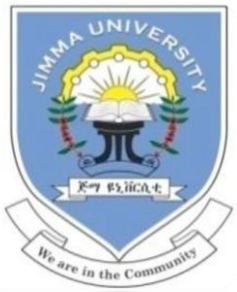

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By

Demissie Kanssa

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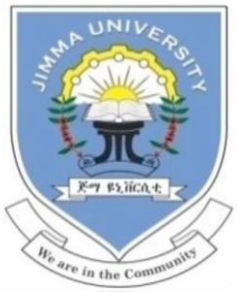

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By

Demissie Kanssa

A Thesis Paper Submitted to the Department of Biology, College of Natural Sciences, Jimma University, in Partial Fulfillment the Requirement for the Degree of Masters of Science in Biology (Applied Microbiology).

We, the advisors, approved submission of this Thesis for public defense.

Signature Date

Principal Advisor: Ketema Bacha (PhD, Professor) ---------- ----------

Co- Advisor: Mesert Guta (MSc, Asst. Prof.) ---------- ----------

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Name Signature Date

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Research Advisors

Principal Advisor: Ketema Bacha (PhD, Professor) ---------- ----------

Co- Advisor: Mesert Guta (MSc, Asst. Prof.) ---------- ----------

Examiners

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Declaration

I declare that this thesis entitled "Microbial and Physicochemical Analysis of *Kammerra* Traditional Fermented Alcoholic Beverage in Konta Special Woreda, Southern Ethiopia", submitted for the Award of Degree of Masters of Science in Applied Microbiology to Jimma University, is my original work. The content of this thesis is based on the experiments that I have performed myself. This thesis has not been submitted for any degree to other University.

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List of Acronyms/Abbreviations

AM: Ameya

AMB*: Aerobic Mesophilic Bacteria*

BT: Betseti Xanga

CD: Chida Edeget

CL*: Coliforms*

CR: Chabara shasho

CSA: Central Statistical Agency

CT: Cheta Kechikecha

DEAS: Draft of East African Standard

EB: *Enterobacteriaceae*

GG: Genj genet

GIS: Geographical Information System

KB: Kerbella Bedegucha

KC: Kecha Rooba

KK: Konta Koysha

LAB: Lactic Acid Bacteria

MD: MedaYejja

SNNPRS: South Nation Nationalities People Region State

ST: *Staphylococcus*

Y&M: Yeast and Molds.

Abstract

Kammerra is a light yellow traditionally fermented alcoholic beverage commonly consumed in Konta special woreda, southern Ethiopia. This study evaluated microbial loads, physicochemical characteristics and nutritional values of Kammerra. A cross sectional study design was followed to document pertinent information related to traditional preparation techniques and socio-economic significances of the product. Microbiological safety of the samples, microbial succession in due course of controlled fermentation of Kammerra, nutritional dynamics and other related parameters were evaluated under laboratory conditions following standard microbiological techniques. Samples of Kammerra were collected every two week for twelve weeks from ten different kebeles, three samples per selected kebele and vending sites (houses) at the study site. The bacterial profiles of Kammerra samples were dominated by (mean log cfu/ml) Aerobic mesophilic bacteria (7.12±0.46), Enterobacteriaceae (7.07 ± 0.45), LAB (6.99±0.39), followed by coliforms (6.08±0.13), staphylococci (5.02±0.1), and Bacillus (3.61±0.06). The mean counts of yeast and molds were closer to the dominant bacterial profiles. A total of 249 microbial Isolates were characterized and identified at least to genus level following the standard microbiological methods. Among frequently isolated bacterial genera were Lactobacillus 25(10.04%), Lactococcus 24(9.64%), Staphylococcus 22(8.84%), followed by Bacillus spp. 13 (5.22%), Klebsiella 12(4.82%), Enterococcus 10(4.02%), Enterobacter 9(3.61%), Citrobacter 9(3.61%), Leuconostocs 7(2.81%), Pediococcus 7(2.81%), Salmonella 6(2.41%) and other many less frequently encountered genera. Saccharomyces 20 (8.03%) is the only dominant yeast strain encountered in many of the samples. The mean values recorded for temperature, moisture content, total solid, pH, Titratable acidity and alcohol content were 15.74±1.09, 96.74±1.13, 1.00±0.45, 4.29±0.58, 1.36±0.58 & 4.39±0.70, respectively. Furthermore, the mean values for percentage of crude protein, fat, total ash, carbohydrate and nitrogen were 15.42 ±1.46, 1.13± 0.62, 5.29± 2.18, 0.12± 0.07, 73.39± 2.46, and 0.18±0.09, respectively. Detection of some food borne pathogens in Kammerra samples calls for improvement of handling practice as the detected pathogens could be post production contaminants as the physicochemical properties of the ready -to-consume Kammerra are stringent for most microbes.

Key Words: *Fermentation, foodborne pathogen, Kammerra, Microbial load,*

1. Introduction

1.1 Background of the Study

Fermentation is one of the effective and the most economical methods of processing and preserving of beverages and foods acceptable to human being. The method is easily adaptable and applicable at home level in traditional communities (Bacha *et al.*, 1999).

Fermentation has many advantages such as inhibition of growth of spoilage and pathogenic microorganisms by producing organic acids and other compounds, improving *organoleptic* properties by producing different flavor compounds and often improving the nutritional value of the original foods (Wilson *et al*., 2012).

Beverage is any food intended to be taken in liquid form which includes juices, coffee, tea, soft drinks etc. several kinds of beverages are consumed not for food value but rather for thirst quenching properties and for their stimulating effects (Badau *et al*., 2018).

Traditional fermented beverages are traditionally fermented product based on the skills of household occupants by indigenous knowledge systems and is produced from a variety of locally and industrially available ingredients using indigenous techniques by people of the area themselves (Abawari, 2013).

Alcoholic beverages have an indispensible relationship with the life of mankind in history. Accordingly, the preparation and drinking of alcoholic beverages are ways of enhancing the nutritional significance of the raw materials used for the making of beverage as well as social relationships for human beings. Locally or indigenously fermented alcoholic beverages and foods are used for their nutritional value in different regions of Ethiopia and the fermentation process is accomplished by indigenous knowledge of the people of the area (Lee *et al*., 2015).

Almost in all areas of the world, some types of alcoholic beverages native to their regions were prepared and consumed. In Africa, fermented alcoholic beverages are consumed in different occasions such as marriage, naming and rain making ceremonies, at festivals and social gatherings, at burial ceremonies and settling disputes. Some information is available on the microbiology and biochemical properties of a variety of African traditional fermented beverages such as the Ethiopian *tella (*Sahle and Gashe 1991), *borde* and *Shamita* (Ashenafi and Mehari 1995, Bacha *et al*.1998 and Bacha *et al*.1999), the Nigerian *pito* and *burukut (*Achi 2005), the Zimbabwean *chibuku* and *mahewu*

*(*Chamunorwa,2002*),* the Kenyan *bussaa (Nout,1980),* the Ugandan *bushera (Muyanja et al.,2002),* Zimbabwean *masvusvu* and *mangisi (*Zvauya *et al.*,1997 *),* and the Tanzanian *togwa* and *mbege (*Okafor,2002*).*

Kammerra is one of traditionally fermented alcoholic beverage familiar in Konta special woreda, Southern Nations and Nationalities Peoples Region (SNNPR), Ethiopia. The name "*Kammerra*" is derived from the starter culture yeast used for its making, because yeast is called '*Kammerra*' in the study area. It is widely consumed beverage in different kebeles of the woreda (district). It is alcoholic and people use as a under fermented honey wine. Most people who use *Kammerra* do not drink other traditional alcoholic beverages like *borde, tella, teji* and *areke*, because *borde, tella, teji* and *areke* are unacceptable in the locality from religion point of views.

They accept *Kammerra* as non alcoholic because it is free from malt. Therefore most of the people who follows protestant religion in the area thinks for *Kammerra* as an alternative drink and religiously acceptable. The basic ingredients used for the making of *Kammerra* are barley, yeast (*Saccharomyces cerevisiae*), sugar, honey, water and Egg yellow powder, locally called *malk*. *Malk* is used as coloring agent to change the colour of *Kammerra* to mead or *teji* colour.

There were two ways of preparation of *Kammerra*: The first preparation process uses barley cleaned, dried, milled, and socked in water. The yeast was mulched on barely, and then, sugar, and honey added to the mixture which are mixed in one fermenting vessel (bucket or pot). The mixture is homogenized for few minutes. Then, the homogenized mixture is allowed to ferment for 24 to 48 hrs under sealed condition. The next day, after adding egg yellow powder to the fermented mixture in order to change the colour in to yellow, the product is ready for use. The second way of preparation of *Kammerra* follows the same procedure but avoid the cooking step. But this study was based on the first one. The consuming period of *Kammerra* normally lasts for 24-48 hrs. After 48 hrs of serving, the ready- to- use *Kammerra* losses the usual taste and sweetness. However, it can be used by adding additional sugar (according to local informant).

The preparation period of *Kammerra* vary in different parts of the woreda based on weather condition and seasons of the year: it will take 24hrs in temperate area, 48 hrs in medium temperate area and 72 hrs in cold temperature area. In general, the process of *Kammerra* preparation can be managed within two to three days. But the process of preparation, duration of fermentation and its alcoholic contents vary in different regions/districts of the Woreda according to the local informants.

The main goal of *Kammerra* processers/fermenters was to gain economic benefit generating income by selling the products. However, the microbial quality, safety and nutritional values of ready-to-use *Kammerra* is unknown as there is no scientific report made so far. To this effect, the objective of this study was, to analyses and characterizes the microbial quality, safety, physicochemical characteristics, and nutritional value of *Kammerra*, a traditional fermented beverage and to report information to support strategies to control their contamination and growth.

2. Objectives

2.1 General Objective

The general objective of this study was to assess the microbial quality and physicochemical characteristics of *Kammerra*, a locally fermented alcoholic beverage

2.2 Specific Objectives of the Study

- To determine the microbial load of *Kammerra* samples and those prepared under laboratory condition,
- To assess the prevalence of pathogens in *Kammerra* samples :-
- To determine the physicochemical characteristics (pH, Alcohol content, Temperature, Moisture content, Titratable acidity and Total solid) of *Kammerra* samples and change in those parameters in the course of *Kammerra* fermentation
- To evaluate nutritional value of *Kammerra* and that of laboratory fermented *Kammerra*

3. Literature Review

3.1 Fermentation

Fermentation is one of the oldest and most economical methods of producing and preserving food and beverages. In addition, fermentation provides a natural way to reduce the volume of the material to be transported, to destroy undesirable components, to enhance the nutritive value and appearance of the food, to reduce the energy required for cooking and to make a safer product (Blandino *et al.*, 2003).

The application of microorganisms, such as bacteria, yeasts and principally fungi, by the food industry has led to a highly diversified food industry with relevant economical assets. Fermentation, with special reference to the production of alcoholic beverages, ethyl alcohol, dairy products, organic acids and drugs which also comprise antibiotics are the most important examples of microbiological processes (Izabel *et al*., 2012).

Fermented products can play an important role contributing to the livelihoods of rural and peri urban dwellers alike, through enhanced food security, and income generation via a valuable small scale enterprise option. There is such a diversity of fermentable substrate available year round, that the activity can provide a regular income. Although harvesting or substrate may be seasonal, fermentation itself is largely independent of weather, and byproducts can be recycled into livestock fodder. Fermentation activities are highly combinable with a variety of other traditional and domestic activities, and can make a particularly important contribution to the livelihoods of women, the disabled and landless poor who, with appropriate training and access to inputs, can increase their independence and self-esteem through income generation (FAO, 2012).

Fermentation is commonly used to produce food materials (beverages, dairy products), renewable fuels (hydrogen, ethanol), pharmaceuticals (antibiotics), and industrial chemicals (acetate, butyrate). In industrial fermentation, specialized pure microbial cultures are normally used to generate specific products.

This requires expensive, sterile production conditions with high-quality raw materials. In contrast, mixed culture fermentation (MCF) uses environmentally ubiquitous organisms to produce a mixture of products depending on the environmental conditions (Zuhaida *et al.*, 2016). Fermentation methods for traditional alcohols are inexpensive and adaptable at household level in traditional communities.

Its process involves chemical change in bringing organic substrates through the action of biochemical catalysts, called enzymes (Yohannes *et al*., 2013).

Alcohol fermentation results in the production of ethanol, and yeasts are the predominant organisms (e.g. wines and beers). *Lactic acid* fermentation (e.g. fermented milks and cereals) is mainly carried out by *lactic acid* bacteria. A second group of bacteria of importance in food fermentations are the acetic acid producers from the *Acetobacter* species. *Acetobacter* convert alcohol to *acetic acid* in the presence of excess oxygen. Alkali fermentation often takes place during the fermentation of fish and seeds, popularly used as condiment (Blandino *et al*., 2003).

In different country, fermented alcoholic beverages are consumed in different occasions such as marriage, naming and making ceremonies at festivals and social gatherings at burial ceremonies and settling disputes. They are also used as medicines for fever and other ailments by adding barks or stems of certain plants. Fermented beverages produced from cereals usually referred to as beers while those produced from fruits are classified as wines (Tafere, 2015).

3.2 Traditional Fermented Food and Beverages

Fermented foods and beverages constitute a very important component of the people's diet in the world. There are several different fermented products, which have been reported (Adesokan, 2013). Fermented beverages are defined as products obtained through desirable biochemical changes caused by the action of microorganisms. They are typically unique and vary according to regions due to the variation in climate, social patterns, consumption practices and most importantly the availability of raw materials (Getnet and Berhanu, 2016).

A wide range of grains, fruit and vegetables are used to manufacture beverages, both thirst quenching products (mostly nonalcoholic), and those which are generally alcoholic and consumed on special occasions, including festivals. The former include tea, coffee, juices, nectars, syrups, and carbonated soft drinks (FAO, 2012).

The nature of fermentation in Ethiopia is not complex and does not required expensive equipment. During production of traditional fermented food products in Ethiopia, it is common to use and follow controlled natural fermentation process with no defined starter cultures used to initiate it. Ethiopian local fermented foods and beverages are products of acid-alcohol type of fermentation. The preparation of many local fermented foods and beverages is still practiced at household. It is known

that a wide variety of fermented foods and beverages are consumed in Ethiopia (Mulaw and Tesfaye, 2017).

The major indigenous fermented beverages and foods which are produced in Ethiopia are *Borde, Shamita, Tella, Tej, Araki, Korefe, Keribo and Duka* (Wassie, 2016), *and* injera, ergo, Ititu, ayib, qibe, arrera, kocho, tella, awaze, borde and tejj. Thus, at different time different researchers were conducted studies on the mentioned local traditional fermented food and beverage products. Bearing in mind the rich diversity in fermented food and beverage types in the country; few studies were carried out in widely different parts of Ethiopia, and included the major ethnic groups (Mulaw and Tesfaye, 2017). Some of traditionally fermenting beverages of Ethiopian are described as follows.

3.2.1 *Tella*

Tella is one of the Ethiopian traditional beverages, which is prepared from different ingredients. It is by far the most commonly consumed alcoholic beverage in Ethiopia. It is assumed that over two million hectoliters of *tella* is brewed annually in households and drinking houses in Addis Ababa alone. There are different types of *tella* depending on type of cereal ingredients. Corn is the most popular, but in some areas barley, millet or sorghum can be used. The method of preparing *tella* differs between ethnic groups and depends on tradition and the economic situation. Even if it may have different names at different localities; fermentation is basically carried out by the activity of *S. cerevisiae* (Andualem *et al*. 2017).

3.2.2 *Borde*

Borde is a cereal-based traditional fermented beverage and is widely consumed in the southern and western parts of Ethiopia. Ethiopian *borde* is a spontaneously fermented, low or non-alcoholic cereal beverage. It is produced by spontaneous fermentation using rudimentary equipment. *Borde* is an opaque, effervescent, whitish-grey to brown coloured beverage, with a thick consistency and a sweet-sour taste. It is an important product because both adults and children often consume it as a low-cost meal replacement (Abegaz *et al*., 2002). In most open markets in southern Ethiopia *borde* is available for purchase (Ashenafi and Mehari, 1995).

3.2.3 *Keribo*

Keribo is an indigenous traditional fermented beverage produced and consumed in different parts of the country, mostly south west Ethiopia. It is produced mainly from barley and sugar. Fermented *Keribo* constitutes a major part of the beverages being served on holidays, wedding ceremony and

also as sources of income of many households in different regions in Ethiopia. The popularity of this traditional fermented beverage is more reflected among the religious groups and those do not like alcoholic drinks. Being considered as a non- or low- alcoholic beverage, *Keribo* is popular among both adults and children. It has poor keeping quality with shelf-life of not more than a day or two and it has a pronounced characteristic of the deteriorating beverage at the end of 48 h of fermentation (Tafere, 2015).

3.2.4 Areki

Areki is a distilled alcoholic beverage. It is a colorless, pungent odder traditional alcoholic beverage which is distilled from fermentation products prepared in almost the same way as *Tella* except that fermentation mass in this case is more concentrated and distillation process is different from *Tella* (Yohannes *et al*, 2013).

