EXTRACTION OF VINEGAR FROM WASTE BANANA PEEL *(Musa sapientum)* BY USING BREAD YEAST (*Saccharomyces cerevisiae*) AND UNPASTEURIZED VINEGAR

BY: SISAY BEKELE HUNDE

A THESIS SUBMITTED TO

THE DEPARTMENT OF APPLIED CHEMISTRY

SCHOOL OF APPLIED NATURAL SCIENCE

PRESENTED IN PARTIAL FULFILMENT OF THE REQUIRMENT

FOR THE DEGREE OF MASTER OF SCIENCE

IN APPLIED CHEMISTRY

OFFICE OF GRADUATE PROGRAM

ADAMA SCIENCE AND TECHNOLOGY UNIVERSITY

JANUARY, 2019

ADAMA, ETHIOPIA

EXTRACTION OF VINEGAR FROM WASTE OF BANANA PEEL *(Musa sapientum)* BY USING BREAD YEAST (*Saccharomyces cerevisiae*) AND UNPASTEURIZED VINEGAR

BY:-SISAY BEKELE HUNDE

MAJOR ADVISER:-GEMECHU DERESSA (PhD)

CO-ADVISER:-FEDLU KEDIR (PhD)

A THESIS SUBMITTED TO
THE DEPARTMENT OF APPLIED CHEMISTRY
SCHOOL OF APPLIED NATURAL SCIENCE

PRESENTED IN PARTIAL FULFILMENT OF THE REQUIRMENT
FOR THE DEGREE OF MASTER OF SCIENCE
IN APPLIED CHEMISTRY

OFFICE OF GRADUATE PROGRAM
ADAMA SCIENCE AND TECHNOLOGY UNIVERSITY

JANUARY, 2019
ADAMA, ETHIOPIA

ACKNOWLODGEMENT

First of all I would like to thank the almighty God for His interminable help. Without His help this research could not have been realized.

I am grateful to my advisors, my major advisor, Dr. Gemechu Deressa and my co-advisor Dr.Fedlu Kedir for their willingness to supervise my research, committed and motivated genuine professional and valuable guidance to successfully complete this research work.

I would like to express my indebtedness to ASTU social service students' dormitory Mr. Lema, analytical chemistry laboratory technician Mr. Guta and biology laboratory technician Mr. Iticha for their professional assistance in all aspects during experimental procedure.

I am also grateful to ASTU for offering me postgraduate study grant and financial assistance and thank all my friends especially Mr. Takalgn Misganu and Mr. Markos Addisu for their sharing experiences and expertise.

I would like to express my gratitude and love to my wife Ms. Tsehay Mekonin Jote with whom I share all ups and downs of life and acknowledge her concern about my education and initiation she took to let me go to school, from idea initiation to covering necessary costs concerning my education.

Finally, I would like to thank all my families for their love encouragements, moral supports and patiences have made this work all possible.

DECLARATION

I, Sisay Bekele, Hunde hereby declare that this M.Sc. thesis entitled as “extraction of vinegar from waste banana (*Musa sapientum*) peel by using commercial bread yeast (*Saccharomyces cerevisiae*) and unpasteurized vinegar “is the outcome of my effort and study and that all sources of materials used for the study have been duly acknowledged. I have produced it independently except for the professional guidance and valuable suggestion of the research advisors. This study has not been submitted for any degree in this University and any other Universities in Ethiopia. It is offered for the partial fulfillment of M.Sc. in applied chemistry.

Name Sisay Bekele Hunde_____

Signature _____

Date of submission _____

ADVISOR'S APPROVAL SHEET

To: Applied Chemistry Department

Subject: Thesis Submission

This is to certify that the thesis entitled as **"**Extraction of vinegar from wastes of banana peel by using commercial bread yeast (*Saccharomyces cerevisiae*) and unpasteurized vinegar (acetic acid)", is to be submitted in the partial fulfillment of the requirement for the degree of Master of Science in applied chemistry, to the graduate program of the department of applied chemistry and has been carried out by Sisay Bekele Hunde with ID.NO: GSS/0223/05 under our supervision. Therefore, we recommended that the student has fulfilled the requirements and hence hereby he can submit the thesis to the department.

Gemachu Derassa (PhD)	_____	_____
Name of Major Advisor	Signature	Date.
Fedlu Kedir (PhD)	_____	_____
Name of Co-Advisor	Signature	Date.

CONTANTS

LIST OF TABLES

List of figures

LIST OF ABBRIVATIONS

AAB	Acetic Acid Bacteria
AC	Ash Content
ADH	Alcohol Dehydrogenase
ALDH	Aldehyde Dehydrogenase
AOAC	Association of Official Analytical Chemist
ASTM	American Society for Testing Materials
CABI	Center for Agriculture and Bioscience International
ECAE	Ethiopian Quality Assessment Enterprise
ESA	Ethiopian Standard Agency
ES	Ethiopian Standard
FAO	Food and Agricultural Organization
FC	Fixed Carbon Content
FDA	Food and Drug Administration
IJSBAR	International Journal of Sciences, Basic and Applied Research
INIBAP	International Network for the Improvement of Banana and Plantain
ISBN	International Standard Book Number
ISO	International Organization for Standardization
LAB	Lactic Acid Bacteria
MC	Moisture Content
MTCC	Metro Toronto Convention Center
NAD	Nicotinamide adenine dinucleotide
NDF	Non-Deliverable Forward
RDA	Recommended Daily Allowance
SAD	Sobourand Dextrose Agar
TS	Total Solid
WHO	World Health Organization
VM	Volatile Matter

ABSTRACT

Banana cultivated in most of the countries in the world at large and specifically in most regions in our country Ethiopia. People use fruit part through its peel everywhere which causes environmental pollution and become a traffic problem as it is slippery. This study becomes a remedy for this problem by converting banana peel into vinegar. The methodology applied was fermentation for seven days by using commercial yeast Saccharomyces cerevisiae to extract ethanol and oxidation of ethanol to vinegar (acetic acid) by acetous fermentation and physicochemical characterization of both ethanol and vinegar the proximate analysis of the peel was also done to determine the organic quality of the banana peel under study. The product having 49.22%(v/v) ethanol strength, density of 0.9340 g/ml, 0.9332 of specific gravity, 4.72 of pH and 0.0058 total solid in % (m/m) for extracted ethanol were comparable with the literature report and the density 1.0013g/m l,1.0028 of specific gravity, 3.3400 of acid concentration, 2.6200 of pH and 0.0650 of total solid were also comparable with the commercial vinegar selam vinegar from these result it is possible to conclude that waste banana peel can be a good substrate for production of ethanol and vinegar.

Key words: *Banana peel, Fermentation, Ethanol, Vinegar, Physicochemical characterization*

CHAPTER ONE

1. INTRODUCTION

1.1. Back Ground Of The Study

Banana belongs to the family of *Musaceae and* scientifically known as *Musa Sapientum.* Banana is the main fruit in international trade and one of the most consumed fruits in the world. Today, banana is the fourth most widespread fruit crop in the world being produced mainly in tropical and subtropical regions. Banana is the second largest produced fruit after citrus, contributing about 16% of the world's total fruit production. India is largest producer of banana, contributing to 27% of world's banana production. Three common species of *Musa* (*M.cavendishii, M. paradisiacal spp.* And *M. sapientum.spp.*) are widely grown in the world.*M.cavendish*known as dessert banana is sweeter and less starchy than. *Paradisiaca*, while *M. sapientum*, is known as true banana usually eaten raw when matured. Both *M.paradisiaca* and *M.sapientum* are characterized by higher starch concentration compared to pure *acuminate* group. Cooking banana has predominant *M. balbisiana* genes (Robinson and Galan, 2010).There is a great diversity of dessert bananas in terms of plant statures, fruit size and colors (yellow, green, red, and orange),namely *M. nana Lour* for Dwarf Cavendish, *M. rubra* Firming vonWall for red banana, *M.corniculata Lour* for horn plantain, and many others Valmayor *et al*, 1999).

Banana peels are readily available agricultural waste that is under-utilized as potential growth medium for yeast strain, despite their rich carbohydrate content and other basic nutrients that can support yeast growth,(Essien *et al*., 2005).The antifungal, antibiotic properties of banana peel can put to be good use. Bagavan, (2011) studied the effects of banana peel on androgen induced enlargement of accessory reproductive organs in castrated mice. Fruit consumption has increased worldwide because of taste, disease prevention and health benefits due to the presence of nutrients such as vitamins, minerals, fiber and other bioactive compounds needed by the human body for a healthy life. However, the increase consumption of these fruits also implies an increment in the volume of waste generated especially peels and seeds (Damila *al.*, 2017). In general banana peel was abandoned as a solid waste. When the peel was decomposed, it produces noxious gases such as hydrogen sulphide and ammonia (Bardiya *et al*, 1996). Concentration of phosphorous increase during the composting processes of wastes of banana peel and the water solubility of phosphorous decrease with humification so that the

solubility of phosphorous during decomposition can be subjected to further problem for microorganism living in the soil (Shymala and Belagal, 2012). The mature phase the banana peel which is organic material continue to decompose and are converted to stable form, that increase in the value of nitrogen at the end of composting period that might occur due to the usage of nitrogen by microorganism to buildup cells, thus reducing and some of the organisms will eventually die, which is recycled as a nitrogen and thus contribute to increase the value (Jusoh *et al*, 2013).

Acetic acid (vinegar) is the predominant flavorings and antimicrobial components in vinegar. The following review will focus on the importance of an acetic acid as a direct food additive or more recently as a food processing aid, to decontaminate food prior to distribution and consumption. Vinegar has been made from a variety of agricultural materials and has been used since around 3000 BC in Asian, European, and other traditional cuisines of the world (Hashimoto *et al*, 2012). It is a traditional fermented food with a sour flavor and is widely used as an acidic seasonings, preservative, beverages, and dressing. It contains specific volatile and nonvolatile compounds, including organic acids, sugars, amino acids, and esters *Saccharomyces cerevisiae* is the main organism used for the production of ethanol from a variety of substrates. It produces ethanol in a large quantity and has the advantage over other organisms of resisting multiple inhibitors such as furans, phenolic compounds and organic acids. The result obtained from the glucose analysis showed a great promise of producing bioethanol from all the pretreatment techniques employed (Hahn-Hägerdal *et al*, 2007). A study shows that the percentages of ethanol production from banana peels at 24 hours interval for seven days by S. cerevisiae strains at pH 6 and at 30ºC increase consistently from 3.645%v/v to 7.923%v/v (Sharaghat *et al*, 2010). The presence of fermentable sugars adhering to banana fruit processing residues (peels) can make them ideal substrates for alcoholic fermentation of fruit juice and subsequent secondary fermentation into vinegar. Even though a number of studies on the extraction of vinegar from banana have been done, extraction of vinegar from waste of banana peel have not been done in Ethiopia, this research aimed for extracting vinegar from very ecofriendly and low cost banana peel and characterization physicochemical properties (density, specific gravity, acid concentration, total soluble solid and pH) of the extracted vinegar and comparing the values with the value obtain by previous findings**.**

1.2. Statement of the problem

There is considerable waste of the banana fruit especially those that do not properly managed. This is due to the fact that the banana fruit is biologically active and carries out transpiration, ripening and other biochemical activities even after harvests. This phenomenon is turned to deteriorate the quality of the fruit and finally makes it unmarketable. Wastage of the banana fruit due to poor post-harvest handling or over-ripening remains a major problem today. The conversion of banana peel into other valuable products before spoilage has been the driving force behind this study knowing enough bananas is wasted after harvesting. Banana peel is generally considered as waste material and do contribute to environmental pollution upon decay as it contain nitrogen and phosphorous compounds, therefore the need to convert the peel into a value-added commodity became imperative. Despite the fact that banana is available in almost all parts of Ethiopia, banana peel are disposed as a waste product causing environmental pollution and becoming wastage of the product. The banana peels waste is normally disposed in municipal landfills, which contribute to the existing environmental problems. These problems can be recovered by utilizing its high value-added compounds, including the dietary fiber fraction that has a great potential in the preparation of functional foods from waste banana peel.

1.3. Objective Of The Study

1.3.1. General objective of the study

The general objective of the research is "extraction of vinegar from waste of banana peel by using commercial bread yeast (*Saccharomyces cerevisiae*) and unpasteurized vinegar".

1.3.2. Specific objectives of the study

- To extract ethanol from the waste of banana peel by using commercial bread yeast(*Saccharomyces cerevisiae*)
- To oxidize the extracted ethanol to acetic acid(vinegar) by secondary fermentation (acetfication) using unpasteurized vinegar
- To characterize physicochemical properties(density, specific gravity, pH and Ethanol concentration,% (v/v/) and total dissolved solids) of extracted ethanol and comparing the values with the previous study
- To characterize physicochemical properties (density, specific gravity, pH, acetic acid concentration total dissolved solids) of the extracted vinegar and comparing the values with that of literature reports.

