100	75	67.5	17.5	16.5
14	15	62.5	95	45	17.5	19.35
20	16	100	85	90	17.5	17.75
12	17	100	85	67.5	20	16.58
10	18	100	85	67.5	15	17.15
5	19	62.5	85	45	15	19.75
24	20	62.5	95	67.5	20	20.75
22	21	62.5	95	67.5	15	21.5

8	22	62.5	85	90	20	20.82
23	23	62.5	75	67.5	20	19.9
21	24	62.5	75	67.5	15	21
25	25	62.5	85	67.5	17.5	21.2

4.3.1 Experimental Design

The experimental result was analyzed using Box- Behnken (BBD) of Design-Expert software to develop a single model equation that can describe the significance of the extraction variables. The suggested choice of model was a quadratic model that fits the data.

Table 13: Build Information

File Version	11.1.0.1		
Study Type	Response Surface	Subtype	Randomized
Design Type	Box-Behnken	Runs	25
Design Model	Quadratic	Blocks	No Blocks
Build Time(ms)	4.00		

Table 14: Factors

Factor	**Name**	**Units**	**Type**	**Minimum**	**Maximum**	**Coded Low**	**Coded High**	**Mean**	**Std. Dev.**
A	mixing ratio	%	Numeric	25.00	100.00	-1 ↔25.00	+1 ↔100.00	62.50	26.52
B	Temperature	^{o}c	Numeric	75.00	95.00	-1 ↔75.00	+1 ↔ 95.00	85.00	7.07
C	Time	min	Numeric	45.00	90.00	-1 ↔45.00	+1 ↔ 90.00	67.50	15.91
D	wort gravity	^{o}p	Numeric	15.00	20.00	-1 ↔15.00	+1 ↔ 20.00	17.50	1.77

From the above table an appropriate point of Gesho to hop mixing ratio, wort boiling temperature and time to give maximum valu of beer bitterness.

Table 15: Responses

Response	Name	Units	Observations	Analysis	Minimum	Maximum	Mean	Std. Dev.	Ratio	Transform	Model
R1	Beer bitterness	BU	25	Polynomial	16.5	24.3	20.16	2.24	1.47	None	Reduced Quadratic

Table 16: Model summary

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	**< 0.0001**		**0.9246**	**0.9047**	**Suggested**
2FI	0.6413		0.9177	0.8581	
Quadratic	**0.0031**		**0.9736**		**Suggested**
Cubic	0.9986		0.8937		Aliased

4.4 Statistical Analysis of the Experimental Results

4.4.1 Analysis of variance (ANOVA)

A relevant model was the variance analysis of the quadratic regression model. The 106.14 Model F-value means that the model is significant. Due to noise, there is only a 0.01 percent probability that an F-value this high will occur. P-values of less than 0.0500 shows that model terms are significant. Based on the result one -way ANOVA analysis , the important model words are A, B, C, D, and A^2 have a significant effect ($P<0.05$). Values greater than 0.1000 mean the terms of the model are not relevant. If there are several minor model terms (not counting those needed to encourage hierarchy), the reduction of the model can enhance your model. Skimming down the last 2 columns of Table 4.10, it can easily be seen that the model is lower than 0.05 for the p-value (<0.0001) to be statistically important. 'p-value' values of less than 0.05 imply that the terms of the model are significant. Hence, the model terms considered for this study (A, B, C, D, and A^2) are relevant in this case. This shows that Gesho to hop mixing ratio, wort boiling time and wort gravity, the relationship between Gesho to hop

mixing ratio, wort boiling time and wort gravity affect bitterness of beer significantly. And the Gesho square to hop mixing ratio influences the bitterness of the beer.

Table 17: Analysis of variance (ANOVA) for Response Surface Quadratic Model

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	117.67	7	16.81	106.14	< 0.0001	Significant
A-mixing ratio	97.87	1	97.87	617.98	< 0.0001	
B-temperature	1.05	1	1.05	6.63	0.0197	
C-time	10.68	1	10.68	67.43	< 0.0001	
D-wort gravity	3.20	1	3.20	20.23	0.0003	
AC	0.6806	1	0.6806	4.30	0.0537	
AD	0.5476	1	0.5476	3.46	0.0804	
A^2	3.64	1	3.64	22.99	0.0002	
Residual	2.69	17	0.1584			
Core Total	120.36	24				

Table 18: Model adequacy measures

Std. Dev.	0.3980		**R^2**	0.9776
Mean	20.16		**Adjusted R^2**	0.9684
C.V. %	1.97		**Predicted R^2**	0.9555
			Adeq Precision	33.7525

The coefficient of regression (R^2) measures the association between the experimental data and the expected responses quantitatively. The 0.9555 Predicted R^2 in reasonable agreement 0.9684 Modified R^2 ((R^2= 0.9776 and Adj-R2=0.9684), suggesting the RSM's achievement. because the difference is less than 0.2. Since the R^2 value is closer to 1.0, it means that the line of regression matches the data perfectly. Similar to that, the R^2 obtained in this investigation

was 0.9776, which was close to 1. The signal to noise ratio is determined by Adeq Precision. It is ideal to have a ratio greater than 4. The level of fit of the model was evaluated by a regression coefficient (R^2). The value of the coefficient in this case ($R^2 = 0.9776$). The obtained R^2 values suggest good adjustments to the experimental results. The ratio of 33.7525 indicates an adequate signal. This model can be utilized to explore the plan space. Thus, it tends to be inferred that this model can be utilized to explore the plan space. Three tests were done to decide the satisfactory of model for all reaction. These included Successive model Sum of Squares, and Model Summary Statistics. Select the most noteworthy request polynomial where the extra terms are critical and the model isn't associated P-esteem < 0.0001.

The regression coefficients and the corresponding 95% CI (Confidence Interval) High and Low were presented in table 4.6 below. If zero was in the range High and Low 95% Confidence interval, the factors has no effect. From the 95% CI High and Low values of each model term, it could be concluded that the regression coefficients of wort boiling temperature, time, mixing ratio and wort gravity have highly significant effect in beer production process.