3.3 Microbiology of Fermented Foods and Beverages

Fermented foods are the centers of consortia of microorganisms, which transform the chemical constituents of raw materials of plant/animal sources during in situ/ex situ fermentation, thereby enhance the nutritional value with health-promoting bioactive compounds to consumers. Common *genera* of the *lactic acid* bacteria isolated from various fermented foods globally are *Alkalibacterium, Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Oenococcus, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus* and *Weissella* species of *Bacillus* are reported for alkaline-fermented foods of Asia and Africa (Tamang, *et al.,* 2017).

The fungi genera *Aspergillus, Paecilomyces, Cladosporium, Fusarium, Penicillium* and *Trichothecium* are the most frequently found in certain products. The common fermenting yeasts are species of *Saccharomyces*, which results in alcoholic fermentation. The type of bacterial flora developed in each fermented food depends on the water activity, pH, salt concentration, temperature and the composition of the food matrix (Blandino *et al*., 2003). The sources of the microorganisms are usually the ingredients and the traditional utensils used for fermentation processes (Tafere, 2015).

3.4 Nutritional Values and Safety of Fermented Foods and Beverages

Fermented foods can be more nutritious than their unfermented complements. This can come about in at least three different ways. Microorganisms not only are catabolic, breaking down more complex compounds, but they also are anabolic and synthesize several complex vitamins and other growth factors. The second important way in which fermented foods can be improved nutritionally has to do with the liberation of nutrients locked into plant structures and cells by indigestible materials (Hasan *et al*., 2014).

Fermentation can enhance food safety through the inhibition of pathogenic bacterial growth, toxin degradation, and the improvement of the shelf life and digestibility of raw food materials. The preservative nature of *LAB* species involves the ability to block the growth of pathogenic microorganisms by nutrient competition and bacterial inhibit or production. Some of the inhibitors include organic acids, hydrogen peroxide, and bacteriocin. lactic and acetic acids are particularly effective at inhibiting the growth of gram negative bacteria, where by hydrogen peroxide has strong oxidizing effect on most pathogenic bacteria (Borresen *et al*., 2012).

3.5 Economic and Social Values of Fermented Foods and Beverages

Preparation of many indigenous or traditionally fermented foods and beverages are remains today as a house art. They are produced in homes, villages and small-scale industries. On the opposing, the preparation of others, such as soy sauce, has evolved to a biotechnological state and is carried out on a large commercial scale. In the distant past, there was no verified data on the economic, nutritional, technical, and quality control implications of the indigenous fermented food (Blandino *et al*., 2003).

Alcoholic beverages are a part of human dietary culture and have an inseparable relationship with the life of mankind in history. The making and drinking of alcoholic beverages has nutritional significance as well as enhancing social relationships (Belete *et al*., 2017). Its consumption is a part of the culture and daily life worldwide, and plays an essential role in business and social activities (Kido *et al*., 2016).

Fermentation of row plant and animal material is one of the best and earliest methods of food preparation and preservation. Thus, fermented foods have a role in social functions such as marriage, naming and rain making ceremonies, where they are served as weaning foods. In addition, fermentation delivers a natural way to reduce the volume of the material to be transported, to

extinguish undesirable components, to improve the nutritive value and appearance of the food, to reduce the energy required for cooking and to make a safer product (Mulaw and Tesfaye, 2017).

3.6 *Kammerra*

There is no enough information to read and organize about *Kammerra* production and fermentation. However, *Kammerra* as an indigenous fermented beverage was started in 1981 E.C.in Chabara Shasho and Konta Koysha kebeles of Konta Special Woreda according to personal communication made with local inhabitant (Woju Habte pers. comm. 2017).

The aim of *Kammerra* fermentation was to gain economic benefit and secondly the people those who follow protestant religion are not to drink other traditional beverages (*Borde, Tella, Areke, and Teji*) were to use *Kammerra*. According to local informants, most of the people who use the *Kammerra* continuously were showing unbalanced characters after use of the beverage. Therefore, when after use of *Kammerra* they participate/ involves in different types of criminals and also exposing to various health problems. To this effect, the study is intended to analyses and characterizes the microbial, physicochemical and nutritional value of *Kammerra* traditionally fermented alcoholic beverage.

3.6.1 Preparation Procedures of *Kammerra*

Preparation of *Kammerra* has been done following traditional recipes/procedures. This includes collection of the necessary materials (jar, bucket, beaker, bowl and others) and ingredients (yeast, sugar, honey, water, barely, and egg yellow powder).The necessary ingredients are added into a bucket of water step by step and homogenized. Then, the homogenized mixture is allowed to ferment for 24 to 72 hrs under sealed condition. Egg yellow powder is added to the fermented mixture in order to change the colour of the fermenting mix making it appear like local *teji*, after which the product is ready for use (Fig, 1).

3.6.2 Safety of Local drinks and the producers' knowledge

The right to use safe drink is the basic human needs, but some drinks can cause different problems on social and economic activities of the community besides affecting the consumer's human health. However, the people of the study area have no enough knowledge on the positive or negative effects of consumption of *Kammerra*. The local fermenters are preparing *Kammerra* depending on their economic interest or benefits. The processing or preparation was traditional in its approach and the

hygienic status of their product is unknown. The users choice of the same product depends on affordability of its cost as *Kammerra* is less costly compared to other traditional drinks according to briefing by the local informants.

3.6.3 Contamination and Social problems associated with Kammerra production and consumption

Food contamination is defined as the presence of harmful chemicals and microorganisms in food and beverages which can cause consumer illness. These chemicals and organisms may be introduced into food at various stages: at production, processing, packing, transporting and/or marketing. Because of its adverse health effects, food contamination has become a matter of serious concern all over the world especially in the developing countries (Rafiq and Shah, 2015).There are limited studies carried out on the Knowledge, Attitude, Beliefs and Practices of people on beverages and food safety issues. A study carried out to assess the attitudes and practices regarding causes of diarrhea in rural community of India revealed that a majority of the mothers were not aware of the precautionary measures to be taken to prevent diarrhea (Sudershan, *et al.*, 2009). Likewise, the main problem associated with *Kammerra* production and utilization is socio-economic problem as most people who used to drink *Kammerra* were socially unstable and disturb their family and the community at large besides participation in different types of criminal acts. They also not use their time properly for their basic job as most of them waste their time at *Kammerra* vending houses. Another problem is the hygienic problems during processing as its production do not follow basic hygienic steps.

3.6.4 Microbiology of *Kammerra*

Microbiology of *Kammerra* could depend on the microbes of its ingredients and facilities used for its preparation. Accordingly, microorganisms of *Kammerra* fermentation could be determined by the microbes associated with its ingredients, namely yeast, honey, sugar, egg yellow powder, and hygienic quality during its handling and making (preparing) process.

3.7 Microbiology of Honey

Honey is the natural product produced from the nectar of flowers and other sources, represents an important source of energy, being widely consumed. There is a high interest in the number and type of microbes in honey, because it is used as food or as an ingredient in the food, pharmaceutical and cosmetic industries, as well as in alternative medicine (Sereia *et al*., 2011). Organisms found in the

environment around honey (i.e. bees, hives, pollen, flowers, soil, etc.) are likely to occur in honey (Snowde and Cliver, 1996).

Two main groups of bacteria, classified as *Gluconobacter* and *Lactobacillus*, are present in ripening honey. A third bacterial group, classified as *Zymomonas*, and several types of yeast are occasionally isolated. *Lactobacillus* and *Gluconobacter* disappear after minimum moisture (about 18%) is reached, but the former does so sooner than the latter (Argueso and Navarro, 1975).

The *Apismellifera* honey has a peculiar microbiological pattern due to its physicochemical composition, with a high degree of resistance to microorganisms' proliferation. Its microbial flora consists of common microorganisms, such as *Bacillus, Penicillium, Mucor* and *Saccharomyces*, which can negatively influence its final quality as they multiply in honey exposed to action of adverse external factors, such as handling conditions, spores contamination, high temperature storage and high relative humidity. The primary sources of microorganisms are: pollen, bee's digestive system, dust, air and flowers (Sereia *et al*., 2011).

3.8 Microbiology of Sugar

Ensiling sugarcane without additives typically results in alcoholic fermentation from the growth of yeasts. This results in a loss of nutritional value as the total concentration of sugars and sucrose decline. *Lactic acid* bacteria have been extensively evaluated as inoculants in silage. Their function is to increase the number and competitiveness of beneficial bacteria in the silage mass, which increases *lactic acid* production and inhibits the growth of undesirable microorganisms (Santos *et al*., 2015).

3.9 Egg yellow

The pigment found in egg yolk determines the acceptability of the product by the consumer who prefers yellow-orange egg yolk. The yellow pigment is mainly explained by the presence of *xanthophylls* derived from *carotenoids. Carotenoids* are important in the poultry nutrition because they are controlled by the diet. Some ingredients used for eggs enrichment with polyunsaturated fatty acids are natural sources of *carotenoids* and their use in diets of laying hens promotes greater yolk pigmentation. yellow corn based diets contribute to the production of eggs with yellow yolks whereas diets with low corn pigmentation, sorghum grain and wheat, with no added dyes, produce eggs with a low yellow pigmentation (Barbosa *et al*.,2011).

3.10 Physicochemical characters of *Kammerra*

Physicochemical characteristics of *Kammerra*, such as alcohol content, (ethanol level), pH, Titratable Acidity, Moisture content, Total solid, and temperature were determined based on physicochemical characteristics of ingredients used to prepare *Kammerra* and the product itself following standard procedure. The next few sections present the physicochemical properties of Kammerra ingredients.

3.11 Physicochemical characters of honey

The quality of honey is determined by their sensory, physical and chemical properties. Its physical and chemical properties depend on the nectar, pollen floral source, color, flavor, moisture, protein content and sugar. All honeys share certain general characteristics, including moisture content below 20%, a sugar content of 70-80%, an ash content ranging from 0.1% to 0.2%, and a pH between 3.8 and 4.7 (Da Silva *et al*., 2017).

Proteins, free amino acids (principally *proline*), organic acids, *aromatics*, and vitamins and mineral are minor components and several enzymes are important components of honey such a *α-glucosidase, β-glucosidase, amylase* and glucose oxidase. The specific percentages of all these different components may vary depending on the plant origin, the geographical location, the season in which the honey was collected, the treatment of honey since its harvesting, and its age (Da Silva *et al*., 2017).

Honey is a substance that has been used for centuries to make beverage which can be fermented to produce different types of mead and spirits that may have different flavors depending on the floral source of honey and additives and yeast used in fermentation (Da Silva *et al*., 2017).

3.12 Physicochemical characters of egg yellow

Eggs contain proteins of high biological value as compared with other dietary proteins. Egg proteins possess desirable functional and nutritional properties and therefore are widely used in many food products. Egg yolk is widely used in the food industry due to the excellent functional properties of its *lipoproteins*, such as flavor, aroma, color, viscosity, emulsifying, and foaming (Lai *et al.*, 2010).

Yolk colour in laying hens is primarily determined by the content and profile of pigmenting *carotenoids* present in their feed and can easily be adapted via feed ingredients. *Carotenoids* are

yellow, orange, and red pigments soluble in fats. *Carotenoids* are divided into two main groups - *carotenes* and *xanthophylles. Xanthophylles* like *lutein* and *zeaxanthin* have the greatest influence on the yolk colour. *Beta carotene* as a representative of *carotenes* is present only in small amount. Each pigment has unique properties, e.g. colour hue and deposition efficiency. The proportion of the dietary intake of *carotenoids* that are absorbed and deposited in the egg yolk determines its real colour varying from pale yellow to dark orange (Bovskova *et al*., 2014).

3.13 Microbiological Quality/ Safety Indicators

3.13.1 *Aerobic Plate* Count

The *aerobic* plate count (APC) is important in food microbiology as an indicator of the microbiological quality as well as a measure of sanitation used during preparing and handling of food and beverages. APC determines counts of the non-fastidious *aerobic* bacteria. In some foods and traditional beverages, high APC may indicate poor quality. Higher bacterial numbers spoil the food faster and result in loss of quality. Food which appears normal may have high APC, indicating that the food is about to spoil (Jacob, 2010).

3.13.2 *Coliforms* count

The term *coliform* does not have taxonomic value. It represents a group of species from several bacteria namely, *Escherichia, Enterobacter, Klebsiella*, *Citrobacter*, and probably *Aeromonas* and *Saerratia* (Feng, *et al., 2001*).

The main reason for grouping them together is their many common characteristics. They are all gram negative, non spore forming rods, many are motile, are facultative anaerobes resistant to many surface-active agents, and ferment lactose to produce acid and gas within 48h at 32 or 35°C(Yousef and Carlstrom, 2003).

Some species can grow at higher temperature (44.5°C), while others can grow at 4 to 5°C.all species are able to grow in food and food products except those that are at pH less than or equal to 4.0 and water activity less than or equal to 0.92, they are sensitive to low heat treatments and are killed by pasteurization (Yousef and Carlstrom, 2003).

Since *coliforms* are common inhabitants of the intestinal tract, their presence in food may indicate fecal contamination. The specificity of *coliforms* as an indicator of fecal contamination for raw food is reduced since large numbers of *coliforms* in the food may result from growth of small non fecal inoculums. In contrast, in heat processed (pasteurized) food products, their presence are considered as an indicator of post heat treatment contamination from improper sanitation (Ray, 2004).

3.13.3 *Enterobacteriaceae*

Enterobacteriaceae family contains a large number of genera that are biochemically and genetically related to one another. This group of organisms includes several that cause primary infections of the human gastrointestinal tract. Members of this family are major causes of opportunistic infection (including septicemia, pneumonia, meningitis, and urinary tract infections). Examples of genera that cause opportunistic infections are: *Citrobacter*, *Enterobacter*, *Escherichia*, *Hafnia*, *Morganella*, *Providencia* and *Serratia (SPB, 2016).*

3.13.4 *Lactic Acid* Bacteria Count

Lactic Acid Bacteria are gram-positive usually non-motile, non-spore-forming rods and *cocci*. They lack the ability to synthesize cytochromes and porphyrins (components of respiratory chains) and therefore cannot generate ATP by creation of a proton gradient. The *LAB* can only obtain ATP by fermentation, usually of sugars. Since they do not use oxygen in their energy production, *lactic acid bacteria* happily grow under anaerobic conditions, but they can also grow in oxygen's presence (Khald, 2011).

3. 13.5 *Staphylococcus* Species count

Staphylococcus species is the normal flora of the living human and animal on the skin and can cause poisoning contaminating foods consumed by human. These bacteria are gram positive, *cocci* shape, non motile, non spore former, facultative anaerobic, Catalase mostly positive.

3.13.5 Bacillus Species count

Gram positive and aerobic spore-forming *bacilli* belonging to the *genus Bacillus* and other related species play important roles in food poisoning and spoilage (Aruwa *and Olatope* 2015).

4. Materials and Methods

4.1 Description of the Study area

Konta special woreda is one of the four special woredas in the South Nation Nationalities and Peoples Regional State (SNNPRS); Ethiopia, and located in between 6º, 46'-7', 27'' North latitude and 36º, 32'-36', 87'' East longitude (Fig.1). The woreda is located 464 km away from Addis Ababa, the capital city of Ethiopia, and 372 kms away from Hawassa, the regional state's capital city; and 110 kms away from Jimma town. Based on the CSA population projection report made in 2013 (CSA, 2013), the total population of the district in 2017 is 115,898 of which 56,656 are males and 59,242 are females. Among these, the urban inhabitants are 17,584 of which females are 9,333.

Konta has a population density of 51.4 people per square kilometer. Administratively, the district (woreda) has 9 towns and 41 rural kebeles. The familiar traditional beverages of the area are Tella, Borde, Areke (Katikala), Tej, Songe, Bushbush and Kammerra. *Kammerra* has been started since 1989 (according to *local* informants) and commonly consumed as traditional beverage in the study area since then.

Although practice in many kebele's of the district, Kammerra is well known and commonly produced and consumed in Konta Koysh, Delba Genet, Chebera Shasho, Chata Kechikecha, Ameya, Bedhet Xanga, Genji Genet, Chida Ediget, Kerbella Bedegucha and Kecha Roba (Fig 2).