1.4. Significance Of The Study

Banana cultivated in most of the countries in the world at large and specifically in most regions in our country Ethiopia hence it can be obtained easily with low price providing good nutritional values and medicinal values. A wide range of seasonal fruits causes problems for farmers through low prices and post-harvest losses. One option to better make use of these fruit is to produce good quality competitively priced banana fruit peels vinegar for the local market. Only a small production unit is required; costing equipment, materials and working capital is needed to produce vinegar**.** But the peels are thrown as waste and consequently these thrown waste peels causes environmental damages as it contain nitrogenous compounds and become traffic problem as it is slippery, so the present study could avoid those problems by extracting vinegar which is very important food preservation and used by all peoples and moreover to indicate that banana peels thrown as waste have many traditionally and medicinal benefits. The exploration of potential benefits of banana peel as a source of starch which was converted to ethanol which was oxidized to produce vinegar may significantly improve the price value of banana and avoid an environmental pollution.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. Morphology Of Banana Plant

The banana plant is a medium tree-like perennial herb. It is an herb because the aerial parts of the parent plant die down to the ground after the growing season. The variability observed in morphological traits is used to characterize banana plants (Robinson and Galan, 2010).The number of banana fruit in one bunch, the length of one banana fruit and the thickness of banana plant, are slightly differ from place to place, varieties and other geographical factors that influence the growing, quality and yield of the fruit. Although in Ethiopia, the living standard of the local value service providers (cooperatives, transporters) has substantially improved in recent years, they still face many challenges in the production or cultivation is still mostly traditional in many parts of Ethiopian For instance, yield per unit area of land is declining due to an improper agronomic technical problems such as overstocking, lack of soil amendments, improper irrigation techniques and mono cropping (Zenebe, *et al*, 2015).

2.2. Requirement For Productivity Of Banana Plant

Like a large grass, the banana tree does not offer much resistance to tropical storms or to fungi and insects. Nevertheless, one of its advantages is that it grows very rapidly: flowers appear by the sixth month and harvesting can start after nine to twelve months of growth. Therefore, it is a bulb and not a seed that bears the fruit. On average, a banana plantation survives for six to seven years. The bananas can be harvested without interruption. This capacity for rapid production is a welcome attribute because, banana is eaten in all seasons, with peaks in the winter season, and is a foodstuff that cannot be stored. Banana production in the world is under great demand in terms of productivity (Anhwange *et al*, 2009)

2.3. Chemical Composition Of Banana Fruit

Most parts of banana plants, including its fruit, leaves, stems, and peels, have been used by the community in some parts of the worldwide. However, banana peel is often considered as a waste material of this plant, being disposed as an organic waste or used as an animal feed for goat and cow. In fact, banana peel accounts for approximately one-third (30- 40%) of the total weight of banana fruit. Banana peel is nutritionally a rich source of starch (13%), crude protein (6-9%), crude fat (3.8-11%), total dietary fibers (43.2-49.7%), and polyunsaturated fatty acids, pectin, essential amino acid, micronutrients (K, P, Ca, Mg)Eventhough different

banana peels have slightly different nutritional values, generally, banana contains a high concentration of sugar and many acids and also contains antioxidants, vitamin A, sucrose, glucose and fructose are all the principal sugars in ripened banana, with large amounts of cellulose, starch and pectin. Bananas fruit can be consumed raw after harvesting. The surplus amounts can be preserved in the form of banana puree/pulp, fruit bar, canned pulp and also as juice and powder (Mohapatra *et al*, 2011). It confers protection for eyesight, healthy bones, kidney malfunctions, morning sickness, itching and swelling, improves nerve functions and is said to help people to quit smoking (Sampath *et al*, 2012).

2.4. Banana (*Musa Sapientum*) Peel

2.4.1. Chemical compositions of banana peel

The nutritional value of banana peel depends on the stage of maturity of fruit and the cultivar; for example plantain peels contain less fiber than dessert banana peels, and lignin content increases with ripening (from7 to 15% dry matter). On average, banana peels contain 6-9% dry matter of protein and 20-30% fiber (measured as NDF).Green plantain peels contain 40% starch that is converted into sugars after ripening. Eight compounds were identified which include estragole(11.18%), hexadecanoic acid ethylester(9.76%),epicatechin(9.97%),gallocatechin(8.58%),pcoumaricacid ethylester(4.12%),1,2-benzendicarboxylic acidmono (2 ethylhexyl)ester(13.47%), betatocopherol(11.37%) and other vitamin parts as vitamin E(31.35%) Banana peels are used for water purification (Chaparadza, and Hossenlopp, 2012) to produce liquid ethanol, cellulose, as fertilizer and in composting, Lactase,(Vivekanand *et al*., 2011),

Figure 2.1 Structure of Banana *(Musa sapientum)* peels

2.4.2. Adevantages of banana peel for environment

The fruit protected by its peel which is discarded as waste after the inner fleshy portion is eaten. Besides being used fresh, banana is also used to be processed into many products such as juice, jams, chips, puree pulps powder, biscuit etc. (Zhang *et al,* 2005).Different studies show that significant quantities of banana peels equivalent to 40% of the total weight fresh banana are generated as a waste product in many industries that are producing banana based products(Tcehobanoglous et al, 1993).At present, these peels are not being used for any other purposes and are mostly dumped as solid waste at large expense. It is thus significant and even essential to find application for the peels as they can contribute to a profound real environment problem (Zhang *et al,* 2005).Banana peel adds calcium, magnesium, sulfur, phosphates, potassium and sodium in the compost pile. They help the organic materials grow healthy. Additionally, the peel makes the compost maintain water and fertilize soil (Doran et al, 2016).Other benefit to the environment is that removing metals from contaminated water because the banana peel includes nitrogen, sulfur and carboxylic acids. The acids are able to not only remove the toxic metals from the water, also make the better case than the existing expensive technological options. Moreover, it does not need any technical preparations, so it is easy to do. It requires only dried banana peels. Banana peel is an organic waste that highly rich in nutrient especially K, that could support the microbial growth in fermentation phase (Essien *et al,* 2005).

2.5. Application Of Banana Peel

2.5.1. Medicinal qualities of banana peel

Perdue University boldly states that every part of the banana plant has medicinal properties. Among the properties cited; Antifungal and antibiotic principles are found in the peel and pulp of fully ripe bananas (Brooks, 2008).Banana flower is actually richer in both soluble and insoluble fiber than the banana itself. Another reason why eating banana flower is a good idea is that fiber has been shown in countless studies to aid in lowering cholesterol. When you keep your cholesterol levels low, you help to ward off complications from heart disease such as heart attacks and stroke. These banana peel nutrients are extremely beneficial. It is also used to treat dysentery, ulcers, andbronchitis.Cookedflowersare good food for diabetics (Johnston *et al,* 2004).All parts of banana plant have medicinal applications: the flowers in bronchitis and dysentery and on ulcers; cooked flowers are given to diabetics; the astringent plant sap in cases of hysteria, epilepsy, leprosy, fevers, hemorrhages, acute dysentery and

diarrhea, and it is applied on hemorrhoids, insect and other stings and bites; young leaves are placed as poultices on burns and other skin afflictions; the astringent ashes of the unripe peel and of the leaves are taken in diarrhea and used for treating malignant ulcers (Girish and Satish, 2008).The roots are administered in digestive disorders, dysentery and other ailments; banana seed mucilage is given in cases of diarrhea in India (Bhat *et al*, 2010).

2.6. Medicinal (Values) Importance Of Banana Peel

Banana peel for an extra boost of nutrients, fiber, and antioxidants, make sure to boil the peel for 10 minutes. Then toss the peel into your juicer or blender with other fruits in order to mask any bitterness. Clean and remove any pesticides from the peel, which goes the same for any fruit you eat. Bananas are one of the best sources of potassium, an essential mineral to maintain normal blood pressure and heart function Scientists suggest that people with a low amount of potassium in their diet may have an increased risk of stroke, High-potassium foods, like bananas, may lower the risk of stroke, (Loganayaki, 2010).Because the banana is rich in non-digestible fibers, it can help to restore normal bowel activity and help with both constipation and diarrhea. Bananas have long been recognized for their antacid effects that protect against stomach ulcers and ulcer damage. A flavonoid in the banana, leucocyanidin, recently has been found to significantly increase the thickness of the mucous membrane layer of the stomach. Banana and orange extracts on neuron cells and found that the phenolic phytochemicals of the fruits prevented neurotoxicity on the cells (Sampath, 2005).A mashed ripe banana is an extremely simple and healthy baby food as it contains: Potassium, Fiber Calcium, Magnesium, Phosphorus, Selenium, Iron, Vitamins A, B2, B6, C, E, Niacin, Foliate, and Pantothenic Acid. Animal studies have shown that banana has the potential that the dietary fiber component in banana pulp was responsible for its cholesterol-lowering effect (Emaga *et al*, 2008).Bananas contain 25 percent of the recommended daily allowance (RDA) for vitamin B6, necessary for producing antibodies and red blood cells as well as aiding in the metabolism of fat. In addition, vitamin B_6 serves as an immunity booster According to a recent survey undertaken by people suffering from depression, many felt much well after eating a banana (Aurore *et al*, 2009).

2.7. Ethanol

Ethanol has been recognized as an important renewable and sustainable fuel source for modern industries (Lee *et al*, 2012). For example, it can be used as a replacement of gasoline for many internal combustion engines, and it can be mixed with gasoline to any concentration.

Most existing car engines can run on blends of up to 15% bioethanol with petroleum/gasoline, thus it can significantly reduce the dependence on crude oil (Sassner *et al*, 2008).Use of cheaper alternative sources of energy mainly for cooking is now an important issue to for considering from several perspectives. The renewable energy from the identified crops and lignocelluloses residues, agricultural and wood based, are undergoing worldwide economic analysis for case of efficient commercialization. The bioethanol production through microbial fermentation can provide economically competitive source of energy. There is the variety of biotechnological processes and microbial mechanisms for biological fuel (ethanol) production from lignocellulosic and cellulosic materials. Microorganisms are mostly required to produce ethanol from lignocellulosic hydrolysates using an economically feasible process. Different fermentations organisms among bacteria, yeast, and fungi have been reviewed with the emphasis on their good performance over lignocellulosic hydrolysates. Types of the biofuel, derived from these sources are important choices for exploiting alternative energy and reduction of environmental pollution (Sassner *et al*, 2008), the excessive consumption of fossil fuels, particularly in large urban area, has been greatly contributed to generation of high levels of pollution. In this regard, converting a renewable non-fossil carbon, such as organic wastes and biomass consisting of all other growing organic matter (plants, grasses, fruit wastes and algae) to the fuel would assure a continual energy supply (Wyman, 1996).

2.8. Vinegar

2.8.1. Vinegar history in the world

Vinegar is the world's oldest cooking ingredient and food preservation method. Vinegar's use can be traced back over 10,000 years. In fact, flavored vinegars have been manufactured and sold for almost 5,000 years. The wide variety of vinegars available today is nothing new. Until the six century BC, the Babylonians were making and selling vinegars flavored with fruit, honey, malt, etc. In addition, the Old Testament and Hippocrates recorded the use of vinegar for medicinal purposes (Conner and Allgeier, 1976). Vinegar production ranges from traditional methods employing wood casks and surface culture to submerged fermentation in acetators by machine (Morales *et al*, 2001).Vinegar traditionally has been used as a foods preservative. Whether naturally produced during fermentation or intentionally added, vinegar retards microbial growth and contributes sensory properties to a number of foods. The wide diversity of food products that containing vinegar (sauces, ketchup, mayonnaise, etc.) and the current fall in wine consumption have favored an increase in good vinegar production. The

manufacturing methods involve the use of common materials that are almost rich in sugars or starches, such as fruits juices, that go through alcohol fermentation and oxidation of ethanol to acetic acid (Lee, 2012).

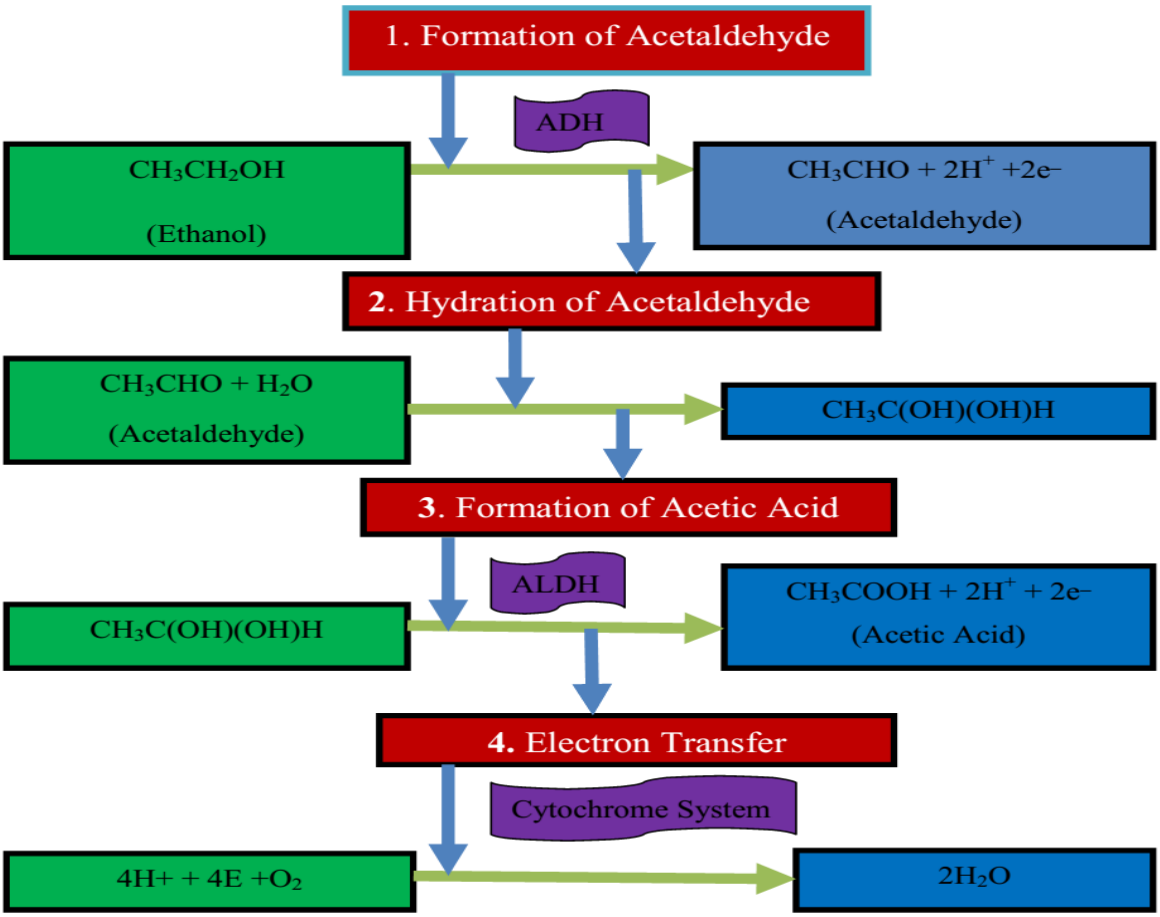
2.8.2. Vinegar production

Vinegar bacteria, called AAB, are members of the genus Acetobacter and characterized by their ability to convert ethyl alcohol (C_2H_5OH) into acetic acid (CH_3COOH) by oxidation as:

$CH_3CH_2OH + O_2 \longrightarrow CH_3COOH + H_2O + 493$ kJ

Ethanol Oxygen Acetic acid Water energy

The studies revealed that there were two steps in the oxidation of ethanol to acetic acid, driven by the enzymes ADH and ALDH. The first step was oxidation to acetaldehyde by ADH, which was further oxidized to produce acetic acid by ALDH as shown in Scheme 2.1below (Suman *et al.* 2014).



