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NON-ALCOHOLIC MALT DRINK PRODUCTION FROM YELLOW MAIZE USING MORINGA LEAF EXTRACT AS PRESERVATIVE AGENT

MSc. Thesis

By

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Non-alcoholic malt drink production from yellow maize using moringa leaf extract as preservative agent

A Thesis Submitted In Partial Fulfillment of the Requirements for the Degree of Master of Science in Chemical Engineering (Process Engineering)

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THESIS SUBMISSION FOR DEFENSE

APPROVAL SHEET – I

This is to certify that the thesis entitled: 'non-alcoholic malt drink production from yellow maize using moringa leaf extract as preservative agent' was submitted in partial fulfillment of the requirements for the degree of Masters of Science with specialization in Process Engineering in the Graduate Program of the Chemical Engineering, College of Engineering, Debre Berhan University and is a record of original research carried out by Tsigie Abera PGR/233/11, under my supervision, and no part of the thesis has been submitted for any other degree or diploma.

The assistance and help received during this investigation have been duly acknowledged. Therefore, I recommend that it be accepted as fulfilling the thesis requirements.

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Name of Co-Advisor	Signature	Date

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THESIS FINAL SUBMISSION

APPROVAL SHEET – II

We, the undersigned members of the board of examiners of the final open defiance by Tsigie Abera have read and evaluated his thesis entitled 'non-alcoholic malt drink production from yellow maize using moringa leaf extract as preservative agent' and examined the candidate. This is therefore to certify that the thesis/dissertation has been accepted in partial fulfillment of the requirements for the degree of Master of Science in Process Engineering.

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DECLARATION

I declare that this thesis entitled 'Preparation of Malt Drink from Yellow Maize and its Preservation Using Moringa Oleifera Leaf Extract' is my genuine work and that all sources of material used for this thesis have been profoundly acknowledged in the text and a list of references given. This thesis has been submitted in partial fulfillment of the requirement for a master of science, M.Sc. at Debre Berhan University and it is deposited at the university library to be made available for users under the rule of the library. I declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

Name: Tsigie Abera (PGR/233/11)

Place: College of Engineering, Debre Berhan University

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ABBREVIATION

AOAC	Association of Official Analytical Chemists
CSA	Central Statistical Agency
EBC	European Brewery Convention
EEA	Ethiopian Economic Association
FCR	Folin–Ciocalteu Reagent
GAE	Gallic Acid Equivalents
MOL	*Moringa Oleifera* Leaves
MOP	*Moringa Oleifera* Powder
MWL	Malting Weight Loss
QE	Quercetin Equivalents
RSM	Response Surface Methodology
TKW	Thousand Kernel Weight
WHO	World Health Organization

ABSTRACT

Non-alcoholic malt drinks with an alcohol content of less than 0.5% are consumed in many countries. Non-alcoholic malt drink is produced primarily by malting yellow maize followed by mashing, wort boiling, maturation and finishing operation. Malt is prepared in five main steps cleaning, soaking, germination, drying, and kilning. After malting, it milling, mashing, mash filter, wort boiling, cooling, maturation, filtration, and packaging operation give the final non-alcoholic malt drink. Microbial spoilage of non-alcoholic beverages is a critical issue, and for this reason, different methods were adapted to reduce spoilage. This study aimed to bring out essential micronutrients and antioxidants of moringa to combat the preservative problem through non-alcoholic malt drink products. Physicochemical and proximate analyses of raw materials (yellow maize and moringa) were performed; i.e. total phenol content and total flavonoid content concentration were found to be 84.10 ± 1.8 mg GAE / g dried and 70.79 ± 0.73 mg QE / g dried, respectively. Germinated yellow maize was a very good source of carbohydrates and energy, but lower content of protein, ash, and fat compared to non-germinated yellow maize. Central composite design (CCD) was used to examine the effects of the three independent variables (Mixing ratio, Temperature, and Time) in two-level combinations with a total number of 20 runs was applied to optimize mash production to conduct with the response variables which was the highest yield of extract. After the optimization of mash had been completed at the mashing step, maize malt was mixed by boiling wort with extracted moringa with the ratio of 5%, 10%, and 15%, and the necessary parameters of the final product were measured. The effect of moringa blended malt drink and storage time on microbial growth was characterized and the result of the analysis was compared with another commercial malt drink product produced in the country; Dashen brewery and the product obtained were tolerated. Storage of malt drink until six months, 10% and 15% of moringa substitution were suitable to minimize the growth of microorganisms and to predict extend shelf life of the product Finally, 5% and 10% of moringa substitution malt drink were acceptable in overall sensory characteristics among given samples.

Keywords: Yellow maize; Optimization; *Moringa Oleifera* leaf; mixing ratio, Temperature, Time, Malt drink; Preservative

CHAPTER ONE

1 INTRODUCTION

1.1 Background

Most food beverages are nutritious as they are either malted or fermented (Ratnavathi & Chavan, 2016). Malt drink is a non-alcoholic beverage obtained from fermentative or unfermented wort. Non-alcoholic malt drinks with alcohol content less than 0.5% are consumed in many countries. The drink has a lot of health benefits such as protection against coronary heart diseases, cancers, and ulcers (Kadivar & Shahedi, 2012). Unlike alcohol, it is used as food for children and patient people. Drinking malt become a mainstream beverage consumed by people of all ages and populations that forbid the drinking of alcohol due to religion and health (Kadivar & Shahedi, 2012; Obuzor & Ajaezi, 2010).

Barley is significantly the main raw material for brewing and malt drink products. It has a short germination period and high starch content that can be converted into higher yields of extracted sugar in the mashing process. However, the price of barley is higher than the alternative raw materials due to importation from a foreign country, so comparatively cost as well as production yield of maize is preferred for malt production (CSA, 2017; Kongkaew et al., 2012; USDA, 2021).

In Ethiopia, improved maize varieties have been linked to significant increases in production and income for adopting farmers. Maize is used in a variety of ways, both as a food source for humans and as a raw material for industrial processing. In Africa, a higher proportion of maize grains are consumed for human consumption, whereas the majority of maize grains are processed industrially in industrialized countries. Maize is a healthy food due to the presence of nutrients and phytochemicals (Alwang et al., 2014; Okoruwa & Kling, 1996; Shah et al., 2016; Teamir, 2019).

Microbial spoilage of non-alcoholic beverages is another critical issue, and for this reason, different methods have been adapted to reduce spoilage. Among these, the employed methods are thermal treatment (pasteurization) and chemical treatments (preservatives) (Ayirezang et al., 2016).

Food preservation is one of the oldest technologies used by humans to avoid food spoilage and protect the consumer from various diseases that come from food spoilage. With the increase in the human population, the demand for food was also increased and efficient food preservative methods were used to preserve the foods (Mubarak et al., 2018). Food prices have risen as a result of the high expense of food preservation, and it has become a burden on people's lives. When plant-originated compounds use as a preservative, they are beneficial to the body, and locally available resources are also utilized (Mashau et al., 2020; Mubarak et al., 2018).

Medicinal plants, which have long been used to treat and prevent diseases, can now be employed as a source of bioactive ingredients for food additives. This is because these medicinal plants are rich in antimicrobial phytochemicals. Although many nations have allowed synthetic antibacterial and antioxidant agents, their use has risen environmental and health concerns, prompting consumers and businesses to seek out natural, safe, and effective preservatives. In this regard, antibacterial activities of Moringa oleifera leaf (MOL) have been discovered (Ayirezang et al., 2016; Faizal et al., 2014).

Moringa oleifera is considered one of the world's most useful trees, and nearly every part of this plant has been used for food and various biological activities. It can be eaten fresh, cooked, or stored as dried powder for many months without loss of nutritional value (Alghamdi & Halaby, 2017; Melo-ruiz, 2016). Moringa leaf extract as a natural antioxidant can be used in beef meatballs (Islam et al., 2018) and rice crackers (Manaois et al., 2013). Moringa oleifera extracts are also employed as sanitizers and preservatives because they suppress the growth of test organisms, which vary from food-borne diseases to spoilage-causing organisms in foods (Bukar, A., Uba, A. and Oyeyi, 2010).

Moringa has not been well researched, and its potential usefulness is not fully known. It is native to southern Ethiopia and was domesticated in the East African lowlands (Seifu, 2014).

1.2 Statement of the Problem

Barley is the major raw material for malt drink and beer production. Barley beer is a widely consumed alcoholic beverage throughout the world and global production. The price of barley is exponentially increasing because of the demand for the product. Additionally, beer barley is not widely cultivated throughout the country, because of the inappropriate environmental conditions. Replacing barely with complimentary cereal grain is like maize is a solution to

overcome this problem. Maize is a very important cereal grain as an alternative for malt production due to being rich in starch, highly cultivated, and low price compared to barley.

In general, the challenges faced with the non-alcoholic product is their rapid deterioration which renders the products to be unacceptable for consumption within two to four days of production, and this is due to over souring and off-flavor caused by the ongoing activities of a microorganism after production. Antioxidants such as vitamin C act as preservatives by inhibiting the effects of oxygen on food, and can be beneficial to health.

Hop is added to provide flavor, bitterness, aroma, and antimicrobial (preservative) properties to the malt. In Ethiopia, pellet and extract hop are a costly ingredient that is imported with the hard currency of the country. Additionally, children are unable to adopt it due to its bitter taste but this hop in malt production can also be substituted by locally available Moringa Oleifera leaf.

However, Moringa is one of the most underutilized and unexplored tropical crops in Ethiopia. It is rich in essential nutrients, for instance, vitamins (such as vitamin A, vitamin C, etc.), minerals, amino acids, beta carotene, antioxidants, and anti-inflammatory compounds. This study is therefore aimed to bring out essential micronutrients and antioxidants of Moringa to combat the preservative problem of non-alcoholic drink products.

1.3 Objectives

1.3.1 General Objective

The main purpose of this study is to develop a malt drink from maize and preserve the product using *Moringa oleifera leaf* extract.

1.3.2 Specific Objectives

- Estimation of physicochemical properties of raw maize, malted maize flours and malt drink incorporated with *moringa oleifera* leaf extract.
- Optimization of mash extraction in the production of non-alcoholic beverages.
- Determining the anti-microbial effect of leaf extract on maize malt drink.
- Evaluating the sensory consent and overall acceptability of malt drinks.

1.4 Significance of the Study

The malt drink made from local yellow maize is highly valuable in semi-arid regions because of its short growing season and higher productivity under heat and drought conditions. For commercial brewing, much of the malt used in Ethiopia country is barely. Since barely cultivation in the Ethiopia region is not generally economically feasible, therefore maize could be an alternative for increasing malt availability for both traditional and industrial use at low cost in Ethiopia. Therefore, the low-price malt drink is the favorite product and the alternative sources of carbohydrates are attractive for the brewery. The potential for the industry to grow has become unquestionable. The challenge however is how the industry can reduce production costs by the use of cheaper sources of raw materials such as malts and moringa Oleifera leaf.

Non-alcoholic malt drink beverages are the new trend that is coming up as a healthier alternative to alcoholic drinks. The study helps to extend the shelf life of malt drink products for storage and distribution without loss of nutritional value of the product by using Moringa Oleifera leaf extract. It creates a moringa-based new economy for local people.

Finally, the study will give information to researchers, students, and teachers in the field on the findings of the study as reference material.

CHAPTER TWO

2 LITERATURE REVIEW

2.1 Overview of Malt Drink Product

Non-alcoholic malt drinks are consumed in many countries. Many people avoid alcohol because of their health. Malt drinks are classified based on the alcohol content as alcoholic (more than 1.2%), low alcoholic (0.5-1.2%), and with alcohol content less than 0.5%. Non-alcoholic malt beverages are produced as non-fermentative or fermentative (Kadivar & Shahedi, 2012).

Malt is one of the main ingredients of malt drink and beer production, which is subjected to a process of germination under controlled conditions. Malting is to develop the hydrolytic enzymes, which are absent in the non-germinated grain (Kumar et al., 2016). Different types of malt can be obtained, with different colors and flavors the malt drink by changing time, temperature, and humidity during the malting process (Paynter, 2015).

Malt is prepared in five steps cleaning, soaking, germination, drying, and kilning. The main objective of malting is to develop the hydrolytic enzymes, which are absent in the non-germinated grain (Kumar et al., 2016). Malting is one of the most effective and convenient ways to the improvement of nutritional value (Bolanle et al., 2012).

The malt production process is performed starting from raw material cleaning, grading, steeping, germination, and kilning (Deme et al., 2020). It is mostly prepared from barley grains but other cereals and starch-containing substances are also used nowadays. These include maize, rice, wheat, sorghum, sweet sorghum, cassava, etc. Qualitatively, the chemical and biochemical components are almost the same in both barley and maize (Guillaume et al., 2018).

The malting process is meant for changes in the biochemical, sensorial, and nutritional characteristics of cereal grains. Malting is a process for weaning food production, based on a reduction in viscosity and an increase in nutrient density in malted flour pastes or beverages (Kongkaew et al., 2012).

2.2 The Main Raw Material for Malt Drink Production

Raw materials required for malt drink production;

A. Malted grains (Starch source)
B. Water
C. Caramel

A. Malted Grains

Barley

Barley is particularly the main raw material for brewing and malt drink products. It has a short germination period, which makes it good for malting. It has a high starch content that can be converted into higher yields of extracted sugar in the mashing process. Barley has a low protein content, which reduces haze in beer, and a high level of diastatic enzymes for starch conversion.

Sorghum

Sorghum has been growing in North Africa since 1000 BC. Sorghum beer is brewed most predominantly in Africa, Mexico, India, and Sri Lanka whose success has stimulated awareness of the brewing potential of sorghum.

Maize

Maize is the most widely cultivated crop and is important for the food security and the livelihoods of the people in sub-Saharan Africa (SSA) (Berge et al., 2019). Whole grain maize or corn consists of essential nutrients such as 76-80% carbohydrate, 9-11% protein, and 4-5% oil. The oil fraction is present in the germ of the maize. Therefore maize is de-germed to limit beer foam damaging effects. During processing to grits or flakes, the protein content is decreased to 7-9%. However, this protein remains largely un-dissolved during mashing free amino nitrogen (FAN) can be a limiting factor when brewing with high maize levels. Maize is processed to make corn grits, maize flakes, refined grits, and corn syrup. The germ or embryo of the maize kernel is high in fat (33.3%) in addition to enzymes and nutrients for new maize plant growth and development. The germ also contains vitamin B complex and antioxidants such as vitamin E.

B. Water

Water is the primary raw material used not only as a component of beer, but also in the brewing process for cleaning, rinsing, and other purposes. In addition, different beer styles require different compositions of brewing liquor. Calcium is perhaps the most important ion in brewing liquor. It protects α-amylase from early inactivation by lowering the pH toward the optimum for enzymatic activity.

C. Caramel

Caramel is produced from the controlled heat treatment of carbohydrates, typically glucose syrup. A valuable brewing ingredient for more than a century, caramel contributes to the character of beer on every continent. Caramel provides color, flavor, and consistency in beer. It also has applications in a variety of related products, including cider, shandy, and non-alcoholic malted beverages. Salted caramel, by weight, is the world's most widely-consumed coloring ingredient in foods and beverages (WHO, 2006).

2.3 Global Production of Maize

Maize or corn (Zea mays L) belongs to the grass family Poaceae (Gramineae). It is an important cereal grain in the world, providing nutrients for humans and animals. Maize is native to America, where it was first cultivated long ago. In Europe, corn was brought by Christopher Columbus in 1493. The maize culture is spread all over the planet, but a ranking has been established, depending on the regions of culture: America; Asia; Africa, and Europe (Soare et al., 2018). Maize has become the second most-produced crop in the world. Specifically, in sub-Saharan Africa, more and more land is used for maize production. From 2007 to 2017, the area in which maize is grown in sub-Saharan Africa has increased by almost 60% (Santpoort, 2020). It is a cereal crop that is cultivated widely throughout the world and has the highest production among all the cereals.

2.4 Maize Production in Ethiopia

In Sub-Saharan countries, Ethiopia has the second rank after South Africa, in yield, and third, after South Africa and Nigeria, in total production has shown substantial progress in maize productivity (*EEA*, 2017).

Maize is the most widely cultivated crop and is of great importance to the food security and livelihoods of the people in sub-Saharan Africa (SSA) (Berge et al., 2019).

Maize is the second most widely cultivated crop in Ethiopia and is grown under diverse agro-ecologies and socioeconomic conditions typically under rain-fed production. As a result, compared to other cereals, maize yield has grown faster in recent years. For instance, from 2003 to 2007, the average maize yield was 1.9 tons per hectare compared to 1.7 tons/ha for wheat, 1.6 tons/ha for sorghum, and 1.2 tons/ha for barley. However, among all major cereals, maize still has the highest potential for additional yield gains From 2003 to 2007, maize yield has averaged 1.9 tons compared to a conservative estimate of yield in farm-level trials of 4.7 tons, giving a difference between potential and actual as 146% of current average yield (Ifpri, 2010)**.**

In 2020, maize production for Ethiopia increased from 971 thousand tonnes in 1971 to 8,600 mill tonnes in 2020 growing at an average annual rate of 7.60% (CSA, 2017; USDA, 2021). According to USDA,(2021); report maize is the most affordable grain for rural communities and poor urban consumers compared to other cereals. Corn is also the most popular crop because of its high demand for animal feed and also a source of fuel for the rural community.

Table 2.1 Cultivation and production of common cereals in Ethiopia MY 2020/21

Crop	Area (1000ha)	Production(1000tone)
Wheat	1850	5100
Corn (Maize)	2340	8600
Sorghum	1850	5000
Barley	1210	2350

Source: (USDA, 2021)

2.5 Significant of Maize

Maize is an amazing plant, with an astonishing capacity to surprise humans. For instance, one seed planted can produce over 500 kernels in return. It is a plant that utilizes sunlight effectively and outstrips the yield/hectare of other grains. Indeed, maize, which is fast becoming an industrial thereby, makes it the first crop to be harvested for food during hunger (Amudalat, 2015). It is an important food staple in many countries and is also used in animal

feed and many industrial applications. The crop has tremendous genetic variability, which enables it to thrive in tropical, subtropical, and temperate climates (Berge et al., 2019).

Whole grain maize or corn consists of 76-80% carbohydrate, 9-11% protein, and 4-5% oil. The oil fraction is located in the germ of the maize. Therefore maize is de-germed to limit Beer foam damaging effects that would otherwise occur. During processing to grits or flakes, the protein content is decreased to 7-9%. However, this protein remains largely un-dissolved during mashing and so free amino nitrogen (FAN) can be a limiting factor when brewing with high Varity maize levels. Maize is processed to make corn grits, maize flakes, refined grits, and corn syrup. The germ or embryo of the maize kernel is high in fat (33.3%) in addition to enzymes and nutrients for new maize plant growth and development. The germ also contains vitamin B complex and antioxidants such as vitamin E (Naves et al., 2011).

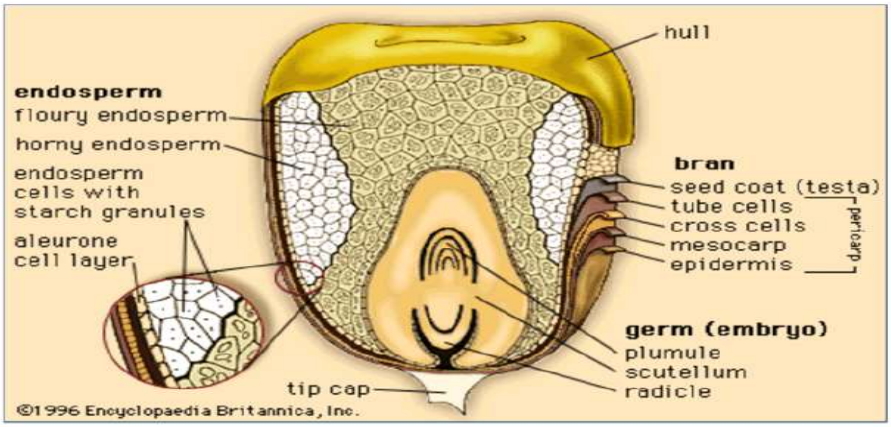


Figure 2.1 Anatomy structure of maize

Source: (Naves et al., 2011)

2.6 Yellow Maize

There are broadly classified into two categories white maize and yellow maize. The choice of variety was depend on the nutritional and physicochemical properties, market requirements, environmental conditions, whether the crop is irrigated and the level of disease resistance required.