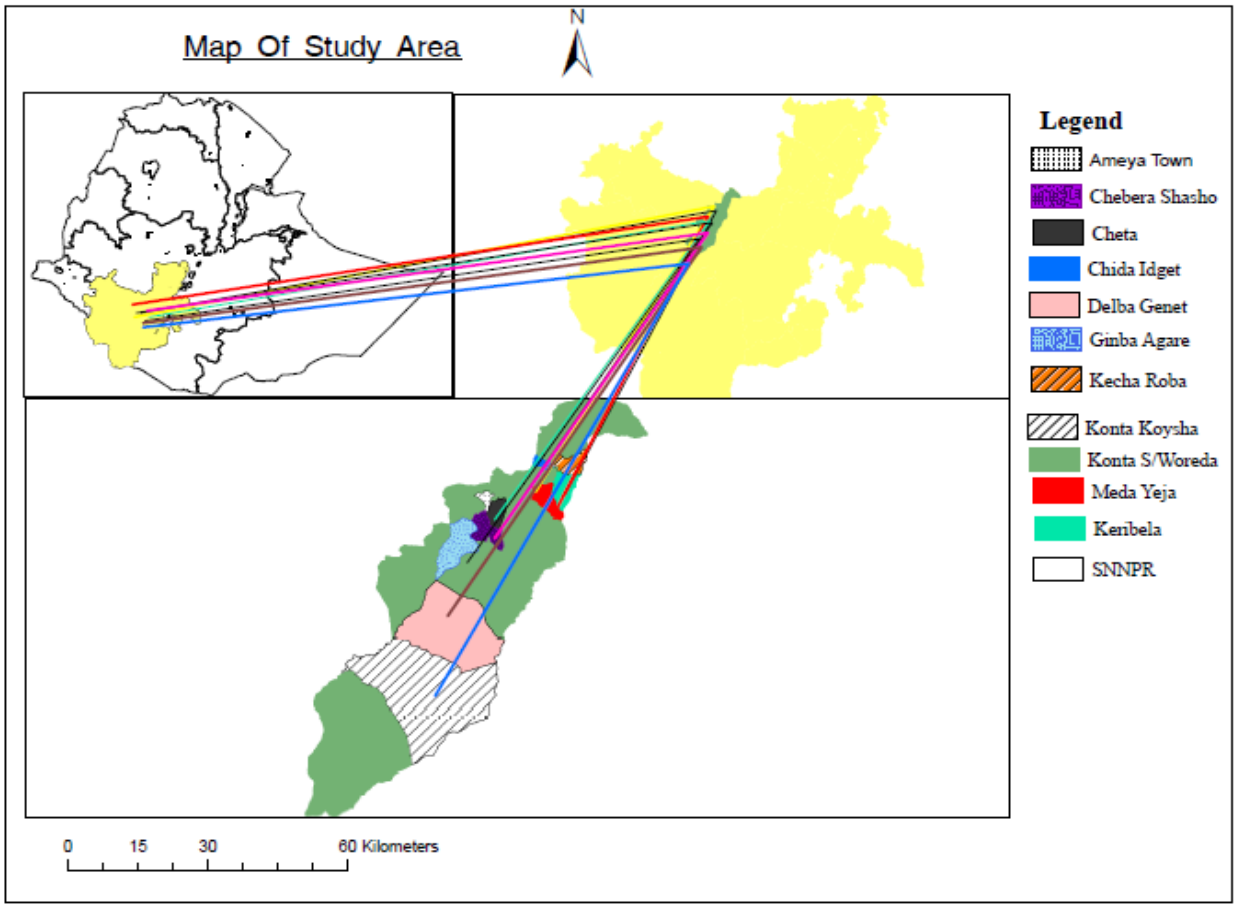

Figure 1. Map of study area. Source: (https://openi.nlm.nih/detailedresul.php?)

4.2 Study design and period

The study had two phases: microbiological and physico-chemical analysis of Kammerra samples as it is made locally available to consumer; and analysis of fermenting *Kammerra* made at laboratory (controlled fermentation) following the traditional recipes(Fig 3.& Fig. 4). Accordingly, Cross-sectional study design was used from May 2018 to February 2019 for evaluation of *Kammerra* samples. The fermentation study was conducted twice under laboratory condition.

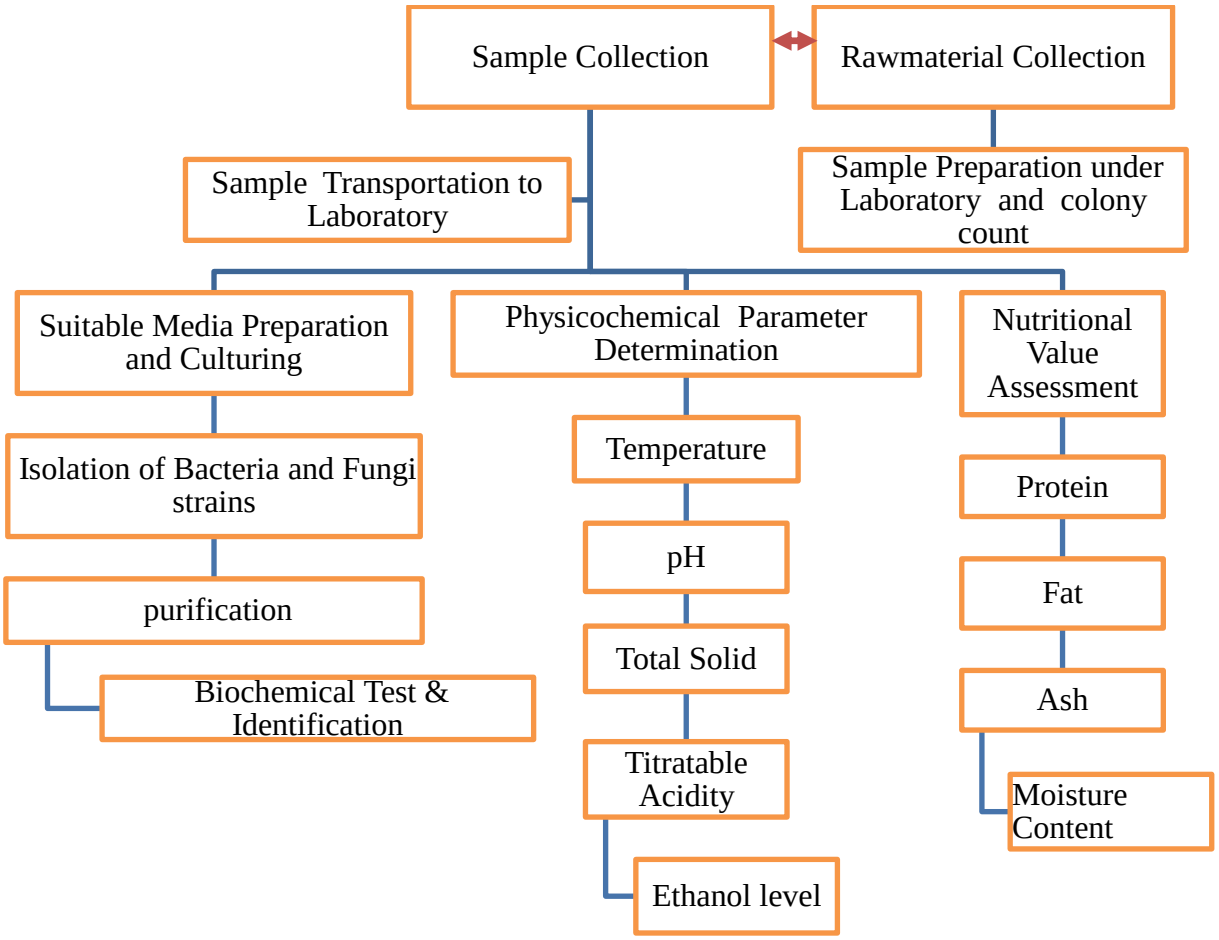

Figure 2. Conceptual frame work of the study

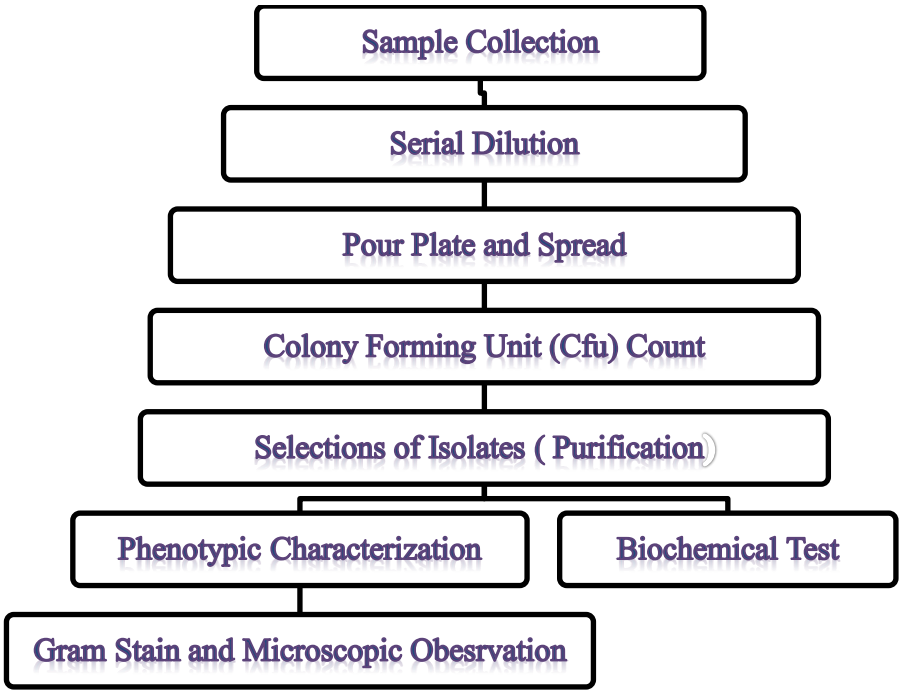

Figure 3.Flow chart of study design for microbiological analysis

4.3 Study population

People who ferment or prepare and consume/drink/ *Kammerra* in Konta Special Woreda were sources of samples and information on traditional fermentation processes.

4.4 Sampling technique

A total of 30 samples were collected randomly depending on the availability of home producers/venders of the same fermented product. The representative samples of *Kammerra* were collected from ten Kebeles of the woreda by clustering the Kebeles according to weather condition.

4.5. Sample collection

Locally prepared 30 samples were collected randomly and based on availability from 10 kebeles (three sample per kebeles) of the woreda (Ameya, Chabara Shasho, Cheta Kechikecha, Genji Genet, Chida Ediget, Meda Yeja, Konta Koysha, Betsti Tsanga, Kecha Roba and Kerbella Bedegucha) in sterile bottles aseptically and transported to Postgraduate and Staff Research Laboratory of Department of Biology using vaccine caring cold chain (ice box). Samples were transported to the laboratory within 6 hrs of collection and have been kept under refrigeration until processed.

4.6 Sample preparation and Microbial Enumeration

Kammerra was prepared in Postgraduate and Staff Research Laboratory of Department of Biology by following traditional technique using the following ingredients: 2000ml tap water, 7gm yeast, 200gm sugar, 14gm honey, 10gm barley and 0.04gm egg yellow powder (Figure4). Materials/ equipments used for preparation of *Kammerra* sample in the laboratory were plastic jar (bucket), Becker, electronics balance, measuring cylinder, spatula and others. Before preparation of *Kammerra* all required materials were cleaned and set/collected.

The measured raw materials were added sequentially and homogenized in one jar/bucket and completely homogenized (Figure5). After homogenization, changes in microbial dynamics were assessed by withdrawing samples intermittently every 6 hrs for 84 hrs. Also pH and temperature were measured by using digital pH meter (Adwa, pH/MV/EC/TDS/Temperature Bench meter, AD 8000, made in ROMANIA) and Titratable (total) acidity of sample was detected by titration against 0.1N NaOH solution, 1% solution of phenolphthalein indicator.

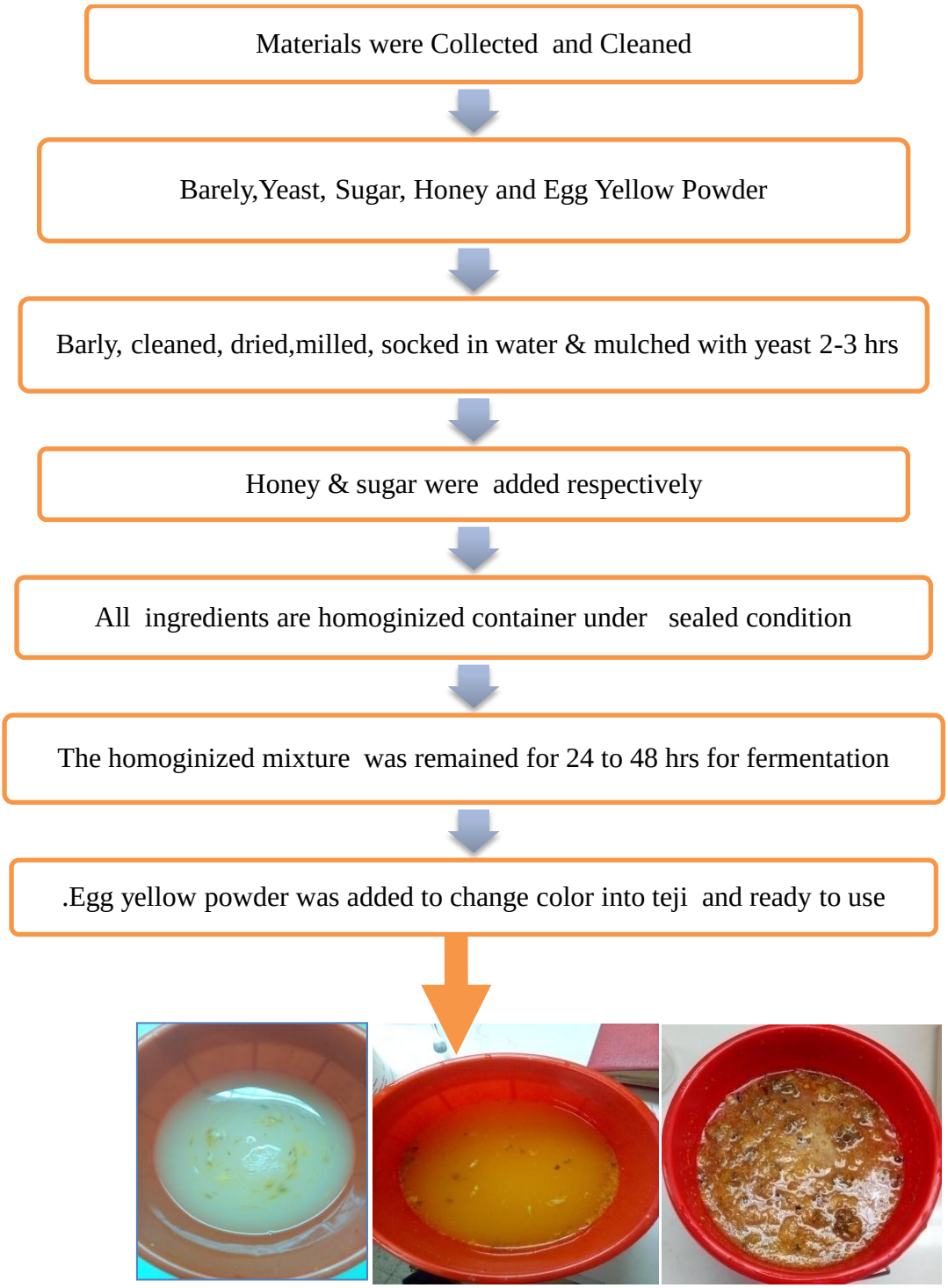

Figure 4. Flow chart of traditional *Kammerra* preparation procedure and product

4.7 Microbial and physicochemical analysis of *Kammerra*

4.7.1 Microbial Enumeration

The collected samples were subjected to microbial and physicochemical analyses. Total of 30 samples, 500ml each, were collected aseptically. Twenty five milliliter of each sample was separately diluted in 125 ml of saline solution. One ml of homogenated solution was introduced into 9 ml of sterile distilled water in test tube and serially diluted to 10^{-1} to 10^{-6} and, 0.1ml of each of the diluted sample was spread plated on a pre-sterilized and surface dried agar plates and processed for count of the following microbiological groups:

4.7.1.1 Count of *Aerobic Mesophilic Bacteria:*

For the count of *aerobic mesophilic bacteria* 0.1 ml of appropriate dilutions were spread-plated in duplicate on pre-dried surfaces of Plate Count Agar (PC). Colonies were counted after incubation at 32°C for 48 hrs.

4.7.1.2 Coliform count

For coliform count 0.1 ml of appropriate dilutions were spread-plated in duplicate on pre-dried surfaces on Violet Red Bile Agar (*Microgen*: DM10495) after inoculating 37°C for 24 to 48 hrs. Red to pink colonies, surrounded by precipitated bile, was counted as coliforms (Weil *et al.*, 2006).

4.7.1.3 Count of *Enterobacteriaceae*

For counting of *Enterobacteriaceae,* 0.1 ml of appropriate dilutions were spread plated in duplicate on pre-dried surfaces of Violet Red Bile Glucose (VRBG) Agar plates and incubated at 30 to 32 °C for 24 hrs. The colonies were counted after 24hrs incubation and recorded as CFU/ml.

4.7.1.4 Count of *Lactic Acid Bacteria*

For count of *lactic acid bacteria*, 0.1 ml of appropriate dilutions was spread plated in duplicate on pre-dried surfaces of MRS agar (Oxoid) plates. Colonies were counted after incubation in an anaerobic jar (Oxoid) at 32 ^{O}C for 48 hrs according to the methods described at (Ashenafi and Mehari, 1995).

4.7.1.5 Bacillus Species Counts

Microbiological analysis for *Bacillus* species were done using 25ml sample mixed with 125ml distilled water and by heating at 80℃ for 10 minutes in water bath and serial dilution technique with spread plating 0.1 ml into plate count agar (Standard Methods Agar, Microgen, DM 1091).
The second and third (10^{-1}, 10^{-2} and 10^{-3}) dilution were used for plating unto the media and incubating for 48 to 72 hrs at 35℃. Colonies were counted after incubation (Aruwa and Olatope, 2015).