Table 19: Regression coefficients and the corresponding 95% CI High and Low

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	20.52	1	0.1104	20.29	20.76	
A-mixing ratio	-2.86	1	0.1149	-3.10	-2.61	1.0000
B-temperature	0.2958	1	0.1149	0.0535	0.5382	1.0000
C-time	0.9433	1	0.1149	0.7010	1.19	1.0000
D-wort gravity	-0.5167	1	0.1149	-0.7590	-0.2743	1.0000
AC	-0.4125	1	0.1990	-0.8323	0.0073	1.0000
AD	0.3700	1	0.1990	-0.0498	0.7898	1.0000
A^2	-0.7638	1	0.1593	-1.10	-0.4277	1.0000

The coefficient gauge addresses the normal change accordingly per unit change in factor value when all leftover components are held steady. The block in a symmetrical plan is that the general normal reaction of the multitude of runs. The coefficients are adjustments around that average supported the factor settings. When the factors are orthogonal the VIFs are 1; VIFs

greater than 1 indicate multi-colinearity, the upper the VIF the more severe the correlation of things. As a rough rule, VIFs but 10 are tolerable.

4.4.2 Development of Empirical Models

From the study, empirical models for the maximum beer bitterness table 4.5 in terms of the process parameters in coded factors were developed by using the BBD. A second order quadratic model equations which are shown below were fitted to the data model for predicting response; beer bitterness in terms of coded and actual factors respectively.

Beer bitterness = +20.52-2.86*A+0.2958*B+0.9433*C-0.5167*D-4125AC+0.3700*AD-0.7638A^2 (4.1)

Beer bitterness = +23.68897-0.044330*mixing ratio+0.29583* Temperature+0.072481*time -0.453333*wort gravity-0.000489*mixing ratio*time+0.000543mixing $ratio^2$ (4.2)

Where A, Gesho to hop mixing ratio, B, wort boiling temperature C, wort boiling time and D, wort gravity . the quadratic effect of A, Gesho to hop mixing ratio, B, wort boiling temperature C, wort boiling time and D, wort gravity were significant ($P<0.05$) on bitterness of beer table 4.5. in addition to the interactive term gesho to hop mixing ratio and wort boiling time (AC) and gesho to hop mixing ratio and wort gravity (AD) not show a significant effect ($P<0.05$) This implies that a slight variation in concentration of the individual variables, gesho to hop mixing ratio (A), time (C) and wort gravity (D) could bring change the bitterness of beer. Besides the square term gesho to hop mixing ratio (A2) have a significant effect ($P<0.05$). Bitterness was the response of the study. From 16.6 to 24.3BU, the response output was arranged. The terms 20.52 and 23.68897 above were intercepts of the coded and actual factors of quadratic model, respectively. The aim of the summary of the model fit was to maximize the R-Squared adjusted and R-Squared predicted values. For the model and model variables, model relevance was tested. Linear model factors such as mixing ratio (A), temperature (B), time (C) and (D) gravity of the wort, and quadratic model factors such as pure quadratic terms (A2, B2, C2) and quadratic terms of interaction (AB, AC, BC) based on F and p values were

tested. It is found from the table that the quadratic model is the best fit model for responses in terms of its importance and for this experimental disparity. The "Pred R-Squared" of 0.9555 is in reasonable agreement with the "Adj R-Squared"0.9684 “because the difference is less than 0.2. The “Pred R-Squared is the measure of the extent this developed model can be used to predict ranges of data this study has not considered, which, therefore, means that this model has 95.55% precision in fitting to overall ranges of data. The Rsquared, on the other hand, shows how much of the difference in the outcome is explained by the model. This model is, therefore, almost is good experimental values in accomplishing this task as demonstrated through its high value (97.76%). “Adeq Precision" measures the signal-to-noise ratio. A ratio greater than 4 is desirable. In this case, the ratio of of 33.752 indicates an adequate signal. The table 4.9 shows that the quadratic model is the best model for responses in terms of its importance and the second order (quadratic) for this experimental design (quadratic). For optimization of the beer production process, the established models are further used

4.4.3 Adequacy Check for the Developed Models

The analysis of variance or ANOVA to show the effect of each independent variable individually and the interaction between each variable to obtain maximum yield of beer bitterness by significant model was seen in detail. The adequacy of the model was checked by examination of fluctuation (ANOVA) and some analytic plots. Examination of change (ANOVA) is utilized to test the importance of the created models. The detail ANOVA for one reaction is given table 4.5. The F-esteem is proportion of variety of the information about the mean. For the most part, the determined F-worth ought to be a few times more noteworthy than the organized esteem, if the model is a decent forecast of their exploratory outcomes and the assessed factors impacts are genuine. Anticipated Residual Error Sum of Squares, which is a proportion of how the model fits each point in the plan; the R-Squared, proportion of the measure of variety around the mean clarified by the model; Adj R-Squared that is a proportion of the measure of variety around the mean clarified by the model, Pred R-Squared, a proportion of the measure of variety in new information clarified by the model, and Adequate Accuracy, this is a sign to unsettling influence proportion because of irregular mistake, introduced in the table 4.4, beneath, are utilized to choose whether the model can be utilized or not.

In the present study, empirical models for the output responses (bitterness level in terms of the process parameters in actual and coded factors were developed by using the GFD. The sequential model fitting for bitterness level of the samples prepared are given at. Two tests were carried out to determine the adequate model. These included Model Summary Statistics and Sequential model Sum of Squares. From Table 3.9 and Table 3.10, it was found that quadratic model was the most suitable model for the present study, because quadratic model had highR2, adjusted R2, predictedR2 and low PRESS for bitterness responses. The Sequential model Sum of Squares also showed that quadratic model was the highest order polynomial where the additional terms were significant as the PRESS value of cubic model could not be defined in the Model Summary Statistics for responses. Besides, it can be observed that quadratic model had close proximity or reasonable agreement of Predicted R-square is with that of adjusted R-square. The regression coefficients of the developed model are determined from the regression analysis. The regression coefficients of the developed model are determined from the regression analysis. From the two tables it is observed that the quadratic model is the best fit model for responses in terms of its significance and for this experimental design, the second order (quadratic) model is suggested, as the p-value of this model is also smaller than that of other. The two developed equations regardless of significant terms for bitterness level were given at table. The developed models are further used for optimization of the beer bitterness.