A. White maize	B. Yellow maize

Figure 2.2 Maize varieties (A. White maize; B. Yellow maize)

Table 2.2 Some physicochemical properties of maize varieties

Properties	**White Maize**	**Yellow maize**
Moisture (%)	12	11.4
Thousand corn weight (g)	240	245
Total nitrogen (%)	1.73	1.63
Crude protein (N×6.25%)	10.8	10.2
Broken kernels (%)	1.4	1.2
Germinate energy (%)	95	97
Germinate capacity (%)	97	99
Physical appearance	Good	Good

Source: (Hailu Demeke, 2018)

Table 2.3 Nutritional compositions of yellow maize varieties

Varieties	Moisture (%)	Total Ash (% DW)	Crude Protein (% DW)	Crude Fat (% DW)	Crude Fiber (% DW)	Carbohydrate (% DW)	Energy Kal/100g (DW)
CML 161	11.31	1.4	12.03	5.13	2.22	67.91	365.93
Melkasa-1q	9.42	1.53	13.11	7.22	1.62	67.1	385.82
BHQMY 545	10.62	1.74	15.3	6.71	3.45	62.13	370.27
CML 165	9.93	1.62	9.69	6.14	2.63	69.99	373.98
Melkassa-7	9.91	1.63	11.92	4.98	2.46	69.1	368.9
Gibie Awash	11.01	1.46	10.27	6.05	2.26	68.95	371.33

Source: (Hailu Demeke, 2018)

According to Akonor & Tortoe, (2014), Yellow maize is recommended for malt production due to low malting loss and required low germination time, therefore yellow maize variety Melkassa-7 was selected for the production of malt beverage. And Melkssa-7 variety has a low content of oil and protein compared to others (Table 2.3).

2.7 Melkassa-7 Based Non- Alcoholic Product

At present, the beverage industry is interested to produce gluten-free, low alcoholic drinks with increased shelf life. Barley and maize are the most commonly used adjuvants in Europe as partial substitutes for malt. Barley beer is a widely consumed alcoholic beverage throughout the world and global production figures have shown an increasing trend during the last decade. For a long time, adjuncts such as maize (corn), rice, un-malted grain, wheat starch, and sorghum have been utilized by the breweries to give fermentable carbohydrates to the yeast. There are many purposes behind the utilization of adjunct in addition to, or instead of, barley malt, including better accessibility of the materials for processing, sensory modification of the non-alcoholic beer, and probably the most basic one, a lower cost. Supplanting barley malt with adjuncts is generally cost-saving.

It has been proven that the use of a 30% of corn adjunct can give an 8% reduction in total production costs (Le & A, 2017).

Qualitatively, the chemical and biochemical components of barley and maize are almost the same (Dabija et al., 2021; Ore et al., 2018). In addition, malted maize could subsequently improve the color and enhance the flavor, aroma, and other sensory characteristics of its non-alcoholic beverage. However beers are derived from barley or wheat is not recommended for people suffering from coeliac disease; however, everybody can consume the maize-based beverage because it is free from gluten.

2.8 Overview of Moringa Plant

2.8.1 Species of Moringa

There are thirteen species of Moringa. These include *Moringa Oleifera, Moringa Arborea, Morillga Borziana, Moringa Concanensis, Moringa Drouhardii, Moringa Hildebrandtii, Moringa Longituba, Moringa Ovalifolia, Moringa Peregrina, Moringa Pygmaea, Moringa Rivae, Moringa Ruspoliana, and Moringa Stel1opetola*. Out of these thirteen species Moringa oleifera is grown in many parts of the world including Africa and is the most studied. *Moringa oleifera* leaves are superior to that the other varieties of micronutrients and amino acids (Matshediso, 2014).

2.8.2 The Growth of Moringa Oleifera

Moringa oleifera grows in many tropical and subtropical regions with peculiar environmental features, namely, dry to moist tropical or subtropical clime, with annual precipitation of 760 to 2500 mm (it requires less than 800 mm irrigation) and temperature between 18 and 28 °C. It grows in any soil type, but heavy clay and waterlogged, with pH between 4.5 and 8, at an altitude up to 2000 m (Leone et al., 2015). It grows best in well-drained soils, and it does not grow in waterlogged or swamp soils.

2.8.3 The Nutritive and Medicinal Value of *Moringa Oleifera*

Nowadays, with continuously changing socio-economic status, people have become more concerned about their health. There is immense scope for foods that can impart health benefits beyond traditional nutrients. *Moringa oleifera* (Drumstick tree) is one such tree having enormous nutritional and medicinal benefits (Melo-ruiz, 2016).

Almost all parts of the *Moringa oleifera* which include root, bark, seed, flowers, pods, seed oil, leaf, and resin have potential food, agriculture, and industrial uses. It has versatile utility as medicine, functional food, nutraceutical, and water purifying properties and is thus considered to be the world's most useful plant (Sahay et al., 2017).

Figure 2.3 Moringa plant in southern Ethiopia (Moringa Leaves and Moringa Leaf Four)

Source: (Seifu, 2014)

Moringa oleifera leaves are the most nutritious part of the plant, being a significant source of B vitamins, vitamin C, provitamin A as beta-carotene, vitamin K, manganese, and protein, among other essential nutrients (Ifeanyi & Sabina, 2020). It is rich in macro and micronutrients like protein, carbohydrate, calcium, phosphorus, potassium, iron, vitamins, beta carotene, and other bioactive compounds which are important for the normal functioning of the body and the prevention of certain diseases (Sahay et al., 2017).

Table 2.4 Nutritional composition and medicinal uses of different parts of Moringa

Part of tree	Medicinal Uses	Nutritive properties
Leaves	Moringa leaves are used to treat asthma, hyperglycemia, Dyslipidemia, flu, heartburn, syphilis, malaria, pneumonia, diarrhea, headaches, scurvy, skin diseases, bronchitis, eye, and ear infections. Also, it reduces blood pressure and cholesterol and acts as an antimicrobial, antioxidant, diabetic, neuroprotectant, and has anti-atherosclerotic agents,	Moringa leaves contain fiber, fat proteins, and minerals like Ca, Mg, P, K, Cu, Fe, and S. Vitamins like Vitamin-A (Beta-carotene), vitamin B-choline, and vitamin B1-thiamine, riboflavin, nicotinic acid, and ascorbic acid are present. Various amino acids like Arg, His, Lys, Trp, Phe, Thr, Leu, Met, Ile, and Val are present. Phytochemicals like tannins, sterols, saponins, terpenoids, phenolics, alkaloids, and flavonoids like quercetin, isoquercitrin, and glycoside compounds are present.
Seeds	Seeds of moringa help in treating hyperthyroidism, and can act as antimicrobial and anti-inflammatory agents.	Contains oleic acid (Ben oil), an antibiotic called pterygospermin, and fatty acids. Phytochemicals like tannins, saponin, phenolics, phytate, flavanoids, terpenoids, and lectins.
Root Bark	Root bark acts as a cardiac stimulant, anti-ulcer, and anti-inflammatory agent.	Alkaloids like morphine, moriginine, minerals like calcium, magnesium and sodium.
Flower	Moringa flowers act as anti-arthritic and treat urinary problems and cold.	Contains calcium, potassium, amino acids and nectar.
Pods	Moringa pods are used to treat diarrhoea, liver and spleen problems, and joint pain.	Rich in fibre, lipids, non-structural carbohydrates, protein and ash. Fatty acids like oleic acid, linoleic acid, palmitic acid and linolenic acid

Source: (Meireles et al., 2020)

2.8.4 *Moringa* Leaf the Best Food Supplement

Supplementation of *Moringa oleifera* powder in cereal porridge has been shown to improve the nutritional value by increasing Moringa leaves containing a rich concentration of vital minerals and vitamins when compared to fruits and vegetables associated with these nutrients. *Moringa oleifera* supplementation has a nutritional purpose, although it can provide other benefits to the product, such as improved digestibility, dough stability, antioxidant capacity, and preservation, among other benefits associated with the plant (Ali, 2018). The application of *moringa* supplementation in different food products was reported in Table 2.5.

Table 2.5 Application of dried moringa Oleifera leaves in different food

Food	Proximate and Minerals						Reference
	Supplementation of MOL	Protein (%)	Fibre (%)	Vitamin A (mg/100gm)	Calcium (mg/100gm)	Iron (mg/100gm)	
complementary (Maize, soybeans and peanut)	5% 10% 15%	16.56 17.08 17.59	2.94 3.68 4.42				(Shiriki et al., 2015)
Pasta (durum wheat and oat)	2.50% 5% 10% 15%	15.56 15.6 16.75 17.651	2.32 2.58 2.91 3.08				(Getachew & Admassu, 2020)
Soy-Mumu (maize and soybean)	10% 15% 20%	18.5 26.2 26.3	4.3 4.6 4.6	2 2.9 4	484.1 587.9 746.2	52.3 61.8 67.3	(Mbanengen et al., 2016)
Bread (wheat flour)	1% 2%	10.17 11.22	2.23 2.46		5.18 7.32	2.76 2.79	(Sengev et al., 2013)

2.8.5 *Moringa* Leaf Extract and its Anti-Microbial Activity

Moringa oleifera leaf extract can exert antimicrobial activity in two ways: by preventing the growth of spoilage microorganisms (food preservation), and by inhibiting/regulating the growth of those pathogenic (POP Anamaria, 2019).

Table 2.6 Activity of moringa extract on organisms

Source of Organisms	Organisms	Aqueous extract (30mg/ml)	Methanolic Extract (30 mg/ml)	Ethanolic extract (30mg/ml)
		Diameter of zone of inhibition (mm)		
Enteropathogens	Escherichia coli (LIO)	0.2	1.1	1
	Salmonella typhi (LIO)	0.5	0.95	1.2
	Staphylococcus aureus(LIO)	0.1	0.65	0.95
	Enterococcus sp. (LIO)	0.1	0.7	0.65
	Pseudomonas aeruginosa(LIO)	0	0.45	0.3
	Klebsiella pneumonia	12	0	0
	Proteus vulgaris	15	0	0
	Providencia stuartii	14	0	0
	Escherichia coli	15	0	0
Orthopaedicswounds	Streptococcus sp.	0	15	0
	Pseudomonas fluorescens	13	17	0
	Acinetobacter baumanii	12	14	0
	Burkholderia cepacia	12	0	0
	Yersinia enterocolitica	15	19	19
	Serratia rubidae	0	13	0
	Salmonella pullorum	15	0	0
	Klebsiella oxycota	15	0	0

Source**:** (Oluduro, 2012).

According to Oluduro, (2012), all the leaf extracts showed little inhibitory effect on the enteropathogens at an extract concentration of 30 mg/ml with a zone of growth inhibition of less than 1.5 mm. The methanolic extract produced inhibition zones ranging from12 to 19 mm with Streptococcus sp, P. fluorescens, A.baumanii, B. cepacia, Y. enterocolitica, P. mirabilis, and S.pullorum but did not inhibit K. pneumonia, P. Vulgaris, P. stuartii, E. coli (ATCC2592), S. rubidae and K. oxycota. However, all the orthopaedics' wounds ethanolic extract inhibited the growth of some of the fungal organisms producing a zone of growth inhibition of 22 mm against T. mentagrophyte, 20 mm on Pullarium sp, and 15 mm each against A. flavus and Penicillium sp (Oluduro, 2012). Organisms developed resistance to the ethanolic extract of the leaves. And, also the ethanolic extract inhibited the growth of some of the fungal organisms producing a zone of growth inhibition of 22 mm against T. mentagrophyte, 20 mm on Pullarium sp, and 15 mm each against A. flavus and Penicillium sp (Oluduro, 2012).

2.8.6 Active Compounds in *Moringa Oleifera* Leaves

Plants contain many active compounds such as alkaloids, steroids, and tannins. glycosides, volatile oils, fixed oils, resins, phenols, and Aavonoids which are deposited in their specific parts such as leaf, flowers, bark, seeds, fruits, root, and others (Matshediso, 2014). Several bioactive compounds were recognized in the leaves of *Moringa oleifera*. They are grouped as vitamins, carotenoids, polyphenol, phenolic acids, flavonoids, alkaloids, glucosinolates, isothiocyanates, tannins, saponins, and oxalates and phytates (Leone et al., 2015). Moringa leaf extract is rich in phenolics are a potent source of antioxidants.

Phenolic compounds are important due to their ability to serve as antioxidants which are widely found in secondary products of medicinal plants. Phenolics are chemical compounds characterized by at least one aromatic ring *(C6)* bearing one or more hydroxyl groups. Extraction of phenolic compounds in plant materials is influenced by their chemical nature, the extraction method employed, sample particle size, storage time and conditions, as well as the presence of interfering substances (Matshediso, 2014).

The extraction temperature and the incubation time directly influence the phenolic released. The incubation process is also a time-dependent process. The more time is allowed, the more the available phenolic compounds are oxidized. Matshediso, (2014), explained that with increasing extraction temperature, the phenolic contents also increase. At higher extraction

temperature the water becomes less polar and, hence dissolves more compounds that are less polar. More, phenolic content would be extracted at moderately high temperatures.

Table 2.7 Phenolic contents of the 0.5 g leaf extracts of Moringa oleifera

Temperature (^{O}C)	Total Phenolic Content (mg kg^{-1}) ± (%RSD)
25	432 ± 1.21
50	507 ± 0.15
100	1757 ± 35

Source: (Matshediso, 2014)

2.9 Malt Drink Production Process

2.9.1 Malting Process

Malting is a five-step process consisting of:

- Cleaning
- Steeping
- Germination
- Drying
- Kilning

2.9.1.1 Cleaning

In the cleaning process, dust, light, and metal particles are removed. The sorting of maize grains by size is of technological importance for achieving uniform steeping and germination to obtain a perfectly homogeneous malt.

2.9.1.2 Steeping

The malting process begins when the grains are steeped for thirty-eight to forty-six hours until they gain almost 50% of their initial weight in water.

Functions of Steeping: It raises the moisture content to initiate germination and wash the grain and remove germination inhibitors as well as all floating material by skimming.

Water is drained and gain is moved to a germination room where they are kept at a constant humidity and temperature for almost four days. They are turned periodically to maintain an even grain bed temperature of 15.6°c to 23.9°c, which promotes germination.

2.9.1.3 Germination

The germination step takes advantage of the plant's natural growth cycle, activating enzymes already present in the grain that begin the process of unpacking and breaking down the proteins and starches at the kernel's center.

The objectives of germination are:

- ✓ To Control the breakdown of cell walls and matrix proteins.
- ✓ Produce optimal levels of hydrolytic enzymes.
- ✓ Minimize loss of potential extract from growth and respiration while achieving optimal modification.
- ✓ Produce balanced, well-modified green malt for kilning.

The degree to which this breakdown occurs is referred to as modification. Most brewing malt produced today is highly modified, meaning a significant amount of enzyme development and starch conversion has taken place, making these essential elements easily accessible to the brewer (Eneje et al., 2004; Kawaguchi & Chief, 1971).

2.9.1.4 Drying

Drying samples take place throughout twenty-four to thirty-six hours at a temperature of 122°F to 50°c, carefully dried to about 4% moisture content (Eneje et al., 2004).

2.9.1.5 Kilning

After drying, some grains are heated in kilns at higher temperatures and for longer periods. This extended kilning gives these malts unique colors and flavors. Malts that have undergone kilning or roasting are called specialty grains.

2.9.2 Milling Process

After the grain is dried the next step is milling. Milling is carried out for the reduction and monitoring of particle size. The objectives of milling the malted grain are to split the husk to expose the endosperm of the kernel, crush the entire endosperm, and also to keep the amount of flour produced in the milling process to a minimum size (Uchechukwu, 2020).

2.9.3 Mashing

Mashing is the breaking down or hydrolysis of macromolecules from grain by the activity of inherent enzymes of the malted cereals. The main objectives of this step are to hydrolyze starch, protein, and β-glucans, to release simple sugars (glucose and maltose) as substrate, and other nutrients that can be assimilated by the yeast. It involves mixing milled malt and adjuncts with water (brewing liquor) at a set temperature and volume to continue the biochemical changes initiated during the malting process (Uchechukwu, 2020).

2.9.4 Mash Filter

In place of the lauter-tun, several brewers employ mash filters to separate the mash into solids and liquid. The total mash is transferred into a vertically arranged filter press. The frames are covered on both sides with filter cloth made of synthetic material. At the same time, the air must escape quickly when the homogeneous mash is pumped into the chambers. After yielding the first wort, water is pumped in and the filter compartments of the filter press are pressed together with corrugated steel plates. Since very low volumes of sparging water (0.5 hl/100 kg) are necessary, this facility is very suited for high-gravity brewing (E. Hailu, 2016).

2.9.5 Wort Boiling

The sweet liquid, now called wort, can pass through the screens of the false bottom while the grain stays behind. The wort is then pumped into the kettle.

Once all the wort is in the kettle, the liquid is boiled for 60-90 minutes. This ensures that the wort is sterile. Boiling also evaporates some water, concentrating the wort and intensifying the color somewhat (Uchechukwu, 2020).

2.9.6 Maturation Vessel

The purpose of this vessel is largely to remove a fermentation by-product with a disagreeable toffee/butterscotch-type flavor. The flavor belongs to a diketone compound, 2,3-butanedione. The precursor to this flavor compound, "- acetolactate"', forms during the fermentation. The warm temperature and low pH of the maturation vessel hasten the conversion of α- acetolactate to 2,3-butanedione. Once the flavor is formed it is rapidly absorbed by the low level of remaining yeast cells to produce a compound with no noticeable flavor, 2,3-butanediol (Uchechukwu, 2020).

2.9.7 Packaging

The "package" is used primarily to move products through a distribution system to the consumer, through the use of attractive labels and colors. The consumer is drawn to select one container over another when confronted with two or more different choices of the same product (Le & A, 2017).

2.10 Preservation of Beverages

Preservation is a key element in the industry of beverages. Along with developed preservation methods, a high amount of preservatives have been investigated and applied for ensuring beverage stability and protect from any type of deterioration. Preservatives have been clustered in various groups depending on their source, synthesis method, structure, and role. Beverage industries are always challenged to meet the needs of consumers, namely the demand for safe, healthier, and functional products. Enhancing minimally processed or fresh products intake seems to be the tendency in the market. Thus, food technologists search for compounds from natural resources to be used in beverages, for example, to increase their shelf life, introducing natural preservatives (V.N. Kalpana, 2019).

It is proved that some individual non-thermal methods, as well as natural antimicrobials, are effective in inactivating microorganisms and at the same time do not adversely affect the sensory and nutritional quality of fruit juice and other products.

Moreover, the combination of these techniques could provide synergistic effects on prolonging the fruit juice shelf-life and potentially as a replacement for traditional pasteurization methods (Rupasinghe & Yu, 2015).

2.11 Preservation of Malt Drink

Malt drink is usually pasteurized to kill any remaining live yeasts or other microorganisms and so prolong the shelf life. It is necessary to produce microbiologically safe non-alcoholic beverages supplemented with various functional ingredients (Saritha et al., 2019). Emphasized the addition of moringa extract and had reduced the fungi and coliform growth in the treated pito (non-alcoholic beer) samples than the untreated pito. Moringa is one of such underutilized crops, to promote its utilization and consumption, studies aimed at investigating its antioxidant activity are pertinent (Ifeanyi & Sabina, 2020).

2.12 Response Surface Methodology and Experimental Design

Design of Experiments is a powerful technique used for exploring new processes; increasing knowledge of the existing processes and optimizing these processes for achieving world-class performance. Engineering experimenters wish to find the conditions under which a certain process attains the optimal results. That is, by careful planning of experiments, they want to determine the levels of the design parameters at which the response reaches its optimum. The optimum could be either a maximum or a minimum of a response (output variable) which is influenced by several independent variables (input variables). One of the methodologies for obtaining the optimum results is the response surface methodology. Response surface methodology (RSM) is an effective statistical technique for optimizing complex processes. RSM has been successfully used for various biotechnological and agricultural applications (Kongkaew et al., 2012). Response surface methodology also quantifies the relationship between the controllable input parameters and the obtained response surfaces.

The main advantage of RSM is the reduced number of experimental runs needed to provide sufficient information for statistically acceptable results (Montgomery, 2000).

CHAPTER THREE

3 MATERIALS AND METHODS

3.1 Description of the Study Area

The experiment was conducted at, Debre Berhan University College of Engineering the Chemical Engineering Department laboratory in North Showa Zone, Amara National Regional State, which is the sub town of Debre Berhan and is located 130 km from Addis Ababa.

3.2 Materials

Yellow Maize (Melkasa-7 variety) was received from Melkassa Agricultural Research Center (MARC) which is found Arsi zone near Assela town, 117 km far from Addis Ababa capital city of Ethiopia. A Moringa leaf was bought from Showa Robit town local market.