4.7.1.6 Staphylococci Count

For count of *staphylococci*, Mannitol Salt Agar (Oxoid) was surface plated and incubated at 37°C for 48 hours. Golden yellow colonies were counted as *staphylococci* (Acco, *et al*., 2003).

4.7.1.7 Yeasts and *moulds* counts

From appropriate dilutions, 0.1ml of the aliquot was spread plated on pre-solidified surfaces on Potato Dextrose Agar addition with 0.1 gram chloroanphinicol and incubated at 25°C for 3-5 days. Smooth (non-hairy) colonies without extension at periphery were counted as yeasts whereas hairy colonies with extension at periphery were counted as mold (Spencer *et al*., 2007).

4.8 Purification

Single colonies of representative isolates were purified following the dilution plating technique in the agar medium specified for a particular type of microbial groups. Separated colonies were transferred again to the agar slants. Purification was done by streaking on plated agar and repeated two or three times or until pure cultures were obtained, as confirmed by microscopic examination, are obtained (Chay *et al*., 2017).

4.9 Identification procedure

The identification of the bacterial cultures were confirmed based on their morphological, physiological and biochemical characteristics using Bergey's Manual of Systematic Bacteriology, John Manual of bacterial identification and *Lactic acid* bacteria microbiological and functional aspects. The identification procedures for the yeast isolates were carried out according to the methods (Fadahunsi *et al*., 2013) and others as described below.

4.9.1 Gram stain

Gram staining was performed to identify the morphology, shape and cell arrangements of bacteria. Smears were flooded with crystal violet for one minute and then washed gently in tap water. In the second step, smears were exposed to Gram's iodine for one minute, and then washed with tap water. In the third step, slides were exposed to alcohol for de-colorization (95%) and washed in 30 seconds with tap water. Finally, dilute counter stain was added and washed after one minute. After drying, stained slides were examined under oil immersion (100X) to note Gram reaction, morphology and arrangement.

4.9.2 Endospore Test

Bacterial Endospore test was performed according to Schaeffer and Fulton (1933) method. A smear of isolates were prepared on a clean glass slide and allowed to air-dry. The air-dried smear was fixed. Heat fixed smear was flooded with 0.5%(w/v) malachite green solution and steamed using cotton dipped in 95% ethanol for 5 minutes. After cooling, the slide was rinsed tap water and counterstained with safranin for 30 seconds. The slide was rinsed with tap water and air-dried; finally under the oil immersion lens (100X) the presence of Endospore was examined. Endospore were bright green and vegetative cells were brownish red to pink were recorded.

4.9.3 Physiological determination

The bacterial and yeast strains isolated from *Kammerra* samples were characterized by physiologically by ability to growth in 5, 6.5,7 and 7.5% NaCl, growth at 10 and 45 °C, and 4.4 and 9.6 pH and lacto phenol cotton blue. The lacto phenol cotton blue (LPCB) was used for morphological identification of yeast (Kanghae *et al*., 2016).

4.9.4 Biochemical Test

After the isolation of pure culture from different agar media, the cultures were then preserved and were later subjected to various biochemical tests for the confirmation and identification of the isolates. The isolates were subjected to various biochemical tests and the individual results were recorded. The biochemical tests that were used to identify the unknown cultures were:-Gram stain, Endospore test, KOH test, Catalase test, Oxidation Fermentation test, motility test, Methyl Red (MR)

test, indole test, urease test, TSIA test, SIM test, Motility and carbohydrate fermentation test as sited in (Sawian *et al.*, 2018) as described below.

4.9.5 Potassium Hydroxide (KOH) Test

Potassium hydroxide test of each bacterium isolate was made to distinguish between Gram positive and Gram-negative bacteria. In principle, the application of KOH is meant to dissolve the lipopolysaccharide present in the cell wall of Gram-negative bacteria which is absent in Gram-positive bacteria. The dissolved lipopolysaccharide stretch when pulled with a needle.

The tests were determined by one to two drops of 3% KOH solution was placed on a clean glass slide. A colony of pure isolate was picked with an inoculating loop and stirred in the KOH solution for 5-10 seconds. The loop was then raised slowly from the mass. In Gram-negative isolates, the KOH solution was become viscous and the thread of slime followed the loop for 0.5 to 2cm or more and it was considered as gram negative. In gram-positive there were no slime that follows the loop and recorded as gram positive (Bacha, 1997).

4.9.6 Catalase Test

The Catalase test is primarily used to distinguish among Gram-positive *cocci*. Members of the genus *Staphylococcus* are Catalase-positive, and members of the genera *Streptococcus* and *Enterococcus* are catalase-negative. Catalase test was carried out using a loop full of overnight grown culture. Cultures were smeared on a clean dry, glass slide and 3% H_2O_2 was added and allowed to react. The presence of bubbles was recorded as Catalase positive.

4.9.7 Methyl red Test

MR-VP broth with a pure culture of the microorganisms was under investigation, which is incubated for 4 days at 35 ^{0}C, was used for both MR Test and VP test. Only the addition of a reagent differs, and both tests were carried out consecutively. About 5 drop of the methyl red indicator solution to the first tube and for voges- proskauer test Barrit's reagent (6 drops of 5% alpha-naphthol, and mixed well to aerate, then, 2 dropped of 40% potassium hydroxide and mixed well to aerate) were added to another tube. Positive reactions were indicated by the color of the medium changed to red and remain as it is within a few minutes and the negative reaction was changed to yellow (Agbagwa *et al*.2017).

4.9.8 Oxidation Fermentation Test (O/F)

Glucose metabolism was determined by the oxidation-fermentation test. Ingredients used were:- Casein Peptone, 2.0g; yeast extract ,l.0g; NaCl, 5.0g; K_2HPO_4, 0.2g; glucose, 10.0g; bromothymoleblue, 0.08g; agar, 2.5g; distilled water,1000ml, pH,7.1.The above medium were freshly prepared (in l0 ml amounts in test tubes), cooled down to about 35^0C under tap water, inoculated by stabbing with a straight wire to the bottom and incubated at 32°C.Acid formation, gas production and growth were recorded for 2-5days (Bacha, 1997).

4.9.9 Triple Sugar Iron Agar Test

The triple sugar iron agar was made in such a way that there are slant and the butts. With a sterilized straight inoculation needle, the isolate colony was picked from the solid media and streaked the TSIA up to the butt and then streaked the surface of the agar slant. The cotton was closed and incubated at a temperature of 37°C for 24hours. After the incubation, black precipitates were observed on the TSI, and also reddish or yellow coloration signifies acidic or basic utilization. The black of the butt indicate hydrogen sulfide is produced (H2S), whereas gap spacing of the medium signifies gas formation. The development of black precipitate and gas at the butt indicated the presence of *Salmonella* species ((Agbagwa *et al*., 2017).

4.9.10 Sulfur Indole Motility test (SIM)

Motility test was carried out using semi-solid agar medium in a test tube. The isolates were inoculated with a straight wire making a single stab down the center of the tube to about half the depth of the medium and incubated at 32°C for 24 hr (Agbagwa *et al*.2017).

A positive motility test was indicated by a red turbid area diffusing away from the line of inoculation and a negative motility test was indicated by straight growth along the inoculation line only but no further. Indole test was tested by inoculation of 8 ml of peptone water with bacterial isolate under test and incubated at 37 ^{0}C for 24 to 48 hrs. Adding 0.2 - 0.3 ml of *Kovac's* reagent to 8 ml of a 24 - 48 hours old culture and shaking gently. The formation of red color ring in the layer was indicated a positive reaction. Formation of yellow colour ring was indicating negative test (Agbagwa *et al*., 2017).

4.9.11 Simmon Citrate Test

The Simmons citrate agar was used for the differentiation of gram negative bacteria on the basis of citrate utilization. A purified broth culture was taken by a sterile loop and streaked on slant medium into a test tube and incubated at 35^0C to 24hrs. The positive reactions of acids were changed the color from green to pink, and the negative reactions remain green were recorded as a result (Difco™ & BBL™ Manual).

4.9.12 Carbohydrate fermentation test

The carbohydrate fermentation test is used to determine whether or not bacteria can ferment a specific carbohydrate. Carbohydrate fermentation patterns are useful in differentiating among bacterial groups or species. It tests for the presence of acid and/or gas produced from carbohydrate fermentation. The sterile loop full of pure broth isolates were inoculated in to eight to ten ml phenol red broth base that contains specific carbohydrate 1% for bacteria and 2% for yeast and Small inverted tubes called Durham tube was immersed in the medium to test for the gas production. Carbohydrate fermentation capacity of yeast species isolated from local *Kammerra* samples were conducted using 4 ml phenol red Broth base with Durham tube in addition to different carbon sources (1.0 ml sterile carbohydrate solution (glucose, galactose, lactose, sucrose, and maltose) and incubated at 25ºC for 72 hrs.

4.9.13 Urease Test

Urease test were performed by inoculating the overnight pure isolates from brain heart infusion broth to in the urea broth and incubated at 37℃ and 25°C for 2 to7 days for bacteria and yeast respectively. The tests were taken to be positive there is the formation of a deep red and yellow/pink color for bacteria and yeast respectively and in the broth, which indicates urease activity the procedure described (Barnett *et al*., 2000).

4.10 Growth potential of *Staphylococcus aureus, Salmonella typhimurium* and *E.coli* isolated from Kammerra samples

The growth potential of *Staphylococcus aureus, Salmonella typhimurium and E.coli* were assessed on Kammerra samples. About 200ml of each Kammerra samples were separately homogenized using vortex and steamed at 80°C in water bath for 10 minutes to kill any vegetative cells that might be present in the samples. About 100 ml of each Kammerra samples were challenged separately with 1

ml overnight cultures of the test strains to bring the final inoculums size to the level of 10^2 -10^3 CFU/ ml. Test strains used were *S.typhimurium (ATCC13311*),*S.aureus*(*ATCC25925)& E.coli (ATCC25922).* The challenged Kammerra was incubated at 30-32°C for 24 hours. To determine the initial inoculum level, 10 ml of each inoculated Kammerra was homogenized in 90 ml of BPW and 0.1 ml of appropriate dilution was spread plated on MSA for *Staphylococcus aureus,* XLD for *Salmonella typhimurium* and VRBA for *E.coli*. The pH of each Kammerra sample was measured using digital pH meter from 0 to 36 hours at an interval of 6 hours while assessing the growth potential of the potentially pathogenic test stains (Muleta and Ashenafi, 2001, Girma *et al*., 2014).

4.11 Physicochemical Analysis

Physicochemical properties such as state (solid, liquid and gas) colour, and opacity were determined by simple observation (Basumatary *et al.,* 2014), and alcoholic content (ethanol level), pH, Titratable Acidity, Moisture content and Total solid of the collected and prepared samples of *Kammerra* were determined following standard procedure (Tekluu *et al.,* 2015).

4.11.1 pH Determination

The pH of the each *Kammerra* samples were measured by dipping the electrode of a digital pH meter (microprocessor) Model number Sl-145 Spectronics India, into the samples after a proper calibration of the pH meter with standard solution of pH of 4,7 and 10 buffer accordingly (Yohannes *et al.*, 2013).

4.11.2 Determination of Titratable Acidity

Titratable acidity was measured by a simple titration method using 0.1 N sodium hydroxide solutions. Ten ml of each sample was measured with a 50 ml measuring cylinder into a conical flask and diluted with 30 ml of distilled water. Three drops of phenolphthalein concentration were added and titrated against 0.1 N NaOH. The end point was indicated by a change from a colorless to pink solution. The total acidity was calculated as percentage lactic acid (Zakpaa *et al*., 2010).

$$\text{TA\%} = \frac{\text{0.1N NaOH x Vol. of NaOH(in Liter) x 90.08)} * 100}{\text{weight of the Sample}}$$

4.11.3 Determination of Alcohol (Ethanol Level)

The alcohol content of *Kammerra* samples were determined after sample collection and by using an alcoholmeter (ELS, India).The measuring procedure was direct inserting of alcoholmeter into 250 ml sample of *Kammerra* poured in the measuring cylinder at room temperature and the reading was recorded.

4.11.4 Determination of Moisture Content

Moisture content was determined by using 5ml of each samples drying to constant weight in a ventilated thermostatic oven at 110 °C for 3-5hrs and each samples were cooled in desiccators and weighed, the results were calculated the following formula as sited (Tigu *et al*, 1995).

$$\text{Moisture (\%)} = \frac{(\text{Weight of wet mass} - \text{weight of dried mass}) \times 100}{\text{weight of wet mass}}$$

4.11.5 Total Solid

The total solid (TS) in *Kammerra* samples (mg/ml) were determined by evaporating the liquids and other volatile substance at 110^0C for 3-5 hours. Five ml of each samples were measured to evaporate by heating the liquid part of samples were evaporated and the remained residue of evaporated was weighed and the results were recorded accordingly (Basumatary, *et al*., 2014) estimated formula at (DEAS, 2013).

$$\text{Total solids(\%)} = \frac{100(\text{M2} - \text{M1})}{\text{V}}$$

Where, *M*2 = Mass of the dish with dry sample, *M*1 = Mass of the dish, and *V* = Volume in ml of sample taken.

4.11.6 Determination of *Kammerra* Temperature

The temperature was determined by using bulb of mercury in glass thermometer at 20°C after 18hrs of sample collection. This was done by dipping the bulb of mercury in glass thermometer into the beverages (samples) and the readings (value of measured) were recorded (Umar *et al*., 2016).

4.12 Determination of Nutritional Value

Nutritional value determination was performed by as follows:- Ash content by direct ashing method, crude Protein content by automatic distillation and titration system, Fat content by simple extraction method, carbohydrate content by calculation (Sunano, 2017). By the following formula: - **%Total Carbohydrate = [100 - %(MC%+ Crude Protein + Fat + Ash+ Alcohol)]**

4.12.1 Ash content determination: Direct ashing method:

Ash was determined by igniting a 10ml sample, poured in a crucible, in a muffle furnace 550°C for five hours according to A.O.A.C923.1 (A.O.A.C., 1990). The weight of sample evaporated and loosed after ignition was considered to be the weight of total organic matter in the sample, the remaining being the weight of ash.

Total ash $= \frac{M2-M1}{V} \times 100$ Where, M2= Weight of crucible + dry residue, M1= Weight of crucible, M = volume of sample taken in to test

4.12.2 Crude Protein content determination: Automatic Distillation and Titration system

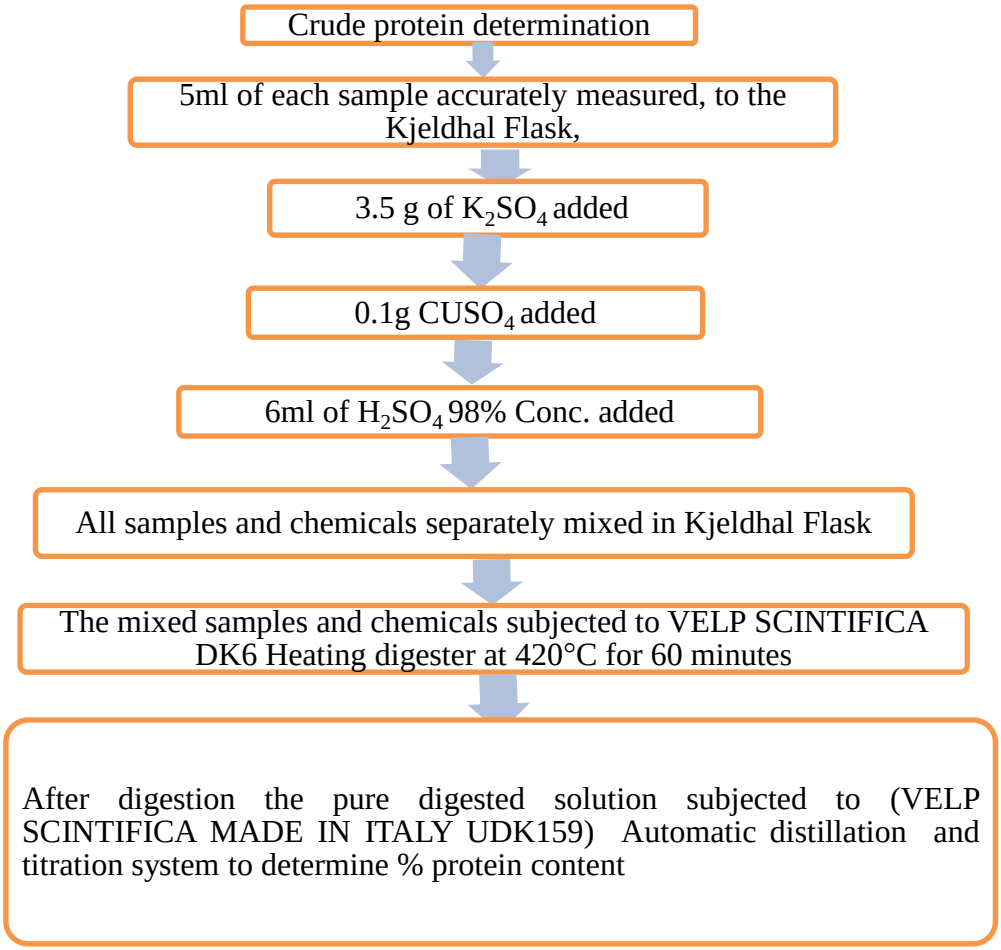

Figure5. Flow chart of protein content determination steps

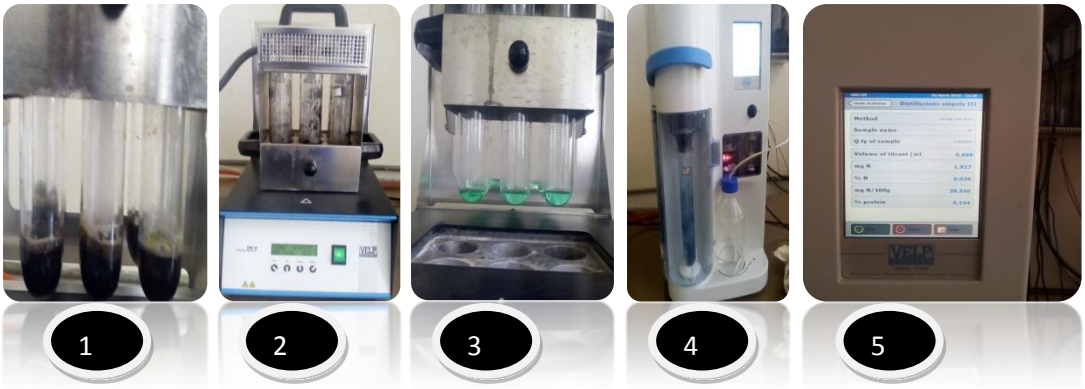

Figure 6.Crude protein determination steps from mixed samples and chemicals to titrated results on the screen