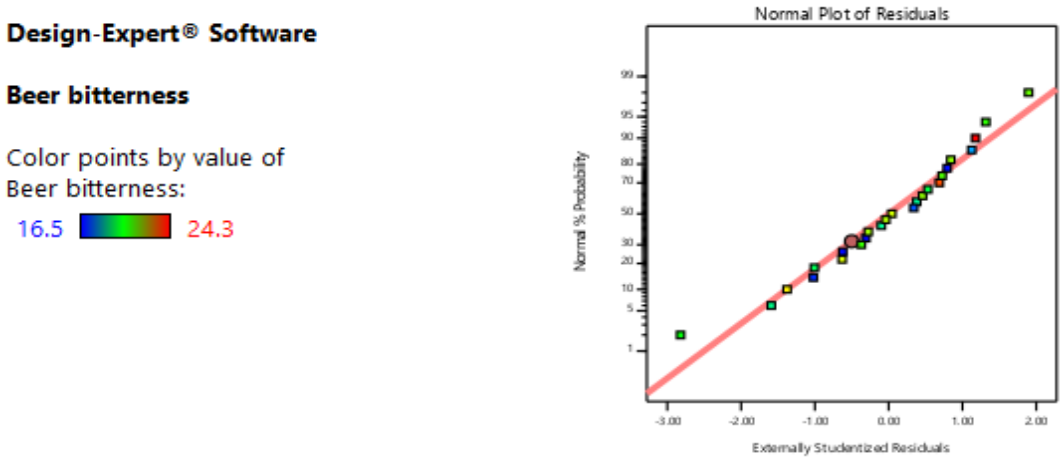


Figure 28: Normal plot of residuals

From the plot as shown above, Residuals are usually considered as components of variations, imprecisely fitted to the model and subsequently it's predicted that they behave according to a traditional distribution feature. the traditional probability plot indicates the residuals following by the traditional in all probability distribution, within the case of this experimental data the points within the graphical plots shows fitted to the line within the figure, this shows that the quadratic model properly satisfies the assumptions analysis of variance (ANOVA) i.e. the error distribution is approximately normal.

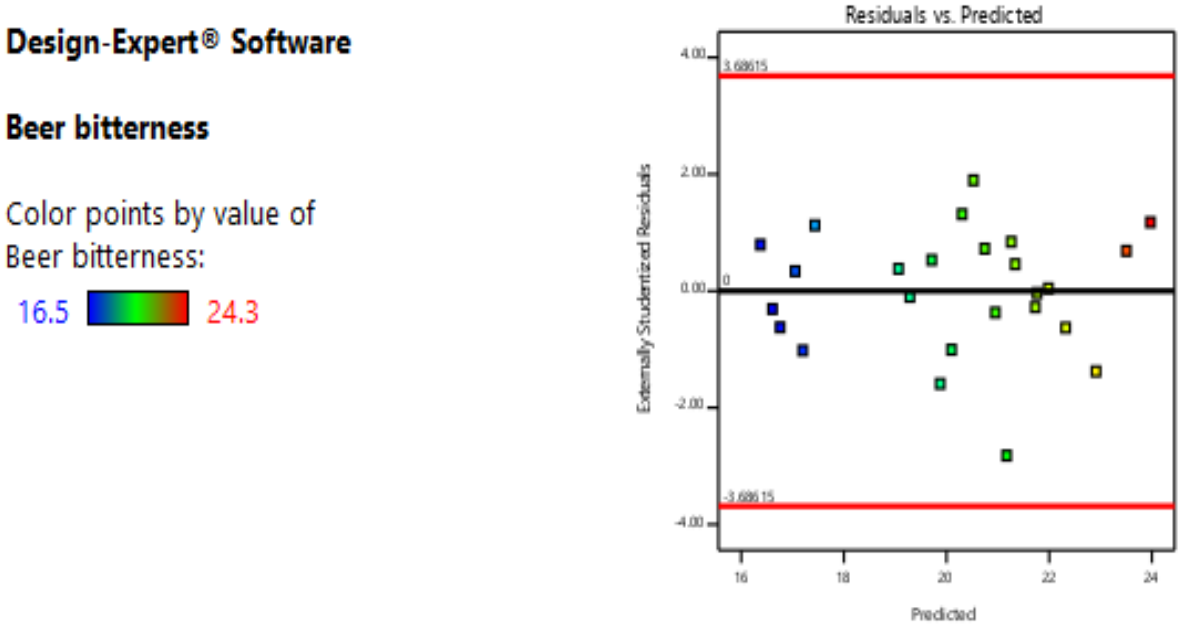


Figure 29: Residual versus predicted values

If the model is correct and therefore the assumptions are satisfied, the residuals should be structure less; especially, they ought to be unrelated to the other variable including the anticipated response. An easy check is to plot the residuals versus the fitted (predicted) values. A plot of the residuals versus the rising predicted response values tests the idea of constant variance. The plot shows random scatter which justifying no need for an alteration to minimize personal error.

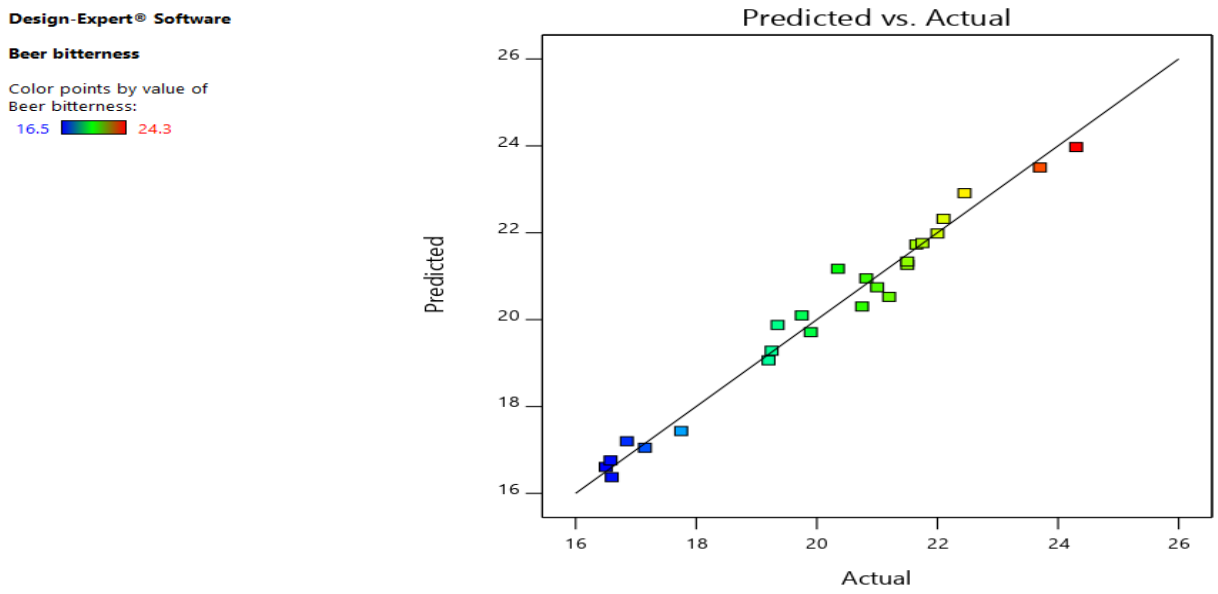


Figure 30: predicted vs actual

The plot of predicted versus actual Figure 4, which is that the graph of the anticipated response values versus the particular response values, is additionally another important diagnostics plot that ought to be taken into consideration. The aim is to detect a worth, or group of values, that aren't easily predicted by the model. Looking into the graph, all values fall along the line, which shows the high power of prediction of the model developed.