Scheme 2.1.Flow chart for routines of conversion of alcohol to acetic acid

2.8.3. The uses (applications) of vinegar

The vinegar can then be used in dressing salads, manufacture of useful medicines, and preservation of food stuffs, provision of antioxidants or as an antibacterial agent (Soltan and Shehata, 2012).Vinegar is a living ingredient created through the process of fermentation. When creating fruit-based vinegar, wild yeasts are added to convert the sugars into alcohol. Vinegar or sour wine is a product of alcoholic and subsequent acetous fermentation of sugary precursor. Food deterioration can be due to a combination of various factors like light, oxygen, heat, humidity and contamination of all possible kinds of microorganisms (Yusuf *et al*, 2012).Among these microorganisms, some having beneficial effect are widely used in biotechnology for production of compound such as vinegar, spirit, wine and antibiotics. This ability allows processing and storage of agro-perishable foods to preserve or to valorize them over a long period of time. That is very necessary for developing countries both economically and socially Vinegar is produced from ethanol fermentation in a process that yields its key ingredient, acetic acid(Seyram *et al*, 2009).

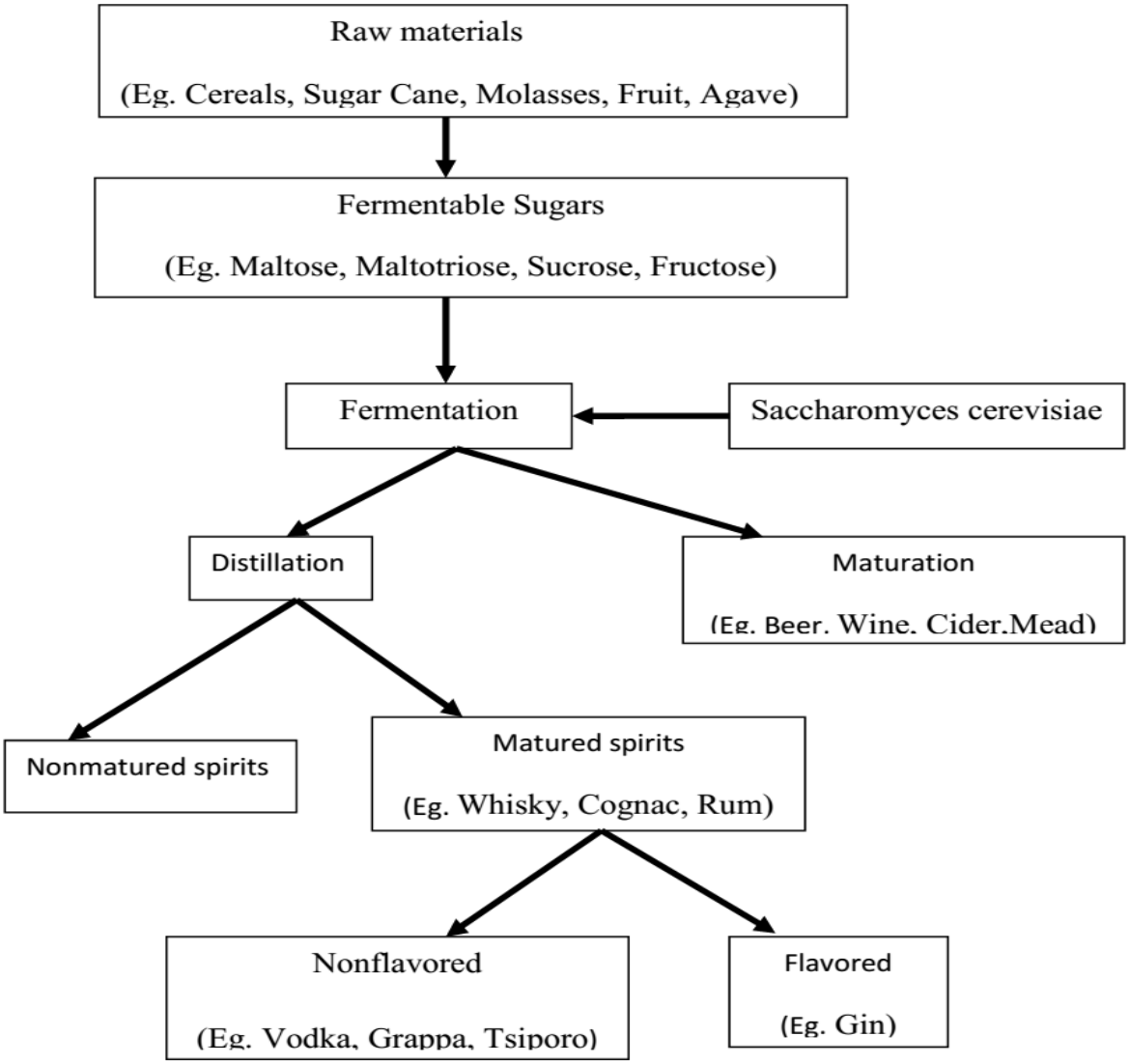
Vinegar is primarily used to flavor and preserve foods (vegetables), meat and as an ingredient in salad dressings and marinades. Vinegar is also used as a cleaning agent. It has very good preservative potentialities and is commonly used as food ingredient but also for its medicinal properties. Moreover, it has physiological effects such as invigorating (Johnston, 2005), and also regulator of blood pressure, appetite stimulator, digestion and absorption of calcium. It is also known to be very effective in cancer osteoporoses and neurological diseases (Xibib *et al*, 2003).Hence it is cost effective or economical to produce vinegar from waste of banana fruit or banana peels. In this study, vinegar, a product of two-stage fermentations (alcoholic fermentation and acetic acid fermentation) produced from banana peel wine. The vinegar production coincides with that of wine making, which has been documented over 5000 years ago. Since then, vinegar has been used as a condiment, in the preservation of food, and as a household cleaning agent. Moreover, vinegar is known for its antioxidant properties and health benefits, including the prevention of inflammation and hypertension (Johnston *et al*, 2013).

2.9. Yeast (*Saccharomyces cerevisiae*) For Fermentation to Produce Ethanol Alcohol

Type of yeast widely used in the conversion of sugar and starch based substrate into ethanol is *Saccharomyces cerevisiae* since this yeast is able to produce high amount of ethanol and has higher tolerance to ethanol and other inhibitor compounds .The ability of each strain in

consuming sugar and producing ethanol was different due to difference in responding to the infuelence of some of the factors such as sugar concentration, ethanol concentration and temperature. The differences in responses were determined by the genetical and physiological stability of each of yeast strains. *Saccharomyces cerevisiae,* the microorganism used in baking industry as leavening agent with the technology advancements in bread industry, has been continuously improvised for decades (Gelina, 2012).

Yeasts are chemoorganotrophs, as, they use organic as source of energy and do not require sunlight to grow. Carbon obtained mostly from hexose sugars, such as glucose and fructose, or disaccharides such as sucrose and maltose. Some species can metabolizes pentose sugars such as ribose, alcohols, and organic. Yeast species either requires oxygen for the aerobic cellular respiration(obligate aerobes) or are anaerobic, but also have aerobic methods for energy production (facultative anaerobes).Unlike bacteria, no known yeast species grow only on an aerobically(obligate anaerobes).Most yeast grow best in a neutral or slightly acidic pH environment. Yeast constitutes an interesting group from a technical and industrial standpoint in the roles of the good microbial world. in the fermentations of *Saccharomyces cerevisiae,*among the known genera and species of the yeast, is used commercially for baking bread and an alcohol beverage production, foodstuffs, animal feed processing and the production of lots of biochemical The yeast, *Saccharomyces cerevisiae* biomass mainly in the form of baker's yeast, represents the largest and abundant of bulk production of any single cell microorganism in the world several million tons of freshly baker's yeast cells are produced for human food use (Fadel and Foda, 2001).Lactic acid bacteria and yeasts are often encountered together in natural ecosystems and are known to compete for the same nutrients For large-scale process of beverage fermentation like in brewing, wine making and distilled for the spirit production, pure cultures of selected strains of *Saccharomyces cerevisiae* yeast enzymes are usually used as shown in scheme 2.2 on page 13 (Hammond, 2016).



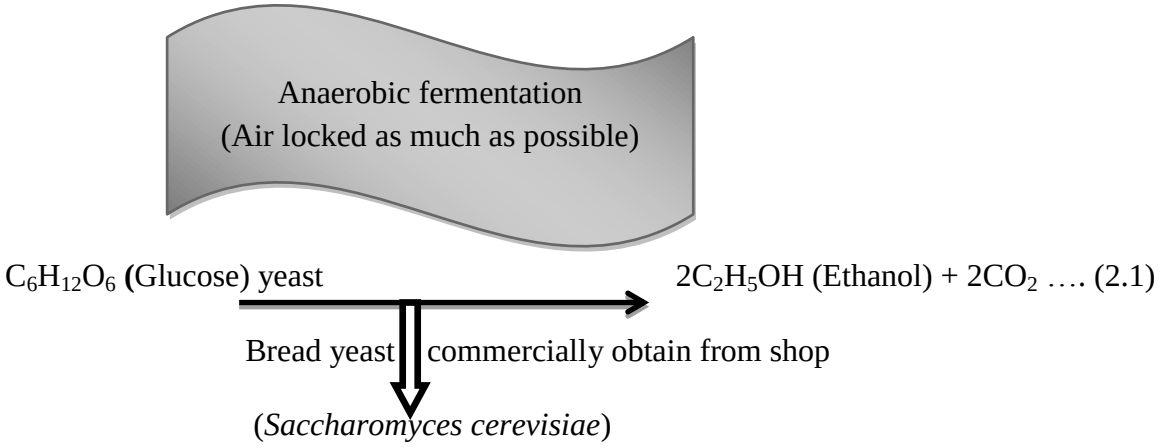
Scheme 2.2.*Saccharomyces cerevisiae,* in production of ethanol during alcoholic fermentation

2.10. Fermentation

2.10.1. Alcoholic fermentation

Microbial species involved in fermentations may range from yeast and LAB to molds and AAB. The microorganisms involved in the elaboration of vinegars are mainly yeasts and AAB. The former being responsible for the alcoholic fermentation and the latter needed for needed for the acetfication (Nanda *et al.,* 2001).Successful fermentations to produce ethanol using yeast require tolerance to high concentration of both glucose and ethanol. These cellular characteristics are important because of high gravity (VHG) fermentations, which are common in the ethanol industry, give rise to high sugar concentrations, at the beginning of the process, and high ethanol concentration at the end of the fermentation. Saccharomyces

cerevisiae is an important micro organism in bio- industry and its tolerance to ethanol is one of main characteristics to decide whether it can be used as bio-fermentation resources (Chiranjeevi *et al*, 2013). Because of yeast fermentative characteristic, there is always a need for yeast strains with better features of fermentation especially high ethanol tolerance for production of ethanol as bio fuel on commercial scale. Yeast strains associated with fruit surfaces are capable of converting wide range of sugars into alcohol. Ethanol tolerance, sugar tolerance and invertase activities are some of the important properties for use in industrial ethanol production (Jimenez and Benetez, 1986). Alcoholic fermentation of carbohydrate is the first critical step in the production of vinegar and takes place under anaerobic condition. In this step sugar is fermented to alcohol by the action of yeast species as shown by scheme 2.3 below (Silva and Swarnakar, 2007).