3.3 Chemicals and Equipment:

Chemicals/ Reagents:

- Distilled Water, Ethanol
- Potassium sulfate
- cycloheximide
- 2-phenyl ethanol
- Quercetin
- Folin- Ciocalteu's phenol
- Formazin solution
- Phenolphthalein, Iodine
- Sodium hydroxide
- Formazin solution
- aluminum chloride
- K_2SO_4, KOH, $NaNO_2$
- CuSO4.5H2O, HCl
- Gallic acid
- Glucose monohydrate
- Raka-ray agar
- Wort agar
- Copper agar
- Bacto yeast extract
- Potato Dextrose agar (PDA)
- Violet Red- Bile agar

Equipment:

- ✓ Miller, Mash bath, pH meter
- ✓ Anton Paar gravity analyzer
- ✓ Spectrophotometer, Turbidity meter
- ✓ Nibem T Foam stability tester
- ✓ Kjeldahl apparatus
- ✓ Weight Balance, Magnetic stirrer
- ✓ Alcoholyzer and Stop watch

3.4 A framework of the Experiment

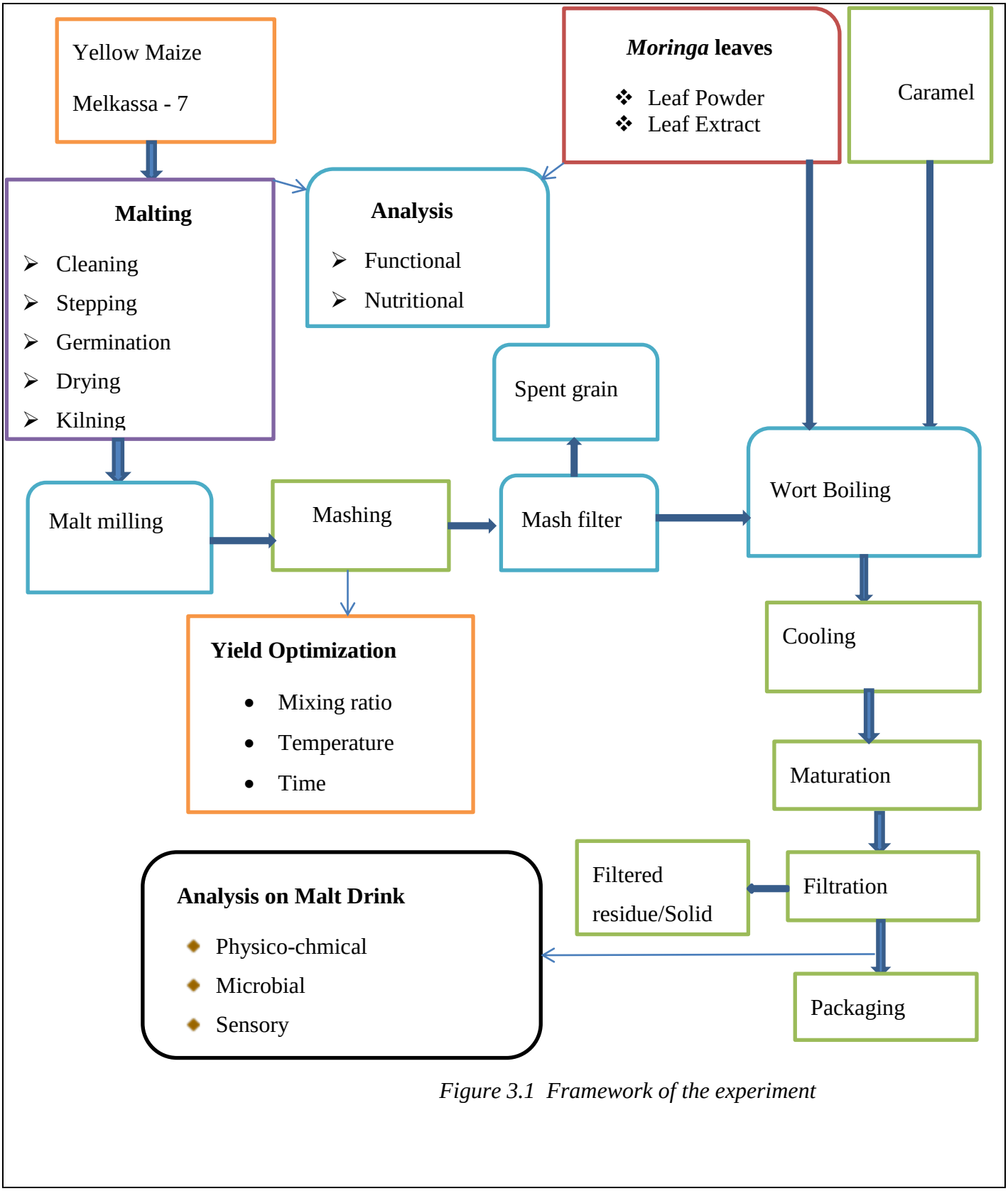


Figure 3.1 Framework of the experiment

3.5 Preparation of the Samples

3.5.1 Maize Malting Process

There are five stages in the process of converting maize into malt product: -

- Maize grading and cleaning: - The maize was received from Agriculture Research Centre. 500 g of maize sample was used in this study. The grain was cleaned through screeners to remove stones, foreign bodies, dust, and straw; then the sample was attained at moisture levels of between 10 and 12%. Finally, the grain was stored in polyethylene.
- Steeping of maize: - Prepared maize was steeped at 25^{o}C room temperature for 48 hours.
- Germination of maize: - After steeping, samples were germinated at 30^{o}C for 6 days in an incubator present at the stated temperature.
- Drying: - Germinated maize was dried at 60^{o}C for 30 hours in an oven, is to about 4% moisture content.
- Kilning: - The sample was kilned at 50^{o}C using a hot air oven for 24 hours,

after kilning the resulting dried malts were polished by removing the sprouts and roots, milled with a grinder, packed in labeled polyethylene bag and stored in a refrigerator (8^{o}C) for subsequent analyses (Deme et al., 2020; Eneje et al., 2004; Ndife & Ugwuona, 2019).

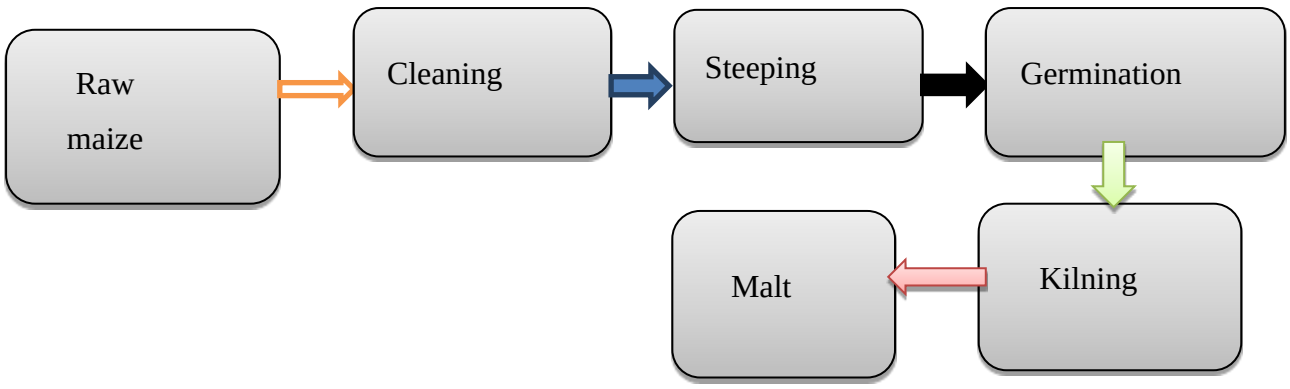


Figure 3.2 Malting Process Steps

3.5.2 Moringa Oleifera Leaf Processing

Moringa Oleifera leaf was collected from the North shoa region community into a clean polyethylene bag. The leaf was washed thoroughly under a tap running water and then air-dried at room temperature (25°C) and relative humidity of 20-30% for 72hrs. The dried leaves were milled into fine powder for 3 minutes and the Moringa powder was collected into a clean airtight bowl, and then stored in a refrigerator at 5°C (Ayirezang et al., 2016; Ndife & Ugwuona, 2019).

3.5.3 *Moringa Oleifera Leaf* powder Extract

Extraction of bioactive compounds was carried out using ethanol solvent according to the following procedure. Twenty grams of the sun-dried and powdered sample were weighed and transferred to a beaker, then 150 ml of the solvent 96% (Ethanol) was added and after agitation was allowed to extract for 72hrs. The mixture was then filtered and the filtrate evaporated and concentrated using a boiling water bath. The crude extract was obtained (Moringaceae et al., 2016).

3.6 Determination of Phenolic and Flavonoids in Moringa Oleifera Leaf Extract

3.6.1 Determination of Phenolic in Moringa Leaf Extract

Total phenolic contents (TPC*)* moringa dried leaves extracts were determined by Folin–Ciocalteu colorimetric method as described by Phuyal et al., (2020) with some modifications. The standard gallic acid solution was prepared by dissolving 5 mg of it in 5mL of methanol (1 mg/ml). Various concentrations of gallic acid solutions in methanol (20, 40, 60, 80, 100, 120, and 140 μg/ml) were prepared from the standard solution. To each concentration, 5ml of 10% Folin–Ciocalteu reagent (FCR) and 4ml of 7% Na_2CO_3 were added making a final volume of 10 ml. Thus, the obtained blue-colored mixture was shaken well and incubated for 30 min at 40°C in a water bath. Then, the absorbance was measured at 760nm against blank. The FCR reagent oxidizes phenols in plant extracts and changes into a dark blue color, which is then measured by a UV-visible spectrophotometer. All the experiments were carried out in triplicates, and the average absorbance values obtained at different concentrations of Gallic acid were used to plot the calibration curve. The samples were prepared in triplicate for each analysis, and the average value of absorbance was used to plot the calibration curve to determine the level of phenolic in the extracts.

The total phenolic content of the extracts was expressed as mg Gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g) (Fachriyaha et al., 2020; Hashemi et al., 2018).

The total phenolic contents in all the samples were calculated by using the formula:

$$C = c*V/m$$

Where: C: Total phenolic content mg GAE/g dry extract

c: Concentration of Gallic acid obtained from calibration curve in mg/ml

V: Volume of extract in ml

m: Mass of extract in grams

3.6.2 Determination of Flavonoids in Moringa Leaf Extract

Total flavonoid content in the extract was determined by aluminum chloride colorimetric assay. Stock solution (4 mg/ml) of Quercetin was prepared by dissolving 4 mg of Quercetin in 1ml of methanol. This standard solution was diluted serially to make various concentrations of 20, 40, 60, 80, 100, 120, and 140 mg/ml solutions. One ml of Quercetin of each concentration was added to the test tube containing 4ml of distilled water. At the same time, 0.3ml of 5% $NaNO_2$ was added to the test tube and 0.3ml of 10% $AlCl_3$ after 5 min. Then, 2ml of 1M NaOH was added to the mixture after 6 min. The volume of the mixture was made to 10ml by immediately adding 4.4ml of distilled water. The total flavonoid content was expressed as Quercetin equivalents using the linear equation based on the calibration curve. The absorbance was measured by a spectrophotometer at 510 nm. Readings were taken in triplicate, and the average value of absorbance was used to calculate the total flavonoid content. The flavonoid content was expressed as Quercetin equivalent (mg QE/g) using the linear equation based on the standard calibration curve (Phuyal et al., 2020).

3.7 Analysis of Proximate Composition

The promotion of yellow maize varieties contains antioxidants that reduce the risk of diseases such as cardiovascular and cancer without altering the intake of other nutrients. According to, Hailu Demeke, (2018)**,** yellow maize varieties (*Melkasa-1q, Melkasa-7,* Gibie Awash, CML 165, CML 161) contain a high amount of nutritional and carotenoid than white maize varieties (BH 660). The raw maize, malted maize, and Moringa dried leaf samples were milled with a laboratory milling machine. The samples were analyzed for their proximate composition (in triplicate) using approved methods of the Association of Official Analytical Chemists (AOAC, 2000).

3.7.1 Moisture Content (Drying Method)

Moisture content was determined using AOAC, (2000). 2.5 g of samples (maize and *moringa* flour) (W_0) were placed in a clean, dried dish (W_1), and then samples were weighed based on their design ratio. The sample together with the dish (which contained the sample) was placed in an oven, which has been preheated to 105 ^{o}C for 3 hours. Then dish was removed from the oven, placed in a desiccator, and cooled to room temperature for 25 min. Then, the dish and content were reweighed (W_2) on an analytical balance. The moisture content was calculated as a percentage of the initial weight of the sample.

$$\boldsymbol{Moisture\ content}\ (\%) = \frac{\boldsymbol{W2-W1}}{\boldsymbol{W0}} * \mathbf{100}\% \qquad \textit{Equation 3.1}$$

Where:

W0: Weight of sample

W_1: Weight of empty drying dish

W_2: Weight of sample with dish

3.7.2 Ash Content

Ash content is determined by high-temperature incineration in an electric muffle furnace according to AOAC, (2000). 2.0 g of dried sampled (W_1) was transferred to a previously ignited and weighed crucible (W_2) and placed in a muffle furnace set at 550°C and heated for 4 hours until the weight becomes stable. The crucible was removed and cooled in desiccators. Then, it was allowed to cool and then weighed (W_3). Weight is expressed as a percentage.

$$\boldsymbol{Ash\ content}\ (\%) = \frac{\boldsymbol{W3-W2}}{\boldsymbol{W1}} * \mathbf{100}\% \qquad \textit{Equation 3.2}$$

Where: W_1 - Weight of sample

W_2-Weight of crucible

W_3 -Weight of ash with crucible

3.7.3 Crude Fat

The crude fat content of the samples is measured by AOAC, (2000), the method by using the Soxhlet- extraction system. Five grams of dried sample (W_1) obtained from the moisture determination was transferred to a 22 x 80 mm paper thimble. A small ball of cotton wool or glass wool was placed into the thimble to prevent the loss of the sample.

One hundred fifty milliliters (150 ml) of petroleum spirit B.P. 60-80^{o}C was added to the flask and the apparatus was assembled. The flask and fat/ oil were heated for 30 min in an oven at 103^{o}C. Then, the flask and contents were cooled to room temperature in desiccators and are weighted (W_3). Finally, the flask is weighed (W_2) accurately and the weight of fat collected is expressed as the percentage.

$$\boldsymbol{Crude\ Fat} = \frac{\boldsymbol{W3-W2}}{\boldsymbol{W1}} * \mathbf{100}\% \qquad \textit{Equation 3.3}$$

Where: W_1 = Weight of sample flour

W_2 = Weight of the extraction flask

W_3 = Weight of the extraction flask plus the dried crude fat

3.7.4 Crude Protein

Kjeldahl method of nitrogen analysis was used for the determination of crude protein (AOAC, 2000). 0.5g of sample was weighted (W_0) into Kjeldahl digestion flasks and 1g of catalyst mixture (K_2SO_4, and $CuSO_4.5H_2O$ in 10:1) was added to each digestion flask. 5ml of concentrated $H_2SO_4$was added and the digestion flasks were placed in the digester and heated at 350°C for about 3 hours until the solution is clear white. The flasks were removed from the digester and allowed to cool and then 50ml of distilled water and 50 ml of 40% NaOH were added. The contents were distilled by inserting the digestion tube line into the receiver flask containing 25ml of 4% boric acid solution. The distillate was titrated by standardized 0.1N HCl until the end of the titration. And then the volume of HCl consumed to reach the titration end point is noted and the nitrogen content was calculated using equation 3.4. And finally, the protein content was calculated by 6.25 times % of nitrogen (Deme et al., 2020).

$$\boldsymbol{Nitrogen}\ (\%) = \frac{\boldsymbol{V2-V1}}{\boldsymbol{W}} * \mathbf{14} * \mathbf{100}\% \qquad \textit{Equation 3.4}$$

Where: V_1= volume (ml) HCL solution used in the titration of the blank

V_2= volume 25 (ml) HCL solution used in the titration of the sample

W= sample weight and 14 is the molecular weight of nitrogen

The protein content is calculated from the equation

$$\textbf{Protein content}\ \left(\%\frac{\mathbf{w}}{\mathbf{w}}\right) = \mathbf{6.25} * \%\textbf{Nitrogen} \qquad \text{Equation 3.5}$$

3.7.5 Crude Fiber

The crude fiber content of the samples is measured by AOAC, (2000) method. 2.0 g of the sample was added into a beaker containing 180 mL preheated 0.128 M H_2SO_4 and boiled for 30 min using a water pressure filter system. The moisture was filtered and the residue was washed 3 times with hot water. The residue was collected at 150 mL preheated 0.22 M KOH was added and boiled for another 30 min. The mixture was filtered and the residue was washed on the water pressure system 3 times with acetone. The residue was collected in a crucible, dried at 130°C for 1 h, and weighed. It was washed in a muffled furnace for 3 h at 500°C and later weighed after cooling. Percent crude fiber was calculated (Adeniyi & Ariwoola, 2019).

$$\%\boldsymbol{Crude\ Fibre} = \frac{(\boldsymbol{A}-\boldsymbol{B})}{\boldsymbol{C}} * \mathbf{100}\% \qquad \textit{Equation 3.6}$$

Where: A = weight of crucible with dry residue (g)

B = weight of crucible with ash (g)

C = weight of sample (g)

3.7.6 Carbohydrate

This was determined by subtracting the values of the moisture content, ash, crude fat, crude fiber, and crude protein parameters from 100 (i.e. by difference method) (Adeniyi & Ariwoola, 2019).

Carbohydrate = 100 – (%Moisture + %Ash + %Crude Fat + %Crude Fiber + %Crude Protein)

Equation 3.7

3.7.7 Determination of Gross Energy Value

The gross energy values of the samples were estimated using the factors for protein (4Kcal/g), fat (9 Kcal/g), and carbohydrate (4 Kcal/g) (Hailu Kassegn, 2018).

The equation is:

$$Gross\ Energy\ Value\ (Kcal) = \%Crude\ Protein * 4 + \%Crude\ Fat * 9 + \%Carbohydrate * 4 \quad Equation\ 3.8$$

3.8 Physicochemical Analysis

3.8.1 Germination Energy

According to Frančáková et al., (2018) method with slight modification, 500 grains of sample raw maize were transferred into a funnel standing in tap water at 20^{o}C. The water was removed after steeping for 3 hours. The steeping was repeated for 2 hours after 20 hours from the beginning of the test. After 72 hours from the beginning of the test, the funnels were emptied and the number of non-germinated grains was counted. The average result of the two counts after 72 hours was obtained. The formula below is used to calculate the Germination Energy:

$$\boldsymbol{Germination\ Energy\ (GE) = \frac{500-N}{5}} \qquad \textit{Equation 3.9}$$

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

3.8.2 Germination Capacity

Two hundred uniform-sized and clean grains were hand-picked and steeped in a 500 ml beaker containing 200 ml of 0.75 % hydrogen peroxide (H_2O_2) solution and incubated for two days at a temperature of 21^{o}C.

At the end of 2 days, the grains were strained and steeped again in 200 ml of H_2O_2 at 21^{o}C for further 24 hours. The germinated grains were then counted and the germination capacity was calculated using the formula (Frančáková et al., 2018):

$$\boldsymbol{Germination\ Capacity\ = \frac{200-N}{2}} \qquad \textit{Equation 3.10}$$

Where: N = grains that did not show roots.

3.8.3 Thousand Kernel Weight (TKW)

Grains separate into 5 trays so that the lightest grains are in tray No. 1 and the heaviest grains in tray No. 5. After completing this step, separate random samples from the grains of trays 1 and 5 from each experimental unit for the calculation of thousand-grain weights. Then, separated a thousand grains from each sample by using the seed counter set, and weighed (Moshatati & Sciences, 2016).