4.4.4 Interpretation of the developed models

In the subsequent headings, whenever direct effect, interaction effect or a comparison between any two input parameters is being discussed and therefore the third parameter would get on its center point. General Factorial Design was wont to estimate the effect of the three process variables on the worth of bitterness level.

Direct effects

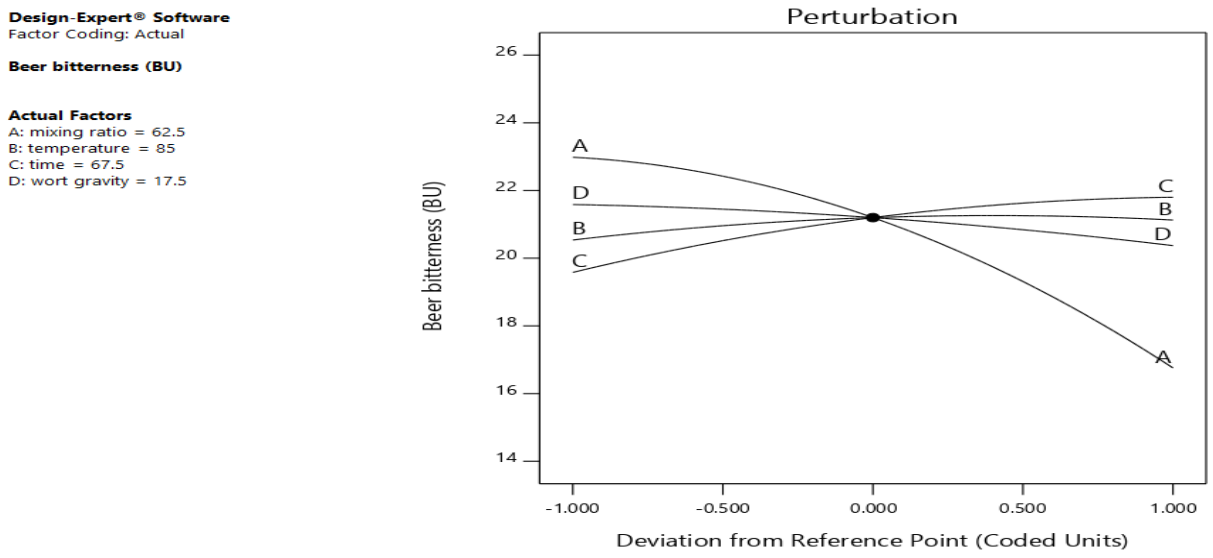


Figure 31: Perturbation plots comparing the effect of all factors at appoint in the design space

The perturbation plot figure, which helps to match the consequences of all the factors at some extent within the design space. The boiling temperature and time features a positive effect on bitterness level. The upper the wort boiling temperature and time, the better to interrupt the molecule of the Gesho .as a result, the bitterness level also gets high. But, within the case of wort gravity and mixing ratio inversely proportion with beer bitterness. Thus, increasing in wort temperature and time resulted in a rise of bitterness level of the Gesho beer. The response is plotted by changing just one factor over its range while holding all the opposite factors constant. A steep slope or curvature during a factor shows that the response is sensitive thereto factor. A comparatively flat line shows insensitivity to vary therein factor. If there are quite two factors, the perturbation plot might be wont to find those factors that the majority affect the response. During this case, because it are often seen from the plot, the rise in four of the factors has increasing effect beer bitterness. This is often very true until the point of reference is reached. Past the point of reference, however, the effect of every factor on the beer bitterness yield is reverted.

Interaction effects

Interaction between Process Variables can affect the performance of finished product. During this unit, the subsequent effects are getting to be seen statistically and graphically.

The effect of temperature variable on yield of extract

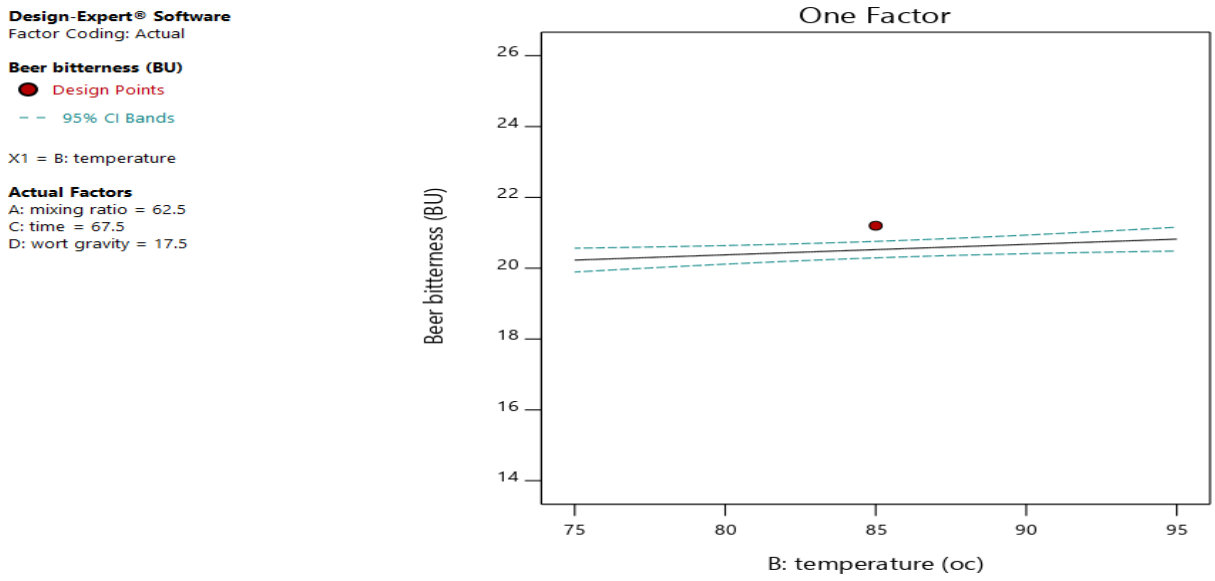


Figure 32: The effect of temperature variable on yield of extract

Figure 4.10 Show that the gesho isomerization temperature significant effects during which as increase the beer bitterness are going to be slightly increased or almost constant reading. Just in case the graph shows that the slight increasing the beer bitterness can continue because the hard and soft resins in Gesho which given bitter beer are soluble when temperature increasing. The simplest optimum temperature for the isomerization of Gesho is 85 OC because at this temperature high bitterness of product was seen 24.BU.