Scheme 2.3. Conversion of sugar to ethanol

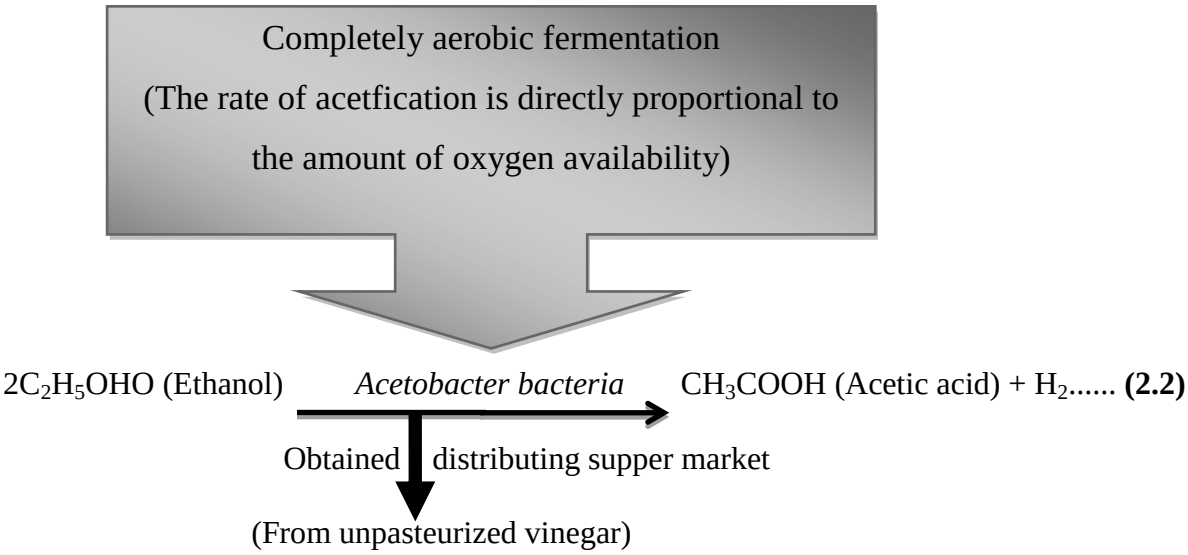
2.10.1.1. Separation of ethanol by distillation

Ethanol is produced by yeast fermentation. Although yeast mainly produces ethanol, it also produces by-products. These by-products need to be removed to obtain pure ethanol. There are mainly two kinds of by-product sources, starch and lignin. Starch derived by-products include esters, organic acids, and higher alcohols. Lignin derived by-products include cyclic and heterocyclic compounds. Fermentation by-products are mostly removed by distillation. However, volatile by-products tend to lodge more in ethanol (Sree *et al*, 1999).Distillation exploits the differences in the volatility of solution's components, which means that every compound has a different boiling point and starts to vaporize (change from its liquid to gaseous state) at a different temperature. When distilling, you heat up the solution so that the component with the lowest boiling point evaporates first, leaving the other solutes to late

behind. The vaporized component in the gaseous state can then be collected in a different container by condensation and is called distillate. Changing the distillation temperature, one can separate many different substances according to their different volatilities. Distillation is the most dominant and recognized industrial purification technique of ethanol. It utilizes the differences of volatilities of components in a mixture (Oleskowicz *et al*, 2008).

2.10.2. Acetic acid fermentation

The acetic fermentation was able conducted by seeding the wine obtained during the alcoholic fermentation with acetic acid bacteria from a non-pasteurized alcohol vinegar/strong vinegar. The oxidation of an alcohol to acid is the second major step in the production of vinegar and is aerobic process. In this step alcohol is oxidized to acetic acid by the action of acetic bacteria; the species of Acetobacter bacteria as shown by scheme 2.4(Xibib *et al*, 2003).



Scheme 2.4. Conversion of ethanol to acetic acid by acetous fermentation

CHAPTER THREE

3. MATERIALS AND METHOD

3.1. Instruments

Instruments employed in this study were, measuring digital balance (model-Sartorius with 0.0.1mg sensitivity and model EP421C), pychnometer (Gay-Lussac, ISO 3507), an incubator (model D.91126 schwabach FRG made in Germany) pH meter (pH meter 3310, JENAY Model Hanna instruments),electrical oven (model 105,Industrial estate, Ambala cantt made in India), shaker (EXCELLAE24R), beakers, crucible, autoclave (model LXB75,made in China), muffle furnace mortar and pistol, desiccators, muslin cheese cloth, hot plate, dropper thermometer, water bath, glass road, scissor, 250 ml conical flask, 250 ml Erlenmeyer flask, Alcoholmeter, Microscope, chemical hood, refrigerator, Lunge-Rey Pipette.

3.2. Chemicals

Sodium bisulphate ($NaHSO_4$), ammonium phosphate $(NH_4)_3PO_4$), sucrose ($C_{12}H_{22}0_{11}$) citric acid, ($C_6H_8O_7$) Ethanol(C_2H_5OH), acetone(C_3H_6O), bread yeast (*Saccharomyces cerevisiae*), commercial unpasteurized vinegar, distilled water, Sobourand Dextrose Agar, lactopherol, sodium hydroxide solution, phenolphthalein indicator solution, 60% ethylene alcohol where all the chemicals were of analytical grade.

3.3. Plant (Waste Banana Peel) Collection And Authentication

Fully ripen yellow banana fruits were collected from shop in Adama Science and Technology University compound and taken to analytical chemistry laboratory; peeled off hand; weighed to be 6 kg and saved for the next work as shown in Figure 3.1 below.

Ripen banana fruits

peel peeled off by hand

Figure 3.1. Material preparation for fermentation

3.4. Experimental Procedure For Proximate Analysis Of Banana (*Musa Sapientum*) Peel

The procedures for the proximate analysis were carried out starting from collecting yellow ripen clean banana fruit and followed by peeling off, washing by tape water, air drying for 1 hour, oven drying at 105℃ for 2 hours and finally grinding very small size in mortar as shown by the four Figures below.

Banana peel air drying banana peel oven drying banana peel ground banana peel

Figure 3.2. Proximate analysis procedures in general

3.4.1. Determination of total solid (TS)

The total solid was determined according to the standard method (ASTM D 3174-89; AOAC 2000) given to analyze the total solids of banana peels under study. 20 gm of the samples was taken in clean and dried crucible and dried at 105 ℃ for 4 hours in oven drying until its weight was becomes constant. The hot crucible was removed from the oven and kept in desiccators until it cool and total solid was calculated by using the equation given below (Abdullah *et al*, 2014,).

$$\textbf{TS (\%)} = \frac{w2}{w1} x100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \textbf{(3.1)}$$

Where TS (%) is the percentage of total solid of the sample under study

W_1 is the weight of the sample plus crucible before oven drying

W_2 is the weight of the sample plus crucible after oven drying

Weighing ground sample, oven drying of sample cooling in desiccators

Figure 3.3. Procedures for determination of total solid

3.4.2. Determination of moisture content (MC).

The percentage of moisture content of the banana peel sample under study was determined by the standard method (ASTM D 2867-99 form AOAC, 2000) method using the oven dry

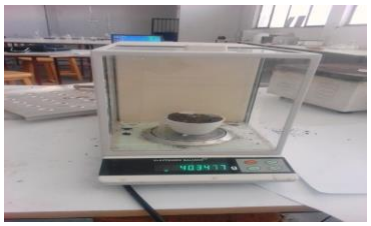
method. Clean dishes were placed in a drying oven at105℃ for one hour to dry then transferred into desiccators until they cooled and 30g of the ground banana peel was taken in a clean and dried crucible and dried in an oven at a temperature of 105℃ for 6 hours and the weight was taken after every 2 hours. All procedures were, repeated until constant weight of the samples was obtained. After each of 2 hours, the sample was removed from the oven and cooled in desiccators for 30 minutes. The sample was finally removed and reweighed. The percentage loss of the sample weight gave the percentage of moisture in sample (Abdullah *et al*, 2014).The percentage of moisture content of the sample is calculated as:

$$\%MC = \frac{W1-W2}{W1} \quad \text{(3.2)}$$

Where, %MC is percentage of moisture content the sample

W_1 is the original weight of the sample taken

W_2 is the weight of the sample after drying.



Weighing ground sample, oven drying of sample cooling in desiccators

Figure 3.4. Procedures for determination of moisture content

3.4.3. Determination of volatile matter (VM)

The percentage of the volatile matter of the banana peel sample was determined by the standard method (ASTM D 3175-89; AOAC 2000) .4gm of the dried sample after moisture removal was taken in a crucible and placed in electrically heated oven at a temperature of 920 ℃ for 10 minute and then it was cooled in desiccators for 30 minute. It was then removed and reweighed. The percentage of weight loss gave the volatile matter content. (Abdullah *et al,* 2014).The percentage of volatile matter is calculated from the equation give below as:

$$\%VM = \frac{W1-W2}{W1-W3}X100 \quad \text{(3.3)}$$

Where: %VM is percentage of the volatile matter of the sample

W_1 is the original weight of the sample plus weight of the empty crucible

W_2 is the weight of the crucible plus weight of the ash

W_3 is weight of the empty crucible.

Weighing ground sample   oven drying sample   cooling in desiccators

Figure 3.5. Procedures for determination of volatile matter

3.4.4. Determination of ash content (AC)

The ash content is defined as the inorganic residue that remained after the organic matter was burnt away. The percentage of ash content of the banana peel sample in study was determined by the standard method (ASTM D 3174-89; AOAC 2000).The porcelain crucible was washed thoroughly and then dried in the oven at 105 ℃ for 60 minutes and then weighed after being cooled in the desiccators.2 gm of the sample was weighed and placed for 2 hours in a muffle furnace which was set at 550℃ . After ashing, the sample was removed from the furnace. The crucible was cooled in desiccators and then reweighed (Abdullah *et al*, 2014). The ash content obtained was calculated by the using the following equation:

$$\%\mathbf{AC} == \frac{W1-W2}{W1} X100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \mathbf{(3.4)}$$

Where: **%** AC is percentage of the ash content of the sample

$W1$ is the original weight of the sample plus weight of the crucible

$W2$ is the weight of the crucible plus weight of the ash

Weighing ground sample,   heating in muffle furnace   ash after burned

Figure 3.6. Procedures for determination of ash content

3.4.5. Determination of fixed carbon content (FC)

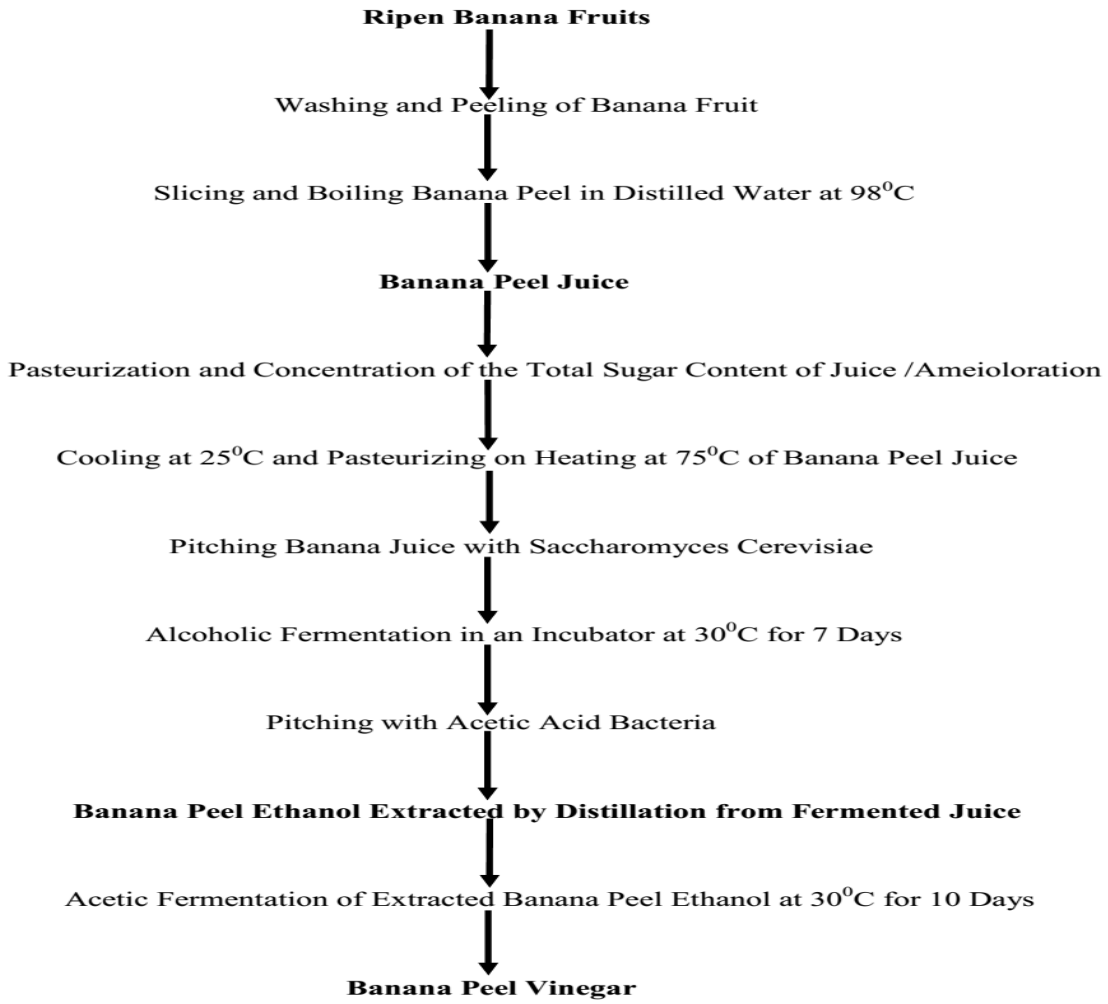
The fixed carbon is the content that remains after complete volatilization. It is the residue left after the moisture, volatile and ash was given up (Abdullah *et al.,* 2014).The fixed carbon content of the sample was calculated by subtracting the sum of moisture content (%), ash content (%) and volatile matter (%) from 100 as given below:

% FC = 100- (moisture content (%) + volatile matter (%) + ash content (%))…… (3.5)

Where FC % is the percentage of fixed carbon content of the sample

3.5. Procedures For Alcoholic Fermentation

All the procedures used were briefly provided in the schematic representation below



Scheme 3.1.Brief representation of the whole procedures of vinegar extraction

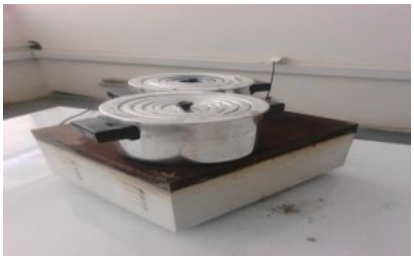
The peel (4kg) was washed thoroughly by using first tape water and then distilled water to remove dust particle and unwanted microorganism on the surface of peel and dried on clean table at room temperature for about three as shown in Figure 3.7 and then sliced into very small size using steel knife which was sterilized by ethanol and flame


Drying washed banana peel sliced banana peel to get ready for boiling

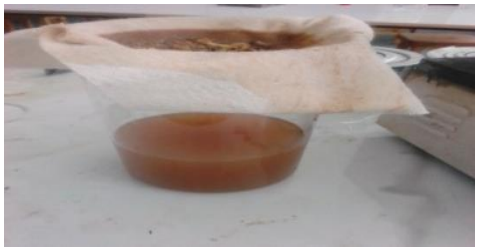
Figure 3.7. Preparation of banana peel for fermentation

3.5.1. Preparation banana peel juice (must)

The sliced peel was again washed thoroughly distilled water, added to boiler heated at 98℃ in order to highly gelatinize the banana peels in 1 liter of distilled water for 30 minutes as shown in Figure 3.8. The boiled peel mixture was cooled to room temperature, 25℃; filtered using a folded white muslin cheese cloth as shown in Figure 3.8 and the residue of first filtrate was again further re-extracted at 98℃ in 1.5liter of distilled water for 20minutes (De OryL *et al,* 1999).



