ETHYL ALCOHOL CONTENT OF EXTRACTS FROM UBE (*Dioscorea alata*), KAYOS (*Dioscorea hispida*), BAONG (*Dioscorea sp.*) AND BANAYAN (*Dioscorea luzonensis*)

A Research Paper
Presented to
The Faculty of Philippine Science High School Western Visayas
Bito-on, Jaro, Iloilo City

In Partial Fulfillment
of the Requirements for
SCIENCE RESEARCH 2

by

Noel Francis G. Belleza
Fiel Christian P. Mamon
Roberto G. Tubongbanua Jr.
Fourth Year – Graviton

February 2008

APPROVAL SHEET

This Research Paper Hereto Entitled:

"Ethyl alcohol content of extracts from ube (*Dioscorea alata*), kayos (*Dioscorea hispida*), baong (*Dioscorea sp.*) and banayan (*Dioscorea luzonensis*)"

Prepared and submitted by Noel Francis Belleza, Fiel Christian Mamon and Roberto Tubongbanua Jr. in partial fulfillment of the requirements in Science Research 2, has been approved and is recommended for acceptance and approval.

ROWENA M. LABRADOR
Science Research 2 Adviser

Approved by the Science Research Committee with a grade of PASSED on February 2008.

EDWARD C. ALBARACIN
Member

WILLIAM A. LARIDE
Member

HAROLD P. MEDIODIA
Member

MAJAL ABE B. MIRASOL
Member

ZENNIFER L. OBERIO
Member

Accepted in partial fulfillment of the requirements in Science Research 2.

JOSETTE T. BIYO, Ph. D.
Director III – PSHSWVC

ACKNOWLEDGEMENT

First of all before we thank anybody else, we would like to thank God for providing us support spiritually and giving us strength to continue doing our research even though we're already very tired and lacked sleep. We love you God.

We thank our parents namely: Mr. and Mrs. Noel Belleza, Mr. and Mrs. Modesto Martin Mamon and Mr. and Mrs. Roberto Tubongbanua Sr. for providing us the financial support that we really needed for the success of this research.

We thank Analyn Mamon for sacrificing her time in going to Los Baños, Laguna to buy the needed materials.

We express our heartfelt gratitude to the family of Alvin Chua Jr. for being so rich and hospitable. Thank you for the free accommodation, food, transportation and work place for our entire stay in Bacolod City. We would also like to include Nong Oting for being patient with us throughout our research.

To the agriculturists in the Provincial Capitol of Iloilo, we are very grateful that all of you helped us in identifying and acquiring the wild ube that we needed.

We thank our Science Research Adviser Mrs. Rowena Labrador for helping us in our research especially during the time that we are still making our proposal.

To the staff and especially to Ma'am Melanie of Kooll Company, we are really thankful that you welcomed us in your laboratory and lend your hand.

We thank Angela Bilbao for helping us during the extraction process of our research. Your help really mattered.

To all those who supported us mentally, spiritually and physically we thank all of you guys.

Belleza, Noel Francis G., Mamon, Fiel Christian P., Tubongbanua, Roberto Jr. G. "Ethyl alcohol content of extracts from ube (*Dioscorea alata*), kayos (*Dioscorea hispida*), baong (*Dioscorea sp.*) and banayan (*Dioscorea luzonensis*)" Unpublished Research. Philippine Science High School Western Visayas, Bito-on, Jaro, Iloilo City. February 2008.

ABSTRACT

Ethanol is a clean-burning, high-octane fuel that is produced from renewable sources. These sources are divided into sugar-containing materials, starchy materials and cellulosic materials. In this study, different varieties of ube namely ube (*Dioscorea alata*), kayos (*Dioscorea hispida*), baong (*Dioscorea sp.*) and banayan (*Dioscorea luzonensis*) were used as sources of starch for processing into ethanol.

The different varieties of ube underwent starch extraction, starch hydrolysis, fermentation and distillation. German thermohydrometer was used in order to determine the ethyl alcohol content.