4.12.3 Fat content determination: simple extraction method:

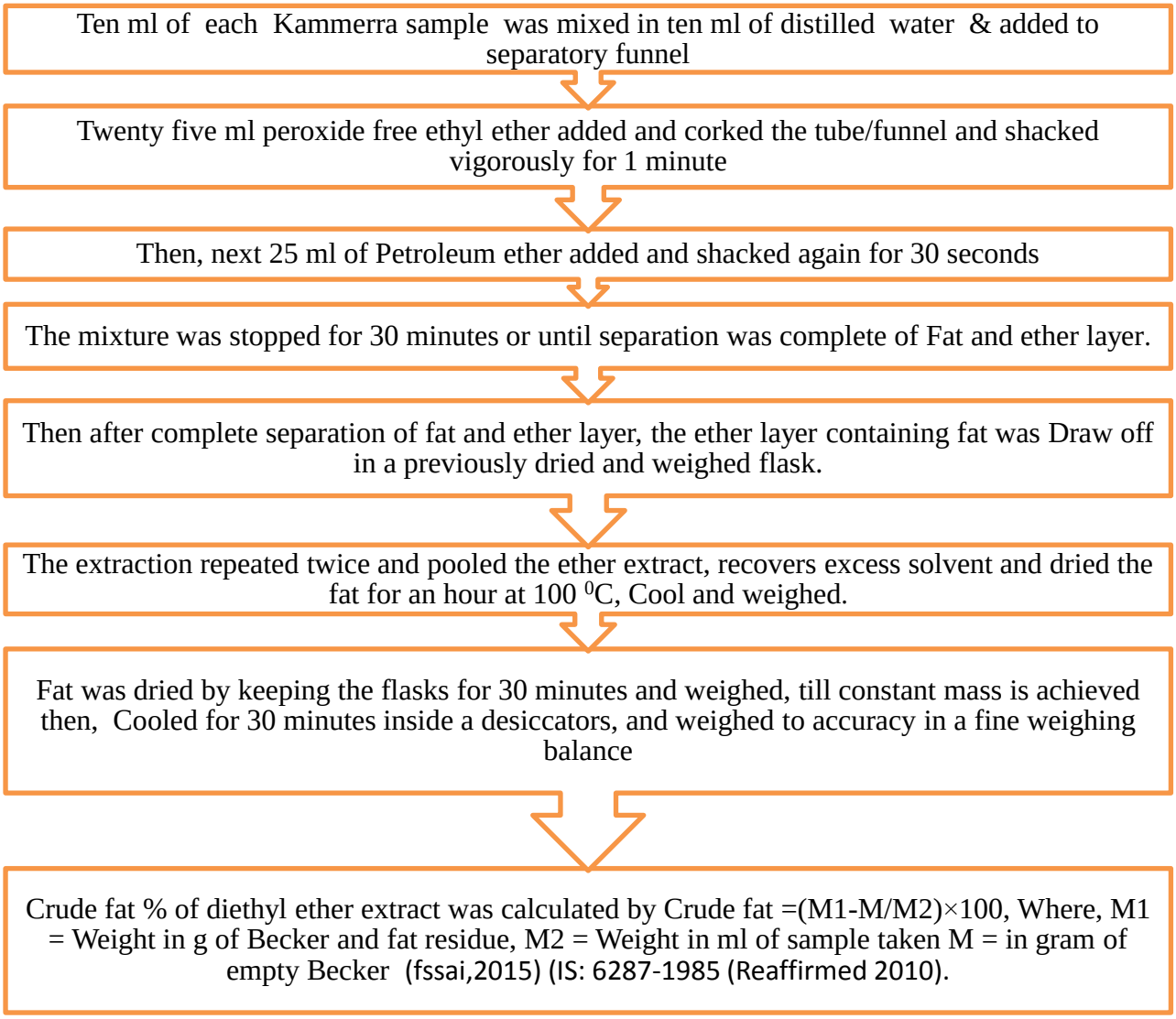

Figure 7. Flow chart of fat determined steps.

4.12.4 Carbohydrate content determination:

Total carbohydrate content was determined by subtracting the known amounts (g) of moisture, protein, fat and total ash from the total weight of the sample. It is to be noted that moisture was calculated by subtracting vehicle dry weight (g) from the wet weight of the sample (Sunano, 2017).

%Total Carbohydrate = [100 - %(MC%+ Crude Protein + Fat + Ash+ Alcohol)]

4.12 Risk assessment

The potential risk factors associated with *Kammerra* could be biological, or chemical. Accordingly, the hygienic practices, the quality of raw materials used for *Kammerra* preparation, problem related with storage and serving of the same product were assessed on site using structure questionnaire (Annex14). Additionally, microbial quality of risk factors of Kammerra was assessed by standard methods.

4.13 Data analysis

Data were analyzed using SPSS statistical software (version 20.0). Descriptive statistics were used to describe the occurrence of microbial load, physicochemical characters and nutritional values of *Kammerra* samples.

4.14 Ethical considerations

Official letter of permission was obtained from Research and Ethical Review Board of College of Natural Sciences, Jimma University. The respondents and woreda administrators were informed about the objective of the study prior to data collection and their permission (consent) were obtained before *Kammerra* sample collection and assessing the possible risk factors.

5. Results

5.1 Socio-Demographic Characteristics the study population

A total of 164 respondents, 102 (62.2%) males and 62(37.8%) females responded to the questionnaire designed to study about *Kammerra* including its production techniques, raw materials used, and handling practices among others. The respondents' age ranged between 20-55 years (mean age of 32.71±6.658). A total of 34 *Kammerra* venders, 18 female and 16 male, were engaged in Kammerra business (Table 1 & 2). Almost half (48.78%) of the *Kammerra* vendors are uneducated, married (71.95%), and majority (91.46%) are followers of protestant religion. Occupations of Kammerra venders and users are from almost all walks of life, majority of which are farmers (42.07%), merchants (24.39%), and house wives (22.56%) (Table1).

Table1.Socio-Demographic Characteristics of study Population of *Kammerra* Consumers and Venders among Konta Special Woreda Southern Ethiopia, 2018/2019.

Characters `	Alternatives	Number of respondent	Percentage (%)
Age	20-30	58	35.37
	31-40	61	37.2
	41-50	30	18.3
	51-55	15	9.15
Sex	Male	102	62.2
	Female	62	37.8
Marital status	Single	38	23.17
	Married	118	71.95
	Divorced	6	3.66
	Separated by death	2	1.22
Religion	Orthodox	14	8.54
	Protestant	150	91.46
	Muslim	-	-
Occupation	Farmers	69	42.07
	Merchants/business runner	40	24.39
	Government Employee	1	0.61
	House wife	37	22.56
	Student	-	-
	Jobless	17	10.37
Educational status	Unable to read or write	80	48.78
	Only read and write	84	51.23

5.2 Habits of Kammerra consumption in study area

Among consumers' reasons of drinking of Kammerra were to quit thirsty (69.2%), for leisure (16.2%), to get happiness (13.1%), and to get energy or power (1.5%). According to the respondents' opinion, people usually drink Kammerra infrequently (only some time) (64.6%) and the amounts used at one go is one to two bottles (42-46%) (Table2).

Table 2. Habits of Kammerra consumption in study area

Characters	Alternatives	No. of respondent	Percentage (%)
Reason of drinking of Kammerra	For thirsty	90	69.2
	Give power	2	1.5
	Make happy	17	13.1
	As leisure	21	16.2
How often did they drink Kammerra?	Usually	27	20.8
	Sometimes	84	64.6
	In market day	19	14.6
Amount of Kammerra used (bottles)	500ml	60	46.15
	1000ml	55	42.31
	1500ml	13	10
	2000ml	2	1.54
How long have you been drinking Kammerra (years)?	1-5	79	60.77
	6-10	41	31.55
	>10	10	7.7

5.3 Knowledge and practice of producing Kammerra in the study area

All the 164 people who participated in this study were familiar with Kammerra production consumption. The sources of knowledge for Kammerra production are elders in the family (52.9%) and/or relatives (47.1%). All those who produce and vend Kammerra (n=34, 100%) rely on same type of raw materials for Kammerra production: yeast, sugar, honey, barely and egg yellow powder. The common sources of water used for Kammerra production are tap water (91.2%) and spring water (8.8%), most likely related to the producers access to the type of water sources. Usually Kammerra is prepared three times a week (67.6%), as responded by the respondents, and vended at individuals home (Table 3).

Table 3. Knowledge and practice of Kammerra production in the study area

Characters	Alternatives	No. of respondent	Percentage (%)
Kammerra producers	Male	16	47.06
	Female	18	52.94
Kammerra users	Male	86	66.15
	Female	44	33.85
Familiar with Kammerra use and fermentation	Yes	164	100
	No	-	-
Sources of knowledge for Kammerra production	Training	-	-
	From family	18	52.9
	From relatives	16	47.1
Types of raw materials used to prepare Kammerra	Yeast	34	100
	Sugar	34	100
	Honey	34	100
	Barley	34	100
	Egg yellow powder	34	100
Sources of raw materials to prepare Kammerra	Shop	2	5.9
	Market	-	-
	Shop and market	32	94.1
Types of water used to prepare Kammerra	Tap water	31	91.2
	River water	-	-
	Spring water	3	8.8
Rounds of preparing Kammerra in a weak	Once	3	8.8
	Twice	8	23.5
	Three times	23	67.6
Place of Kammerra selling	Home	27	79.4
	Market	7	20.6

5.4 Hygienic practices, and materials used during Kammerra preparation and serving.

All *Kammerra* venders have good cleaning habits of serving materials and service places although they vary in frequency of cleaning as some clean it only once (32.35%), twice (41.18%) or thrice (26.47%). All *Kammerra* producers and venders have no habits of wearing overcoat, while more than half 20(58.82%) wear hair cover and only 14(41.18%) of them not. Furthermore, all producers have good habit of hand washing before and after preparation of *Kammerra* and serving even though there was limitation of nails trimming as only 22(64.71%) practice it regularly (Table4). *Kammerra* was storing as it is for 24 to 48 hours. Most of them 29(85.3%) store *Kammerra* with all other home materials and directly pitching from bucket by pitcher and dispense to bottle and vend for user while only 5(14.7%) of them use local shelves for temporary storage using pitcher, bottle and plastic Beaker (Table4).

Table 4. Hygienic practices during Kammerra preparation/vending and serving

Character	Alternatives	No. of respondent	Percentage (%)
Cleaning habits of material and selling place	Yes	34	100
	No	-	-
Times of cleaning	Once	11	32.35
	Twice	14	41.18
	Three times in a day	9	26.47
Reagents used to clean materials	Yes	31	91.18
	No	3	8.82
Types of reagent used to clean material	Water and Omo / Ajax soap	31	91.18
	Water only	3	8.82
Habits of wearing over coat	Yes	-	-
	No	34	100
Habits of wearing hair cover	Yes	20	58.82
	No	14	41.18
Habits of hand washing	Yes	34	100
	No	-	-
Nails of *Kammerra* venders short trimmed and clean	Yes	22	64.71
	No	12	35.29
Storage condition of *Kammerra*	Refrigerator	-	-
	Shelves	5	14.7
	With bucket in Homeroom	29	85.3
Materials used to sell *Kammerra*	Bottle	34	100
	Plastic Beaker	34	100
	Pitcher	34	100

5.5 Microbial Enumeration

The mean counts (log_{10}(cfu/ml ± standard deviation) of *Aerobic Mesophilic* Bacterial, *Bacillus species, Enterobacteriaceae, Lactic acid bacteria*, *Staphylococcus* spp, *Coliforms*, yeast and mold count of *Kammerra* samples were 7.06 ± 0.46, 3.61 ± 0.06, 7.16± 0.15, 7.06± 0.39, 5.02 ± 0.1, 6.08 ± 0.13, and 6.98± 1.28 respectively (Table5 & Appendix7). The ranges of counts for *Aerobic Mesophilic* Bacterial, *Bacillus species, Enterobacteriaceae, Lactic acid bacteria*, *Staphylococcus* spp, *Coliforms*, yeast and mold were 5.93 to 7.41, 2.6 to 3.73, 6.79 to 7.39, 6.79 to 7.39, 4.77 to 5.18, 5.84 to 6.33 and 6.2 to 7.31 log10 (cfu/ml), respectively (Table5& Appendix5). There was statistically significant differences between groups ($p<0.05$) (Appendix 6) in mean count of *Mesophilic* Bacterial, *Bacillus species, Enterobacteriaceae, Lactic acid bacteria*, *Staphylococcus* spp, *Coliforms* and yeast and mold in all *Kammerra* samples (Table6).

Table5. The mean count (log10 cfu/ml ±SD) of different microbial groups in fermenting Kammerra samples, Konta special woreda, South Ethiopia, 2018/2019

Log10(CFU/ml)	Microbes	N	Mean± Std. Deviation	Minimum	Maximum
	AMB	30	7.12±0.46	5.93	7.41
	B	15	3.61±0.06	3.52	3.73
	EB	30	7.16±0.15	6.79	7.39
	LAB	30	7.06±0.14	6.79	7.36
	ST	30	5.02±0.1	4.77	5.18
	COL	30	6.08±0.13	5.84	6.33
	YM	30	6.98±0.29	6.20	7.31

AMB= aerobic mesophilic bacteria, B= bacillus spp., EB=Enterobacteriaceae, LAB=lactic acid bacteria, ST= staphylococcus spp., CL= coliforms, YM=yeasts and molds

In *Kammerra* sample prepared under laboratory condition, the mean microbial counts of Yeast and Molds, *Lactic Acid* Bacteria, *Aerobic Mesophilic* Bacteria, *Enterobacteriaceae*, *Coliforms*, *Staphylococcus*, and *Bacillus spp.*, respectively, were 6.53, 6.4, 5.51, 5.33, 4.49, 4.26 and 2.74. Accordingly, Yeast and molds had the highest mean count (6.53) while the least was recorded for *Bacillus* spp.(2.74). The mean and standard deviation of microbial counts of prepared sample was 5.51±0.57, 2.74±0.42, 5.33±1.25, 4.49±0.84, 6.4±1.07, 4.26±0.81 and 6.53±0.85 respectively (Table 6).

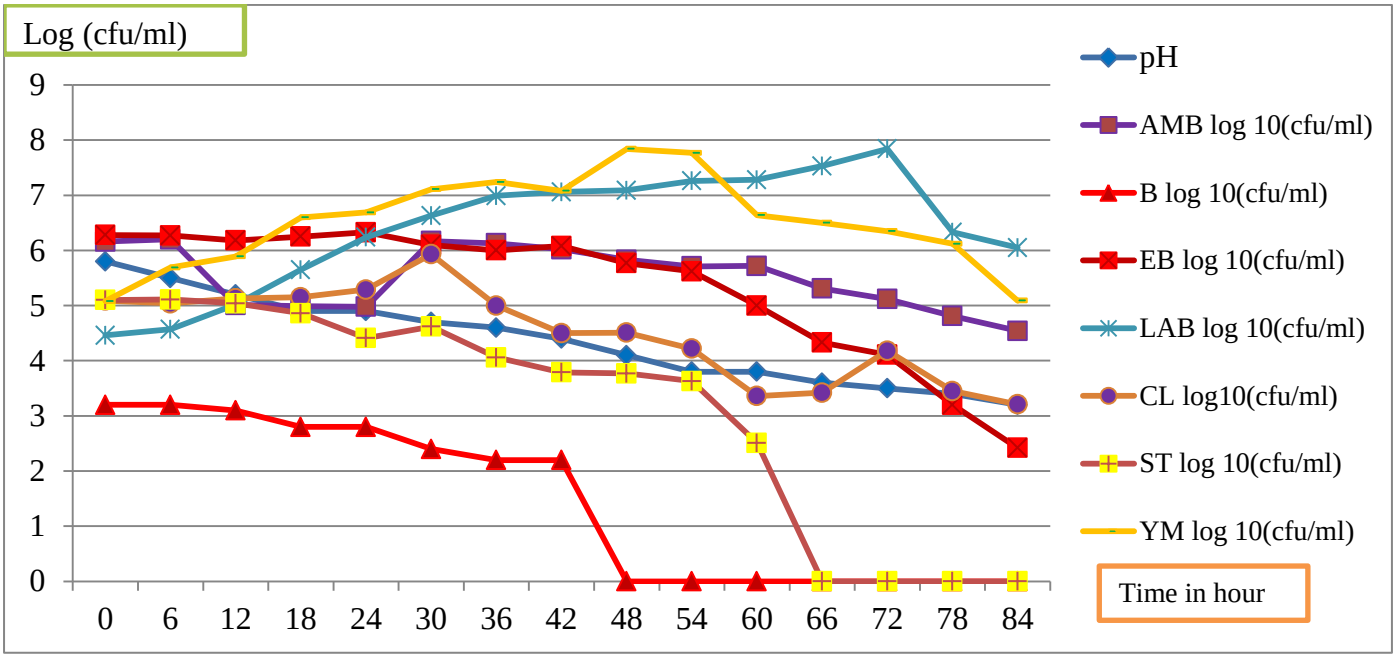

Figure 8. The count (log10 cfu/ml) of Microbial Growth under laboratory prepared Kammerra during Fermentation Time.