Boiling sliced banana peel



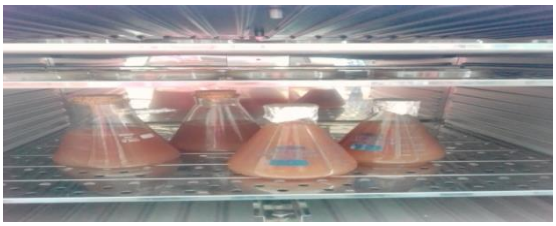
filtering the boiled mass

Figure 3.8 Gelatinizing of banana peel by boiling

3.5.2. Must inoculation for alcoholic fermentation in vinegar extraction

The first and second filtrates was then mixed and filtered to remove small particle of the first filtrate and 1.5 liter of distilled water was added in four different flasks and kept for 48 hours in incubator at 37℃ after 48 hours 100gm of sucrose for amelioration of sugar levels, 2gm of salt sodium bisulphate to inhibit the contamination of microflora ,2ml of chromic acid was added in each fermentation flask, air-lock each flasks and kept in incubator at 30℃ for an aerobically fermentation for 7 days as shown below in Figure 3.9 on page 22.(for maximum ethanol production) (Johnston *et al*., 2004).

Banana peel juice after filtration



filtered banana peel juice kept in incubator

Figure 3.9. First start of fermentation of banana peel juice

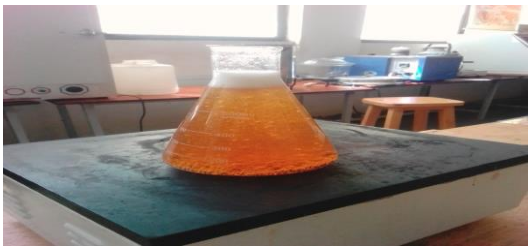
3.5.3. Yeast inoculation for alcoholic fermentation

The preparation of the yeast for alcoholic fermentation is of a great importance for the overall processes, which must include two stages: laboratory and industrial. The conditions of the

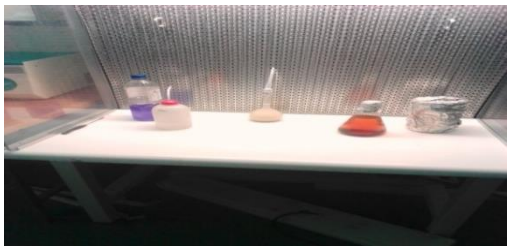
yeasts cultures leave can determine to a great extent of the technological processes and qualitative indicators of the alcohol obtained (Bambalov *et al*, 2000).

3.5.3.1. Sobourand dextrose agar (SDA)

The process was carried out by dissolving 65g of commercially powdered SDA and 0.50g of chlorophenical powder into 2 liters of distilled water into conical flask. It was shaken and corked with cotton wool. The autoclave was then used to sterilize it at 121℃ for 15 minutes. It was taken out of the autoclave and allowed to cool between 450℃ and 50℃.Eight Petri dishes was then washed and dried using hot dry oven at 150℃ for 1 hour. They were also allowed to cool. The liquid SDA were then dispersed into eight Petri-dishes aseptically and allowed to solidify. The fermentation process is started by mixing a source of sugar, water and yeast in an oxygen free environment. This anaerobic environment forces the yeast to shut down the "burning" of sugar and allows fermenting to alcohol (Sharaghat *et al*, 2010).



Boiling SDA for yeast culturing

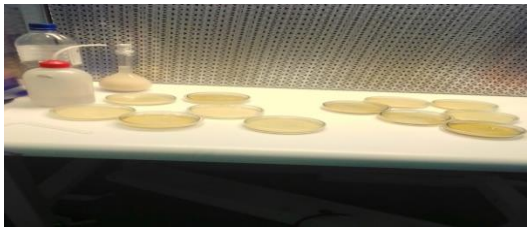


chemicals for yeast growing

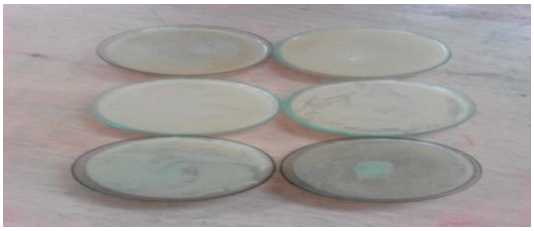
Figure 3.10. Preparation of media for yeast inoculation

3.5.3.2. Inoculums preparation

After solidifying, the stock yeast was prepared and taking aseptically using a disposable syringe and needle. For each Petri dish 2ml of stock yeast will be inoculated in it by pouring it on top of the medium (SDA) with a sterilized wire loop. After inoculation it was incubated at 35℃ for 48 hours. After a while, growth was observed and recorded in a table (Sharaghat *et al*, 2010)



Inoculated yeast in chemical hood



inoculated yeast ready to grow

Figure 3.11. Yeast inoculums preparation

3.5.3.3. Microscopic observation of the grown of *Saccharomyces cerevisiae*.

In microscopic determination, lactopherol (2ml) was poured in to the top of a clean slide and sterile wire loop was used to pick the yeast from the agar, placed on a glass slide cover slip. It was then viewed under the microscope at 40 times magnifying objective. The observations were then recorded. These procedures were then carved out on the two remaining Petri dishes. The result was also recorded and compared (Sharaghat *et al*, 2010).

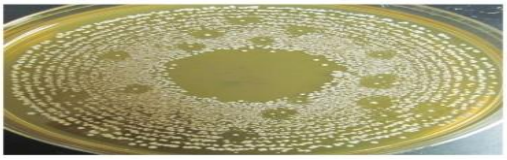


Figure 3.12. Visualization of cultured *Saccharomyce.cerevisiae* production on SDA

3.5.3.4. Colonial morphology of yeast grown

Colonial morphology of the grown yeast in the plate after 48 hours was observed and recorded as follows in the table below:

Table 3.1.Colonial morphology of the activated yeast for fermentation under microscope

Color in sight	Constituency	Edge	Odor	Shape
Creamy	Mucoid	Spherical	Fermented odor like that palm	Oval

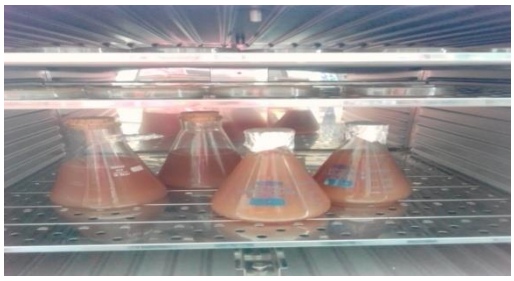
3.5.4. The adjustment pH during six successive days of primary fermentation

For the adjustment of pH of the fermenter (must) before addition of any microorganism, yeast to the must, pH of the must had to be adjusted; otherwise the *Saccharomyces cerevisiae* was died in the hyper acidic and hyper basic state. A pH of around 5.0-5.5 was maintained. The banana peel juice (must) was primarily checked for pH by using digital pH meter. The pH was adjusted to 5.0-5.5, when the pH becomes below the range of 5.0-5.5, aqueous sodium hydroxide was added drop wise to the fermenting flasks with constant stirring until the pH becomes within the required range of (5.0-5.5) and when pH of must becomes beyond the interval of 5.0-5.5 range, concentrated sulfuric acid was added drop wise to maintain the pH of banana peel juice within the ranges favorable for *Saccharomyces cerevisiae* (Asli, 2010).

3.5.5. Simple distillation to extract ethanol from fermented banana peel juice

The alcohol produced by fermentation process was concentrated from the fermented solution in which the fermentation takes place by distillation. Simple distillation works particularly

well on mixtures of two liquids which have a very large boiling point difference. The lower boiling component distills off first and can be collected. In 1000 ml round bottom distillation flask, 500 ml of fermented banana peel juice was added and the setup of the simple distillation case (as shown in Figure below) was corrected started to boil and alcohol started to drop at 78℃ and continued to 80℃.All the first distillation processes were carried out at temperature of 85℃ and a distillation time of 6 hours for single distillation set up (Sree *et al*, 1999).



Fermented banana peel juice in incubator

set up of simple distillation of ethanol alcohol

Figure 3.13. Extraction of ethanol alcohol from fermented juice

3.5.6. Double distillation process to optimize concentration of ethanol extracted

The all the nine distillates collected by the first simple distillation were redistilled carefully to optimize the concentration of bioethanol for all samples. Both the volumes and the % v/v of ethanol extracted by double distillation were recorded and compared with that of the ethanol extracted by simple distillation (Aresta *et al*, 2001).

3.6. Characterization Of Physicochemical Properties By Ethanol Extracted

3.6.1. Determination of density of ethanol extracted

Density at 20℃ of a material is the mass unit volume of the material at 20℃. It is expressed in gram per milliliter. Density of the ethanol extracted was determined at Addis Ababa, ESA, in ECAE chemical laboratory, by Ethiopian Standard ES ISO 758:2001-reaffirmed in 2017 Pychnometer, flask, type 3(Guy-Lussac, ISO 3507 identical with ISO 758: 1976), made of glass and of a size and type which is suitable for use with the material under use. Density determination by pycnometer is a very precise method. This uses a working liquid with well-known density, such as water. The pycnometer is a glass flask with a close-fitting ground glass stopper with one capillary hole through it. This fine hole releases a spare liquid after closing a top-filled pycnometer and allows it for obtaining a given volume of measured and/or working liquid with a high accuracy. Clean and dry Pycnometer flask and weigh it with its stopper to the nearest 0.001g filled with freshly distilled and cooled distilled water, and

determine the apparent mass of the content, previously brought to 20 ± 0.1℃ in the water bath and then empty, clean and dry the flask, fill it with the sample under test and determine in a similar manner the apparent mass of sample contained in the flask at 20℃.the density of the sample at 20℃, in gram per milliliter is given by the formula:

$$\mathbf{PEtOH} = \frac{M1+A}{M2+A} \text{ ..... (3.6}$$

Where: PEtOH is density of ethanol analyzed in g/ml

M1 is apparent mass, in grams of the sample required to fill the flask at 20℃

M2 is apparent mass, in grams of the water required to fill the flask at 20℃

P is density of water at 20℃ = 0.9982g/ml

A is buoyancy correction = p_a X m_2, Where p_a is the density of air = 0.0012 g/ml

3.6.2. Determination of specific gravity of ethanol extracted

Specific gravity (relative density) SG is the ratio of the density of a substance to the density of reference substance normally water. Specific gravity of the ethanol extracted was determined at Addis Ababa, ESA, in ECAE chemical laboratory, by (Ethiopian Standard ES ISO 758: 2001-reaffirmed in 2017 and AOAC 2000), in a similar manner with that of density with the relationship that can exists between density and specific gravity (Pychnometer flask, type 3(Guy-Lussac), ISO 3507 identical with ISO 758:176). Specific gravity is dimensionless unit define as the ratio of density of a substance to density of water *a*t a specified temperature 20℃ in air and can be expressed as:

$$\mathbf{SG_{EtOH}} = \frac{\rho EtOH}{\rho H2O} \text{ ..... (3.7)}$$

Where: SG_{EtOH} is specific gravity of the substance in air at 20℃

ρ_{EtOH} is density of the extracted ethanol in g/ml

ρ_{H2O} is density of water normally in air at 20℃ in g/ml

3.6.3. Determination of dry residue (total dissolved solid) of ethanol extracted

Dry residue (total dissolved solid) of the ethanol extracted was determined at Addis Ababa, ESA, in ECAE analytical chemical laboratory by the Ethiopian Standard ES ISO 759:2001-reaffirmed in 2017, AOAC 2000.The test portion ,100 ml the ethanol sample to be tested was taken and introduced to platinum dish of capacity 150 ml heated in water bath maintained at temperature appropriate to the boiling point of the product under examination for 2 hours on an electric oven capable of being maintained at 110±2℃.The process was

repeated until constant mass was obtained; the dry residue obtained from sample under study is given by the formula given below (Johnston *et al*, 2004).

$$TDS = m_1 - m_2 \qquad (3.8)$$

Where: TDS is the total dissolved solid of ethanol sample

m_1 is the mass, in gram of empty dish

m_2 is the mass, in gram, of dish plus residue

3.6.4. Determination of pH of extracted ethanol

The pH of ethanol extracted was determined according to Association of Analytical Chemists (2000 AOAC), at Addis Ababa, in ECAE chemical laboratory, by the method of Ethiopian Standard, ES 827:2002-reaffirmed in 2012. The pH was determined instrumentally with digital pH meter (Model Hanna instrument), monitoring of the percentage of the alcohol strength %(v/v) (Silva and Swarnakar, 2007).