Results of the study showed that the ethyl alcohol content of extracts from different varieties of ube ranged from 3.90% to 4.15% with banayan yielding the least amount of ethyl alcohol and ube with the greatest amount of ethyl alcohol content.

One-way ANOVA showed a significance level of 0.257. This means that there is no significant difference between the ethyl alcohol content of the different varieties of ube.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

CHAPTER I
INTRODUCTION

A. Background of the study

Ethanol is a clean-burning, high-octane fuel that is produced from renewable sources. At its most basic, ethanol is grain alcohol, produced from crops such as corn (www.wikipedia.com). It is a clear, colorless liquid with a characteristic, agreeable odor. Ethanol, also known as ethyl alcohol or grain alcohol, can be used either as an alternative fuel or as an octane-boosting, pollution-reducing additive to gasoline. In dilute aqueous solution, it has a somewhat sweet flavor, but in more concentrated solutions it has a burning taste.

Ethanol when compared to petroleum is better because, it does not cause air pollution or any environmental hazard. Ethanol is an octane booster and anti-knocking agent. It is an excellent raw material for synthetic chemicals. It is a renewable fuel made from plants. It is not a fossil-fuel: manufacturing it and burning it does not increase the greenhouse effect. It provides high octane at low cost as an alternative to harmful fuel additives. Ethanol blends can be used in all petrol engines without modifications. (http//:www.journeytoforever.com).

The top ethanol producing countries as of 2005 are the following: USA, Brazil, China, India, France, Russia, Germany, South Africa, Spain, UK, Thailand, Ukraine, Canada, Poland, Indonesia, Argentina, Italy, Australia, Saudi Arabia, Japan, Sweden, Pakistan, Philippines, South Korea, Guatemala, Ecuador, Cuba, Mexico, Nicaragua, Zimbabwe, Kenya, Mauritius and Swaziland. USA and Brazil are the top producers of ethanol; producing 70% of the world's ethanol in 2005. The Philippines produced a total of 22 million gallons of ethanol in 2005. (http://www.ethanolrfa.org/industry/statistics/#E)

Ethanol can be produced from varieties of raw materials. Types of raw materials can be usefully divided into sugar-containing materials, starchy materials and cellulosic materials. Starch is abundant in the seeds of cereal plants and in bulbs and tubers. Cellulosic materials contain complex carbohydrates like starch. (Reed G. Production of Fermentation Alcohol as a Fuel Source, Microsoft Encarta Premium 2007)

Ethanol may be produced from any sugar-containing fruits, fruit juices, or extracts, such as grape juice, apple juice, honey, or sugar containing effluents of canaries. Such sucrose is usually too costly in comparison with sugar beets, sugar cane, or sweet sorghum. Total production of sugar in developing countries in 1975 is estimated at 36 million MT (40 million tons), and projected production in 1985 is 47 MT (52 million tons). At a yield of 0.9 MT (1 ton) of ethanol from 2.02 MT (2.25 tons) of sugar, the increased production could yield about 4.5 million MT (5 million tons) of ethanol or about 6 billion liters (1.6 billion gal.). The major sugar-producing countries are Brazil, Cuba, Mexico, and the Philippines, in that order.

In the Philippines there are many tubers such as corn, ube, tugui, cassava, potatoes, and gabi. These plants are considered as high value commercial crops. It also means that there is mass production of these plants all over the country.

Based on the information provided by an agriculturist in the Province of Iloilo, there is an availability in the commercial scale of ube (*Dioscorea alata*) in places like Iloilo of Region 6, Leyte of Region 8 and Bohol of Region 7. It is in demand in the making of delicacies, candies and flavoring for ice cream and breads.

However, there are wild varieties of ube that have no commercial value. They just grow anywhere as long as it is a forested area. Kayos, baong and banayan are wild varieties of ube that grow in forested areas in different conditions. Since they are hardly eaten and have no commercial use, they might be better as sources of ethanol. Kayos (*Dioscorea hispida*) has to be intricately prepared to remove the toxin present in it. Banayan could hardly be harvested whereas baong has thorns.