Where, *AMB = Aerobic mesophilic bacteria; B= Bacillus; EB= Enterobacteriaceae; COL= Coliforms; LAB= Lactic acid bacteria; ST= Staphylococci; YM= Yeast and Molds*

5.5.1. Microbial analysis

From the total of Kammerra samples analyzed, 249 bacterial isolates were characterized and grouped into more than 29 genera using standard references of Bergey's Manual of Systematic Bacteriology volume 3. Analyses of Microbial growth were by using plate count agar. Then after, one milliliter of each sampled Kammerra drink was poured in to 9ml of sterile distilled water in sterile test tubes, shaken and then serially diluted. From the appropriate dilution, 0.1ml was inoculated separately on to PCA, MRS agar, VRBG agar, MacConkey agar, Nutrient Agar and Potato Dextrose Agar plates and spread evenly using sterile bent glass rod. The inoculated microbes were incubated at 30ºC to 37ºC for 24 and 48 hours respectively and after growth morphologically, physiologically and biochemically characterized.

Table 6.The isolated microorganisms in 30 samples of *Kammerra* locally collected, from Konta special woreda, South Ethiopia, 2018/2019.

Isolates	No. of isolates	% of Isolates
Lactobacillus	25	10.04
Lactococcus	24	9.64
Staphylococci	22	8.84
Saccharomyces	20	8.03
Streptococci	15	6.02
Bacillus	13	5.22
Klebsiella	12	4.82
S.aureus	11	4.42
Enterococcus	10	4.02
Citrobacter	9	3.61
Enterobacter	9	3.61
Pseudomonas	8	3.21
Moellerella	7	2.81
Leuconostocs	7	2.81
Pediococcus	7	2.81
Escherichia	6	2.41
Salmonella	6	2.41
Paenibacillus	6	2.41
Micrococci	5	2.01
Proteus	4	1.61
Yersinia	3	1.2
Erwinia	2	0.8
Pantoea	2	0.8
Providencia	2	0.8
Serratia	2	0.8
Tatumella	2	0.8
Leminorella	1	0.4
Xenorhabdus	1	0.4
Kluyvera	1	0.4
Other Entric genera	7	2.81
Total	249	99.97

5.5.2 The growth potential of *Staphylococcus aureus, Salmonella typhimurium* and *E.coli* in Kammerra samples

The count of *Staphylococcus aureus* was >10^6cfu/ml within 12hours in all Kammerra samples tested. Counts increased by about 1.5log units first 6hours and 1.7log units within next 12hours and showed a steady growth thereafter. Count of *Salmonella typhimurium* was > 10^6 cfu/ml within 18hours in all Kammerra samples tested and showed a steady growth after that. The count of *E.coli* was > 10^6 cfu/ml within 24hour and showed a steady growth thereafter and the growth was increased by about 1log units first 6 hours and 0.9 and0.7 units next 12 and 18 hours respectively in all tested Kammerra samples (Figure9).

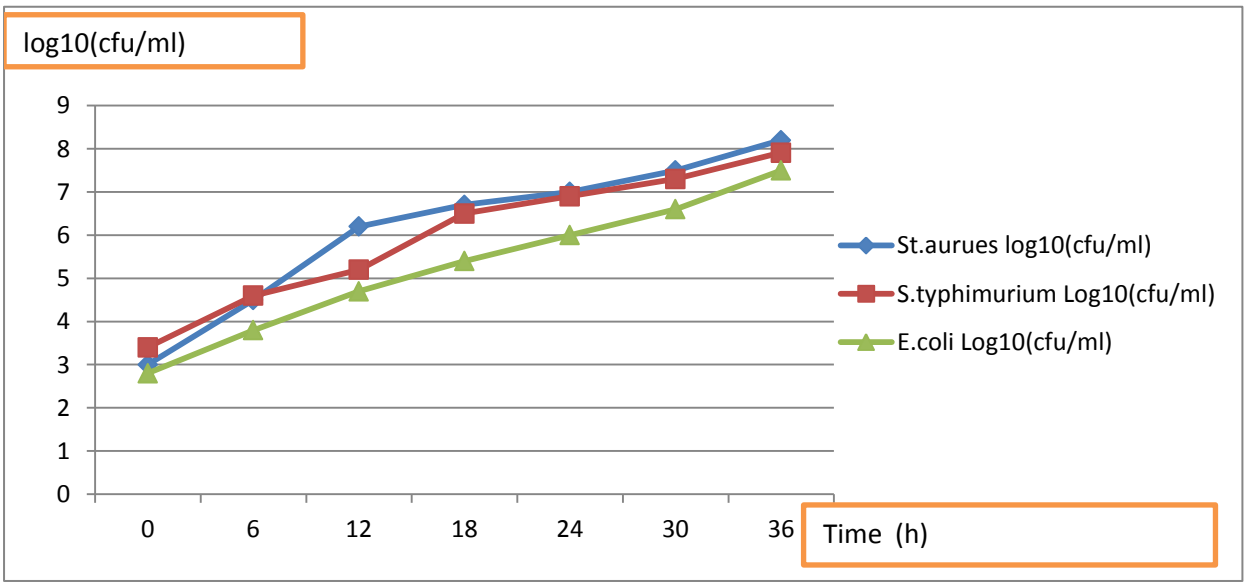

Figure 9. Count of growth potential of log cfu/ml of *Staphylococcus aureus*, *Salmonella typhimurium* and *E.coli* in fermented Kammerra, samples in Konta special woreda, 2018/2019

5.6 physicochemical characters of locally collected and prepared Kammerra samples

Determined physicochemical characters of locally collected and prepared *Kammerra* samples were Temperature, Moisture content, total solid, pH, TA, Alcohol contents. The range between physicochemical characters of locally collected *Kammerra* samples of lowest and highest, T°C, MC%, TS%, pH, TA% and alcohol content were 14&17, 94.78&98.8, 0.34&2.18, 3.3&5.5, 0.9&2.46 and 3.3 &5.5 respectively. The mean ± standard deviation of T°C, MC%, TS%, pH, TA% and alcohol content were 15.74±1.09, 96.74±1.13, 1.00±0.45, 4.29±0.58, 1.36±0.58 & 4.39±0.70 respectively (Table.9 &Appendix12).

Table 7.The mean and standard deviation of physicochemical characters of *Kammerra* samples, collected from Konta Special Woreda, South Ethiopia, 2018/2019

	MC in %	TS in %	pH	TA%	Alcohol%
Mean±Std.dev.	96.74±1.13	1±0.45	4.29±0.58	1.36±0.28	4.39±0.7
Minimum	94.78	0.34	3.3	0.9	3.1
Maximum	98.8	1.8	5.5	1.9	5.5

5.7 Nutritional Value Analysis Results

Nutritional value analysis of locally collected *Kammerra* samples and prepared under laboratory condition were the range between highest and lowest of Moisture%, Crude protein, Fat, Ash, Carbohydrate and Nitrogen respectively were 19.3&10.9, 2.41 & 0.24, 11.3 & 1.28, 0.35 & 0.01, 78.63 & 70.32, 0.39 & 0.04 and the remaining were intermediates. The highest and lowest contents were determined from samples code of CD3 & CT1, KC2 & BT1, CT1 & AM2 AM3, CR2 & KC2, AM2& MD3 and KC2 &BT1 respectively (Table13). The mean and standard deviations of each component were 15.38±1.46, 1.14± 0.62, 5.29± 2.18, 0.12± 0.07, 73.94± 2.31, and 0.18±0.09 (Table 10 & Appendix13).

Table 8. Nutritional value of mean± std.dev. (Mean ±Std. Dev) minimum, & maximum, of locally collected Kammerra samples, from Konta special woreda, south Ethiopia, 2018/2019

	MC%	Crude P%	FAT%	ASH%	CHO%	N%
Mean ±Std. Dev.	15.38±1.46	1.14± 0.62	5.02±1.93	0.12±0.07	73.94±2.31	0.18±0.09
Minimum	10.9	0.24	1.28	0.01	70.32	0.04
Maximum	19.3	2.41	8.3	0.35	78.63	0.39

Where, MC, Moisture content; P, protein; CHO, Carbohydrate; n, nitrogen

6. Discussion

Kammerra is a traditional alcoholic, whitish yellow colored fermented beverage commonly consumed in rural and urban areas of Konta special woreda, southern Ethiopia, some similarity with *Tella, Teji, Areki and Korefe* of Ethiopia (Yohannes *et al.*2013, Getent & Berhanu, 2016).

It is produced by 24 to 72 hours fermentation of yeast, barley, honey, sugar, and egg yellow powder with water. The socio-demographic characteristics of this study showed that the majority of Kammerra fermenters/ venders were females. It is similar with the Chukuezi (2010) in Nigeria reported that majority (66.67%) of the street vendors were females.

The consumption of *Kammerra* local fermented alcoholic beverages among the Konta special woreda kebeles was widespread. This local beverage has been associated with irresponsible behaviors and poor health, especially among men and women in the districts of Konta special woreda (Table1).

Socio-demographic characteristics of home venders of *Kammerra* in this study indicate that the majority of home producers of *Kammerra* were females and from the consumers males were higher than females. Total of 34, *Kammerra* producers/venders 18 female and 16 male 52.94% and 47.06% were respectively. In this study from total participated 130 consumers, 42 females and 85 males 33.07% and 66.93% were participated respectively. This indicates that the majority of *Kammerra* producers were females and the consumers were males (Table3).

From total of 164, people participated in this study were familiar with *Kammerra* consumption. Sources of knowledge for *Kammerra* production were from family and relatives 52.9% and 47.1% respectively. According to local informants, types of raw materials used to produce *Kammerra* were 100% of yeast, sugar, honey, barely and egg yellow powder and Sources of water used to produce *Kammerra* were tap water 91.2% and spring water 8.8% and also, Sources of raw materials to prepare *Kammerra* were shop only 5.9% and both shop & market were 94.1%(Table4).

High counts of *Aerobic Mesophilic* Bacteria, *Enterobacteriaceae*, Staphylococcus and *Coliform* due to production and handling contamination. The occurrence of *Enterobacteriaceae*, *coliform* and *Staphylococcus* are evidences of poor hygienic conditions of Kammerra samples. These organisms may be contaminants of unsafe water used either to dilute the ready-to-use Kammerra or wash utensils. The utensils used for preparation of Kammerra and serving are made low quality plastics and necked bottles that are difficult to be cleaned (Abawari, 2013).

Although, there are no microbiological standards set for the traditional fermented food/beverages of Ethiopia, the mean counts of *Staphylococci, Enterobacteriaceae, yeast and molds* observed in the samples of Kammerra were on the lowest margin of the standard set for fruit juices served in the gulf region, indicating the maximum count permitted for total colony count of coliforms, yeasts and molds are $1x10^{4,}$ 100 and $1x10^{3}$ cfu/ml respectively (Gulf standards, 2000). However, the means counts of *aerobic mesophilic* bacteria and *aerobic spore formers*, on the samples were 4.96log cfu/ml (with maximum count of 7.97 log cfu/ml) and 2.34log cfu/ml (with maximum count of 8.31log cfu/ml), respectively.

On the basis of gulf standards, it is clear that the colony counts of *AMB, LAB* and *bacillus spp*. in Kammerra samples greater than the standard by considerable margin. From long history of its safety, the high counts of *LAB* may not pose hazard to the health of consumers. The low mean counts of *staphylococci* also avoid the risk of enterotoxin production as toxin production among these groups is possible after the counts greater or equals 10^{6}cfu/ml (Jemes, 2000). High counts of *aerobic mesophilic bacteria* may trigger health problems provided that there are potential pathogenic strains among the strains including *E.coli* and *salmonella* species.

Microbial counts of banana beer reported by Wilson et al. (2012) were higher than microbial count of *Kammerra* samples of current study. In other hand, mean log 10 cfu/ml of Microbial counts, of *LAB, AMB, EB, Staphylococcus* spp., *coliforms*, yeast and molds reported by Nemo *et al*, (2013) and Abawari, (2013), of street vended food and fermented *Keribo* were smaller than microbial counts *Kammerra* samples of current study.

For microbial identification of other all were different biochemical tests used and the used biochemical tests were:- Gram stain, Endospore test, KOH test, Catalase test, Oxidation Fermentation test, motility test, Methyl Red (MR) test, indole test, urease test, TSIA test, SIM test, and carbohydrate fermentation test were based on the references as sited in (Barnett *et al*., 2000, Agbagwa *et al*.2017,Difco™ & BBL™ Manual, Bacha, 1997, Sawian *et al.*, 2018, Kanghae *et al*., 2016, and Fadahunsi*et al*., (2013), Wassie (2016) and Bergey's Manual of Systematic Bacteriology, John manuals of bacterial identification and Sampo *et al*.,(2012), *Lactic acid bacteria* microbiological and functional aspects were used as reference). Total of 249 microbial isolates were characterized and identified to genus level from thirty *Kammerra* samples. Morphological, physiological and biochemical characteristics of isolated genus were grouped into respective genera. The cell morphology of all isolates was evaluated through microscopic observation total of 249, 115 were found to be *cocci* and the 114 were rod and 20 were ovoid/ egg shaped.

According to (Yohannes *et al.*, 2013). Some of physicochemical characters such as: - pH value of filtered *Tella, Tej* and *Areke* reported were almost similar with *Kammerra* of present study. The average pH of *Kammerra* current study and filter *tella, tej* and *areki* from Yohannes et al., 2013 were 4.29, 4.67, 3.95 and 4.44 respectively. The pH of *Kammerra* was greater than *tej* and less than *tella* and *areki*. The ethanol level/ alcohol content of *Kammerra* samples collected and measured were high in MD3 and low of LA1. The alcohol content of *Kammerra* les than the alcohol contents of *areki* and *tej* according to (Yohannes *et al.*2013). The mean physicochemical characters of commercial Local Alcohol available beverages such as Tej, Araki, Tella, Checka and St. George Beer of pH, TA and Alcohol contents reported by Haftu (2018) were 2.85, 3.9, 3.28, 3.66 and4.02 for pH, 0.009, 0.012, 0.0102, 0.015 and 0.0108 for TA and 7.85, 24.54, 4.8, 3.66 and 4.02 for Alcohol content and the pH, TA and alcohol content of Kammerra of current study was 4.29, 1.36 and 4.39. This shows that the pH& TA of *Tej, Araki, Tella*, Checka and St. George Beer reported by Haftu (2018) were smaller than the pH& TA, of current study of Kammerra and also, the alcohol content reported by Haftu (2018) of Checka and St. George Beer were smaller than Kammerra of this study. The alcohol content reported by Haftu (2018) of Tej, Araki and Tella were greater than alcohol content of Kammerra of this study. This indicates that the physico-chemical parameters of Kammerra are under the level of consuming. The mean and standard deviation of alcoholic content of *Korefe* reported by Getnet & Berhanu, (2016), was 2.706± 0.7 and *Kammerra* of this study was 4.39±0.7, this indicates the alcohol content of *Kammerra* is higher than that of *Korefe*.

The nutritional values assessed from *Kammerra* samples were crude protein, fat, ash, nitrogen, moisture, and carbohydrate. From the nutritional value analysis of locally collected *Kammerra* samples were high of carbohydrate content and low of ash content. According to (Bhuyan *et al* 2014) biochemical and nutritional value analysis of rice beer of north India was higher than biochemical and nutritional value analysis of *Kammerra* samples of Konta special woreda, south Ethiopia. Similarly, Getnet & Berhanu, (2016), nutritional and physicochemical properties of *Korefe*, traditional, fermented alcoholic beverage reported was higher than *Kammerra* of current study except alcoholic content. Nutritional value of *Korefe* reported by, Getent & Berhanu, (2016), crude fat and ash of mean± standard deviation was7.01± 0.28 and 4.73±0.37, Nutritional value crude fat and ash of *Kammerra* of mean± standard deviation was 5.02 ± 1.93 and 0.12± 0.07. Nutritional value of crude protein and ash determined from *Korefe* were higher than nutritional value of crude protein and ash determined from *Kammerra*. This indicates that the consummation significance of *Kammerra* is less/small with comparing different investigations.