Effect of Time and mixing ratio on beer bitterness Yield

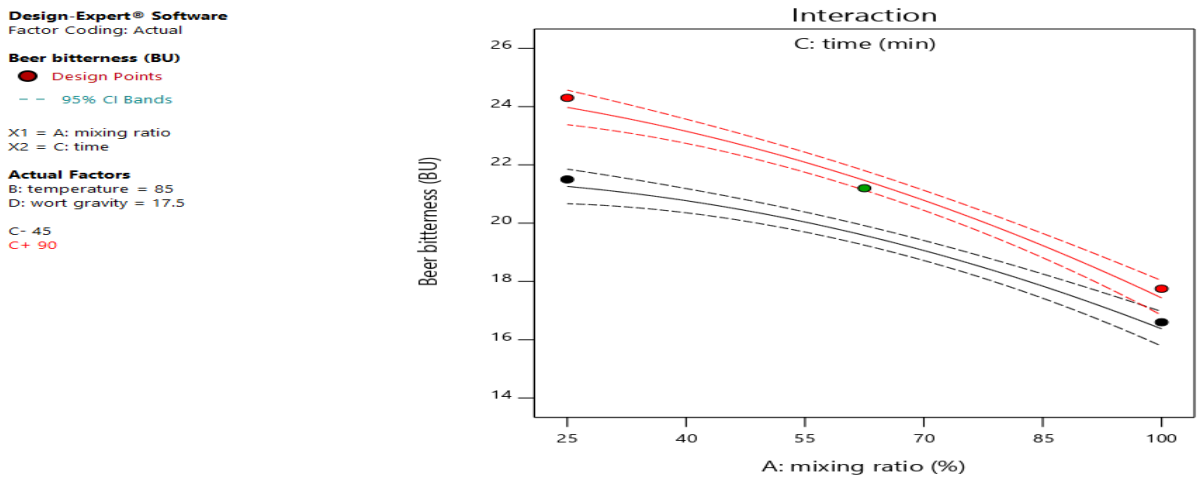


Figure 33: Effect of Time and mixing ratio on beer bitterness Yield

From the graph, it can be seen that the lines are going to meet at some point this shows that there is significant interaction effect between mixing ratio and time.

Effect of wort gravity and mixing ratio on beer bitterness Yield

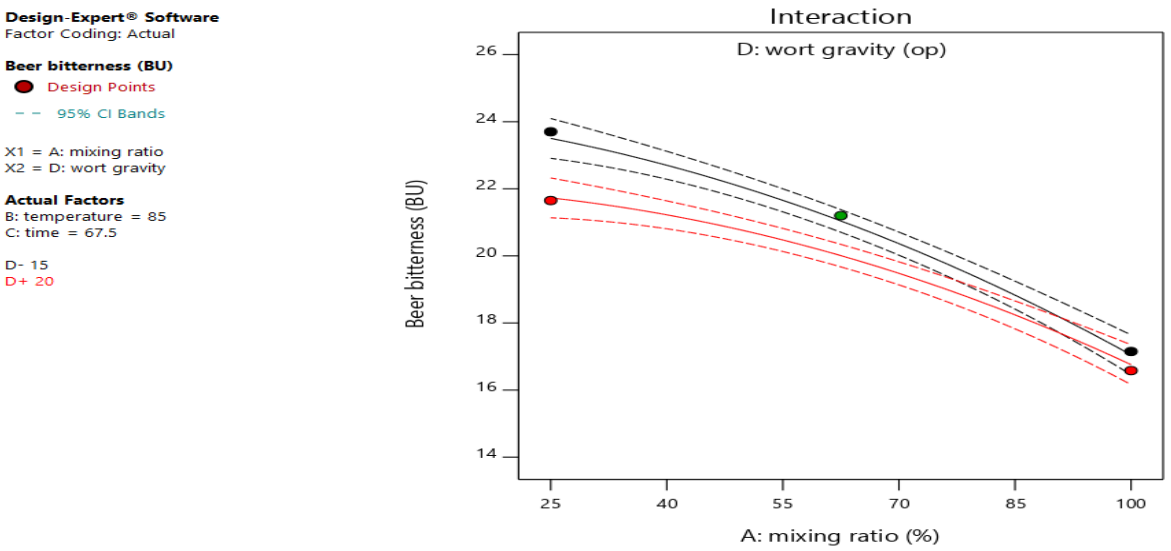


Figure 34: Effect of wort gravity and mixing ratio on beer bitterness Yield

Effect of mixing ratio and Time on beer bitterness Yield

The below Figure 4-7 shows how the interaction of extraction time and temperature affected the results of beer bitterness when the quantity of temperature and wort gravity were kept at a continuing value of 85 oC and 17.5 Op respectively. The plot, therefore, shows that the yield increased until it reaches the worth 24.3 BU. as both the extraction time and temperature increased. This is often because both time and temperature are key variables for the isomerization of gesho. A temperature of 85 °C and 17.5 oplato gave an optimum condition for the extraction solvent to act on the ester bonds that crosslink the extractives to the lignocellulosic material. Because it was illustrated during the discussion on the effect of one-factor section, the rise of your time (45 -90 min) and temperature (25 -100oplato) could only increase the yield to only over 21.2BU and 24.3 BU respectively. But when these independent factors interact, their effect is maximized to possess a further increment value to rise the extract yield to around 24.3 BU.