Therefore, in this study it has been decided to use different varieties of ube namely ube (*Dioscorea alata*), kayos (*Dioscorea hispida*), baong (*Dioscorea sp.*) and banayan (*Dioscorea luzonensis*) as sources of starch for processing into ethanol. These all have the adaptation to grow all year long.

B. Statement of the Problem

What is the ethyl alcohol content of extracts from ube (*Dioscorea alata*), kayos (*Dioscorea hispida*), baong (*Dioscorea sp.*) and banayan (*Dioscorea luzonensis*)?

C. Objectives of the Study

Objective 1: To measure the alcohol content of extracted alcohol obtained from ube (*Dioscorea alata*), kayos (*Dioscorea hispida*), baong (*Dioscorea sp.*) and banayan (*Dioscorea luzonensis*)

Objective 2: To compare the alcohol content of extracted ethanol obtained from ube (*Diocorea alata*), kayos (*Dioscorea hispida*), baong (*Dioscorea sp.*) and banayan (*Dioscorea luzonensis*)

D. Hypothesis of the Study

There is no significant difference in the alcohol content of extracted ethanol obtained from ube (*Dioscorea alata*), kayos (*Dioscorea hispida*), baong (*Dioscorea sp.*) and banayan (*Dioscorea luzonensis*).

E. Research Paradigm

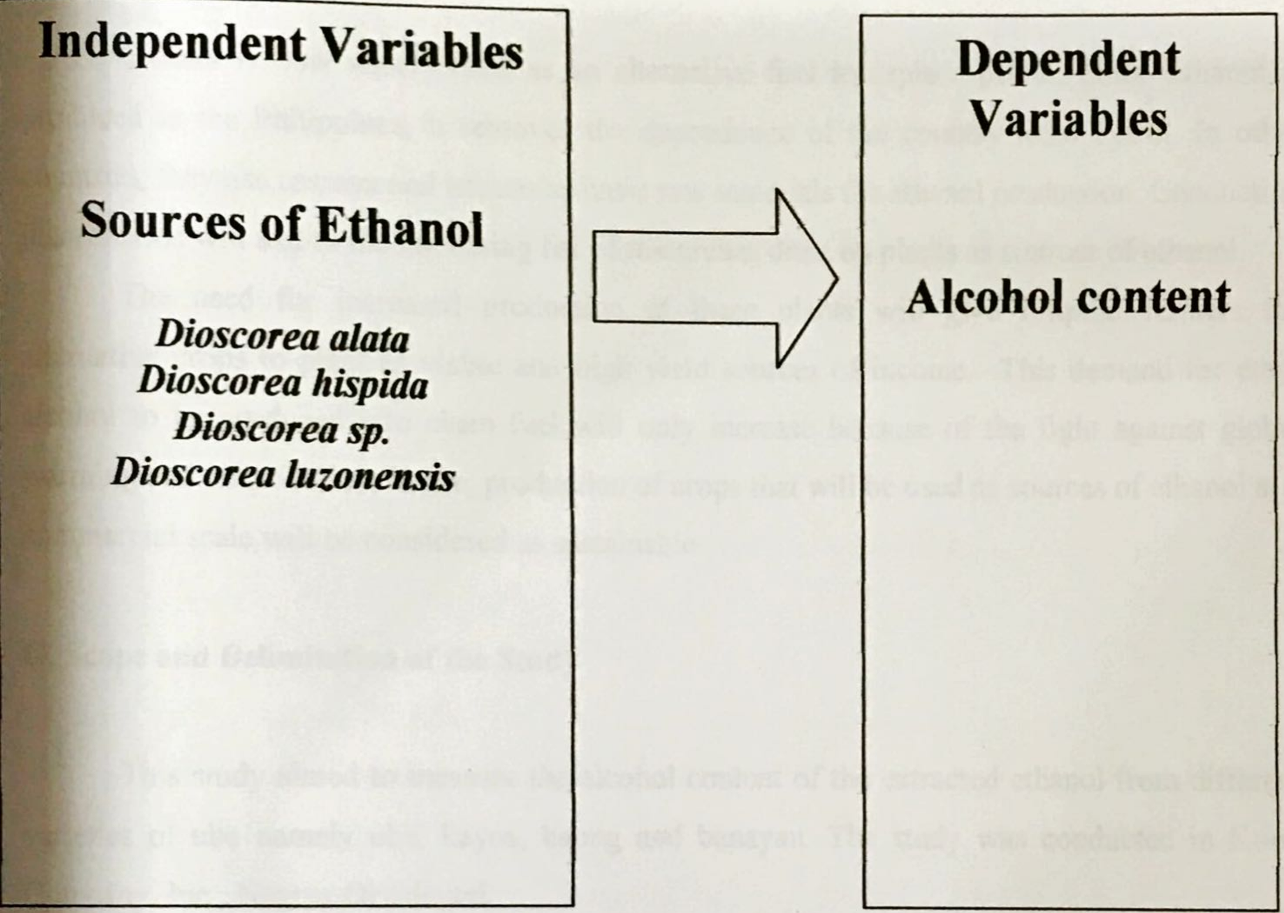

Figure 1. The Research Paradigm

F. Significance of the Study

Ethanol is now widely used as an alternative fuel to replace petrol. Since Ethanol is produced in the Philippines, it removes the dependence of the country from Petrol. In other countries, they use cassava and banana as basic raw materials for ethanol production. Conducting this research will add to the increasing list of researches done on plants as sources of ethanol.

The need for increased production of these plants will give Filipino farmers the alternative crops to plant as viable and high yield sources of income. This demand for ethyl alcohol to be produced into clean fuel will only increase because of the fight against global warming and air pollution, hence, production of crops that will be used as sources of ethanol at a commercial scale will be considered as sustainable.

G. Scope and Delimitation of the Study