3.7. Secondary (Acetous Fermentation) To Produce Acetic Caid (Vinegar)

The extracted banana peel ethanol was subjected to an aerobic fermentation for seven days by adding commercial 30ml "selam acheto"to 600ml of extracted ethanol as a soure of acetic acid producing bacteria.The solution was kept in an incubator at 30℃for 10 successive days in a clean and cotton-closed galss bottle by permtting the enterance of suficeint oxygen as acetobacteria needs oxygen to properly oxidize ethanol to acetic acid (Xibib *et al*, 2003).

3.8. Characterization Of Extracted (Vinegar) And Commercial Vinegar (Selam Acheto)

Physicochemical properties of extracted acetic acid(vinegar), density at 27℃ in g/ml, specific gravity in air at 27℃, acid concentration, total dissolved solid and pH were characterized at ESA, Addis Ababa in ECAE laboratory by specification/test method: Indian Standard(IS 695-1986) for both acid concentration as acetic acid by mass % (m/m) and total dissolved solid %(m/m), Indian Standard(IS 82-1973) for the specific gravity in air at 27℃,the density by pycnometer and pH of the solution by digital pH meter.

3.8.1. Determination of acetic acid content (acid concentration, % by mass)

Acetic acid concentration was determined at Addis Ababa, ESA in analytical laboratory of ECAE by Indian standard specification for acetic acid, third edition, IS: 695-1986, Appendix A(Method of test for acetic acid).phenolphthalein indicator solution by dissolving 0.1gm of phenolphthalein in 100ml of 60% ethylene alcohol and adding solution of sodium hydroxide (1N) until the color turns faint pink. Find the mass of acetic acid on the basis that, 1ml of

sodium hydroxide solution is equivalent to 0.06005g of acetic acid (CH_3COOH) and deduct the acetic acid content equivalent of any formic acid present as determine formic acid test specification as:

$$\textbf{Acetic acid } (CH_3COOH)\textbf{, percent} = \frac{6.005VN}{M} - 1.299S \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.9)$$

Where V= volume in ml of the standard sodium hydroxide solution used,

N =normality of standard sodium hydroxide solution,

S = percentage of formic acid as determined in determination of formic acid

M = mass in gram of the material taken for the test

3.8.2. Determination of total dissolved solids in both the extracted and selam (vinegar)

Total dissolved solid,% by mass, of extracted vinegar was determined at Addis Ababa, ESA in analytical laboratory of ECAE by Indian standard, specification for acetic acid, third edition, IS: 695-1986, Appendix A (Method of test for acetic acid).

3.8.3. Determination of pH and density of extracted vinegar

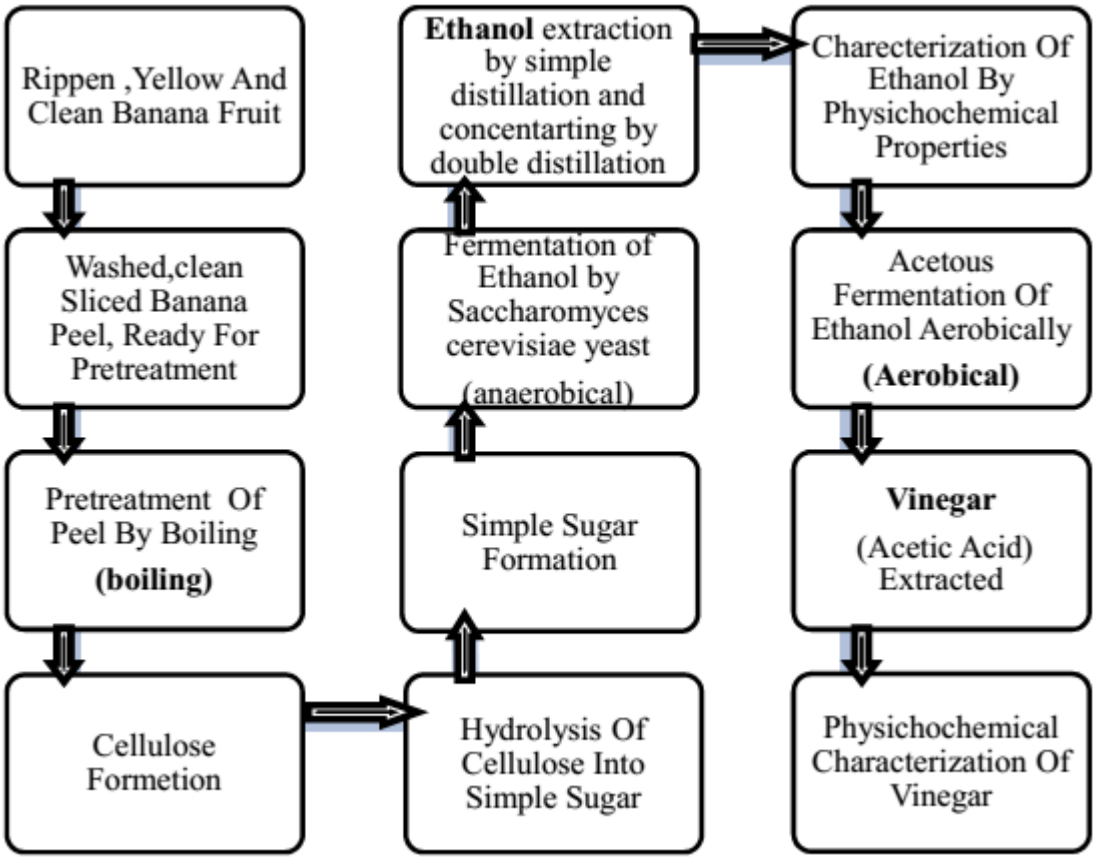
The pH and density of extracted acetic acid (vinegar) were determined at Addis Ababa, ESA, in analytical laboratory, ECAE by digital pH meter and pycnometer respectively in the same as in the case of extracted ethanol.

3.8.4. Determination of specific gravity of extracted vinegar

The specific gravity of the extracted acetic acid (vinegar) was determined at Addis Ababa, ESA in analytical laboratory of ECAE by Indian standard specification for acetic acid, third edition, IS: 82-1973, Appendix A Method of test for acetic acid)

3.9. The Basic Steps For Conversion Of Sugar To Vinegar (Acetic Acid)

The conversions of cellulose polymers to ethanol involves two different processes converting cellulose to glucose units via hydrolysis and the sugars produced from the hydrolysis process can then be converted to ethanol, fermentation using *Saccharomyces cerevisiae*. (Endo *et al*, 2008).



Scheme 3.2.Conversion of sugar from waste of banana peel to vinegar (acetic acid)

CHAPTER FOUR

4. RESULT AND DISCUSSION

4.1. Characterization Of Banana (*Musa Sapientum*) Peel

4.1.1 Characterization of proximate analysis of banana peel (*Musa sapientum*)

Table 4.1. The result proximate analysis of banana (*Musa sapientum*) peel sample

S.NO:	Proximate composition Of the sample of banana peel	Results of proximate analysis and their references			
		Present study	Previous studies		
			Anhwange, 2009	Abdullah, 2014	Tumutegyereize, 2011
1	Total solid (%)	10.89±1.65%	10.23±2.50%	9.46±1.20%	13.87-22.41%
2	Moisture content (%)	9.45±1.89%	6.70±2.22%	11.56±0.06%	8.75-11.25%
3	Volatile matter (%)	84.64± 1.33%	88.02±1.33%	85.26±1.12%	83.66-91.90%
4	Ash content (%)	8.35±1.46%	8.50±1.52%	9.28±0.22%	7.89-11.90%
5	Fixed carbon content (%)	2.45±0.49%	2.70±0.78%	2.70±0.07%	0.99-.540%

According to different previous three researchers: Anhwage (2009), Abdullah,(2014) and Tumutegyereize (2011); the total solid, moisture content, volatile matter, ash content and fixed carbon content of different banana peels were reported as given in Table 4.1. The results of these studies are in a comparable range with the literature values as reported by the above mentioned three different researchers. Hence these shows that the banana peels under study can be concluded of good quality which can be a substrate as extraction of both ethanol and vinegar. Moisture content is a measure of the amount of water in the banana peel. Moisture content analysis used for the determination of the rough proportionalities of solids to liquids ratios in the pretreatments and hydrolysis process and increasing moisture content affects the product quality. The moisture content analysis is again is used to determine other properties such as, ash content, volatile matter and fixed carbon content. The sample of banana peel with higher moisture content needs more heat of moisture vaporization. Ash is a measure of

inorganic impurities in the banana peel. Ash is the mineral residue obtained after combustion of organic materials. The composition of ashes depends on the source, species of plant material and the nature of the soil where the plants grow a range of 6.3 to 12.0% ash contents of banana peels were observed in some varieties of *Musa* species (Babeyemi *et al,* 2010). Finally, fixed carbon content (FC) if the carbon found in the in the mineral, which is left after volatile matter are driven off, which is used in the determination of carbon in the banana peel (Motoya and Gomez, 2014).

4.1.2. Extraction of ethanol alcohol

4.1.2.1. Amount of sugar released during primary fermentation

Table 4.2. Amount of sugar released during successive days of primary fermentation

Number of days during primary fermentation	D1	D2	D3	D4	D5	D6
Amount of sugar released in g/100gm	4.4	5.7	6.1	7.6	8.1	5.2

During primary fermentation (Saccharification), the amount of sugars released was observed and found, from day one to fifth day increase in the release of sugar was a rapid conversion of sugar to ethanol by the yeast *Saccharomyces cerevisiae* during primary fermentation of the substrate due to favorable condition. This drop released was attributed largely to the uncontrolled accumulation of other metabolites unknown, which might have interfered with the production of reducing sugars which is in line with the study by Gaddafi *et al*, (2016).

4.1.2.2. Amount of ethanol produced during fermentation

Table 4.3. Amount of ethanol produced during six successive days of primary fermentation

Number of days during fermentation	D1	D2	D3	D4	D5	D6
Ethanol produced during fermentation in g/100gm	4.2	5.5	6.1	7.5	8.1	7.1

During the primary fermentations (Saccharification), the amount of ethanol alcohol produced was observed and found to be, from day one through day five, there was a steady increase in releases of ethanol productions were observed during Saccharification with the highest amount of ethanol amount on the fifth day and a slight decrease was observed of the sixth day. The result of the total ethanol alcohol produced in terms of g/100gm during primary fermentation was recorded in Table 4.3 as shown above.

4.1.2.3. The results of pH values during primary fermentation

Table 4.4. Results of pH values recorded during six successive days of primary fermentation

Number of days for primary fermentation	D1	D2	D3	D4	D5	D6
pH during successive days of primary fermentation	5.3	5.4	5.14	5.2	5.4	5.2

The pH was set to 5.0–5.5 (which is the optimum pH for the activity of *S. cerevisiae*) by the addition of 1 M NaOH and conc. H_2SO_4. The samples were checked every day by adjusting pH to be between 5.0 and 5.5, which was in line with Sharma *et al*, (2007). The pH in the range of 5.0-5 5 was suitable for the production of ethanol under anaerobic condition as tested by Mohapatra *et al.(*2010).Therefore; the result of the ethanol produced during six successive days of fermentation of this study was in a comparable range of the previous studies.

4.1.2.4. Extracting and concentrating of ethanol distillation

Distillation is one of the oldest and still most common methods for both the purification and the identification of organic liquids. It is a physical process used to separate chemicals from a mixture by the difference in how easily they vaporize. As the mixture is heated, the temperature rises until it reaches the temperature of the lowest boiling substance in the mixture, while the other components of the mixture remain in their original phase in the mixture. The resultant hot vapor passes into a condenser and is converted to the liquid, which is then collected in a receiver flask. The other components of the mixture remain in their original phases until orderly the most volatile substance has all boiled after the fermentation; distillation is the most important step for the quality of distilled beverages and according to the components' volatility power. It is possible to isolate the volatile (water, ethanol and others) components from the non-volatile ones (suspended solids, minerals, yeast cells, non-fermentable sugars, proteins, (Aresta, 2001).