3.8.4 Malting Weight Loss (MWL)

According to Hailu (2018), the malting weight loss of each variety was determined by calculating the dry matter-based 1000-kernel weight of the dried polished malt and subtracting this from the dry matter-based 1000-kernel weight of the intact dry grain.

$$\textbf{\textit{Malting Weight Loss}} = \frac{TKWG - TKWM}{TKWG} \qquad \textit{Equation 3.11}$$

Where: TKWG: Thousand Kernel Weight grain

TKWM: Thousand Kernel Weight malt

3.9 Malt Drink Process

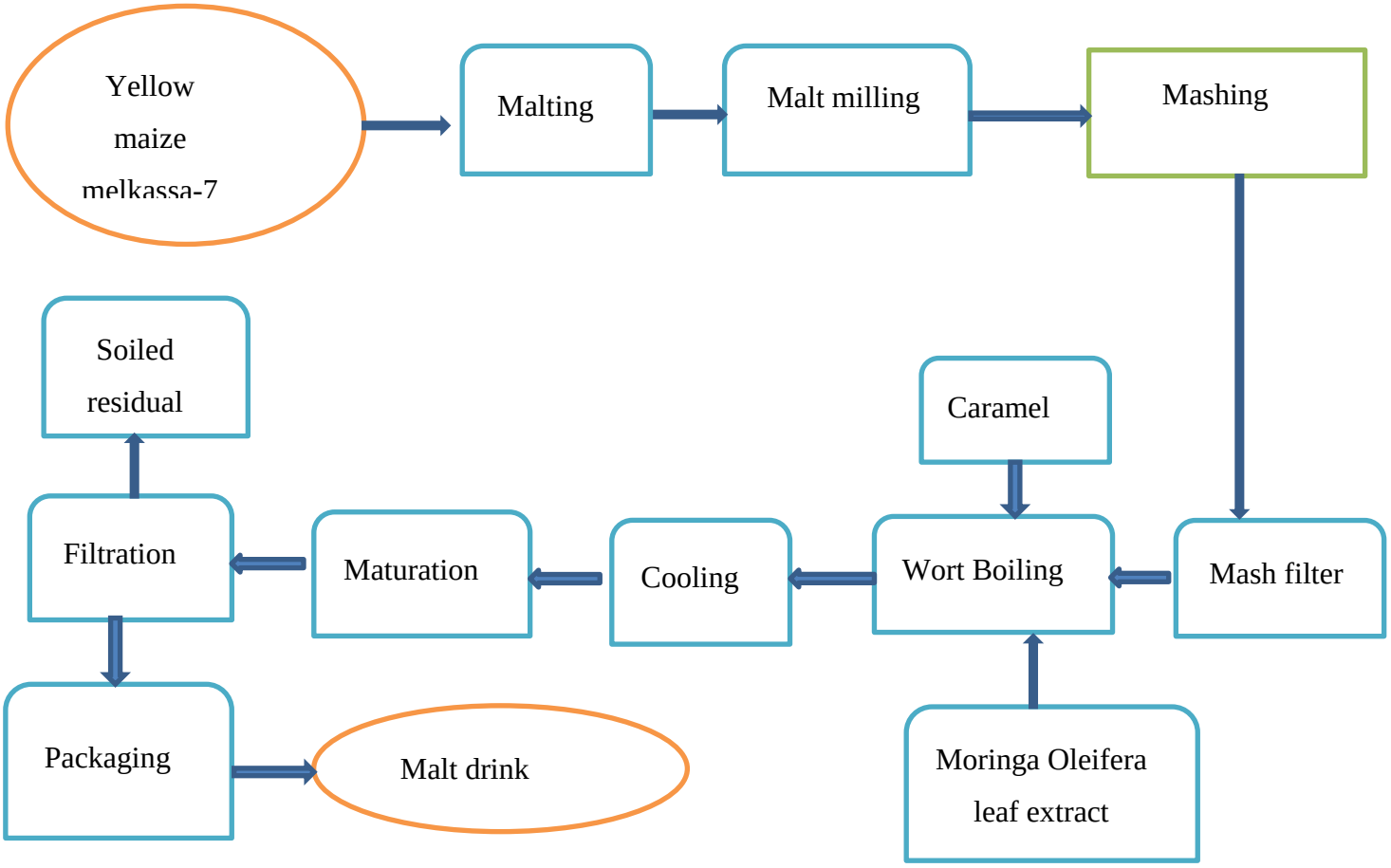


Figure 3.3 Malt drink process follow diagram

3.9.1 Optimization and Mashing process

During optimization of process variables in the mashing step, analysis was performed to determine the mash extract of 20 treatments, which was done by design expert software and as part of extract analysis, to monitor mashing process conditions.

Maize malts are milled in a laboratory disc mill with a 0.1 mm milling gap. Then mixed with water in the mash tun, the malt grits to water ratio of 1:3 (100 g grits and 300 mL water) to 1:7 (100 g grits and 700 mL water) was used for the mashing process. The mash obtained was boiled between 30 min to 60 min and at a temperature range between 60oC to 80^{o}C. The process of mashing refers to the conversion of brewing material in the presence of enzymes into the fermentable extract. During mashing, an Iodine test is performed. In this step, starch must be completely degraded, so a sample was taken to determine whether it was degraded or not. I added a drop of iodine solution to the sampled mash. If it gives a blue-black color, it indicates incomplete conversion, whereas color less indicates complete conversion into fermentable sugars (Ayirezang et al., 2016; Elgorashi et al., 2016; Ndife & Ugwuona, 2019).

3.9.2 Extract Content (EBC Method 4.6, 2004)

The mash in the beaker was filtered by emptying into a pleated filter paper (Whatman No 4) in the funnel and then measured the extract using Anton Paar gravity analyzer, expressed in degrees Plato (P) and converted to a % dry matter basis as follows:

$$\text{Extract as wet basis } (wt\ \%)E1 = \frac{\text{P (M+800)}}{100-M} \qquad \text{Equation 3.12}$$

$$\text{Extract as dry basis } (\%)E2 = \frac{\text{E1} * 100}{100-\text{M}} = yield\ extract \qquad \text{Equation 3.13}$$

Where: P= extract content of mash, in o Plato (deg.plato)

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

E_1 = malt Extract in wet basis % (m/m).

E_2= malt Extract in dry basis % (m/m).

800=the amount of distilled water added per 100gm of the mash.

Figure 3.4 Anton paar gravity analyser: Model no. 500

3.9.3 Mash filter

At the end of mashing, wort and spent grain are separated by using a filter aid (watsman paper no 4) to remove insoluble components of the cereals.

3.9.4 Wort Boiling

After the spent grain is separated from the wort, caramel is added as a coloring agent to color the wort. The amount of color was added at 5ml per 1000 ml of wort. The purpose of adding caramel is to impart a brownish color to the malt drink.

This involved the addition of Moringa extract to malt drink at 5, 10, and 15% replacement levels to obtain a final volume of 1000 ml. Thus, for 5% (950 ml of malt drink and 50 ml Moringa extract), 10% (900 ml of malt drink and 100 ml Moringa extract), and 15% (850 ml of malt drink and 150 ml Moringa extract). Pure wort (100%) is used as a control, and the final product is an analysis of consumer acceptance and antimicrobial effects of the added Moringa on the growth of fungi and coliforms in malt drinks (Ayirezang et al., 2016).

The wort was boiled for 60 min at (90°C up to 100°C) (Le & A, 2017).

Changes during Wort Boiling

- ✓ Sterilization of wort: many bacteria, if they are not destroyed make malt drink sour and undrinkable
- ✓ The Concentration of wort:
- ✓ Isomerization of bitter substance:
- ✓ Reduction of wort nitrogen level: this is important to coagulate nitrogen compounds that cause haziness in the malt drink.
- ✓ Wort boiling is important to stabilize the malt drink.
- ✓ Reduction of volatile compounds: during boiling volatile compounds are escaped.

3.9.5 Maturation

After the end of the wort boiling, the malt drink stayed in the Carlsberg, which was made from stain-less steel, and the temperature was adjusted to a lower temperature (below 0°C) to be saturated. During this time, it was necessary to settle down haze materials.

3.9.6 Filtration

Finally, malt drink products are filtered to improve stability. Filtration is the process in which a turbid liquid or unfiltered liquid is quality and other non-fermentable sugar neglects the clarity. The common method of removing these impurities was filtration with watsman paper no 4 and kieselguhr (filter aid)

3.9.7 Pasteurization

The final finished products were pasteurized for 1hr at 60oC by using a water bath.

3.9.8 Bottling

After filtration, the malt drink was filled into a sanitized bottle, and the bottles had the right amount of malt drink to provide the necessary headspace to allow the bottle to be conditioned and immediately capped with corky. Then the bottles have been labeled with a product type and production date. Bottles were permitted to be conditioned at room temperature.

3.10 Physico-Chemical Analysis of Malt Drink

The characteristic of the finished product was evaluated by chemical and physical methods. All samples for analysis were filtered by using filter papers together with filter aids; whereas filtration is not needed for the finished product. And also each sample was adjusted to room temperature.

3.10.1 Chemical Parameters of Non-Alcoholic Beer (EBC Alcoholyzer Method 9.3-9.4, 2010)

The most important parameters of non-alcoholic malt drink were determined by using Anton Paar alcoholizer. Anton Paar calibration and cleaning were performed daily with a constant frequency. The non-alcoholic malt drink was filtered and degassed at room temperature to remove the carbon dioxide. The parameters determined by the instrument were the alcohol content (%v/v), alcohol content (%w/w), original gravity (^{o}p), apparent extract (%w/w), real

extract (%w/w), specific gravity, total Nitrogen, head retent oxygen (DO_2) and carbon dioxide. Before measuring the parameters the Anton Paar device is calibrated and cleaned. Then, 100ml of the sample was filtered and injected into the device while the result is displayed on the screen after five minutes.

$$Real\ Extract\ (RE) = 0.251 + 1.2980 \times S + 0.1179 \times R \qquad \textit{Equation 3.14}$$

$$Alcohol\ Content\ (AC) = 0.323 - (2.774 \times S + 02691 \times R) \qquad \textit{Equation 3.15}$$

$$Original\ Gravity\ (OG) = \frac{(2.0665 \times AC + Real\ extract)}{100 + (1.0665 \times AC)} * 100 \quad Equation\ 3.16$$

Where:

S: Specific gravity

R: Refractive index

Figure 3.5 Anton Paar alcoholizer: Model no. 4500

3.10.1 Physical Parameters of Malt Drink

P^H (EBC Method 8.17, 2010)

One hundred mL of filtered sample was collected. The collected sample was degassed and transferred to a 50mL vial. The calibrated pH meter electrode was immersed in the sample and the temperature was adjusted to 20^{o}C. Finally, the value was displayed on the screen.

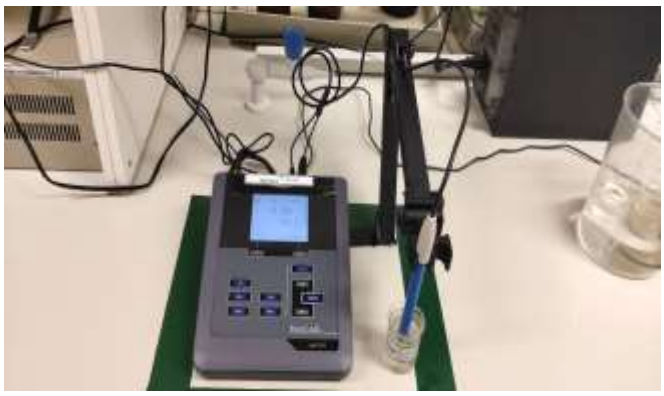


Figure 3.6 PH meter: model no. PH 7310 & PH 7310 P

Color (EBC Method 8.5, 2010)

For non-alcoholic malt drinks, 100ml of the sample was filtered and carbon dioxide (CO_2) was removed by shaking or degassing. But in the case of malt drink, the sample was centrifuged at 3000rpm for 10 min. Suspended solids were then settled to the bottom of vials. The absorbance was measured at 430nm using a UV-VIS spectrophotometer.

Result expression

$$\boldsymbol{Color(C) = 25 * A * F} \qquad \textit{Equation 3.16}$$

Where:

C = color of the sample in EBC

A = absorbance of sample at 430 nm in 1.00cm cuvette

F = dilution factor

25 = multiplication factor

Figure 3.7 UV-VIS spectrophotometer: Model no.840-210600

The haziness of Non-Alcoholic Beer (EBC 9.29, 2010)

Haze can be due to the suspension of grain solids that were transferred over from the mash to the finished non-alcoholic malt drink. It is a result of the interaction of proteins and polyphenol (tannins) that form particles that can scatter light. Non-alcoholic beer colloidal haze is generally the result of protein molecules within the non-alcoholic beer joining with polyphenol to form molecules large enough to cause turbidity. The bright non-alcoholic malt drink sample was taken in a glass beaker and degassed at room temperature. Then the haziness was measured by a haze meter which was calibrated with 1 EBC standard formazin solution. Finally, the result was reported in the EBC unit.

Figure 3.8 Turbidity meters: Model no. Haffmans Vos Rota 90/25

Head Retention (Nibem.T Method) EBC Method 9.42, 2010

The foam stability of non-alcoholic beer was determined by using NIBEM.T / TPH foam stability tester. The malt drink was temperate to 20°C in a sealed container and dispensed through a foam flashing Device (Flasher) in which beer was forced under carbon dioxide pressure. This was produced by a standard glass of beer foam. A movable plate containing three electrodes was lowered so that it just rests on the surface of the malt drink foam. As the foam was collapsed the electrodes become reduced. The plate was moved to maintain contact with malt drink foam. Then value was reported in seconds.

3.11 Microbial Analysis and Media Preparation (EBC Method 9.45, 2010)

Microbial activity was involved in every step of production and defined many sensory characteristics that contribute to final quality. Most of these activities were desirable, as non-alcoholic malt drink is the result of non-fermented, but others represent threats to the quality of the final product and must be controlled actively through careful management.

Raka-Ray Agar

Suspended 77.1g (disco) of raka ray agar medium and 10mL of tween 80 were added in 1L distilled water. Then pH was adjusted to 5.3. The selectivity of the medium was increased by the addition of 3g of 2-phenyl ethanol and 7mg of cycloheximide (actidione) per liter before autoclave. The solution was stirred and heated after the solution was dissolved; the molten medium was dispensed into final screw-cap containers and sterilized in an autoclave for 15minutes at 121^{o}C. The medium was cooled to 45^{o}c and poured into sterile Petri dishes. Alternatively, it was held as a solid agar and melted before use or it was used immediately after cooling to 45^{o}c in pour plate determination.

Wort –Agar

The wort was filtered from the production process by keslguhur and the strength of the malt drink was adjusted to 10^{o}p with distilled water. The pH of the solution was measured and adjusted to 5 with 1N of HCl or with 1N NaOH solution. 20g of bacto agar was added to this 10^{o}p solution (pH= 5.0) and boiled with constant stirring until the bacto agar was dissolved.

The molten medium was dispensed into final screw cap containers and sterilized in an autoclave for 15 min at 121^{O}C. For the spread plate for the membrane filtered sample, the medium was cooled to 45^{o}C and poured into sterilized Petri dishes.

Copper Agar

Copper sulfate solution was prepared by dissolving 1.60g of $CuSO_4.5H_2O$ in 10mL distilled water. The solution was sterilized by membrane filtration (0.45μm filter). Next prepared basal medium composition of Malt extract 3g,bacto yeast extract 3g,bacto peptone 5g, Glucose monohydrate 10g,bacto Agar 20g, and distilled water 1000mL. For the preparation of the medium first, the ingredients of the basal medium were added. Then the medium was mixed well by stirring and distributed into containers.

The prepared solution was then sterilized by autoclave at 121°C, for 15 minutes. Cooled to 50°C and 0.3mL of sterile copper sulphate solution was added aseptically per 100mL medium. Therefore mixed well and poured about 20mL into sterile Petri dishes. The plates were solidified and dried in an incubator at approximately 30°C. For higher levels of $CuSO_4$ (a maximum of 300 mg/L was permitted) adjusted the volume of copper sulphate solution appropriately. The plates were then incubated at 30°C for 3 days.

Yeast and Mould Count

Samples of the wort were serially diluted following similar methods as for total bacterial count but dilutions surface plated on Potato Dextrose Agar (PDA). The dried plates were then incubated at 25^0C for 3 to 5 days. Colonies with a blue-green color were counted as yeasts and molds (Yousef and Carlstrom, 2003).

Total Coliform Count

From each previously prepared serial dilution of the samples, portions of 0.1 ml of appropriate dilutions were inoculated in duplicate on Violet Red-Bile Agar (VRBA); Petri dishes were incubated at 32°C for 24hrs and counts were made on typical dark red colonies normally measuring at least 0.5 mm in diameter on un-crowded plates. Finally, the plate counts were calculated as N, the number of colony-forming units of coliforms per ml of wort sample using (Marth, 1978).

Escherichia coli count

To isolate Escherichia coli for each sample, dilutions were made by aseptically withdrawing 1 ml of each sample into 9 ml of 0.1% sterilized buffered peptone water, then serial dilutions were prepared. A 10 ml was drawn from appropriate dilutions and was plated on MacConkey Agar. The sterile glass beads were used to spread the sample on agar, and plates were incubated at 37 ºC for 24 hrs. The positive colonies which showed pink color were subcultured to obtain pure colonies (Addo et al., 2011).

3.12 Shelf-Life of Malt Drink (EBC Method)

Haze stability of malt drinks is a very important measurement. Clear filtered malt drink in time loses its brilliance until it finally becomes hazy (turbid). It is therefore to know as soon as possible if the malt drink was stable for a long time or if it will soon form a haze. Consequently,

methods have been developed which will predict, after only a few days, the tendency of a non-alcoholic beer to form a haze. The most important method is the forcing test. The finished bottled product was allowed to cool at 0^{o}c overnight and the haziness was measured in the morning at 0^{o}c by calibrating the haze meter with 1 EBC formazin solution. The bottles were placed in a water bath in an upright position at 60^{o}C for 48 hours without agitation. Again the samples were placed in the refrigerator to cool at 0^{o}C and the final haziness was measured. Therefore the initial and final results mean values were reported in the EBC unit. The result was more in agreement with practice and was obtained by storing the sample at a temperature related to the likelihood of trade for 6 months and above. (EBC 9.30, 2010)

3.13 Sensory Evaluation and Description (EBC Method)

Sensory analysis of non-alcoholic malt drink products was conducted by participating six professional tasters from Dashen brewery who had enough experience with the malt drink flavor and beer acceptability taste. The comment on flavor (color, bitterness, fruity, oxidation, sweetness, sourness, and diacetyl taste) was conducted on 3 malt drink samples with (M5, M10, and M15) leaf extract. The malt drink sample was served using pure use and through the plastic at a low temperature (5^{o}C) to protect from contamination and to detect the natural flavor of non-alcoholic malt drinks. One score sheet and pure rinsing water for all assessors were placed on each table to easily give a score for each parameter. The assessors were instructed how to give a score and rank the given non-alcoholic malt drink samples within 30minute. Therefore the statistical data for the selected basic parameters were collected from the numerical result of sensory analysis. (EBC 13.0-13.14, 2010)

3.14 Experimental Design

Design-expert software (Trial Version 7.1.3, Stat-Ease Inc., U.S.A.) was used for the experimental design, regression, and graphical analyses of the data. Analysis of Variance ANOVA is conducted on the mean values to determine the significance of any differences between samples, and the statistical significance is set at a 5% level (Bolanle et al., 2012). Face center central composite design (FC-CCD) was used to examine the effects of the three independent variables (Malted grain: water blend (X_1), Temperature (X_2), and Time (X_3)) in two-level combinations with a total number of 20 runs were used to conduct this study, as

presented in Table 3.1. Extract content was expressed as the dependent variable (response) of the mashed product.

Table 3.1 Experimental design formulated for mash optimization

Variable	Unit	Codes	Variable level	
			-1	1
Malted grain : water	gm.: ml	X_1	100:300	100:700
Time	(Min)	X_2	30	60
Temperature	(^{O}C)	X_3	60	80

The quadratic model for predicting the optimal point is expressed according to the Equation:

$$Y = \beta o + \sum_{j=1}^{k} \beta i Xi + \sum_{j}^{k} \beta ii X_i^2 + \sum_{i\,(j=1}^{k} \beta i Xi\, Xj$$

Where Y is the response calculated by the model;

X_i and X_j are the coded variables; and

βi, βii, and βij are the coefficients estimated by the model and present the linear, quadratic, and interaction effects of each factor, respectively.

CHAPTER FOUR

4 RESULTS AND DISCUSSION

4.1 Physicochemical Analysis Moringa

Total phenolic content of Moringa leaves was determined by Folin–Ciocalteu (F–C) method using Gallic acid as the standard which methods provided in section 3.3.4 and the results were displayed in Table 4.1. The absorbance values recorded at different concentrations of Gallic acid were used for the plotline of the calibration curve (Appendix 1). Total phenolic content of the extracts was calculated from the regression equation of calibration curve ($y = 0.0051x + 0.0357$; $R^2 = 0.9896$) and expressed as mg Gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g).

The results showed that total phenol content was obtained using a concentration of ethanol solution of 96 %, with a total phenol content of 84.10 ± 1.8 mg GAE / g dried. According to Phuyal et al., (2020), report the total phenolic content value was observed for the fruits was 226.3 ± 1.14 mg GAE/g. however; the present result was in close agreement with Series & Science, (2019), a report that was finding of total phenol content was 87.11 ± 0.81 mg GAE / g dried leaves.

The total flavonoid content of the extracts was calculated from the regression equation of the calibration curve ($y = 0.0034x + 0.0371$) and expressed as mg Quercetin equivalents (QE) per gram of sample in dry weight (mg/g). The result of total flavonoids content was indicated that 70.79 ± 0.73 QE / g dried (Table 4.1). In a previous study, Series & Science, (2019), reported that the highest flavonoid content (75.79 ± 0.73 mg Quercetin/ g of dried leaves) is quite higher than that of the present study. The concentration of phenols and flavonoids also depends on the polarity of the solvents used for extraction

Table 4.1 The physicochemical analysis of Moringa extracted

Content	***Moringa* extracted sample**
Total phenolic content	84.10 ± 1.8 mg GAE / g dried
Total Flavonoids content	70.79 ± 0.73 mg QE / g dried

4.2 Proximate Composition of Raw Materials

Proximate analysis is a crucial part of raw material quality assessment in the food industry. Maize and *Moringa Oleifera* leaves are the main raw material in this study. Hence, the values of moisture, ash, crude protein, crude, fiber, crude fat, carbohydrate, and gross energy of flours were evaluated for the leaf powder, raw, and germinated maize and presented in Table 4.2.