This study aimed to measure the alcohol content of the extracted ethanol from different varieties of ube namely ube, kayos, baong and banayan. The study was conducted in Kooll Company, Inc., Negros Occidental.

The ube, kayos, baong and banayan was gathered from the forest of Badiangan. The bulk of the ube, kayos, baong and banayan were used to produce extracted ethanol. The fermentor of the root crops was the yeast (*Saccharomyces cerevisiae*) bought from UP Biotech, Los Baños Laguna.

In extracting ethanol from ube, kayos, baong and banayan, the following processes was conducted: Starch hydrolysis, followed by fermentation, distillation and measurement of parameters.

The parameter measured was the ethyl alcohol content from extracts of ube, kayos, baong and banayan by the use of Alcoholometer.

There were three (3) replicates for each procedure.

H. Definition of terms

Alcohol – term applied to members of a group of chemical compounds and, in popular usage, to the specific compound ethyl alcohol, or ethanol.

Alcoholometer – instrument used to determine the purity of the extracted alcohol.

Alcohol content- is an indication of how much alcohol (expressed as a percentage) is included in an alcohol sample.

Brix – percent by weight of dissolved solids on a sugar solution as indicated by a brix hydrometer or other densimetric device.

Ethanol – clear, colorless liquid, with a burning taste and characteristic, agreeable odor. Ethanol is the alcohol in such beverages as beer, wine, and brandy.

Fermentation – chemical changes in organic substances produced by the action of enzymes.

Heart Alcohol – final alcohol, distillate relatively fine from fusel oil and aldehyde.

Mash – grain and water mix: a fermentable mixture of hot water and grain, usually barley or wheat, from which alcohol is brewed or distilled.

Proof - measure of an alcohol sample's strength.

Pycnometer – instrument use to determine the alcohol yield by means of specific gravity.

Slurry- a liquid mixture of water and an insoluble solid material

Tuber- a short, thickened portion of an underground stem, as in the potato.