4.1.2.5. The effect of distillation type on yield of ethanol strength (%v/v)

Table 4.5. Distillation type and the corresponding yield of ethanol (v/v %)

Samples distillate	S1	S2	S3	S4	S5	S6	S7	S8	S9
ethanol yield in simple distillation	5.4	4.8	7.4	6.5	5.8	8.8	7.8	7.4	9.8
ethanol yield in double distillation	6.8	6.4	8.7	7.4	6.4	10.5	13.5	12.8	14.8
Temperature in ℃	77-79	77-79	77-79	77-79	77-79	78-79	78.5	78.3	78
Time in minutes	60	60	60	60	60	45	40	30	30

From Table 4.5, one can see that as the concentration of bioethanol produced by simple distillation was consistently increased from 5.4% to 9.8% (v/v), the concentration of ethanol produced by double distillation from fermented banana peel also correspondingly increased consistently from 6.8% to 14.8%, this is because ,as the produced distillate by the simple distillation was redistilled by double distillation process, the amount of water contents of the distillate was decreased, and correspondingly ,decreasing the amount of amount of water in the distillate results in increasing of the concentration of the bioethanol of the distillate, and thus ethanol content(yield of ethanol v/v% in double distillation) of each sample after double distillation were greater than that of simple distillation (Bruno *et al*, 2007).The distillate shows a high alcoholic content (65-70% v/v) at the beginning of the process and a separation of 5-10% of the total theoretical volume of spirit by the initial distillation is recommended. This is known as the "head" distillate, rich in aldehydes, ethyl acetate, fatty acids, ethyl caprate and ethyl caprylate (Bruno *et al*, 2007) and other volatile compounds that have greater affinity for ethanol than for water, a factor of greater significance than the individual boiling temperature (Leaute,1990). Other undesirable secondary products in concentration higher than the recommended formed during the fermentation or inside the alembic itself (Belincanta *et al*, 2006). The "heart fraction" represents about 80% of distillate volume. Since it contains the smallest amount of undesirable substances, this is best fraction. In practice, usually around 45-50% volume ethanol can be obtained by double distillation (Leaute, 1990).

4.1.3. Physicochemical characterization of extracted ethanol

The result of the physicochemical analysis of extracted ethanol including pH, density (g/ml), specific gravity, ethanol strength % (v/v) and total dissolved solid % (m/m) which were

analyzed at ESA in the laboratory of Ethiopian Conformity Assessment Enterprise, ECAE, at Addis Ababa as tabulated below..

Table 4.6. Result of Physicochemical Characterization of Ethanol Extracted from Banana Peel

S/NO:	Physicochemical characteristics tested	Specification or test method	Test result
1	Density in air at 20℃, in g/ml	ES ISO 758	0.9340
2	Ethanol strength, %(v/v)	ES 615(annex A) and ES 827:2002	49.2200
3	Residue on evaporation (total solid),%(m/m)	ES ISO 759	0.0058
4	Specific gravity	ES ISO 758	0.9332
5	Ph	pH meter	4.7200

The strength,% (v/v) of ethanol extracted by double distillation from fermented banana peel in the study was determined by Ethiopian Conformity Assessment Enterprise, ECAE, at Addis Ababa by standard method ES 615:2001(Annex A) and ES 827;2002 of ethanol specification The density of the extracted ethanol 0.9350 g/ml is comparable with the density of ethanol extracted from fruit which is 0.9320 comparable with the study by Abdullah *et al*, (2014) the specific gravity 0.9332 is also comparable with the specific gravity of ethanol produced from fruit which is 0.9285 comparable with the study by Bruno, (2007). The pH of the extracted ethanol 4.7200 is in a comparable range of pH of ethanol from fruit which is 4.00 to 7.00 comparable with the study by Abdullah *et al*, (2014).the total dissolved solid of the extracted ethanol 0.0058 is insignificant and can be consumable as from FAO. And the strength of ethanol 49.22 % (v/v) is also in a comparable with the commercial ethanol which was acceptable as Leaute, (1990).

4.1.4. Characterization of extracted (vinegar) and commercial vinegar (selam acheto)

Physicochemical properties of the extracted acetic acid (vinegar), like density at 27℃ g/ml, specific gravity in air at 27℃, acetic acid concentration, total dissolved solid and pH were characterized at ESA, Addis Ababa in ECAE laboratory by specification/ test method: Indian

Standard (IS 695-1986) for both acid concentration as acetic acid by mass % (m/m) and total dissolved solid% (m/m) Indian Standard (IS 82-1973) for specific gravity in air at 27℃ density by pycnometer and pH of the solution by Hanna instrument, digital pH meter. From 6kg (6000g) banana peel 6Litters (6000ml) banana juice was prepared ,700ml of ethanol extracted and 370ml of vinegar finally obtained.yhe yield of ethanol was 17.8%(v/m) and that of vinegar was 52.8%(v/v) which was in line with the literature.

Table 4.7.Physicochemicalcharacterizations of both extracted and selam vinegars

S/NO:	Physicochemical characteristics Tested	Specification or test method	Comparison of extracted and commercially obtained acetic acids	
			Extracted Acetic acid (Vinegar)	Selam acheto Acetic acid (Vinegar)
1	Density in air at 27℃, in g/ml	By Pychnometer	1.0013	1.0012
2	Specific gravity in air at 27℃	IS 82-1973	1.0028	1.0025
3	Acid concentration (as acetic acid), %(m/m) by mass	IS 695-1986	3.3400	3.3100
4	Total dissolved solid %(m/m) by mass	IS 695-1986	0.0650	0.0572
5	pH of solution	By pH meter	2.6200	2.6500

The pH of the vinegar during the secondary fermentation was observed to decrease from pH 4.72 (of the extracted ethanol in primary fermentation) to pH 2.65(of the extracted acetic acid in secondary fermentation/acetification); which shows an increase in acidity. This increase in acidity provided optimal growth conditions to initiate acetification. This fall in pH can be attributed to accumulation of acetic acid and other volatile short chain organic acids such as propionic, tartaric and butyric acids, which are important in development of the flavor and aroma of vinegar. The vinegar produced had a pH of 2.26 and 3.34% total acidity (acetic acid). This fell below the 5% to 6% acetic acid for wine vinegars and 4% to 7% total acidity for distilled vinegars as reported by This could be attributed to interruption of air supply

during the oxidation process as acetic acid bacteria are reported to be very susceptible to air interruption during oxidative processes and could also be as a result of incomplete oxidation of the ethanol to acetic acid. It could also be probably dependent on the type of selam acheto used as the acetification starter (as unpasteurized vinegar), may have very less amount of Acetobacteria living cells which are responsible for complete oxidations of ethanol to acetic acid (vinegar) in relation to Byarugaba *et al*, (2014).

On the other hand, previous study reveals that the lowest and highest possible values of pH of vinegar extracted from different fruit at different country by fermentation were 2.64 $\pm$ 0.01 and 3.21 $\pm$ 0.00 respectively and the lowest and highest possible values of acid concentration, % (v/v) of vinegar extracted from different fruits at different countries by acetfication were 2.14$\pm$0.03 and 6.30$\pm$0.06 respectively (Kyung Jang, 2015).Therefore results, pH and acid concentration, of vinegar extracted from banana peel in this study were in a comparable range with the literature values as reported by the above researcher. Even though the fermented banana vinegar yet reached an acid level of 5% to 6% or 4% to 7% and could thus not strictly be called vinegar. The alcohol content in the extracted banana peel, which was 49.22% (v/v) ethanol, was however, high enough to reach sufficient acidity. But probably the time taken for acetification, the selam acheto used as source of acetobacteria and also the methods used were not optima. However, the waste banana peels have potential for vinegar production, if all the process and the sources of Acetobacteria are improved. The rest of the physicochemical properties the acid; density, specific gravity and the total dissolved solid depend on the two main properties of acetic acid (pH and acid concentration).

CHAPTER FIVE

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

The banana ethanol production process took 7 days and had physiochemical characteristics of 49.22 % (v/v) alcohol strength and pH of 4.72 which complied with the standard ranges of banana peel ethanol after complete fermentation. This study therefore, showed that a banana peels can be used as an ideal substrates for production of good quality ethanol. This not only increase the economical and food value of *Musa sapientum* but also provides a way of utilizing banana waste in Ethiopia.

The massive utilization of an ethanol in the world requires that its production technology be cost-effective and environmentally sustainable. The current research tendencies for improving ethanol and vinegar production are linked to the nature of employed raw materials, processing steps, and related process engineering issues. Agro-processing technoeconomic activities are to be generated for conservation and handling of waste agro-perishable agricultural product and make it usable as food. In this present study, efforts were made to extract ethanol and vinegar banana fruit wastes as potential raw material for ethanol and vinegar production and the results showed that banana peel fermentation yield 49.22% of ethanol and similarly 3.34% vinegar. The high non structural carbohydrates, reserve starch content showed the potentiality of bananas peel as a good source of ethanol and vinegar production.

The above results show that agriculture residues including modified banana peels products can be efficiently and cost effectively used ethanol and vinegar production. The use of these agro-wastes in ethanol and vinegar production will not only help in the reduction of environmental pollution associated with them but will also convert them into valuable products which will be helpful in the economic growth of the community at large. Eco-friendly methods of waste management of converting waste of banana peel to value added product like vinegar which is very essential in food preserving and salad dressing and accepted, used by all class of society in any religion could be effectively tuned for the production of commercially important bio products at reduced cost for the benefits of society.

5.2. Recommendation

- Further researches have to be carried out to increase the yield of ethanol from waste of banana peel by the use of other organisms which are capable of converting the 5- and 6- carbon sugar into ethanol by alcoholic fermentation and consequence conversion of ethanol to banana peel vinegar upon continuous acetification.
- Further investigation should be done to analyze the potential of vinegar production from the waste of banana peel using oxidizing agent other than unpasteurized vinegar as it seems to be difficult to easily get unpasteurized vinegar
- Further researches should be done on the residue of banana peel left after pretreatment of boiling to prepare banana peel juice can be effective organic fertilizer as solution and solid form on further treatments

REFERENCES

Abdullah,N.,Sulaiman,F.,Miskam, M. A.,and Taib, R.M.(2014). Characterization of Banana (*Musa spp*.) Pseudo Stem and Fruit Bunch Stem as a Potential Renewable Energy Resource.*International Journal of Biological,Veterinary, Agricultural and Food Engineering*, *8* (8), 712-716.

Anhwange. A, Ugye, J. and Nyiaatagher D. **(2009)**. *Electronic Journal of Environment, Agricultural and Food Chemistry, 8*(6), 437-442.

Aresta, M., Boscolo, M. and Franco, D.W. **(2001).** Copper(II) catalysis incyanide conversion into ethyl carbamate in spirits and relevant reactions. *Journal of Agricultural and FoodChemistry.,49*, 2819-2824.

Asli, M.S**.(2010)**."A study on some efficient parameters in batch fermentation of ethanol using *Saccharomyces cerevesiae* extract from fermented siahe sardasht pomace". *African Journal of Biotechnology , 9* (20), 2906-2912.

Aurore, G., Parfait, B. and Fahrasmane, L.,**(2009).** Bananas, raw materials for making processed food products. *Trends in Food Science and Technology*, *20*(2), 78-91.

Babayemi,J.O.,K.T.Dauda, A.A.A. Kayode, D.O. Nwude, J.A. Ajiboye, E.R. Essien and O.O. Abiona, (**2010).** Determination of potash alkali and metal contents of ashes obtained from peels of some varieties of Nigeria Grown Musa species. *Bioresource, 5*,1384-1392

Bagavan A.,**(2011).** In vitro antimalarial activity of medicinal plant extracts against Plasmodium falciparum. *Parasitology Research*.9,55-62.

Bambalov,G.,Hr.Spasov and E.Kocheva,**(2000).**Comparative study of dry yeast.*Vine-growing and Wine-making, 4*, 10-13.

Bardiya N, Somayaji D and Khanna S. **(1996)**. Biomethanation of banana peel and pine-apple waste. *Bioresour Technol, 58,* 73–76.

Belincanta,J.,Kakuta Ravagnani,T.M. and Pereira, J.A. F.**(2006).** Hydrodynamic and tray efficiency behavior in parastillation column. *Brazilian Journal of Chemical Engineering , 23*, 235-146.

Byarugaba-Bazirake,W Byarugaba,M Tumusiime and D.A Kimono. (January, **2014)**. The technology of producing banana wine vinegar from starch of banana peels. *African Journal of Food Science and Technology (ISSN: 2141-5455) , 5* (1), 1-5.

Bhat, M.S.,Prabhakar, A.,Rama, K. R. R., Madhu, G. M. and Rao, G. H. **(2010)**. Statistical optimization and neural modeling of amylase production from banana peel using Bacillus subtilis MTCC 441. *International Journal of Food Engineering, 56*, 34-45.

Brooks, A.A.**(2008)**. Ethanol production potential of local yeast strains isolated from ripe banana peels. *African Journal of Biotechnology, 7*, 3749-3752.

Bruno,S.N.F.,D.S.Vaitsman,C.N.Kunigami and Brasil,M.G.**(2007)**.Influence of the distillation processes from Rio de Janeiro in the ethyl carbamate formation in Brazilian sugar cane spirits,. *Food Chemistry., 104* (4),1345-1352.

Chaparadza and JM Hossenloppt.**(2012).** adsorption kinetics, isotherms and thermodynamics of atrazine removal using a banana peel based sorben. *Water Science Technology, 65*(5), 940-947.

Chiranjeevi,T.,Hari Prasad,O.,Navya, A.,Praveen,C.V.R.,Nanda,K.,Ismail,S.M.,Uppu,V. P., Sudheer,A.,Narasimha,V.K.,Matcha, B. **(2013).** Isolation and characterization of ethanol tolerant yeast strains. *J. Bioinformation., 9* (8), 421-425.

Cho and D.G.Peterson, **(2010).**"Chemistry of bread aroma: A review ".*Food Science and Biotechnology,19* (3), 575–582.