7. Conclusion and Recommendation

7.1 Conclusion

The microbiological, physico-chemical and nutritional value during a traditional preparation of *Kammerra* in Konta special woreda were evaluated in this study. The presence of high numbers of yeast and *lactic acid* bacteria shows that the natural fermentation process is a mixed alcohol and *lactic acid* fermentation.

The results reported could serve as a starting point to understand the microbiological, physico-chemical and nutritional value in *Kammerra* production with the aim of improving the efficiency of the process.

Traditional fermented *Kammerra* is produced at the household level in study area and the microbial, physico-chemical and nutritional value have not been investigated & reported before.

On the other hand, there were not significant that, from *bacteria Enterobacteriaceae, Staphylococcus aureus, Salmonella, E.coli, lactic acid bacteria* and from fungus detected one only *Saccharomyces servisiae* were associated with fermentation and the microbes isolated from *Kammerra* samples were found to be within the non permissible limits. But, it can also be concluded that lactic acid bacteria and yeast were dominant microorganisms during *Kammerra* fermentation. However, these potential pathogenic microbes, found in countable limits, from the fermenting samples could not value public health attention. Therefore, periodic showing of *Kammerra* and its producers/fermenters, not joined with education on the maintenance of recommended guidelines concerning beverage/drink production is not encouraged.

Finally, this study may serve as a basis for further studies on the process of optimization of *Kammerra* and its production. Therefore, it requires further study on the effect of producing and handling process of *Kammerra*.

7.2 Recommendation

These findings demonstrated that homemade *Kammerra* prepared for consumers at home comprises a likely potential hazard to a human health.

The isolation of *Enterobacteriaceae* in ready- to- use *Kammerra* good indicator of producing and handling contamination. Therefore, producer's discussion should be on personal hygiene, and how to improve *Kammerra* quality and safety was necessary.

The study has shown that infected *Kammerra* handlers may be at risk of developing illness themselves, and may cause a threat to the health of the consumers. For example all bacteria discussed in this study were transmitted via the direct contact with infected *Kammerra* handlers or by cross-contamination to healthy consumers. Thus *Kammerra* producers/ fermenters should have to improve *Kammerra* processing/ producing and storing practice.

Health sectors, municipality and Nongovernmental organizations should work together to improve the sanitary facilities of the community and in turn protect the health of consumers to reduce the public and economic burden caused due to foodboren diseases.

Kammerra producers/venders, those having direct contact with *Kammerra* need to have periodic medical examinations including stool test for enteric pathogens at least twice a year to prevent the problems they might face and avoid transmission of these pathogens through contaminated *Kammerra*.

It is strongly recommend, to the Konta special woreda head office of health, primary hospital, health keeping centers, head office of administrative and all stakeholders it must be necessary to aware people those who produces *Kammerra* of the woreda in ways of processing and handling practice.

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9. List of Appendices

Appendix 1.Kammerra producers/ venders Descriptive Statistics and one way ANOVA

	N	Mean	Std. Dev.	Variance	Minimum	Maximum
Age1	34	32.71	6.658	44.335	20	45
Age2	130	33.68	8.810	77.616	20	55
How much liter do you sells in a day	34	67.38	46.027	2118.486	10	250
How many customers did you have	34	77.06	61.785	3817.390	15	220

Appendix 2. Kammerra producers/ venders one way ANOVA

		Sum of squares	df	Mean square	F	Sig
Age1	Between Groups	420.844	1	420.844	12.922	.001
	Within Groups	1042.215	32	32.569		
	Total	1463.059	33			
Age2	Between Groups	690.638	1	690.638	9.483	.003
	Within Groups	9321.793	128	72.827		
	Total	10012.431	129			
How much liter did you sells in a day	Between Groups	1108.092	1	1108.092	.515	.478
	Within Groups	68801.938	32	2150.061		
	Total	69910.029	33			
How many customers did you have?	Between Groups	3374.132	1	3374.132	.881	.355
	Within Groups	122599.750	32	3831.242		
	Total	125973.882	33			

Appendix 3. Bacterial identification

Gram stain	+	+	+	+	+	-	+	-
Shape	Rod	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Rod
Motility	-	-	-	-	-	-	-	+
Endospore	-	-	-	-	-	-	-	-
Catalase	-	-	-	-	-	-	+	+
MR							-	-
VP							-	+
Citrate	+	+					-	+
Indole	-	-					-	-
G/F. of acid or to a +g	+/-	++	-			++	-	+
O/F	+	-	+/-			+	-	-
Urease	-	-	-	-	-	-	+	-
Fermentation of acid								
Lactose	+	+	+				-	-
Sucrose	+	+	+				-	-
Mannitol							-	-
Growth Temp.								
10°C	+/-	+	+	+	-	+/-		
45°C	+/-	+	-	-	+/-	+/-		
Growth NaCl								
5%	+/-	+	+/-	+/-	-	+/-		
6.5%	+/-	+	+/-	+/-	-	+/-		
7%	-	-	-	-	-	-	-	-
Growth at pH								
4.4	+/-	+	+/-	+/-	-	+		
9.6	-	+	-	-	-	-		
Lactobacillus	X							
Enterococcus		X						
Lactococcus			X					
Leuconostocs				X				
Streptococcus					X			
Pediococcus						X		
Micrococcus							X	
Pseudomonas								X

Note: + = positive - = negative, ± = some of them positive and varies between isolates.

Appendix 4. Bacterial identification

Gram stain	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Aerobic growth	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Anaerobic growth	+			+	±	±	F	-	+	±	±	±	±	F	F	F	+		+
Shape	C	R	R	C	R	R	R	R	R	R	R	R	R	R	R	CB	R	R	R
Motility	+	-	±	-	+	+	-		+	-	-	+	-	-	+	-	-	+	+
Endospore	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
MR	-	-	+	+	+	+	+	+	±	+	+	-	+	+	-	+	-	+	±
VP	+	+	-	-	-	+	-	-	±	-	-	+	-	-	+	-	-	-	±
Citrate	+	+	+	+	+	+	+	-	±	-	+	+	+	+	+	-	-	+	±
Indole	-	-	+/-		-	-	-	+	±	-	+	-	+	-	-	-	-	+	±
TSIA	A^+K^-	A^+K^-	A^- K^+G^-		$A^-K^+G^-$										A^+K^- G^- H_2	A^+K^+ G^-H_2S	$A^-K^+G^-$	A^-K^+ G^-	
Urease	±	±	+	±	+	-	-												
Acid production																			
D-glucose	+	+	++	+	+	+									+	++	++	+-	
Maltose	+	+	+	+	+	-									+	+-	+-	+-	
Lactose	+	+	+	+	+	-									+	+-	+-	+-	
Sucrose	+	+	+	+	+	+									+	+-	+-	+-	
Mannitol	+	+	+	+	-	+									-	+-	+-	+-	
Enterobacter	X																		
Klebsiella		X																	
Citrobacter			X																
Staphylococcus				X															
Proteus					X														
Bacillus						X													
Paenibacillus							X												
Escherichia								X											
Salmonella									X										
Moellerella										X									
pantoea											X								
Erwinia												X							
Providencia													X						
Leminorella														X					
Serratia															X				
Yersinia																X			
Xenorhabdus																	X		
Kluyvera																		X	
Other enteric genera																			X

R= Rod shape, C = *Coccus*, CB = *Coccobacillus* shape

Appendix 5. Mean microbial count, Descriptive statistics for different microbial groups isolated from Kammerra samples, in Konta special woreda, south Ethiopia, 2018 /2019

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
CFU/ml	AMB	30	1.61E+07	6.90E+06	1.26E+06	1.35E+07	1.86E+07	8.47E+05	2.56E+07
	B	15	3.87E+03	1.13E+03	2.93E+02	3.24E+03	4.50E+03	4.00E+02	5.35E+03
	EB	30	1.52E+07	4.62E+06	8.44E+05	1.35E+07	1.69E+07	6.15E+06	2.48E+07
	LAB	30	1.21E+07	3.73E+06	6.81E+05	1.07E+07	1.35E+07	6.10E+06	2.28E+07
	ST	30	1.31E+05	1.43E+05	2.60E+04	7.80E+04	1.84E+05	1.23E+04	8.65E+05
	COL	30	1.22E+06	4.31E+05	7.86E+04	1.06E+06	1.38E+06	1.31E+05	2.13E+06
	YM	30	1.18E+07	5.47E+06	9.99E+05	9.75E+06	1.38E+07	1.59E+06	2.05E+07
	Total	195	8.69E+06	7.80E+06	5.59E+05	7.58E+06	9.79E+06	4.00E+02	2.56E+07
Log	AMB	30	7.0599	.45649	.08334	6.8894	7.2303	5.93	7.41
(CFU/ml)	B	15	3.6075	.06257	.01616	3.5729	3.6422	3.52	3.73
	EB	30	7.1580	.14656	.02676	7.1033	7.2127	6.79	7.39
	LAB	30	7.0617	.13834	.02526	7.0100	7.1133	6.79	7.36
	ST	30	5.0157	.10311	.01883	4.9772	5.0542	4.77	5.18
	COL	30	6.0820	.12963	.02367	6.0336	6.1304	5.84	6.33
	YM	30	6.9767	.28664	.05233	6.8696	7.0837	6.20	7.31
	Total	195	6.3319	1.11331	.07973	6.1747	6.4892	3.52	7.41

Appendix 6. One way ANOVA result of different microbial groups isolated from Kammerra samples, in Konta special woreda, south Ethiopia, 2018 /2019

		Sum of Squares	df	Mean Square	F	Sig.
CFU-ml	Between Groups	8.53E+15	6	1.42E+15	81.523	.000
	Within Groups	3.28E+15	188	1.74E+13		
	Total	1.18E+16	194			
Log_CFUml	Between Groups	230.000	6	38.333	689.358	.000
	Within Groups	10.454	188	.056		
	Total	240.454	194			

Appendix 7. Mean microbial count, standard deviation and Variance for different microbial groups isolated from Kammerra samples, in Konta special woreda, south Ethiopia, 2018 /2019

Types of Bacteria		Cfu/ml	Log 10 (cfu/ml)
AMB	Mean	16050900	7.0599
	N	30	30
	Std. Deviation	6898298.853	.45649
	Variance	4.75865E+13	.208
B	Mean	3870	3.6075
	N	15	15
	Std. Deviation	1134.806466	.06257
	Variance	1287785.714	.004
EB	Mean	15186666.67	7.1580
	N	30	30
	Std. Deviation	4623868.61	.14656
	Variance	2.13802E+13	.021
LAB	Mean	12068666.67	7.0617
	N	30	30
	Std. Deviation	3727470.931	.13834
	Variance	1.3894E+13	.019
ST	Mean	131210	5.0157
	N	30	30
	Std. Deviation	142581.9023	.10311
	Variance	20329598862	.011
COL	Mean	1220700	6.0820
	N	30	30
	Std. Deviation	430741.3937	.12963
	Variance	1.85538E+11	.017
YM	Mean	11797833.33	6.9767
	N	30	30
	Std. Deviation	5472166.513	.28664
	Variance	2.99446E+13	.082
Total	Mean	8685832.564	6.3319
	N	195	195
	Std. Deviation	7800429.69	1.11331
	Variance	6.08467E+13	1.239

Appendix 8. The isolated microorganisms in 30 samples of *Kammerra* locally collected, from Konta special woreda, South Ethiopia, 2018/2019.

Contaminated Samples Code	Isolates	No. of isolates	% of Isolates
AM1,BT1,CT1,KK1, AM2, CR2,CT2,KB2, MD2, KC2, GG2, CD3, AM23, CR3, GG3,	*Lactobacillus*	25	10.04
CD1,AM1,GG1,KB2, CD2,MD2, AM3, KK3, CR3,	*Lactococcs*	24	9.64
CR1,KK1,CD1,BT1, KC2,KB2,MD3,GG3, AM3	*Staphylococci*	22	8.84
AM1,GG1.KK1,CR1,CT2, GG2, MD2,CD2, KC2,BT2, KB3, KC3, KK3, GG3	*Saccharomyces*	20	8.03
CR1,KK1,CT1,AM2,GG2, MD2, CD3, CR3	*Streptococci*	15	6.02
CD1,AM1,GG1,KB2, CR2, CD2,MD2, AM3, KK3, CR3,	*Bacillus*	13	5.22
KK1,CR1,CT1, AM2,CD2,Bt2, KK3, CR3, GG3, MD3	*Klebsiella*	12	4.82
AM1,BT1, CD1, MD1, GG1, CR2,CT2, KK2, KB2,KC2, AM3, GG3, MD3, CR3,CT3	*S.aureus*	11	4.42
CD1,AM1,GG1,KB2, CD2,MD2, AM3, KK3, CR3,	*Enterococcus*	10	4.02
KK1, CR1, CT2, AM2,GG2, MD2,	*Citrobacter*	9	3.61
AM1,GG1.KK1,CR1,CT2, GG2, MD2,CD2, KC2,BT2, KB3, KC3, KK3, GG3	*Enterobacter*	9	3.61
GG1, AM1, MD1, CR1, CT1, BT2, KC2	*Pseudomonas*	8	3.21
KC1, KK1,CD2,KC2,BT3,CD3	*Moellerella*	7	2.81
MD1,GG1,KC1, BT3, CD3, MD3	*Leuconostocs*	7	2.81
GG1,KC1, CT2,KB2, MD2, CR3, GG3,	*Pediococcus*	7	2.81
AM1, CR1, KK2, GG2, MD3,CD3	*Escherichia*	6	2.41
AM1, GG1, MD1, CD2, BT2, KB2, KC2, CR3	*Salmonella*	6	2.41
CD1,AM1, GG1, AM2, CR2,CT2, KK3, CR3	*Paenibacillus*	6	2.41
AM1, KK1,KC1, AM2	*Micrococci*	5	2.01
CR1, CT1, KK1, AM1, GG2, MD2, CD2, KB2, BT3, KC3	*Proteus*	4	1.61
MD1,KB2,KB3	*Yersinia*	3	1.2
BT1, KB3	*Erwinia*	2	0.8
GG2,AM3	*Pantoea*	2	0.8
CR2, CT2	*Providencia*	2	0.8
GG3, MD3	*Serratia*	2	0.8
CT1,CR3	*Tatumella*	2	0.8
CT3	*Leminorella*	1	0.4
KK2	*Xenorhabdus*	1	0.4
MD2	*Kluyvera*	1	0.4
CD1, MD1, AM2, CR2,CT2,KB2, KK3, CR3, GG3	*Other Entric genera*	7	2.81
Total		249	99.97

Appendix 9. Physicochemical characters of *Kammerra* samples, collected from Konta Special Woreda, South Ethiopia, 2018/2019

Code	**T °C at 23°C**	**MC in %**	**TS in %**	**pH**	**TA%**	**Alcohol%**
AM 1	16.5	96.64	1.5	3.8	1.71	4.2
AM 2	16	97	1.8	4.6	1.26	3.8
AM 3	15.8	98.6	1.55	4.1	1.35	4
BT1	14	95.39	1.6	4.2	1.44	3.4
BT2	14	98.8	1.1	4.5	1.26	4
BT3	15	96	1.55	3.6	1.8	3.8
CD1	16	96.5	0.34	4.5	1.35	5
CD2	14	97.4	0.54	3.43	1.89	4.8
CD3	15	98	0.44	4.15	1.71	5
CR1	16	96.66	1.55	5.13	1.08	4.85
CR2	15	97	1.38	4.2	1.53	4.8
CR3	15	98	1.37	5.4	0.99	4
CT1	16	96	0.85	4	1.35	4.6
CT2	16	98	0.75	3.8	1.62	4.2
CT3	16	96	0.95	4.3	1.35	4.4
GG1	17	95.98	0.5	4	1.35	3.4
GG2	17	97.8	0.45	4.2	1.26	3.6
GG3	16.5	97.8	0.5	4	1.35	4.3
KB1	14	94.78	0.9	5	1.17	4.8
KB2	14	96.2	1.34	4.8	1.17	4.6
KB3	15	97	1.42	5.5	0.9	4.8
KC1	17	96	0.75	5.1	1.07	4.3
KC2	17	95	0.95	4	1.35	3.4
KC3	17	96	1.25	4.6	1.27	3.2
KK1	17	94.99	0.35	5.23	1.44	5.4
KK2	17	98.4	0.56	3.6	1.22	5.2
KK3	17	96	0.56	4.2	1.89	5.5
MD1	16	95.5	0.6	3.8	1.9	5.3
MD2	16	98	1.35	4.3	1.2	5.1
MD3	16	96	0.8	3.7	1.02	5.5
LA1	14	97.5	1.5	3.3	0.9	3.1
Min.	14	94.78	0.34	3.3	0.9	3.1
Max.	17	98.8	1.8	5.5	1.9	5.5
Mean	15.74	96.74	1.00	4.29	1.36	4.39
Std. Dev.	1.09	1.13	0.45	0.58	0.28	0.7