The results indicate that moisture, ash content, and carbohydrate were increased significantly as a result of the germination of maize from 9.36% to 10.2%, 1.5% to 2.1%, and 71.22% to 74.48% respectively. The rest of this result is within the finding of Omer et al., (2021). The germination process decreased the protein, fat, and fiber of maize significantly from 12.22% to 8.21%, 3.5% to 3.0%, and 2.2% to 2.01% respectively. The effect of germination on protein, fat, and fiber seems to be a conflicting report by Imran, (2015). Protein, fat, and fiber contents are decreased during the removal of bran and germ from the germinated cereals. Whereas, protein barely is an insignificant difference from maize. Thus, bran and germ are rich in protein, fat, and fiber content (Ikram et al., 2017). This result of protein reduction and insignificant effect of fatwas within the finding of Omer et al., (2021) who found a reduction in the total protein of maize after germination. The value of gross energy is affected by crude protein and crude fat. The proximate composition of Moringa leaves powder is similar to previous observations reported by (Ogbe & Affiku, 2011). The selected *Melkassa* 7 maize variety is a very good source of carbohydrate (particularly starch) with little content of protein, ash, and fat and in carbohydrates; it is more compared to that of barley.

Table 4.2 Proximate and energy composition of maize and Moringa Oleifera leaf powder

Nutrients	Raw Maize	Germinated Maize	Moringa Leaf powder	Barley (Arif et al., 2011)
Moisture (%)	9.36 ± 0.02	10.2 ± 0.05	7.80 ± 0.02	10.42
Crude protein (%)	12.22 ± 0.03	8.21 ± 0.04	25.45± 0.03	12.28
Crude Fat (%)	3.5 ± 0.01	3.0 ± 0.06	2.10 ± 0.01	1.70
Ash (%)	1.50 ± 0.02	2.10 ± 0.03	5.85 ± 0.01	2.45
Crude Fibre (%)	2.20 ± 0.01	2.01 ± 0.03	15.20± 0.02	5.90
Carbohydrate (%)	71. 22 ± 0.09	74.48 ± 0.21	43.6 ± 0.1	67.25
Energy (K.Cal)	365.26 ± 0.5	357.76±0.35	70.79± 0.50	333.42

4.3 Quality of Malt Analysis

The quality of the malt is assessed in terms of thousand kernel weight, Malting loss, Germination capacity, and Germination energy. The malting parameters were calculated by the methods provided in section 3.4. And the results were displayed in Table 4.3. Thousand kernel weights is a measure of seed quality. For the selected maize its value is 273.5 g. and is more compared to that of barley. Malting loss due to growth of root and shoot of germinated Melkassa-7 variety is 13.5%, whereas, for barely it are 20.845. The germination capacity which is the percentage of living corns with in the sample is 95%. The germination energy represents the per cent of grains expected to germinate fully is 98%. High germination energy compared to barley is also a favorable factor in addition to high weight and low malting losses compared to barley.

Table 4.3 Physical and malting properties of Maize

Parameters	Yellow maize	Barley	Reference
Malting Loss (%)	13.5 ± 0.50	8.05 - 20.84%	(Iwouno, J.O., and Odibo, 2015)
Thousand Kernel Weight	273.50± 0.50	271.50± 2.50	(H & Vic tor, 2014)
Germination Capacity (%)	95.00 ± 1.20	> 96%	(D. Kumar et al., 2013)
Germination Energy (%)	98.00 ± 0.20	> 96%	(D. Kumar et al., 2013)

4.4 Optimization of Mashing Process

4.4.1 Saccharification (Iodine Test)

Temperature is one of the critical factors in starch conversion to simple sugars. During mashing, starch must be completely degraded. To know whether it is completely degraded or not iodine test was used. Experiments were conducted at 60^{o}C, 70^{o}C, and 80^{o}C, and the conversion was tested. A colorless result indicates conversion, whereas blue black not. As per the results in Table 4.4, the starch conversion during mashing requires higher temperature (>60^{o}C) to break down starch to maltose and then maltose to α-amylase.

Table 4.4 Iodine test result of starch conversion during mashing

Mashing of Yellow maize malt	Mashing temperature		
	60^{o}C	70^{o}C	80^{o}C
	Blue black	colorless	Colorless

4.4.2 Data Analysis of Yield of Extract

The starch conversion and yield depend on mashing temperature, time, and the volume of water added for 100 g of malt during the mashing process. Finding out the optimum values of these parameters to obtain the maximum yield is an important cost-cutting aspect. Design Expert 7 is used for these studies. As mentioned in Table 4.5, 20 experiments were conducted and the corresponding yield was estimated as mentioned in section 3.5.2, and the result was submitted as a single response (as shown in the last column).

Table 4.5 Experimental design formulated for Mashing Optimization

Run	Temperature (℃)	Time (min)	Mixing (ml)	Extract Yield (%)
1	70.00	45.00	163.63	61.75
2	60.00	30.00	300.00	22.36*
3	60.00	30.00	700.00	25.8
4	70.00	45.00	500.00	84
5	70.00	19.77	500.00	34
6	80.00	30.00	700.00	54.6
7	60.00	60.00	700.00	24.5
8	60.00	60.00	300.00	54
9	70.00	45.00	500.00	85.1
10	70.00	70.23	500.00	78
11	80.00	60.00	300.00	81
12	70.00	45.00	836.36	39
13	70.00	45.00	500.00	85.3
14	70.00	45.00	500.00	87.35**
15	70.00	45.00	500.00	83.1
16	86.82	45.00	500.00	81
17	53.18	45.00	500.00	23.1
18	80.00	60.00	700.00	74
19	70.00	45.00	500.00	78.9
20	80.00	30.00	300.00	42

Note: * Minimum value; ** Maximum value.

Data in Table 4.5 shows that the lowest extract content value of mash, 21.8% was observed at (Experiment No. 17) mashing temperature of 53.18°C, mashing time of 45 min, and 500ml of water. The highest extract content value, 85.8% was observed for (Experiment No. 14) mashing temperature of 70°C, mashing time of 45 min, and 500ml of water.

4.4.3 The analysis of variance or ANOVA

Table 4.6 Analysis of variances (ANOVA) for Quadratic Mode

Sum of Source	**Squares**	**Mean DF**	**F Square**	**F Value**	**Prob > F**	
Model	11418.79	9	1268.75	68.37	< 0.0001	Significant
A	3619.01	1	3619.01	195.01	< 0.0001	
B	1939.24	1	1939.24	104.50	< 0.0001	
C	252.48	1	252.48	13.61	0.0042	
A^2	2092.08	1	2092.08	112.73	< 0.0001	
B^2	1635.21	1	1635.21	88.11	< 0.0001	
C^2	2302.79	1	2302.79	124.09	< 0.0001	
AB	98.42	1	98.42	5.30	0.0440	
AC	125.29	1	125.29	6.75	0.0266	
BC	345.06	1	345.06	18.59	0.0015	
Residual	185.58	10	18.56			
Lack of Fit	144.65	5	28.93	3.53	0.0961	Not significant
Pure Error	40.93	5	8.19			
Cor Total	11604.37	19				

The Model F-value of 68.37 implies that the model is significant. There is only a 0.01% chance that a "Model F-value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case, A, B, C, A^2, B^2, C^2, AB, AC, and BC are significant model terms. Values greater than 0.1000 indicate that the model terms are not significant.

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

The "Lack of Fit F-value" of 3.53 implies there is a 9.61% chance that a "Lack of Fit F-value" this large could occur due to noise.

As presented in Table 4.7, below, the Coefficient of Variation, the standard deviation is expressed as a percentage of the mean.

Table 4.7 Model adequacy measures

Std. Dev.	4.31	R-Squared	0.9840
Mean	59.94	Adj R-Squared	0.9696
C.V. %	7.19	Pred R-Squared	0.8974
PRESS	1190.64	Adeq Precision	21.335

Predicted Residual Error Sum of Squares, which is a measure of how the model fits each point in the design; the R-Squared, measure of the amount of variation around the mean explained by the model; Adj R-Squared, is a measure of the amount of variation around the mean explained by the model. Pred R-Squared is a measure of the amount of variation in new data explained by the model, and Adequate Precision is a signal to disturbance ratio due to random error were used to decide whether the model can be used or not.

The "Pred R-Squared" of 0.8974 is in reasonable agreement with the "Adj R-Squared" of 0.9696. "Adeq Precision" measures the signal-to-noise ratio. A ratio greater than 4 is desirable. In this study, the ratio of 21.335 indicates an adequate signal. Hence, this model can be used to navigate the design space.

The regression coefficients and the corresponding 95% CI (Confidence Interval) High and Low were presented in table 4.8 below. If zero was in the range of High and Low 95% Confidence Interval, the factors have no effect. From the 95% CI High and Low values of each model term, it could be concluded that the regression coefficients of maize percentage and the interaction terms of mashing temperature, time & maize- water mixing percentage have a highly significant effect on the mashing process.

Table 4.8 Regression coefficients and the corresponding 95% CI High and Low

Factor	Coefficient Estimate	Standard Error	95% CI		*VIF*
			Low	High	
Intercept	84.08	1.76	80.16	87.99	
A -Temprature	16.28	1.17	13.68	18.88	1
B –Time	11.92	1.17	9.32	14.51	1
C - Mixing ratio	-4.30	1.17	-6.90	-1.70	1
A^2	-12.05	1.13	-14.58	-9.52	1.02
B^2	-10.65	1.13	-13.18	-8.12	1.02
C^2	-12.64	1.13	-15.17	-10.11	1.02
AB	3.51	1.52	0.11	6.90	1
AC	3.96	1.52	0.56	7.35	1
BC	-6.57	1.52	-9.96	-3.17	1

The quadratic polynomial model equation

Final Equation in Terms of Coded Factors:

$$Extract\ yeild = +84.08 + 16.28 * A + 11.92 * B - 4.30 * C - 12.05 * A2 - 10.65 * B2 - 12.64 * C2 + 3.51 * A * B + 3.96 * A * C - 6.57 * B * C\ .Equation\ 4.1$$

Final Equation in Terms of Actual Factors:

$$Extract\ yeild = -726.47469 + 16.45434 * Temprature + 4.51301 * Time + 0.25452 * Mixing\ ratio - 0.12049 * Temprature2 - 0.047343 * Time2 - 3.16021E-004 * Mixing\ ratio2 + 0.023383 * Temprature * Time + 1.97875E-003 * Temprature * Mixing\ ratio - 2.18917E-003 * Time * Mixing\ ratio$$

Equation 4.2

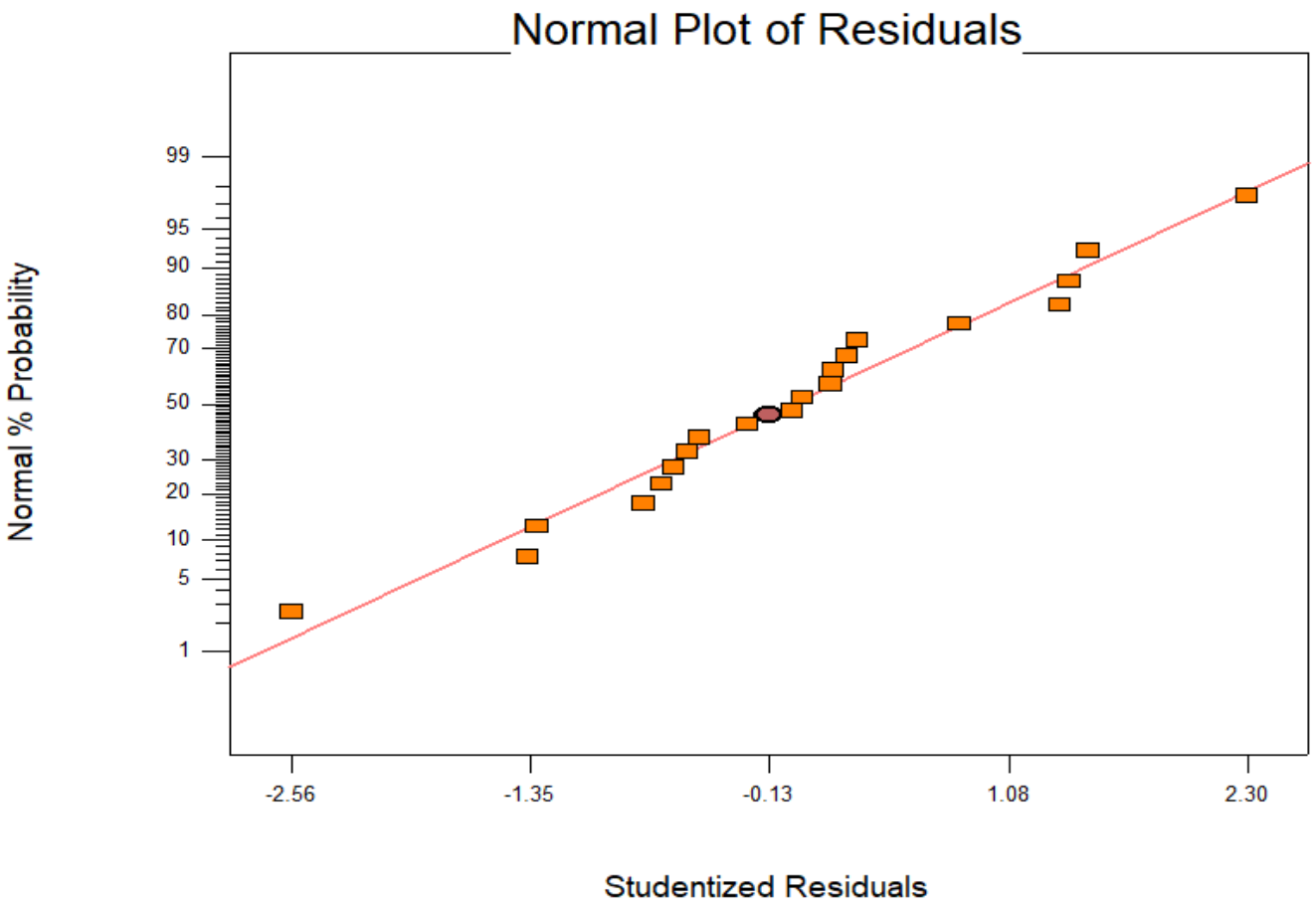


Figure 4.1 Normal plots of residuals for a yield of extract

From the plot as shown above (Fig. 4.1), the plot of the normal probability of Studentized residuals for Extract Yield coefficient. The plot of normal probability indicates that if residuals follow a normal and independent distribution, points must follow a straight line. It is observed that error values related to the response variable of Extract yield coefficient were almost along with the straight normal line and it shows the error distribution is normal.

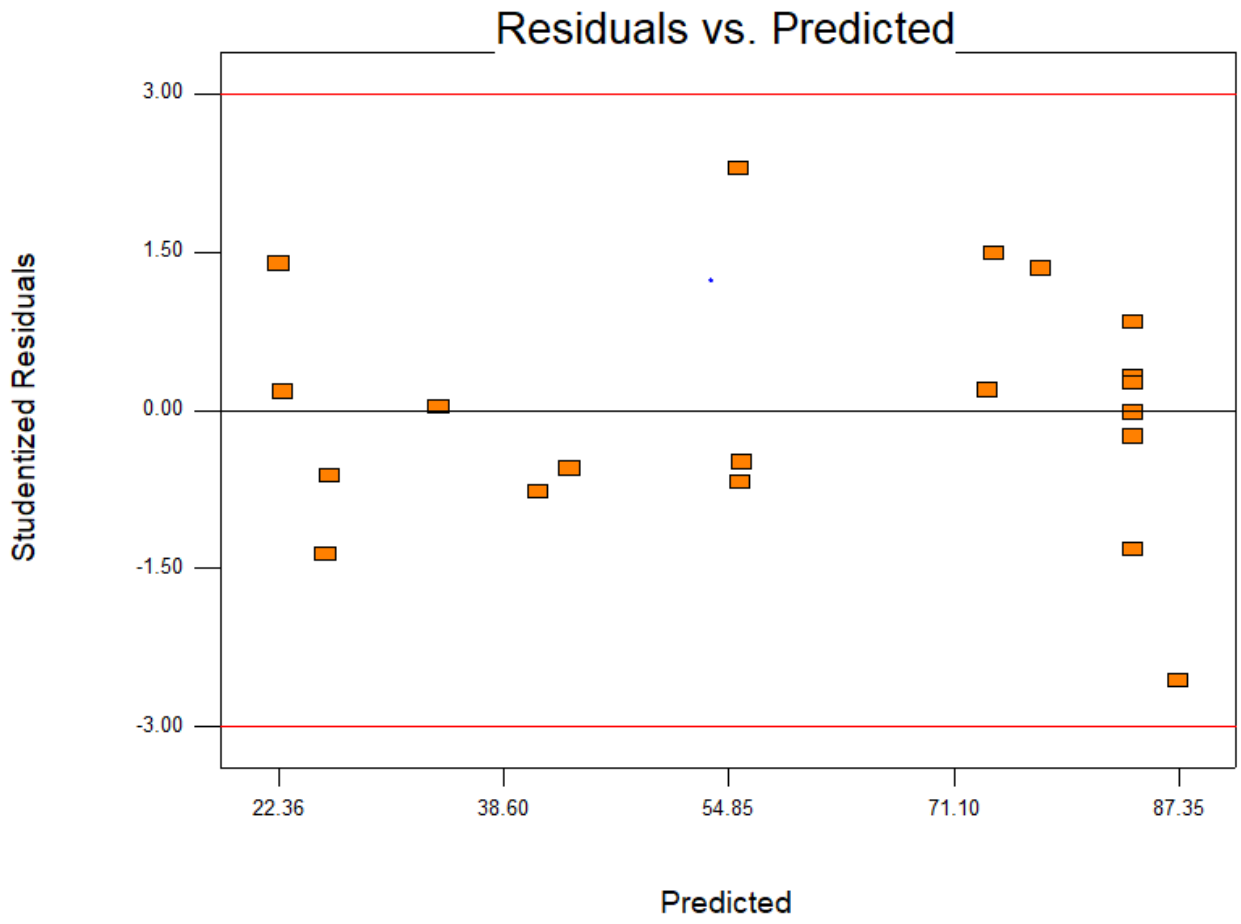


Figure 4.2 Residuals Vs. Predicted

Figure 4.2 indicates the residuals versus predicted values to check for constant error. Residuals are normally distributed with no outliers or grouping in the data. If the model is correct and the assumptions are satisfied, the residuals should be structure-less. They should be unrelated to any other variable including the predicted response. A simple check is to plot the residuals versus the fitted (predicted) values. The plot shows random scatter which justifies no need for any alteration to minimize personal error. Therefore, the residual standard deviation can be used as the measure of the random variability of the process.