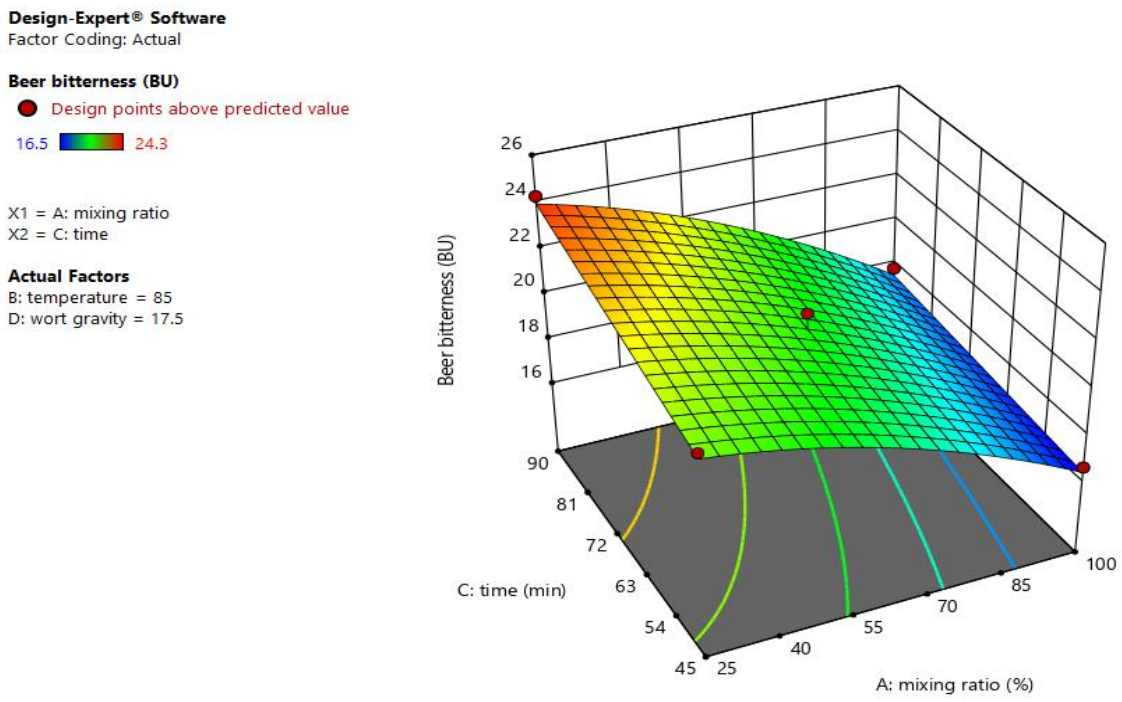


Figure 35: Surface plot of time and mixing ratio interaction effect on yield

Effect of wort gravity and mixing ratio on beer bitterness Yield

The same trend is additionally observed for the interaction effect of temperature and caustic soda concentration on the yield at a continuing value of temperature 85oC and time (67 min): the yield increased from 16.5 to around 24.3 BU as temperature increased from(75-95 OC) and mixing ratio decrease from (100 -25 %) . As discussed on the one-factor effect section, it's indicated that the only effects of caustic soda and extraction temperature could end in a maximum results of about 45. When these two most vital factors interact with one another , as indicated in Figure 4-7, they might raise the extract yield to about 24.3. which suggests that the interaction effect of temperature and hydrolyzing agent concentration is critical .

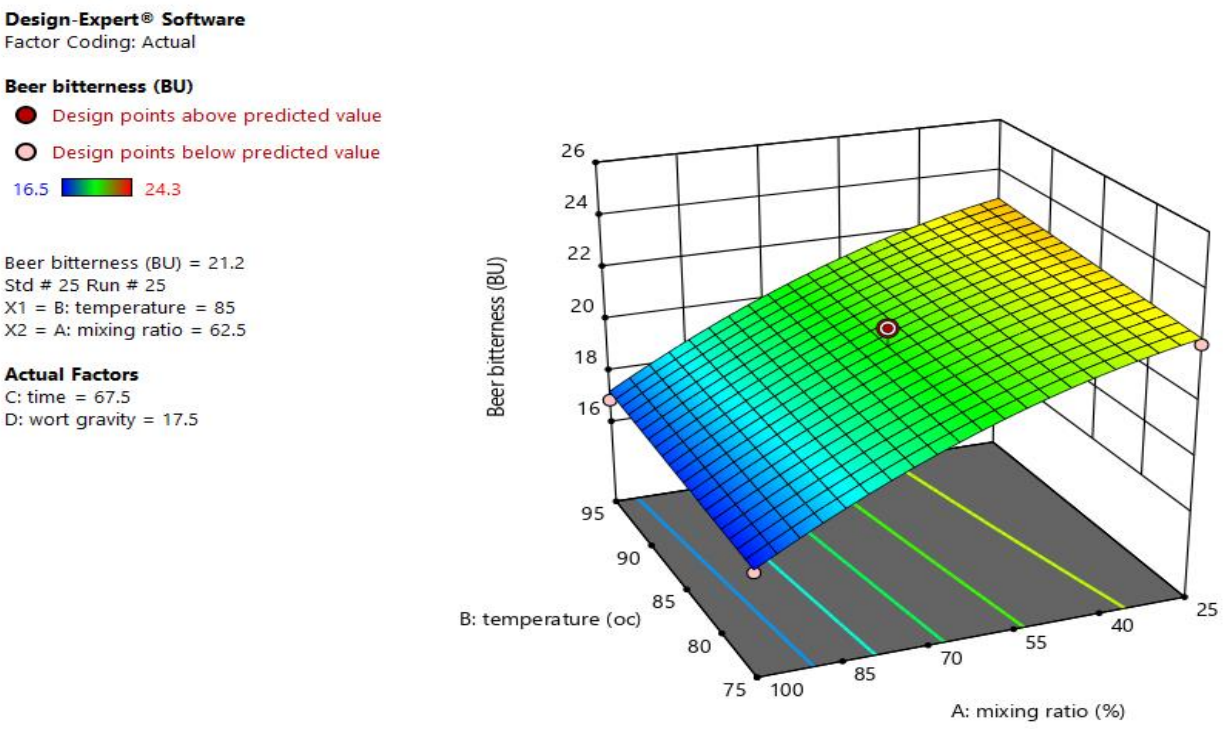


Figure 36: Surface plot of effect of wort gravity and mixing ratio interaction effect on yield

an equivalent trend is additionally observed for the interaction effect of temperature and mixing ratio on the yield at a continuing value of your time 67.5min and wort gravity17.5 op the merchandise yield increased from 16.6 BU to 24.3 BU as temperature and alkali concentration increased from 60-120 min and. As discussed on the one-factor effect section, it's indicated that the only effects of caustic soda and extraction temperature could end in a maximum results of about 22.2 BU. When these two most vital factors interact with one another, as indicated in Figure 39, they might rise the extract yield to about 24.43 BU, which suggests that the interaction effect of temperature and hydrolyzing agent concentration is critical .This will be explained by the very fact that the heat makes the method of attacking the bonds that keep the structure of the beer intact easier, which results in a far better chance of releasing