Colin,M.,Jennife,M.,Lopes,D.E.andMiguel,J.V.**(2006)**. Generation of novel wine yeast strains by adaptive evolution. *Am. J. Enol. Vitic , 57*, 423-430.

Conner Ha.and Allgeier Rj.**(1976).**Vinegar:its history and development. *Adv. Appl. Microbiol, 20*, 81-133.

Damila R. Morais, Eliza M. Rotta, Sheisa C. Sargi, Elton G. Bonafe, Rúbia M.Suzuki, Nilson E.Souza, Makoto Matsushita and Jesuí V.Visentainer.**(2017)**. Proximate Composition, Mineral Contents and Fatty Acid Composition of the Different Parts and Dried Peels of Tropical fruits cultivated in Brazil. *J.Braz.chem.sco., 28* (2), 308-311.

De Ory L, Romero LE. and Cantero D.**(1999)**. Maximum yield acetic acid fermenter. *Bioprocess Engineering, 21*, 187-190.

Doran,I.,San, B. and Kaya,Z.**(2016)**.Journal of Environmental Biology/Academy.*'The Effects Of Compost Prepared From Waste Material Of Banana Plants On The Nutrient*

Emaga,T.H.Robert,C.,Ronkart,S.N.,Wathelet,B.and Paquot,M**.(2008)**.Dietarfiber component and pectin chemical features of peels during ripening in banana and plantain varieties. *Bioresource Technology*., 99 ,4346-4354

Endo, T. Nakamura, A. Ando, K. Tokuyasu, and J. Shima,. **(2008)**. Genome-wide screening of the genes required for tolerance to vanillin, which is a potential inhibitor of bioethanol fermentation, in Saccharomyces cerevisiae. *Biotechnol. Biofuels, , 1* (1), 3.

Essien,J.P.,Akpan,E.J.and Essien,E.P.**(2005).**Studies on mould growth and biomass production using waste banana peels. *Bioresource Technology, 19*, 361-363.

Fadel and M.S Foda.**(2001)**.Anovel approach for production of highly active. Baker'syeast from fodder yeast, abyproduct from ethanol production industry.*Online Journal of Biological Sciences, 1* (7), 614-620.

Gaddafi I.Danmaliki, Auwal M. Muhammad, Abdullahi A. Shamsuddeen and Bashir J.Usman.**(2016).** Bioethanol Production from Banana Peels. *Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT) e-ISSN: 2319-2402,p-ISSN: 2319-2399. 10* (6,ver.II), 56-62.

Gelinas,.**(2012)**."In search of perfect growth media for Baker's yeast ´production: Mapping patents.*" Comprehensive Reviews in Food Science and Food Safety, 11* (1), 13–33.

Girish, H.V and S.Satish.**(2008).**Antibacterial activity of important medicinal plants on human pathogenic bacteria-a comparative analysis. *World Application of Science Journal, 5*(3), 267-271.

Hai-Yan Sun, Li Juanhua, Pingjuan Zhao, Ming Peng **(2011).** Banana peel A novel substrates for cellulase production under solid-state fermentation. *African Journal of Biotechnology*, *10* (77), 1788.

Hahn Hägerdal, K.Karhumaa,C.Fonseca, I.Spencer Martins, and M.F.Gorwa Grauslund,l. **(2007)**. "Towards industrial pentose fermenting yeast strains.,"App. *Microbiol. Biotechnol.,74* (5), 937–53.

Hammond, J.R.M. **(2016)**. Microscopes, microbes and manipulation: 35 years of brewing. *J. Am. Soc. Brew.Chem , 74*, 157–172.

Hashimoto M, Obara K, Ozono M, Furuyashiki M, Ikeda T,Suda Y, Shigehisa H.**(2012)**. Separation and characterization of the immunostimulatory components in unpolished rice black vinegar (Kurozu). *J. Biosci. Bioeng. , 21*, 1729-1734.

Jimenez and Benitez, **(1986).** Characterization of wine yeasts for ethanol production. *Appl. Microbiol. Biotechnol, 25*, 150-154.

Johnston C,Cindy Kim. and Buller A.**(2004)**.Vinegar Improves Insulin Sensitivity to a High Carbohydrate Meal in Subjects With Insulin Resistance or Type 2 Diabetes. *Diabetes care, 27*, 272–290.

Johnston, C. S. **(2005).** Strategies for healthy weight loss: from vitamin C to the glycemic response. *Journal of American College Nutrition,, 24*, 15865.

Johnston C.S., Quagliano S.and White S. **(2013).**Vinegar ingestion at mealtime reduced fasting blood glucose concentrations in healthy adults at risk for type 2 diabetes. *J. Funct. Foods, 5*, 2007–2011.

Jusoh M. L.C., Manaf L. A., and Latiff P.A.**(2013).** Composting of rice straw with effective microorganisms (em) and its influence on compost quality. *Iranian journal. Environ. Health science, 10*(1), 17.

Kalemelawa.E.Nishihara,T. Endo, Z. Ahmad, R.Yeasmin, MM Tenywa, and S.Yamamoto, . **(2012).**"An evaluation of aerobic and anaerobic composting of banana peels treated with different inoculum for soil nutrient replenishment". *Bioresource Technology, 126*, 37.

Kyung Jang, Mee Youn Lee, Hyang Yeon Kim, Sarah Lee, Soo Hwan Yeo, Seong Yeol Baek, and Choong Hwan Lee.**(2015).** Comparison of Traditional and Commercial Vinegars Based on Metabolite Profiling and Antioxidant Activity. *J. Microbiol. Biotechnol. , 25* (2), 217-226

Lee,W. Chen, I.Chang, C. and Yang, S.**(2012)**. Bioethonalproduction from sweet potato by coimmobilization of saccharolytic molds and Saccharomyces cerevisiae. *J.Renewable Energy , 39*, 216-222.

Leaut,**(1990).** Distillation in alambic. *American journal of enology and viticulture, 41*, 90-103.

Loganayaki N.**(2010)**. Antioxidant capacity and phenolic content of different solvent extracts from banana (Musa paradisiaca) and mustai (Rivea hypocrateriformis). *Food and Science Biotechnology*.

Manikandan,V Saravanan and T Viruthagir .(January **2008**). Kinetics studies on ethanol production from banana peel waste using mutant strain of Saccharomyces cerevisiae . *Indian Journal of Biotechnology , 7*, 83-88.

Mohapatra D, Mishra S, Singh CB and Jayas DS. **(2011)**. Post-harvest Processing of Banana. *Opportunities and Challenges Food Bioprocess Technol, 4*, 327–339.

Mohapatra, D.,Mishra, S. and Sutar, N.**(2010)**. Banana and its by product utilization: an overview. *Journal of Scientific and Industrial Research, 69*, 323-329.

Morales Ml, Gustavo A, Gonzalez Jose A, and Troncoso Ana M. **(2001).** Multivariate analysis of commercial and laboratory produced sherry wine vinegar: influence of acetification and aging. *Journal of food technology, 212*, 676-682.

Motoya Gomez.N.**(2014)**.Study of pertreatment for an enhanced bioethanol production from the organic fraction of municipal solid wastes. *global NEST journal , 16* (6), 1091-1099.

Nanda, K., Taniguchi, M., Ujike, S., Ishihara, N., Mori, H., Ono, H and Murooka, Y. **(2001)**. Characterization of acetic acid bacteria in traditional acetic acid fermentation of rice vinegar (komesu) and unpolished rice vinegar (kurosu) produced in Japan. *Applied and Environmental Microbiology , 67*, 986-990.

Oleskowicz-Popiel,.Lisiecki,J.B.Holm-Nielsen,A.B.Thomsen,andM.H.Thomsen.**(2008)**. Ethanol production from maize silage as lignocellulosic biomass in anaerobically digested and wet-oxidized manure. *Bioresource Technology , 99* (13), 5327-5334.

Robinson, and Galán Saúco,V.(2010). *Bananas and plantains.* CABI:Wallingford (GBR).

Rodriguez de Sotillo Hadley M,and Holm T.,**(1999).** *Journal of Food Science, 59*, 1031-1033.

Sampath Kumar .**(2005)**. Bananas,The world's healthiest foods. *online publication. K.P.*2012.

Sampath Kumar KP, Debjit Bhowmik, Duraivel S.and Umadevi M. (**2012)**.Traditional and Medicinal Uses of Banana. *Journal of Pharmacognosy and Phytochemistry, 3*(1), 51-60.

Sassner, Galbe, and Zacchi, G.**(2008).**Techno economic evaluation of bioethanol production from three different lignocellulosic materials. *Biomass Bioenergy , 32*, 422-430.

Serio, Tesse and Santacsaria.,**(2001)**. Akinetic and mass transfer model to simulate the growth of baker's yeast in industrial bioreactors.*Chemical Engineering Journal, 82* , 347-354

Seyram, Ameyapoh,Y. Karou, and De Souza, **(2009).** Study of pineapple peelings processing into vinegar by biotechnologies. *Pakistan Journal Biology Sciences, 12*,859-865.

Sharaghat, H.,Najafpour, G.D., Rezaei, P.S. and Sharifzadeh, M. **(2010)**.Optimal growth of Saccharomyces cerevisiae (PTCC 24860) on pretreated molasses for the ethanol production: The application of the response surface methodology. *Chemical Industry and Chemical Engineering,quarterl,16* (2),19920.

Sharma, K..L.,Kalra, H.S. Oberoi, S. **(2007)**. Bansal Optimization of fermentation parameters for production of ethanol from kinnow waste and banana peels by simultaneous saccharification and fermentation. *Indian J. Microbiol., 47*, 310-316.

Shymala,D.C.,Belagali,S.L.**(2012)**. Studies on variations in physico chemical and biological characteristics at different maturity stages of municipal solid waste compost. *International journal of environmental sciences, 2* (4), 1984-1997.

Silva and Swarnakar R Cashew.**(2007).**Wine Vinegar Production: Alcoholic and acetic fermentation. *Brazillian Journal of Chemical, 24*, 163–169.

Singanusong, R.,Worasit,T.,Teeraporn, K. and Chiraporn, S., **(2013)**.Extraction and properties of cellulose from banana peels. *Journal of Science andTechnology., 21* (3), 201-213.

Soltan sahar S.and Shehata MMEM .**(2012)**. Antidiabetic and Hypocholestrolemic Effect of Different Types of Vinegar in Rats. *Life Science Journal, 9*, 2141–2151.

Souza, C. S.,Oliveira, K.F.,Trevisan, H.C. and Laluce,C.**(2007)**. A strategy to compare yeast strains and improve cell survival in ethanol production processes above 300C, Communic.Current Res. and Educ. *Topics and Trends in Appl. Microbiol., A. Méndez-Vilas(Ed)* , 410-417.

Sree,N.K.,M.Sridhar,L.V.Rao,andA.Pandey.**(1999)**. Ethanol production in solid substrate fermentation using thermotolerant yeast. *Process Biochemistry , 34*, 115-119.

Sulaiman F,Yusof M, Eldeen M, Seow M, Sajak A, Supriatno, Ooi, L, **(2011).** *Journal of Food Composition and Analysis, 24*, 1–10.

SumanVikas Bhat, Rehana Akhtar and Tawheed Amin.**(2014).** An Overview on the Biological Production of Vinegar. *International Journal of Fermented Foods, 3* (2), 139-155.

Tchobanoglous,G.,Theisen, H. and Vigil, S. **(1993)**. Integrated solid waste management: Engineering principles and management issues. *MCGraw-Hill, NewYork* , 3 –22.

Thomas, K.C.,S.H. Hynes, and W.M. Ingledew.**,(2002).** Influence of medium buffering capacity on inhibition of Saccharomyces cerevisiae growth by acetic and lactic acids. *Appl. Environ. Microbiol: , 68*, 1616-1623.

Tumutegyereize., F. I. Muranga , J. Kawongolo and F. Nabugoomu **(2011)**. Optimization of biogas production from banana peels:Effect of particle size on methane yield. *African Journal of Biotechnology , 10* (79), 18243-18251.

Vivekanand, P. Dwivedi, N. Pareek and R .P Singh. **(2011)**. Banana peel:A potential substrates for laccase production by Aspergillus fumigatus in solid-state fermentation. *Applied Biochemistry and Biotechnology , 165* (1), 204-20.

Valmayor R V, Jamaluddin S H, Silayoi B, Kusumo S, DanhL D, Pascua O C. and Espino R C, **(1999).** Banana cultivar namesand synonyms in Southeast Asia, in in and the . Advancing Banana and Plantain R and D. Pacific, Asia.

Wyman CE.(**1996)** .Handbook on Ethanol:Production and Utilization. *Taylor and Francis, Bristol, Paris, France.*, 55-59.

Xibib, S.,Meilan, H. and Moller, H.**(2003)**. *Risk factors for oesophageal cancer in Linzhou, China.* A case-control study.*Asian Pacific Journal of Cancer Prevention, 4, 119-24*

Yusuf, Jibril ,M., Misau, and Danjuma, B.Y.l **(2012).** Production of vinegar from pineapple pee. *Int .Journal of Advanced Sciences Research and Technology, 2*, 656-666.

Zenebe W, Ali M, Derbew B, Zekarias S.and Adam B.,(**2015**). Assessment of Banana Production and Marketing in Ethiopia. *International Journal of Sciences: Basic and Applied Research (IJSBAR), 24*(3), 283-307.

Zhang, P.,Whistler, R. BeMiller, J. N. and Hamaker, B. R. **(2005)**. Banana Starch: production, physicochemical properties, and digestibility. *Carbohydrate Polymer, 59,* 443-458.