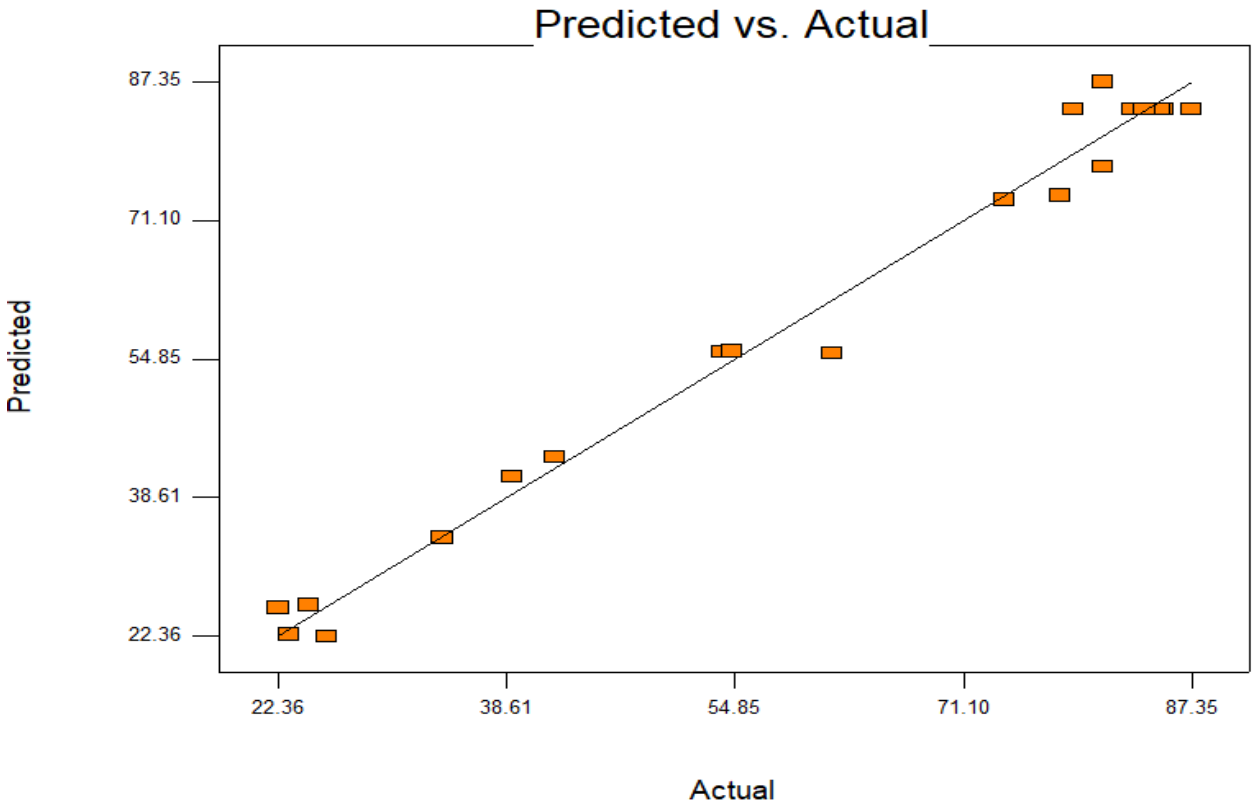
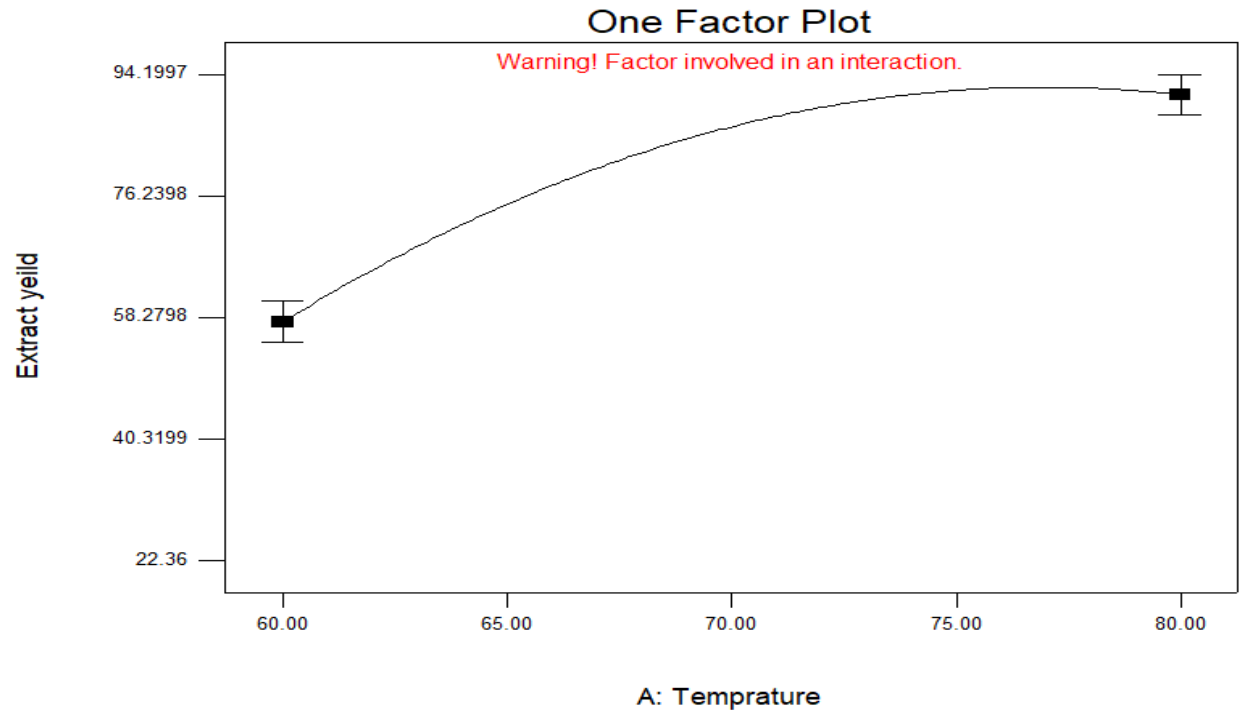


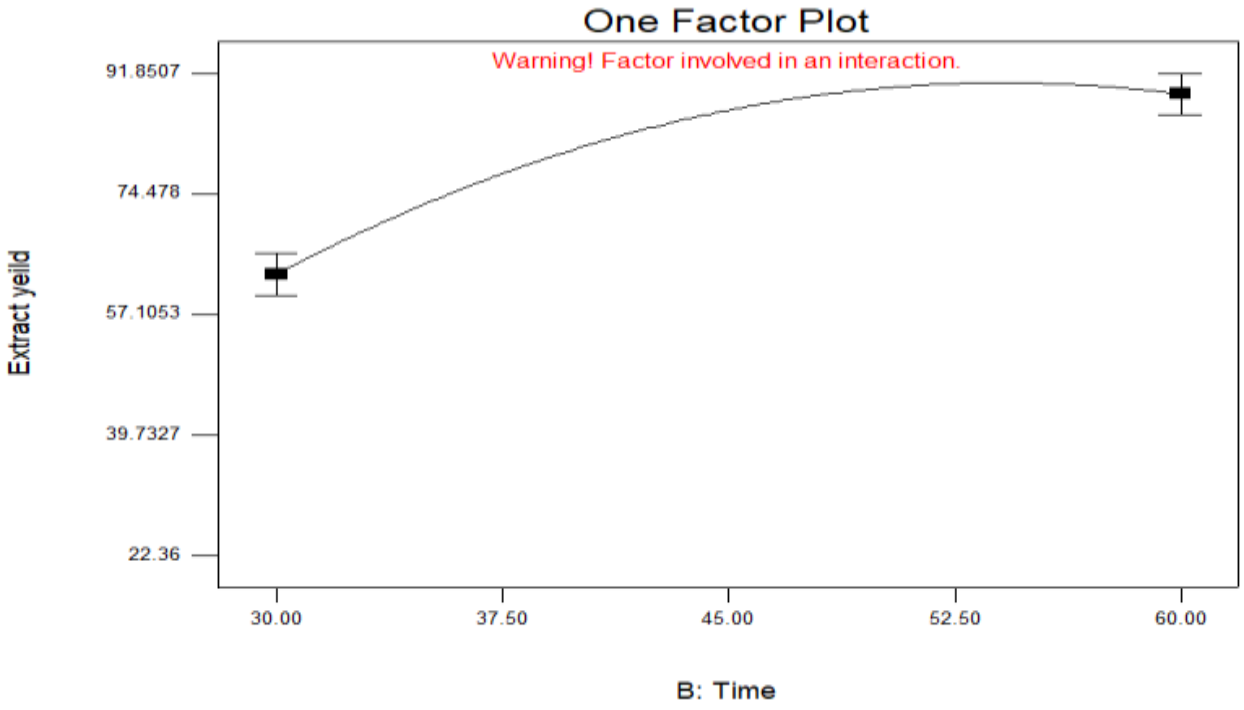
Figure 4.3 Predicted versus Actual for a yield of extract

The relationship between the model and experimental results of Extract yield is provided in Figure 4.3. This figure indicated a good agreement between the model and experimental results, the residuals for the majority of the responses were close to the diagonal line.

4.4.4 Effects of Experimental Variables on Extract Yield

A. The effect of individual variables on the Yield





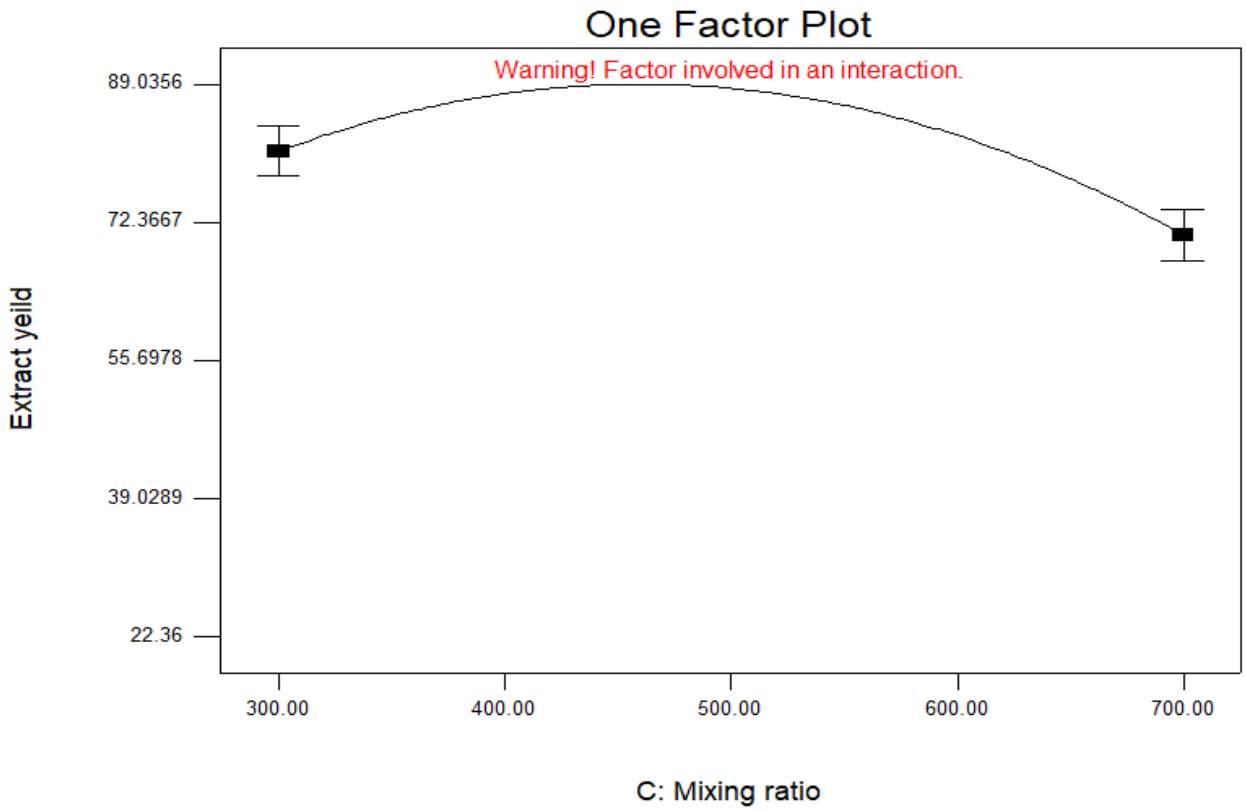


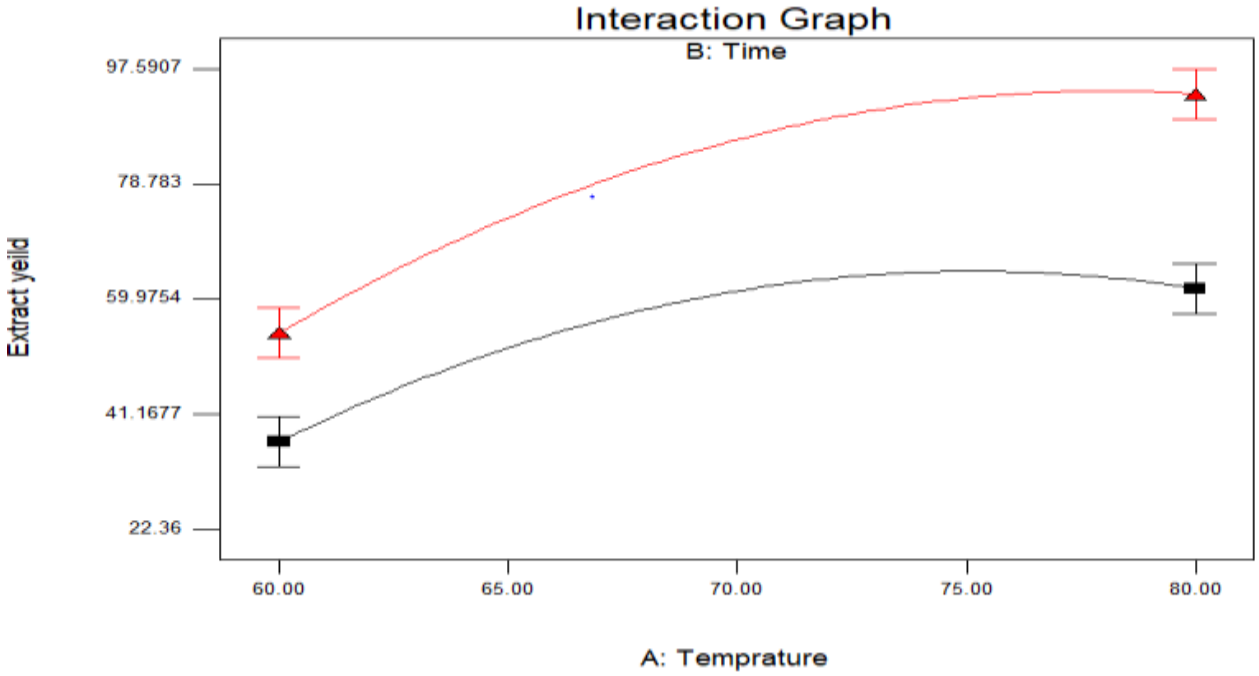
Figure 4.4 Effect of One Variable (A. Effect of mashing Temperature B. Effect of Time, C. Effect of Mixing Ratio)

The effect of mashing temperature on the amount of extract yield (Figure 4.4. A) is found to have a highly significant effect ($p< 0.0001$) (Table 4.6.) on the extract yield of mash. As depicted in the figure above as the mashing temperature increased the extract yield of mash also increased from temperature 60^oC to 70^oC.

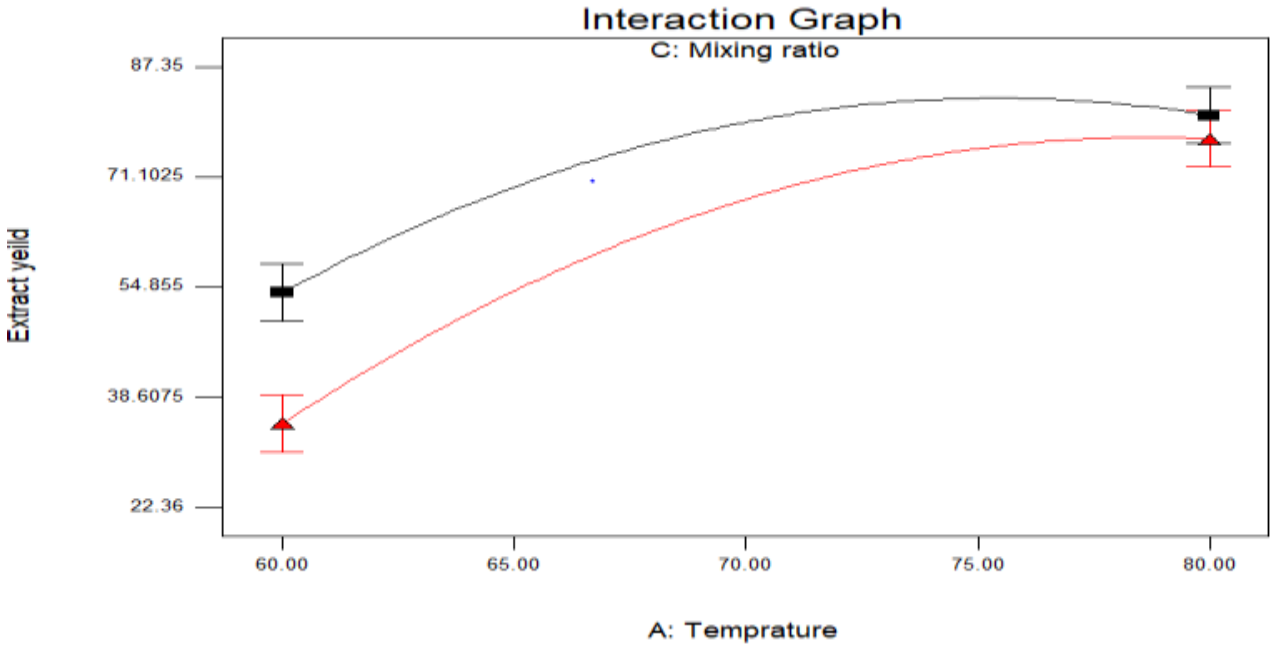
The ANOVA results (Table 4.5) show that mashing time significantly ($P <0.001$) affects the yield of Extract. In this regard, the optimal mashing time for a high mash extract is to be 45 min Figure 4.4 B. Figure 4.4 C showed that the effect of the mixing ratio on the extract yield of mash, was significant ($p< 0.0048$) (Table 4.5). Extract yield is increasing concerning the variable up to a certain extent and then decreasing. It is due to a change in enzyme activity concerning the change in parameters. As the finding in this study suggests, optimal temperature, time, and mixing ratio are better suited for brewing due to the higher extract value of mash. This implies yellow maize starch was adequately scarification at optimal temperature producing higher levels of reducing sugars and extracts mash.

B. Effect of two variables on yield of extract

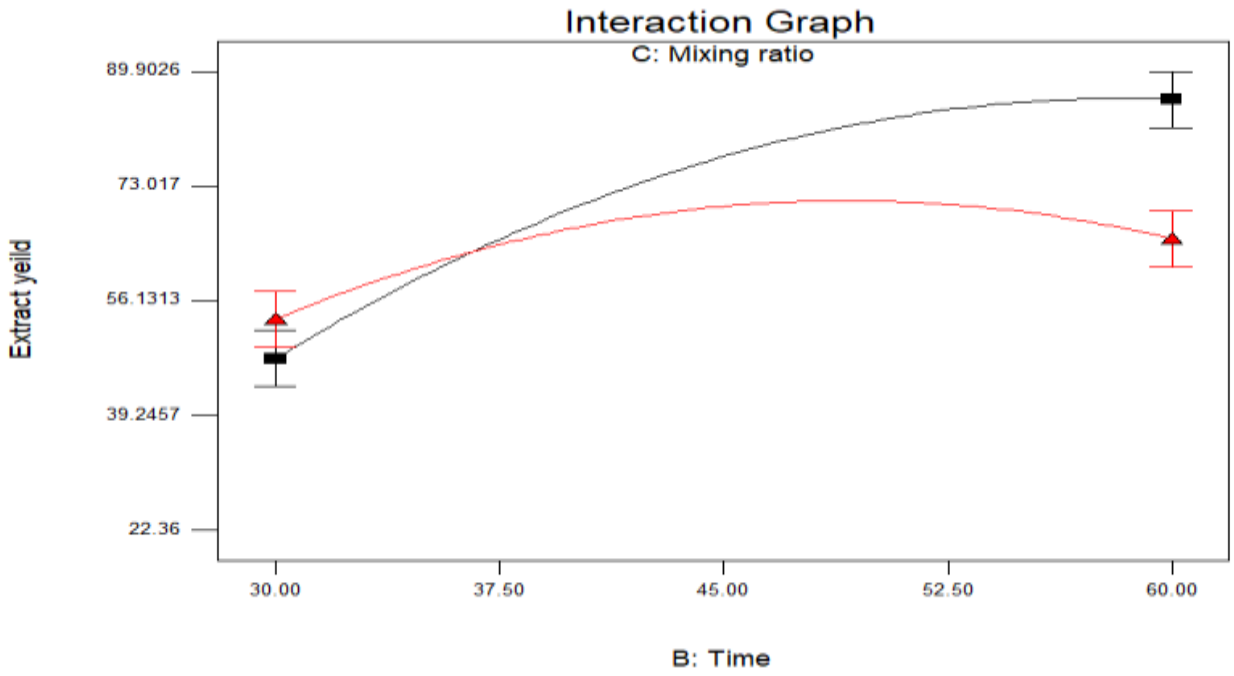
Effect of Temperature and time



A. Interaction of Temperature and Time percentage on the yield of extract of mash, when the mashing time is at the center point



B. Interaction of Temperature and Mixing ratio on the yield of extract of mash, when the Mixing ratio is at the center point.