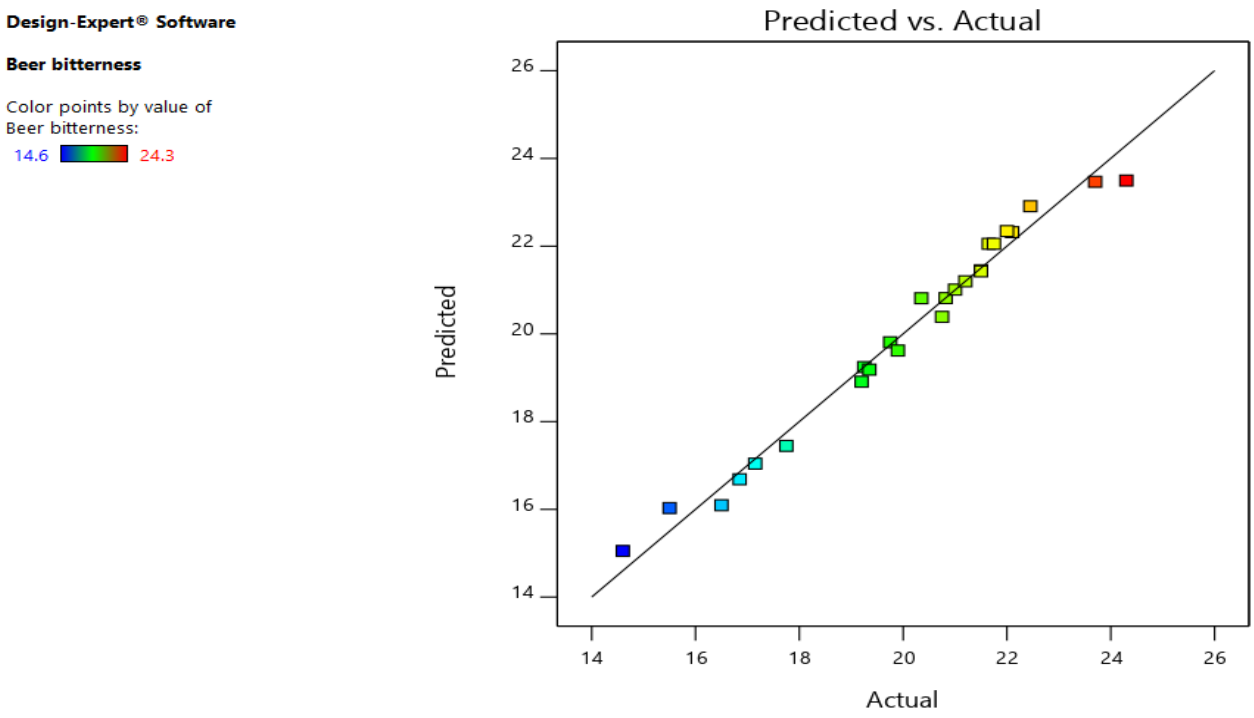


Figure 37: Predicted vs Actual

4.5 Optimization of process parameters

One of the objectives of this study was to seek out the optimal process variables for the simplest final beer bitterness. The method variables like mixing ratio, time, and temperature and wort gravity and were optimized using the numerical optimization function of Design-Expert software. While doing so, all the factors were set to be in range while the beer bitterness was the response that must be maximized. Table 4-5 shows the summary of things, goals and therefore the corresponding set of specific objectives that would optimize the method condition to possess the very best response. One among the first objectives of this study was to seek out the optimum process parameters for maximizing the worth of Bitterness level. The method variables like temperature, time and particle size are optimized using General Factorial experimental design and their output values are executed using design-expert software 11. In optimizing the bitter leaf extraction process, the temperature, time and particle size are a group of process parameters held to be "in range" while the Bitterness level and Iso- alpha definite quantity are set of responses that require to be "maximized". Table 4.13shows the summary of factors/responses and goals and therefore the corresponding set of specific objectives which will optimize the method condition. At the optimal process conditions (mixing ratio 25,

temperature 94.16, time 79.86 min and wort gravity) the bitterness value was 24.45BU. The very best 24.45 BU beer bitterness value was possibly gained when using time 79.86 min, temperature 94.16℃ mixing ratio 25.35 attempt to wort gravity 15.12 ^{o}P.

Table 20: constraints applied for optimization

Factors/response	Ultimate goal	Experimental range				
		Lower limit	Upper limit	Lower weight	Upper weight	importance
Boiling temperature(^{o}c)	In the range	75	95	1	1	3
Wort boiling time (min)	In the range	45	90	1	1	3
Mixing ratio (%)	In the range	25	100	1	1	3
Wort gravity (^{o}p)	In the range	15	20	1	1	3
Bitterness unit	Maximizing	14.6	24.3	1	1	3