Appendix 10. Nutritional value of locally collected Kammerra samples, from Konta special woreda, south Ethiopia, 2018/2019

Code of S	MC%	Crude P%	FAT%	ASH%	CHO%	N%
AM1	15.5	2.26	4.7	0.07	73.27	0.37
AM2	15.9	0.32	1.28	0.07	78.63	0.05
AM3	15.9	0.32	1.28	0.07	78.43	0.05
BT1	15.4	0.24	3.29	0.09	77.58	0.04
BT2	15.3	0.57	4.03	0.06	76.04	0.09
BT3	14.7	1.95	6.12	0.18	73.25	0.31
CD1	14	0.69	2.44	0.11	77.76	0.11
CD2	15	1.31	6.37	0.11	72.41	0.21
CD3	19.3	1.54	2.8	0.1	71.26	0.25
CR1	16.3	0.88	5.3	0.08	72.59	0.14
CR2	16.7	0.61	5.99	0.35	71.55	0.1
CR3	16.2	1.45	1.29	0.06	77	0.23
CT1	10.9	1.14	8.3	0.23	74.83	0.18
CT2	14.7	2.11	6.09	0.06	72.84	0.34
CT3	14.6	1.87	6.57	0.06	72.5	0.3
GG1	16.4	1.54	6.35	0.13	72.18	0.25
GG2	14.3	1.49	7.87	0.11	72.63	0.24
GG3	18.2	0.42	4.09	0.25	72.74	0.07
KB1	14.6	1.41	3.72	0.16	75.31	0.23
KB2	14.7	1.41	5.34	0.05	73.9	0.26
KB3	15.6	1.37	5.19	0.18	72.86	0.22
KC1	14.3	0.82	4.91	0.13	75.54	0.13
KC2	15.6	2.41	6.8	0.09	71.7	0.39
KC3	15.8	0.26	6.24	0.01	74.49	0.04
KK1	16.3	1.31	4.9	0.2	71.89	0.21
KK2	14.1	0.94	7	0.14	72.62	0.15
KK3	15.7	0.69	5.32	0.12	72.67	0.11
MD1	13.9	1.56	4.98	0.08	74.18	0.25
MD2	16.51	0.5	6	0.07	71.82	0.08
MD3	16.33	0.56	7.1	0.19	70.32	0.09
LA1	14.1	1.31	3.89	0.15	77.45	0.21
Minimum	10.9	0.24	1.28	0.01	70.32	0.04
Maximum	19.3	2.41	8.3	0.35	78.63	0.39
Mean	15.38	1.14	5.02	0.12	73.94	0.18
Std. Dev.	1.46	0.62	1.93	0.07	2.31	0.09

Appendix 11. Mean counts (log cfu/ml) of different microbial groups in fermenting Kammerra samples, Konta special woreda, South Ethiopia, 2018/2019

Fermentation period (hrs)	Microbial groups and their counts				
	AMB	Staphylococci	LAB	Enterobacteriaceae	Yeasts and molds
24	7.06	5.02	7.06	7.16	6.97
48	6.44	4.6	7.23	5.11	.7.16
72	6.23	4.22	6.5	4.44	7.1
96	5.34	3.11	6.33	3.2	6.33
120	4.6	-	5.43	-	5.2
144	3.25	-	5.2	-	4.51
Mean	5.49	4.24	6.29	4.98	6.022

Appendix 12. Mean counts (log cfu/ml) of spoilage potentials of different microbial groups in fermenting Kammerra samples, Konta special woreda, South Ethiopia, 2018/2019

Fermentation period (hrs	Microbial groups and their counts				
	AMB	Staphylococci	LAB	Enterobacteriaceae	Yeasts and molds
48	5.2	3.4	6.52	5.55	5.58
72	4.7	3.11	6.44	5.12	5.31
96	3.11	2.6	6.23	4.32	4.52
120	2.5	-	6.1	-	
144	-	-	5.86	-	
Mean	3.88	3.04	6.23	4.99	5.14

Appendix 13. Frequency of isolation (%) of dominant microbial strains in fermenting Kammerra samples, Konta special woreda, South Ethiopia, 2018/2019

Fermentation period (hrs)	**Microbial groups and their counts**					
	Bacillus spp(N=19)	S. aureus(N=11)	Lactobacillus spp.(N=25)	Pediococcus spp(N=7)	Saccharomyces spp.(N=20)	Molds and yeasts(N=9)
24	11(57.89%)	5(45.45%)	2(8%)	1(14.29%)	-	4(44.44%)
48	3(15.79%)	3(27.27%)	3(12%)	1(14.29%)	2(10%)	2(22.22%)
72	3(15.79%)	2(18.18%)	5(20%)	2(28.57%)	3(15%)	2(22.22%)
96	1(5.26%)	1(9.09%)	6(24%)	3(42.86%)	5(25)	1(11.11%)
120	1(5.26%)	-	7(28%)	-	5(25)	-
144	-	-	2(8%)	-	5(25%)	-
Mean	3.8	2.75	4.17	1.75	4	2.25

Note: - the genera of bacteria and yeast given above are examples and the result depends on observation

Appendix-14.

JIMMA UNIVERSITY

SCHOOL OF POST GRADUATED STUDIES

COLLEGE OF NATURAL SCIENCES

DEPARTMENT OF BIOLOGY

Questionnaires

Dear Respondents,

The objective of this questionnaire is to collect relevant information related to *Kammerra* fermentation in order to document the microbial and physicochemical characteristics of locally fermented alcoholic beverage/*Kammerra*/ in Konta special woreda, Southern Ethiopia. The accuracy and honesty of the information you provide will be determine the relevance of data generated and significances of decision to be made on the collected data.

Any information obtained from respondents will be kept confidential and used only for scientific purpose and improvement of *Kammerra* production and safety. So, you are selected as a respondent in this survey just due to chance and have nothing to do with your personal identity. You are kindly requested to answer every question. You have the right not to respond to this questionnaire although you participation is highly appreciated.

Thank you in advance!

Part- I: Personal information

Instruction: Choose from among the alternatives for choice cases and underline the letter of your choice or respond to open ended questionnaire by write on the space provided.

1. Sex – a) Male b) Female

2. Age in years ----------

3. What is your level of educations? A) Illiterate B) literate C) Primary D) secondary E) if others, specify.....

4. Marital status a) single b) married c) divorced d) widow e) separated by death f) if others, specify:.....

5. Are you familiar with *Kammerra* and *Kammerra* fermentation? A) Yes b) No

6. Have you knowledge for Kammerra a) yes b) no

7. If you have knowledge of Kammerra from where did you get a) training b) family c) relatives d) I don't know?

8. When did you started to prepare Kammerra? ----------

9. What types of ingredient did you use to prepare Kammerra? ----------

a) Yeast b) sugar c) honey d) barley e) egg yellow powder f) all

10. From where did you buy raw materials to prepare Kammerra? A) Shop B) Market C) Shop and Market. If other specify ……

11. How much liter do you sell in a day? ----------

12. How many times do you prepare Kammerra in a weak? A) 1 B) 2 C) 3

13. Where do you sell Kammerra? A) In my home B) in the market

14. How many customers did you have? ----------

15. Did you know how many birr do you sell in a day? A) Yes B) no

16. Did you know daily profit? A) Yes B) no

17. Where did you store? A) refrigerator b) with bucket in floor c) shelves

18. Did you use bottle to sell Kammerra? A) Yes B) no

19. Did you use Becker to sell Kammerra? A) Yes B) no

20. Did you clean material and selling place? A) Yes B) no

21. How many times did you clean? A) 1 B) 2 C) 3 d) if other specify

22. Did you use reagents to clean materials and place? A) Yes B) no

23. What reagent do you use to clean material? A) Water and omo B) only water C) water soap D) If others specify.....

24. Did you have vending and selling place? A) Yes B) no

25. Have you wearing coat? A) Yes B) no

Date…….....

Part-II Personal information

Instruction: Choose from among the alternatives for choice cases and underline the letter of your choice or respond to open ended questionnaire by write on the space provided.

1. Sex – a) Male b) Female

2. Age in years ----------

3. What is your level of educations? A) Illiterate B) literate C) Primary D) secondary E) if others, specify.....

4. Marital status a) married b) single c) divorced d) widow e) if others, specify:.....

5. What is your religion a) Orthodox b) Protestant c) Muslim?

6. What is your job? A) Farmer B) merchant C) Government Employee D) Student E) House Wife F) Jobless.

7. Are you drinking *Kammerra*? a) Yes b) No

8. How did you started to drink Kammerra? A) By seen other people drink as a good drink b) after accept of protestant religion C) my relatives drink it and I have started with them

9. Why did you drink Kammerra? A) For thirsty B) give power C) it make me happy d) as the leisure

10. How long have you been drinking Kammerra (years)? .....

11. How often did you drink Kammerra? A) Usually B) sometimes C) in market day

12. From where do you drink *Kammerra*? .....

13. How many bottles did you drink in a day? A) One B) Two C) Three D) If Others, Specify:

14. Did you know your expense of Kammerra? A) Yes B) no

15. Did you know your expense of Kammerra in a day? A) Yes B) no

16. Did you know the expense you pay to the Kammerra sellers in a month? A) Yes B) no

ቀን----------

በጅማዩኒቨሪስቲድሬምረቃት/ቤትየተፍሮሣይንስትምህርትኮሌጅስነ-ህይወትትምህርትክፍል፡፡

<u>መጠየቂያዎች</u>

ውድተሳታፊዎች፡-
የዚህመመረቂያጽሑፍዋናዓላማበወረዳችንለመጠዋአገልግሎትበመዋልላይየሚገኘውንየካሜራመ ጠዋእውነተኛውንናትክክለኛውንመረጃበማሰባሰብናበማደራጀትየመጠጡንየተፈጥሮእናበሰውሰራ ሽነት/
በአሰራርናአዘገጃጀትጉድለትልመጡየሚችሉጥቃቅንተዋስያን፣ፕዝካላዊናኬሚካላዊባህርያትንለ ማጥናትናመረጃውንበማጠናቀርለትምህርታዊአገልግሎትለማዋልይሆናል፡፡በመሆኑምከተሳታፊ ዎችየሚሰጠውማንኛውምመረጃበምስጥራዊነትየሚያዝናለምርምረአገልግሎትብቻየሚውልይሆና ል፡፡ስለሆነምእርሶእንደፅድልሆኖለዚህመጠይቅየተመረጡበመሆኑበማንኛውምመንገድእርሶየሚሰ ጡመረጃበምስጥርየሚያዝስለሆነከየትኛውምጥረጣሬነጻበመሆንመረጃውንእንድሰጡናአልሰጥምየ ማለትምመብትመኖሮንበትህትናእንገልፃለን፡፡ፈቃደኛበመሆኖምበቅድሚያእናመሰግናለን፡፡
ክፍልአንድግለሰባዊመረጃ
ትዕዛዝ፡-
ከዚህቀጥሎከተሠጡትምርጫዎችውስጥትክክለኛውንመልስበመምረጥበስሩበማስመርየሚመለስሲ ሆንክፍትቦታለተሠጣቸውጥያቄዎችበክፍትቦታዎችመልሱንበጹሑፍግለጹ፡፡

1. **ዕታህ) ወንድለ) ሴት**

2. **ዕድሜ----------ዓመት**

3. **የትምህርትደረጃሀ) የተማረለ) ያልተማረሐ) የመጀመሪያደረጃመ) ሁለተኛደረጃሠ) ለላካለጥቀስ.....**

4. **የጋብቻሁኔታህ) ያገባለ) ያላገባሐ) የፈታ/የፈታችመ)በሞትየተለየሠ) ለላካለጥቀስ.....**

5. **ካሜራትጠላለክ/ኳያለሽ? ሀ) አዎለ) አላጠላም**

6. **ስለካሜራዕውቀትአለክ/ሽ? ሀ) አዎአለኝለ) የለኝም**

6. **ስለካሜራዕውቀትካለክ/ሽ? ካለከየትነውያገኘሄው/ሽው? ሀ) በስልጠናለ) ከቤተሰብሐ) ከጓደኛመ) አላቅም**
7. **ስራክ/ሽምንድንነው? ሀ) አርሶአደርለ) ነጋዴሐ) መንግስትተቀጣሪመ) ተማሪሠ) የቤትእመትረ) ስራአጥ**
8. **ካሜራመስራትከጀመርክ/ሽምንያህልጊዜሆነክ/ሽ? .....**
9. **ምንዓይነትጥሬዕቃትጠቀማለክ/ሽ? .....**
9.1 **ሀ) ካሜራለ) ሱካርሐ) ማርመ) ገብስሠ) ቀለምረ) ውሃሸ) ሁሉም**

10. **ጥሬዕቃየሚትገዛውከየትነው?ሀ) ከገበያለ) ከሱቅሐ)ሀናለመ) ለላካለግለጽ**
11. **በቀንምንያህልሊትርትሸጣለክ/ኪያ?**
12.**በሣምንትስንትግዜካሜራይዘጋጃልሀ**) 1 **ለ**)2 **ሐ**)3 **መ**)4
13. **ካሜራየሚትሸጠው/ሸኖውየትነው? ሀ) በቤቴውስጥለ) በገበያውስጥሐ) በሁለቱም**
14. **ምንያህልካሜራተጠቃምደንበኞችአለክ/ሽ?** ……
15. **ካሜራለመሸጥምንዓይነትቁሳቁስትጠቀማለክ/ሽ?ሀ) ጠርሙስለ) ብከርሐ) ብርኖውቆመ) ሁሉም**
16. **መሸጫቁሳቁስናመሸጫቦታታፀደለክ/ጃለሽ? ሀ) አዎለ) አላፀዳም**
17. **በቀንምንያህልግዜታፀዳለክ/ጃለሽ? ሀ**)1 **ለ**)2 **ሐ**)3 **መ) ለላካለ**……

18. **ለፀዳትማፀጃቁሳቁስትጠቀማለክ/ያለሽ? ሀ) አዎለ) አልጠቀምም**

19. **ለፀዳትምንምንማፀጃቁሳቁስትጠቀማለክ/ያለሽ? ሀ) ውሃናኦሞለ) ውሃብቻሐ) ውሃከሳሙናመ) ለላካለ**…….
20. **ለካሜራመሻጫናመስሪያቦታአለክ/ሽ? ሀ) አዎአለኝለ) የለኝም**

21. **ካሜራለመስራትየሚትለብሰውዩኒፎርም/ የራሱልብስአለክ/ሽ? ሀ) አዎለ) የለኝም**

22. **ካሜራበሚትሠራበትጊዜፀጉርመሸፈኛአለክ/ሽ? ሀ) አዎለ) የለኝም**

ቀን----------

ክፍልሁለትግለሰባዊመረጃ

ትዕዛዝ፡-
ከዚህቀጥሎከተሠጡትምርጫዎችውስጥትክክለኛውንመልስበመምረጥበስሩበማስመርየሚመለስሲ ሆንክፍትቦታለተሠጣቸውጥያቄዎችበክፍትቦታዎችመልሱንበጹሑፍግለጹ፡፡

1. ዖታህ) ወንድለ) ሴት

2. ዕድሜ----------ዓመት

3. የትምህርትደረጃህ) የተማረለ) ያልተማረሐ) የመጀመሪያደረጃመ) ሁለተኛደረጃሠ) ለላካለጥቀስ.....

4. የጋብቻሁኔታህ) ያገባለ) ያላገባሐ) የፈታ/የፈታችመ)በሞትየተለየሠ) ለላካለጥቀስ.....፡፡

5. ስራምንድንነው? ህ) አርሶአደርለ) ነጋዴሐ) መንግስትሠራተኛመ) ተማሪሠ) የቤትእመበትረ) ስራአጥ

6. ካሜራትጠላለክ/ጪያሽ? ህ) አዎለ) አልጠላም

7.ካሜራመጠጣትየጀመርከውእንዴትህ)ለሎችሰዎችስጠጡአይቸጥሩነውስለለ) ፕሮተስታንትከሆንኩበኋላ ሐ) ጓደኞቼስለምጠጡከነሡጋርነውየጀመርኩት

8. ካሜራለምንትጠላለክ/ጪያለሽ? ህ) ለጥምለ) ኃይልስለሚሰጥሐ) ደስተኛያደርገኛልመ) ሰዎችጥሩመጠጥነውስለሚሉሠ) ለመዝነኛነት

9. ካሜራየሚትጠጣከሆነከጀመርክምንያህልጊዜ/ዓመትሆነ?.....፡፡

10. በምንሰዓት/ መችመችነውካሜራየሚትጠጣዉ/ጭው? ህ)ዘዎትርለ) አልፎአልፎሐ) በገበያቀንመ) ለላካለጥቀስ.....፡፡

11. ካሜራየሚትጠጣውከየትነው? ……

12. በቀንምንያህልጠርሙስትጠላለክ/ሽ? ህ) 1 ለ) 2 ሐ) 3 መ) ለላካለ……

13. ለካሜራየሚታወጣውንወጪታውለክ/ሽ? ህ) አዎለ) አላቅም

14. ለካሜራበቀንየሚታወጣውንወጪታውቃለክ/ቂያለሽ? ህ)አዎለ)አላቅም

15. ለካሜራበወርየሚታወጣውንወጪታውቃለክ/ቂያለሽ? ህ)አዎለ)አላቅም