C. Interaction of Time and Mixing on the yield of extract of mash, when the Mixing ratio is at the center point

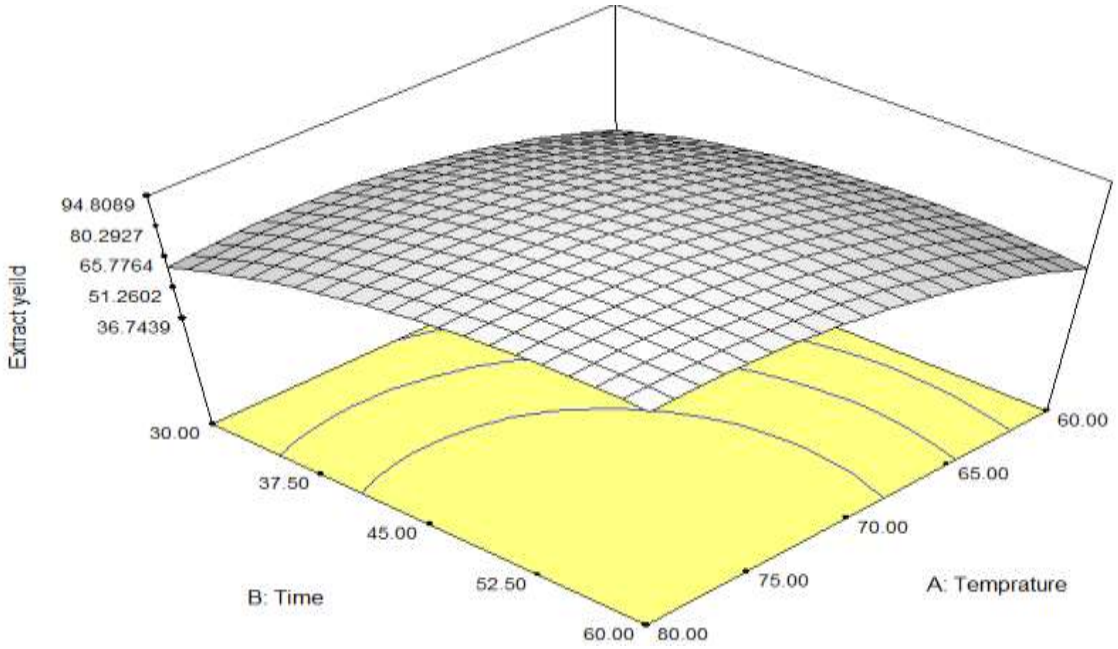
Figure 4.5 Interaction of factors of independent variables (A, B, and C)

The interactive effect of the process variables on the extract yield shows in Table 4.6. The interaction effect between temperature and mashing time (A) was significant with a p-value <0.0379. The interaction effect between temperature and mixing ratio (B) was significant because the p-value was <0.0321 and also the interaction effect between mashing temperature and mashing time (C) was significant because the p-value was <0.0013. In the above figures, 4.5 (A, B, and C) showed that at lower mashing temperature and high mixing ratio the yield of the extract is lower and at higher mashing temperature the yield of the extract is higher in the range of time. This is due to the activity of β-amylase at lower temperatures and higher temperatures. B-amylase is not very active at low temperatures. As a result of this low yield of the extract is observed. From the above plot, we understand that at a higher mixing ratio at a low time the yield of the extract is significantly decreased. This may be because a higher mixing ratio and time limit the breakdown of starch to maltose and create a short time for the rest time for α-amylase.

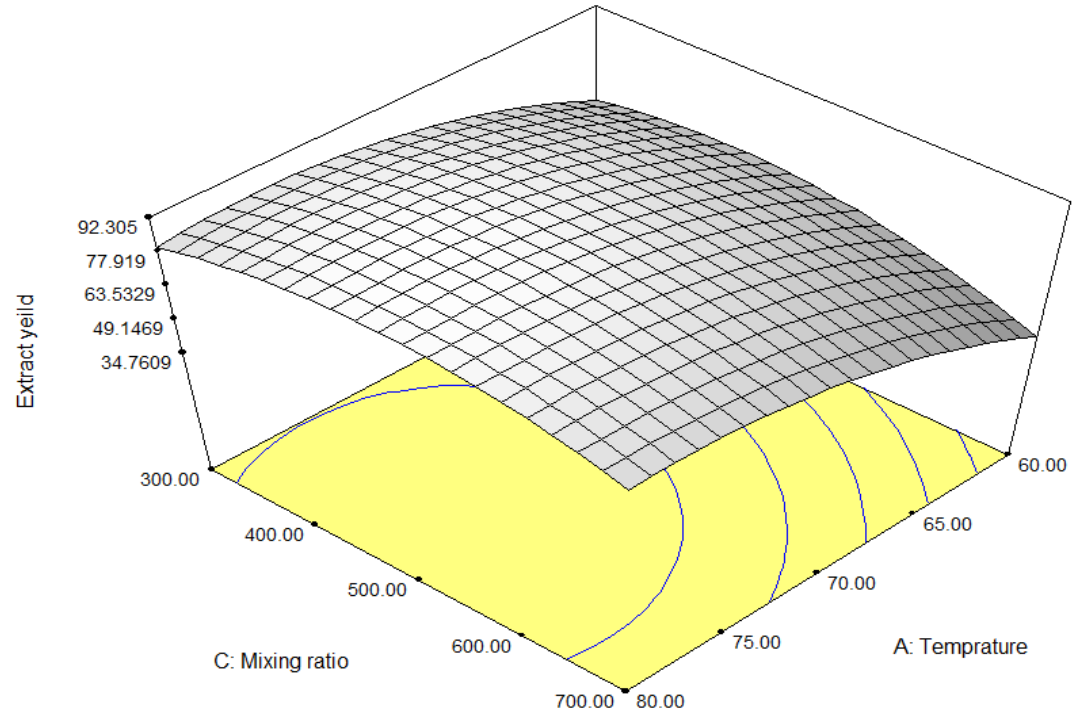
4.4.5 The Three-Dimensional Surface Interaction Effect

The relationship between independent and dependent variables was illustrated in a three-dimensional graph that was generated by the model for extract content. Two variables were depicted in one three-dimensional surface plot, while the other variable was kept constant. For better visualization, 3D response surface plots for extract content are shown in Fig 4.6. (A, B, C).

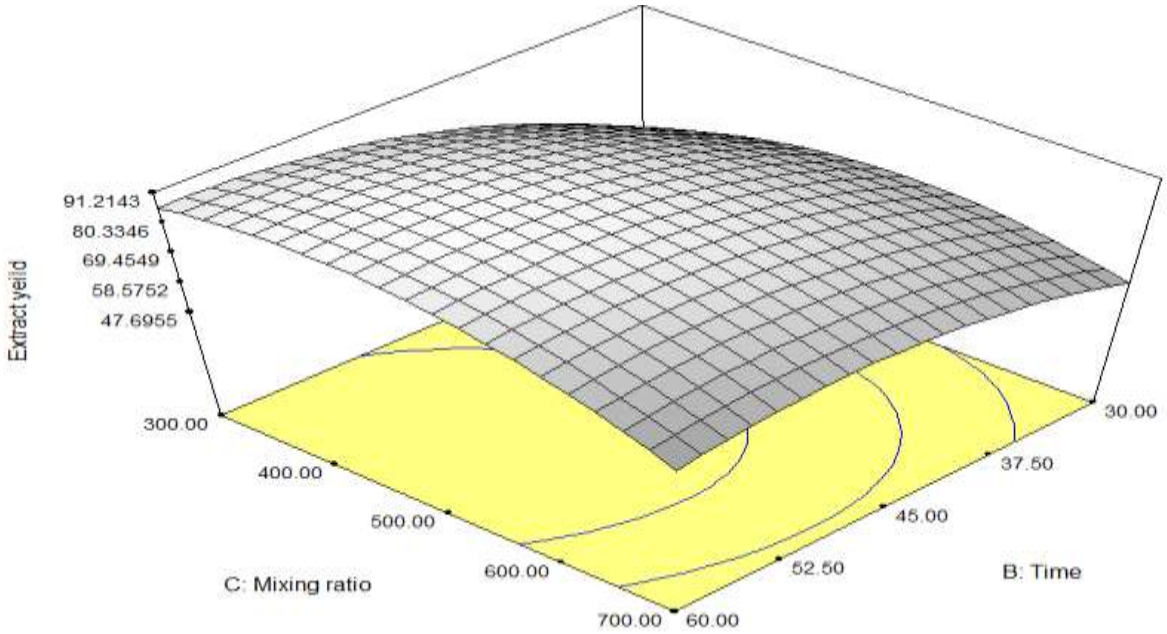
The interaction effects of the temperature and time (A), effects of the temperature and mixing ratio (B), and effects of the time and mixing ratio (C) on the extracted content of mash are shown in Figure 4.7 (A, B, C). This result demonstrates that as the mashing temperature increases the extract in the mash caused a lower mashing time and mixing ratio. From the response surface plot, when the mashing temperature is more than 70^{o}C the extracted content was declined. This might be a result of the high enzymatic activities contributed by the barley malt. And also mashing time is more than 45min the extracted content was gradually decreased. Similarly, when the mixing ratio is greater than 600 ml the extracted content was gradually decreasing.



A.

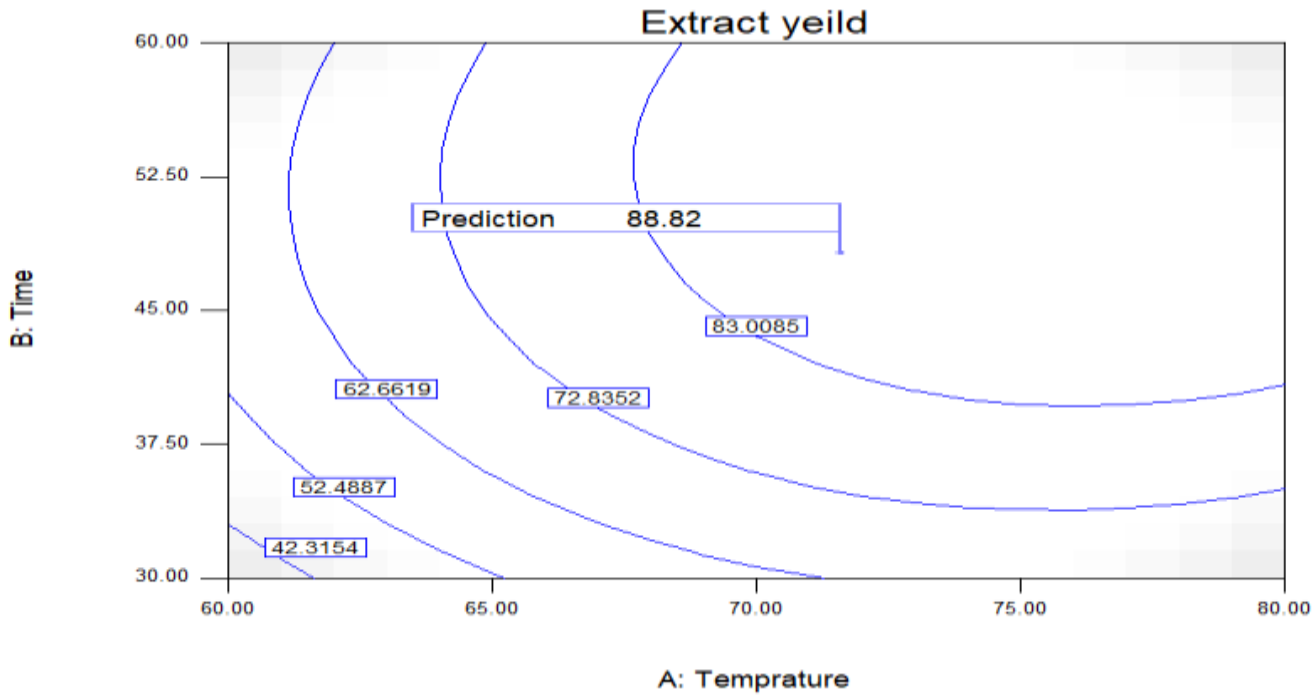


B.

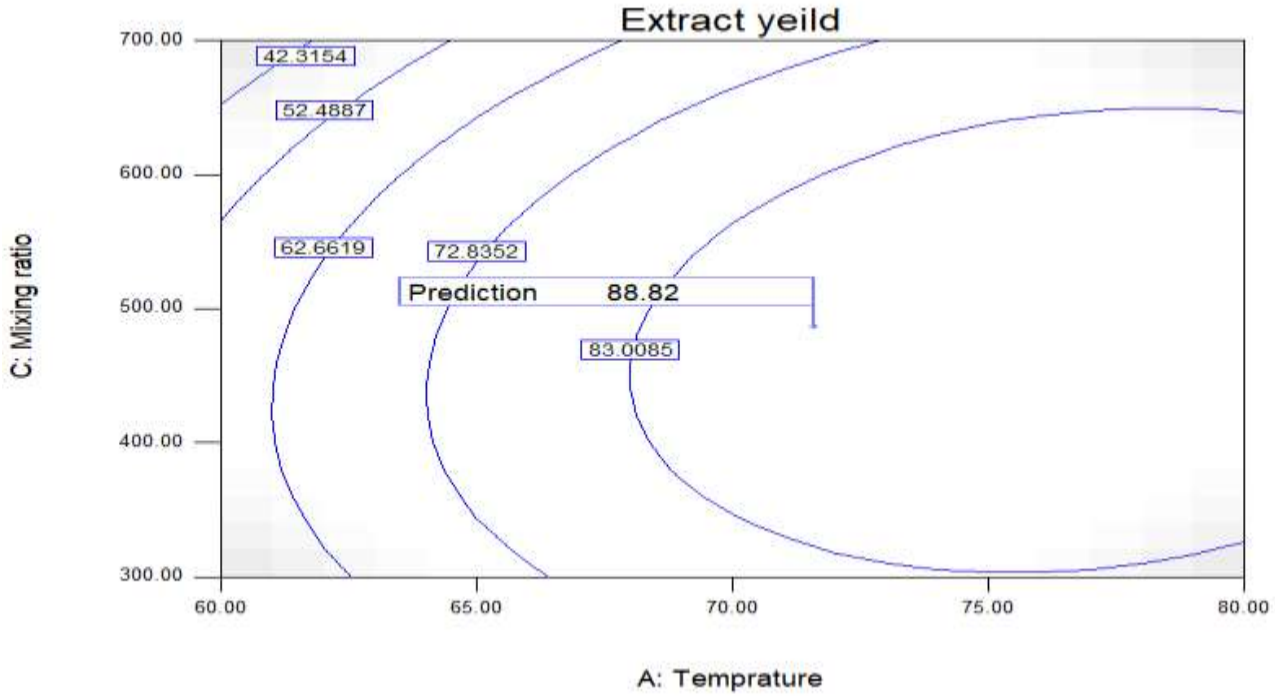


C.

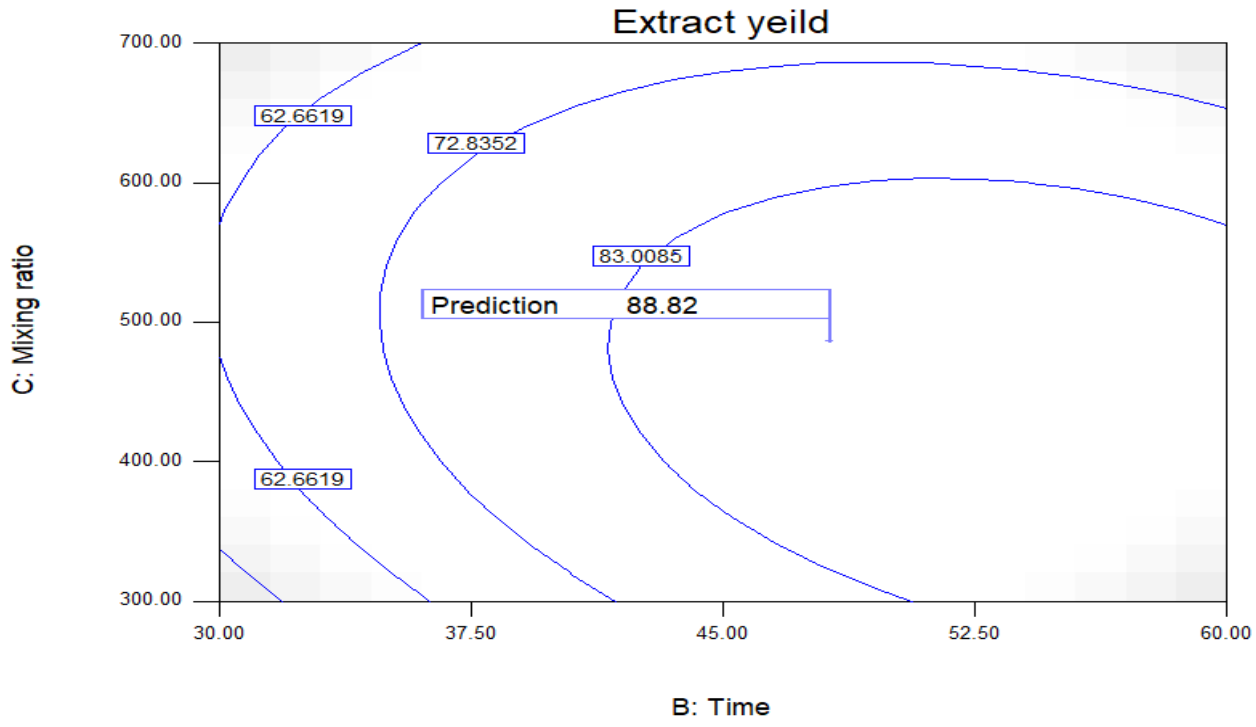
Figure 4.6 3D surface (A, B, C) Response surfaces plot of the effects of mashing Temperature and mashing Time on the yield of extract


Extract yeild
60.00
52.50
45.00
37.50
30.00
B: Time
Prediction 88.82
83.0085
62.6619
72.8352
52.4887
42.3154
60.00
65.00
70.00
75.00
80.00
A: Temprature

A.


Extract yeild
700.00
600.00
500.00
400.00
300.00
C: Mixing ratio
42.3154
52.4887
62.6619
72.8352
Prediction 88.82
83.0085
60.00
65.00
70.00
75.00
80.00
A: Temprature

B.



C.

Figure 4.7 Contour (A, B, C) Response surfaces plot of the effects of mashing Temperature and mashing Time on the yield of extract.

Figure 4.7 showed that the contour and the interaction plot show that the increase in Extract yield is greater when mashing temperature, mashing time, and mixing ratio are (70^{o}C, 45min, and 500ml) respectively higher than the mashing temperature, mashing time, and mixing ratio (60^{o}C, 80^{o}C) and (30min, 60 min) in the mashing process. The mashing temperature is the main effect on the yield of extract and also the graph shows that mashing time had a significant effect on the yield of extract. Generally, the interaction effect of temperature, mashing time, and mixing ratio on extract yield was significant. The effect of the two independent variables on the yield of the extract is the parallel and opposite effect. That means at low mashing time and temperature the yield of the extract is low while at a higher mixing ratio higher yield of extract was observed. But, the curved graph indicates that the activity of the enzyme is limited at a high percentage of mixing ratio. The interaction of independent factors to optimize the response variables extract content value is 88.82.

4.4.6 Optimization Studies

The optimal conditions for malt drink production in this study were selected based on the high extract yield were, yellow maize *(Melkassa 7* varieties), 70^{o}C (mashing temperature), and 45 min (mashing time) were used to brew malt drink using the optimal condition for mashing Extract method.

Table 4.9 Optimization criteria for optimum yield of extract

Constraints	**Goal**	**Lower Limit**	**Upper Limit**
Temperature	is in range	60	70
Time	is in range	30	45
Mixing Ratio	is in range	700	500
Extract yield	maximize	22.36	87.35

Table 4.10 The desirable optimization solution

Number	Temperature	Time	Mixing Ratio	Extract yield	Desirability	
1	71.59	48.19	486.2	88.8177	1.000	Selected

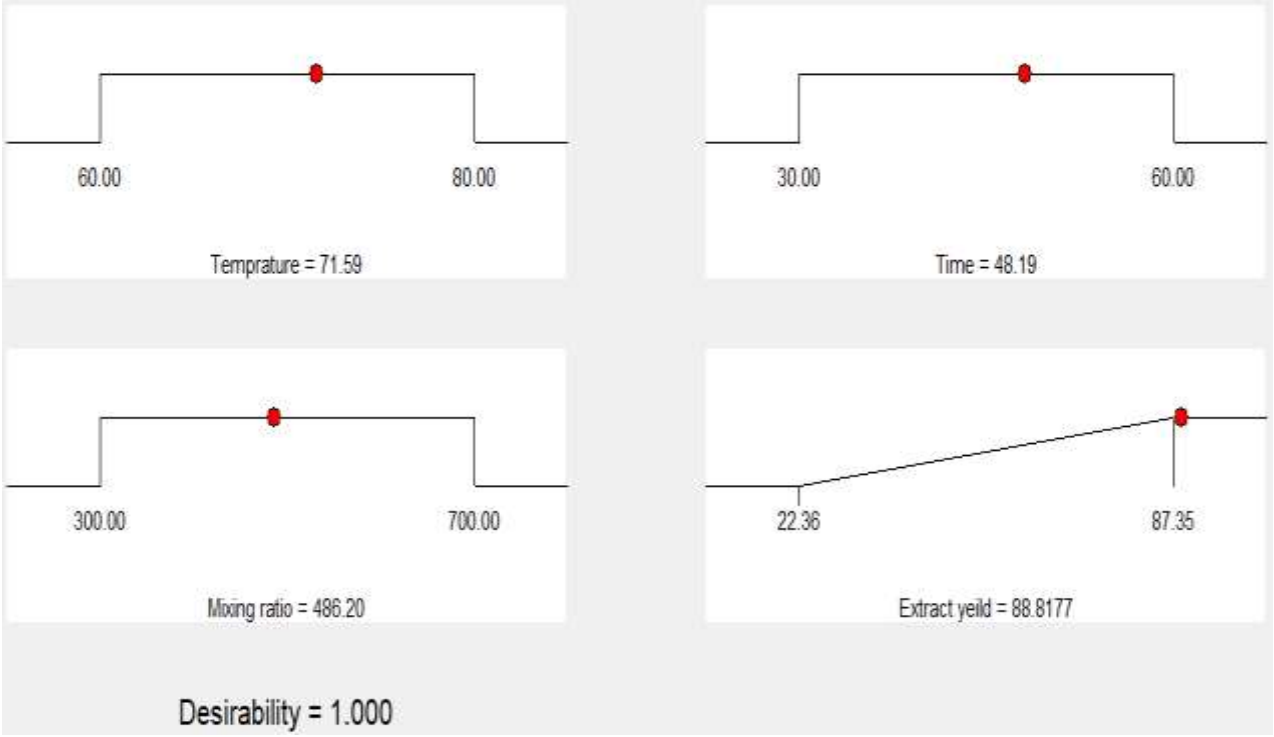


Figure 4.8 Desirability of optimization solution

The optimization result shown in Table 4.10 sorghum varieties of yellow type, mashing time of 48.19 min, mashing temperature of 71.59$^{O}_{C}$ and mixing ratio of 486.2 were optimal for brewing with a high extract yield value of 88.8177. The above optimum operating parameters obtained sufficient sugar extracts for the brewing process.