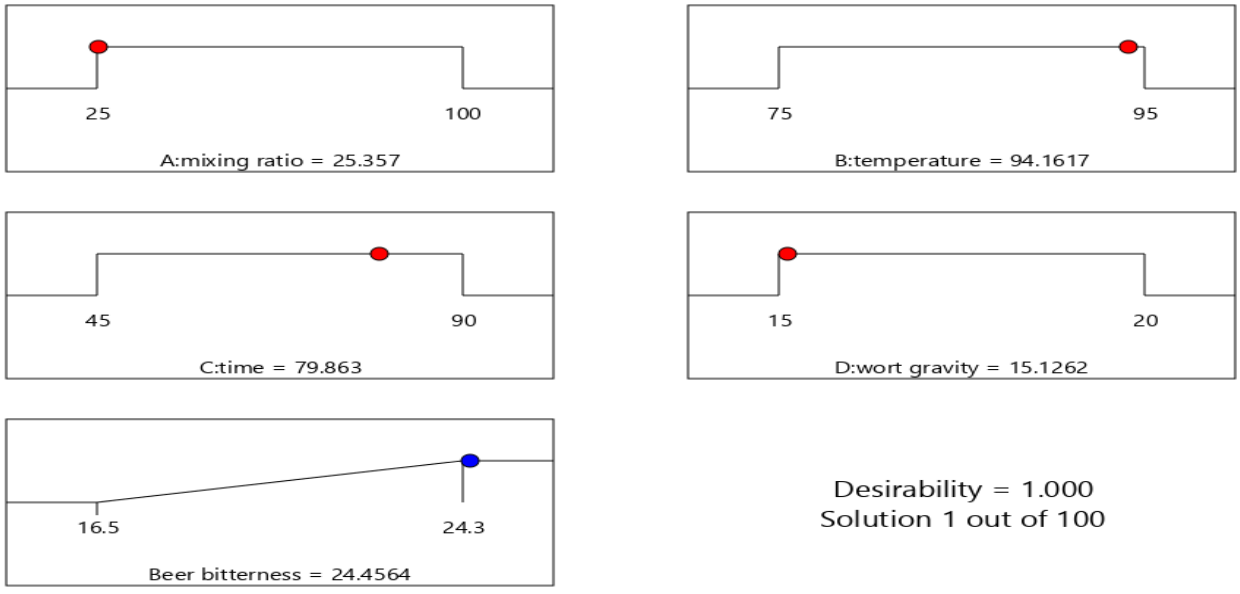


Figure 38: Ramp Plot of Optimization Solution for the Response

4.5.1 Physical and chemical analysis:

My beer follows the physico-chemical quality criteria. These below table 4.15 shows all about the standard requirement of 25% finished beer with comparison thereupon of 100% imported hop and castel standard BGI raya beer. The analysis is analogous quality with the standared castel beer. Within the analysis of beer, indicates PH value 4.6 which indicates the suitable range of beer PH. the colour value of finished beer was 9.5 EBC. Table 4.15 indicates color that the beer produced under different processing conditions had a suitable range of color.

Alcohol (4.46 % wbv) shows the beer alcohol was during a normal range of these findings are in agreement with physico qualitative analysis of castel beer standared under the varied process conditions. This example indicates the efficiency of using proper process variables and quality raw materials. The method parameters were optimized to urge up maximum beer bitterness 24.3 BU. the remainder caractersistics also fulfill the need of the quality beer castel beer.

Colour (9.575EBC) indicates less its targeted value in comparison it. But the sole reason was no caramel utilized in my lab work. Raya Beer Company used caramel so as to extend the color of their beer meaning to realize bright and attractive beer.

Table 21: Physico chemical analysis result of celar (Indicative) beer

Parameter	25% Gesho (Rhamnus priniodes)	100% Imported hop(Herkule)
RI	47	49.80
SG	1.01272	1.01366
Alcohol(%v/v)	7.32	8.04
Alcohol(%w/w)	5.72	6.27
Original gravity (%w/w)	16.58	17.99
Real extract(%w/w)	6.77	6.23
Apparent extract(%w/w)	3.25	3.49
ADOF (%w/w)	80.40	80.62
Bitterness(Bu)	22.7	24.70
Colour(EBC)	12.5	13.8
PH	4.56	4.51
Polyphenol(ppm)	238.12	130.6
Hazenes at 90^o	1.34	N/A
Hazenes at 25^o	0.74	N/A
VDK(ppm)	0.33	0.284

CO2(g/l)	N/A	N/A
F.G(ml/l)	N/A	N/A
Calcium (ppm)	64.128	56.1

Table 22: Physicochemical analysis final (Finished) beer

parameters of beer	25%Gesho beer(Rhamnus priniodes)	100%Imported hop(Herkule)	Castel beer Standard
RI	39.0	39.2	38.90
SG	1.00957	1.00961	1.00940
Alcohol(%v/v)	5.46	5.52	5.45-5.55
Alcohol(%w/w)	4.28	4.33	4.25-4.35
Original gravity (%w/w)	12.65	12.65	12.55-12.75
Real extract(%w/w)	4.38	4.41	4.25-4.45
Apparent extract(%w/w)	2.45	2.46	2.30-2.50
ADOF (%w/w)	80.63	80.73	80.50-81.50
Bitterness(Bu)	17.20	17.40	17.00-19.00
Colour(EBC)	9.575	9.52	11.00-13.00
PH	4.60	4.60	4.30-4.50
Polyphenol(ppm)	180.40	180.80	<120
Hazenes at 90^{o}	1.00	0.89	<1.00
Hazenes at 25^{o}	0.54	0.58	<0.50
VDK(ppm)	0.25	0.20	<0.15
CO2(g/l)	5.5	5.55	5.50-5.70
F.G(ml/l)	0.2	0.17	<1.00
Calcium (ppm)	46.09	40.09	40.00-60.00
Water ratio (%)	32	42	

5. Conclusions and Recommendations

5.1 Conclusions

In this study, the utilization of gesho (Rhamnus prinoides) nearly as good hop replacement was found to be promising or to enhance the beer product. The optimum value of process parameters for beer production were found to be 79.86 min , temperature 94.16 oC, mixing 5ratio 25.35 attempt to wort gravity 15.12 ^{o}P. The very best value beer bitterness content at these optimum conditions was found to be significant 24.456 BU. Beer having 24.456 BU might be produced using 0.377 gram gesho(Rhamnus prinoides) and 0.03086 gram of hop (25:75 %).

In Ethiopia the assembly of Rhamnus prinoides (gesho) can be advantageous for all, for the subsequent reasons:

- ✓ On location, the first staple is cheaper and, otherwise, farmers would be motivated to supply more within the fight against poverty.
- ✓ Craftsmen will make gesho beer.
- ✓ Economic activities and job development will need to be increased, etc. so as to verify the components liable for health benefits in gesho beer.

5.2 Recommendation

The present economic recession demands that researches are geared towards the invention and development of local raw materials to avoid huge waste of hard-earned exchange. Tons of efforts are made within the brewing industry for the substitution of hops with some local substituent. The substitution of hops with local raw materials has not received commensurate attention. So I recommened for Ethiopian commercial beer industries that to use Rhamnus priniodes (Gesho) and to offer specialise in the substitution of the plat by the local row material. Because the 25% of Rhamnus priniodes fulfill standard requirement of beer quality criteria like bitterness, foam, aroma, color, flavor and clarity. The 25% Rhamnus priniodes beer should be used for brewing industries as bittering agent and pasteurized so as to discourage spoilage by microbes and to increase its shelf-life even better.

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