4.5 Physico-chemical Analysis of Final Product

Three types of drinks (M_5, M_{10}, and M_{15}) were prepared with 5%, 10%, and 15% leaf extract, and their characteristics (Table 4.10, I-column) were estimated as per the methods mentioned in section 3.9.3 and the results were displayed in Table 4.10. Alcolyzer (Anton Paar) device was used to measure the alcohol value in terms of %w/w and %v/v, specific gravity, original gravity, apparent extract, and real extract. Total nitrogen, carbon dioxide, oxygen content (DO), and pH values were estimated by the methods mentioned in section 3.10. A decrease in DO was observed with extract concentration. It is due to the increase of anti-oxidants present in the leaf extract.

Whereas an increasing trend was observed for all other parameters. Moreover, all parameter values are within the standard range for all three prepared drinks (POP Anamaria, 2019).

Table 4.10 Physico- Chemical analysis of the final product of malt drink

Property	**Malt drink**			**Standard (Requirement)**
	M_5	**M_{10}**	**M_{15}**	(SEA, 2013)
Original extract (OG) (w %)	12.66 ± 0.05	14.7± 0.06	15.1 ± 0.01	7- 15
Apparent extract (EA) (w %)	8.3 ± 0.02	9.1± 0.11	14.7 ± 0.03	6.5 – 14.5
Real extract (ER) (w %)	12.8 ± 0.01	12.7± 0.23	14.6 ± 0.15	6.5 – 14.5
Alcohol content (w %)	0.025 ± 0.01	0.02± 0.09	0.04 ± 0.04	Below 0.5
P^H	4.49 ± 0.04	4.47± 0.07	4.16 ± 0.01	3.6 – 5.25
Corbondioxide (CO_2), w %	0.42 ± 0.02	0.49± 0.11	0.52 ± 0.02	0.45
Oxygen content (DO_2), mg O_2/L	0.05 ± 0.01	0.047± 0.01	0.035 ± 0.05	0.03-0.05
Haziness/clarity, EBC	0.96± 0.01	0.65± 0.12	0.55± 0.08	< 1 EBC
Color,EBC	120± 0.01	126± 0.15	127± 0.03	110 – 130
Head retention	100± 0.01	120± 0.01	121± 0.06	90 – 140

Where: M_5 -5% moringa, M_{10} - 10% moringa and M_{15} - 15% moringa

4.5.1 Alcohol content

The results are the average value of triplicate analysis ±of the standard deviation. (-) indicates as the standard can derivate or depends on correspondence to the parallel parameters.

In this study, the product is classified under non-alcoholic beer and the taste method was the European brew convention (EBC) method. The alcohol (ethanol) content of the finished beer was adjusted early during mashing. Brewing with low gravity wort can result in low alcohol content. The value in the above Table 4.10 was shown that; the alcohol content of malt drinks (M_5, $M_{10,}$ and M_{15}) was (0.025, 0.02, and 0.04 %v/v) respectively which is within the non-alcoholic beer standard.

4.5.2 Carbon dioxide (CO_2) content

The CO_2 content of malt drink was 3.6g/L which is less than the standard stated for light non-alcoholic beer (minimum 0.45%) (National standard, ES831 in EBC test method). Because of this reason carbonations were performed to increase the value of CO_2. Carbonation is a process of dosing CO_2 into non-alcoholic beer. Carbonation aims to achieve the desired level of CO_2 in bottled malt drinks. CO_2 contributes tingle feel and maintains an aerobic environment that helps in preserving beer. And carbon dioxide was added manually to the malt drink (M_5, $M_{10,}$ and M_{15}) from CO_2 contained cylinder through the hose and the value becomes 4.5g/L (0.42%, 0.49, and 0.52) respectively which is within the national standard.

4.5.3 Head retention/Foam stability of the final beer product

The foam stability of malt drink was below the standard stated by the Ethiopian national standard (90-140 seconds). The head retention (foam) of malt drink (M_5, $M_{10,}$ and M_{15}) was tested by using a NIBEM-TPH foam stability tester and the time taken to collapse was (100,120, and 121) seconds respectively, owing to the low solubility of carbon dioxide in malt drink. If CO_2 can easily dissolve in malt drinks it may increase the foam of the malt drink. Foam is one of the main characteristics of malt drinks to be selected by consumers and the foam character of malt drinks is a very important parameter of customer acceptance. Malt drink foam is a colloidal system, being built from a gas (CO_2) and a liquid (malt drink). It is formed by CO_2 release when bubbles rising in the malt drink are loaded with surface-active substances from the malt drink, which form more or less stable foam when reaching the surface of the liquid. Immediately after formation, the foam starts to collapse, building liquid malt drink again.

4.6 Microbiological Analysis

Three important spoilage microbes yeast, coliform, and Escherichia Coli (E.coli) were used for microbiological analysis. The microbial count of these species for M_5, $M_{10,}$ and M_{15} was estimated at zero, three, and six months. According to the estimated results, as shown in Table 4.11, even though a gradual increment is observed in the growth of each species for M_5, it is very mild. All three drinks are perfectly preserved for a period of a minimum of six months and the count is within the limits (Florence et al., 2016).

Table 4.11 Effect of Morniga blended ratio on microbiological analysis of malt drink

Duration	Property	M_5	M_{10}	M_{15}	Standard
0 months	Yeast mould	1	0	0	<10
	Coliform	0	0	0	<1
	Escherichia coli	0	0	0	<1
3 months	Yeast mould	4	0	0	<10
	Coliform	1	0	0	<1
	Escherichia coli	0	0	0	<1
6 months	Yeast mould	13	0	0	<10
	Coliform	2	0	0	<1
	Escherichia coli	0	0	0	<1

Where: M_5 -5% moringa, M_{10} - 10% moringa and M_{15} - 15% moringa

The antimicrobial studies were further extended for beer spoilage bacteria and aerobic and anaerobic wort bacteria. From the results of this study in Table 4.12, it is observed that all the three microbes were absent for 3 months for all the three beverages, but they were detected for M_5 at a period of 6 months. Based on these anti-microbial studies M_{10} beverage was selected as the best due to its stability at a low concentration of leaf extract and hence used for further studies.

Table 4.12 Microbiological analysis of maize malt drink

Duration	Parameters	Result (growth in CFU)		
		M_5	**M_{10}**	**M_{15}**
Production time	Aerobic wort bacteria	Free	Free	Free
After 3month		Free	Free	Free
After 6 month		Positive	Free	Free
Production time	Anaerobic wort Bacteria	Free	Free	Free
After 3month		Free	Free	Free
After 6 month		Positive	Free	Free
Production time	Wort stability/ beer	Normal (-ve)	Normal (-ve)	Normal (-ve)

	spoilage			
After 3month		Normal (-ve)	Normal (-ve)	Normal (-ve)
After 6 month		Positive (+ve)	Normal (-ve)	Normal (-ve)

Where: M_5 -5% moringa, M_{10} - 10% moringa and M_{15} - 15% moringa

- Normal (-ve) indicates the absence of beer spoilage bacteria and stability of wort
- Positive (+ve) indicates the presence of beer spoilage bacteria and stability of wort
- Free indicates the absence of aerobic and anaerobic bacteria growth

4.7 Comparative Studies

The performance of the as-prepared malt drink (M_{10}) was compared with that of the commercial one, Luse (a standard product of Dashen Brewery). In addition, three other combinations: raw, pasteurized, and Moringa with pasteurized, were also tested. All these five samples were tested for the growth of the same microbial species mentioned in section 4.6. The growth was measured in terms of CFU (Colony Forming Unit) and the results were given in the form of Table 4.13.

The results of S_2 and S_4 conclude that M_{10} is more microbial stable compared to the commercial product. Adding Moringa leaf extract is similar to treating with moringa and pasteurization malt drink as evident from the results of S2 and S3. Therefore if Moringa leaf extract is added pasteurization can be eliminated (results are the same for S_2 and S_3).

Table 4.13 Comparison among types of malt drink

Property	S_1(CFU)	S_2(CFU)	S_3(CFU)	S_4(CFU)	Standard(CFU/100ml)
Yeast and mould	12	0	0	2	< 10
Coliform	3	0	0	1	< 1
Escherichia coli	1	0	0	0	<1

Where: S_1: untreated malt drinks

S_2: treated with *Moringa* malt drink (M_{10})

S_3: treated with moringa and pasteurization malt drink

S_4: commercial malt drinks (Luse) produced Aug /2021

4.8 Shelf Life of Malt Drink Product

Apart from microbial spoilage, there are other factors like pH, redox potential, etc. that affect the stability of the beverages. Shelf life is a crucial factor for the commercialization of any food product. Generally, it is expressed in terms of haziness value. It is estimated as per the procedure explained in section 3.8. As per the EBC recommendation, any product with a haziness value of less than one is stable for up to a minimum of six months (SEA, 2013).

Table 4.14 Prediction of shelf life of the product

Temperature	Duration (Hr)	Actual haze (EBC)	Mean Haze (EBC)	EBC Standard (6 months)
0 ^{o}C	24	0.46	0.61	< 1
60^{o}C	48	0.76		

Further, the leaf extract is proven effective over every single species (yeast, coliform, and E.coli). Therefore, it can protect malt drinks from a wide variety of spoilage organisms. Further, inhibition is very strong at high concentrations of leaf extract. In addition to the anti-microbial test, the shelf life of the sample drink (M_{10}) was estimated in terms of Haziness value. This value was estimated by the EBC method and a value of 0.61 indicates the drink is stable up to a minimum of 6 months

4.9 Sensory Characteristics of Malt Drink

Sensory evaluation is a method that determines, analyses, and interprets responses to products perceived through the senses of sight, smell, touch, and taste, of the consumers. The results of the sensory analysis (Table 4.15) showed that M_{10} was rated close to the samples with 5% substitution malt drink M_5 in terms of bitterness and roasted, but was significantly different from it in terms of color, sweetness, fruity, sourness, and overall impression. However, M_{15} was slightly lower among the given samples. Blended Moringa malt drinks are overall more acceptable than control malt drink, S_1 (free of Moringa). This means the sensory and the nutritional attributes could be varied to obtain nutritional and acceptable malt samples (Sci et al., 2015).

Table 4.15 Effect of Moringa blend proportion on sensory characteristics of malt drink

S/N	Attributes	Score			
		M_5	**M_{10}**	**M_{15}**	**S_1**
1.	Color	3.23	3.11	2.55	2.5
2.	Bitterness	3.61	3.62	3.05	2.89
3.	Sweetness	3.63	3.54	3.26	3.1
4.	Fruity	3.55	3.60	3.61	3.4
5.	Sourness	3.12	3.02	3.06	3.25
6.	Soapy, Diacetyle	2.80	2.85	2.9	2.82
7.	Caramelized, Roasted	3.75	3.74	2.95	3.6
8.	Overall Impression	3.52	3.75	3.03	2.8

Where: M_5 -5% moringa, M_{10} - 10% moringa, M_{15} - 15% moringa, and untreated malt drink

CHAPTER FIVE

5 CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

The study realized that by using local yellow maize and moringa Oleifera leaf extract as preservatives malt drinks were produced. From the study; the investigation has proved that the high starch content, low cost, and good flavor of local yellow maize fall behind that of regular local and imported barley. The raw materials analysis proved that yellow maize varieties had an appropriate composition required for malt drink production. However, optimization results indicated that maize varieties of yellow type, mashing time of 45 min, mashing temperature of 70ºC, and mixing ratio 500 were optimum operating parameters for malt drink with high extract yield value. The mashing procedure was very effective in complete starch gelatinization and saccharification of maize wort and producing sufficient sugar extracts for malt drink production.

The Moringa Oleifera leaf can be used for extra-long periods to produce a stable shelf life of at least 6 months by extracting the active preservative compounds phenol and flavonoids using ethanol extraction.

Further, the finished product from yellow maize was characterized and all product parameters could be tolerated compared to the standard barley. The main parameters for finished malt drink were valued that an alcohol content of 0.02 w %, the color of 126 EBC, and Haziness/clarity of 0.61 EBC.

Storage of malt drink until six months, 10% and 15% of moringa are suitable to minimize the growth of microorganisms and to predict extend shelf life of the product; however, 15% moringa substitution malt drink was slightly lower sensory characteristics among given samples. Generally, the ratio of 10% moringa extracted blended with maize malt drink as a preservative agent is accepted no need for further pasteurization regarding fighting microorganisms and overall acceptable sensory characteristics.

Furthermore, the microbial analysis of the finished malt drink indicated that free of yeast, mold, and spoilage bacteria. Coliform and Escherichia coli

5.2 RECOMMENDATIONS

Our country, Ethiopia can benefit from the malt drink product produced by the replacement of imported or local barley with local yellow maize which is a cheaper plant source that allows more consumers with reduced cost. From the result of the work carried out, I recommend the following:

- Local yellow maize malt drink should be used for brewing industries as a starch source and the final product should preserve with moringa oleifera leaf extract to discourage spoilage by microbes and to extend its shelf-life.
- Second, due to the low cost of maize and abundant availability in the local market, malt drink companies should consider yellow maize as an alternative raw material, to enhance company profitability.

Malt drink production is a non-alcoholic non-fermenting beverage, but it is still used hop for its extended shelf life and bitterness. As a result, using extra sugar to reduce bitterness is not recommended for health and the Muslim religion. So, using moringa Oleifera leaf extract as a preservative is acceptable to all without the addition of sugar.

In our country, there are no such findings in the area of the feeding of Moringa oleifera leaf as a preservative agent for malt drink products to improve shelf life.

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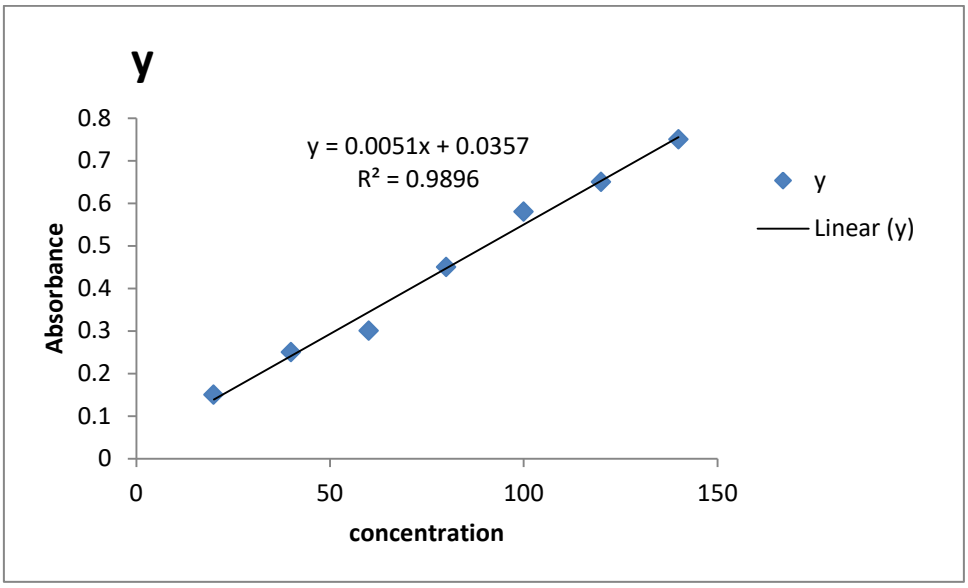
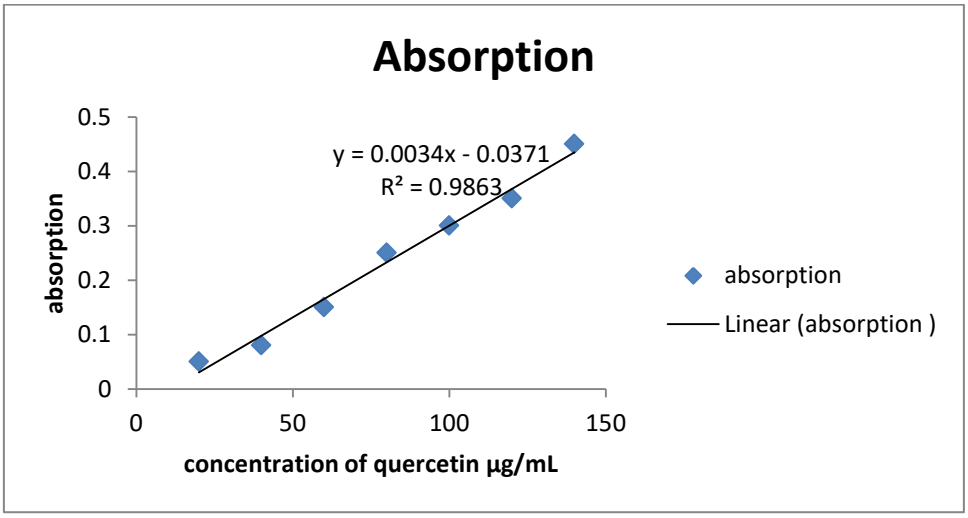
APPENDIXES A

Determination of Phenolic and flavonoids Analysis of *Moringa* extracts



A: Determination of Phenol



B: Determination of Flavonoid

APPENDIX B

Production process

Raw Material Preparation

A. Raw Yellow Maize

B. *Moringa* Leaves

C. Germinated Yellow Maize

D. Malted Maize Flour

E. Filtration of *Moringa* extract

F. *Moringa extract* residual flour

Extraction of *Moringa*

Mashing

Mash filtration

Final product blending

APPENDIX C

Questionnaires for non-alcoholic malt drink Taste

Questionnaire for Yellow Sorghum Beer Sensory Analysis			
Panelist Code		Sample Code	
Age		Gender	

1 How do you rate the **OVERALL APPEARANCE** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
1	2	3	4	5	6	7	8	9

2 How do you rate the **COLOUR** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Slightly	Like Moderately	Like Very Much	Like Extremely
1	2	3	4	5	6	7	8	9

3 How do you rate the **BEATERENESS** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
1	2	3	4	5	6	7	8	9

4 How do you rate the **MOUTH FEEL** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
1	2	3	4	5	6	7	8	9

5 How do you rate the **SWEATENESS** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
1	2	3	4	5	6	7	8	9

6 How do you rate the **FRUITY TASTE** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
1	2	3	4	5	6	7	8	9

7 How do you rate the **OVERALL LIKING** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
1	2	3	4	5	6	7	8	9

8 How do you rate the **SOURNESS** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
1	2	3	4	5	6	7	8	9

9 Is this product **ACCEPTABLE?** _____

APPENDIX D

Dear assessors; this malt drink from local maize and moringa as a preservative is present as a new product on the shelf. You have one score sheet for the represented sample. The ordinal scale (4 levels) was designed as 0 to 4 scores to evaluate the given parameters. And finally please attribute your overall impression. The score

- ✓ 0 is extremely disliked,
- ✓ 1 dislikes,
- ✓ 2 is normal,
- ✓ 3 is like,
- ✓ 4 is like very much.

S/N	Attributes	Score		
		M5	M10	M15
1	Color			
2	Bitterness			
3	Sweetness			
4	Fruity			
5	Oxidized			
6	Sourness			
7	Soapy, Diacetyl			
8	Caramelized, Roasted			
9	Overall Impression			

Name (optional) ----------Date ---------- sign----------

Comment (optional) ----------

Thank you!!!

Sensory analyses of malt drink products

Dear assessors; this malt drink from untreated local maize malt drinks, treated with *Moringa* malt drink (M_{10}), treated with pasteurization malt drink, treated with moringa and pasteurization malt drink and commercial malt drinks (Luse) produced Aug /2021. You have one score sheet for the represented sample. The ordinal scale (4 levels) was designed as 0 to 4 scores to evaluate the given parameters. And finally please attribute your overall impression. The score:

- ✓ 0 is extremely disliked,
- ✓ 1 dislikes,
- ✓ 2 is normal,
- ✓ 3 is like,
- ✓ 4 is like very much

S/N	Attributes	Score				
		S1	S2	S3	S4	S5
1	Color					
2	Bitterness					
3	Sweetness					
4	Fruity					
5	Oxidized					
6	Sourness					
7	Soapy, Diacetyl					
8	Caramelized, Roasted					
9	Overall Impression					

Name (optional) ----------Date ---------- sign----------

Comment (optional) ----------

Thank you!!!