Vats – fermentation vessel.

Weak Alcohol – crude alcohol, more of water about 70-80 proof.

Wort – the diluted molasses solution for fermentation wherein chemicals and other ingredients have been added, except the addition of yeast.

Yeast – greenish yellow fungus, got from fermenting malt liquors and used as fermenting agent, to raise bread.

CHAPTER II
REVIEW OF RELATED LITERATURE

This chapter consists of four topics, namely, (A) Ethanol, (B) Sources of Ethanol, (C) Procedure and (D) Related Studies.

A. Ethanol

Ethanol is also called grain alcohol or ethyl alcohol. It is produced by fermenting biomass. It is flammable, colorless, mildly toxic chemical compound with a distinctive perfume like odor, and it is also the alcohol found in alcoholic beverages. Ethanol, the most widely used biofuel, is made by fermenting biomass in a process similar to brewing beer. Currently, most of the 1.5 billion gallons of ethanol used in the U.S. each year is made from corn and blended with gasoline to improve vehicle performance and reduce air pollution.

Ethanol may be distilled from grain, sugar crops, or almost any starchy plant. Recent studies show that banana, waste potatoes, corn and cassava are positive for ethanol extraction. These are good sources of ethanol because these are sugar-containing materials and rich in starch. Sugar-containing materials can be fermented directly and starchy materials can be easily hydrolyzed by enzymes or acids to fermentable sugars.

The personal care products industry is one of the largest users of industrial ethanol, or ethyl alcohol. Ethanol is also used in many deodorants, lotions, hand sanitizers, soaps, and shampoos.

Industrial alcohols are also used extensively in pharmaceuticals. The characteristics of ethyl alcohol make it a prime carrier for a whole spectrum of medicines including cough treatments, decongestants, iodine solution, and many others. As a solvent for the pharmaceutical industry, ethanol is useful for processing antibiotics, vaccines, tablets, pills, and vitamins.

Many cleaning products contain high volumes of industrial alcohol. A bottle of household disinfectant spray can contain nearly 80 percent ethanol.

Ethanol is used as a solvent in the manufacture of many other substances including paints, lacquer, and explosives. Industrial ethanol is used as a raw material for the production of vinegar and yeast, and similarly in chemical processing as a chemical intermediate.

Even food products like extracts, flavorings, and glazes contain large amounts of alcohol. The ethanol is also used in some liquid animal feed products as energy. (www.wikipedia.com)

B. Sources of Ethanol

B.1 Ube (*Dioscorea alata*)

Ube (*Dioscorea alata*) or purple yam is a vine which produces both aerial tubers called bulbils and underground tubers or roots. Production of ube is quite typical; ube is usually one of the staple crops here. On February 2005, Alaminos city met with the Brgy. Council of Amangbangan to discuss the development of idle lands through ube production, which will also augment the income of farmers and possibly partner with companies which produces ice cream, candies and other delicacies. On the same month, training for Ube production was conducted, participated by farmers from the city's 39 barangays. The first Ube Farm was then launched in Brgy. Amangbangan on May 2005, in a 1 hectare farm. The city provided the ube sets while DMMSU provided the free training for the technology needed for ube production in the city. There are currently ten (10) ube farms in the city located in the following barangays: Inerangan, San Antonio, San Roque, Panagapisan, Mona, Bolaney, Macatiw, Linamnsangan, Pogo, and San Jose.

ANALYSIS OF UBE ROOTS NUTRIENTS COMPOSITION

Moisture 70.0%

Starch 28.0%

Fat 0.1-0.3%

Crude Protein 1.1-2.8%

Sugar 0.5%

Crude Fiber 0.6-1.4%

Ash 0.7-2.1%

Vit. C (mg/100g) 5.8-8.0%

Vit. B1 (mg/100g) 0.09%

Vit. B2 (mg/100g) 0.03%

(http://www.da.gov.ph/tips/ubi.pdf)

B.2 Kayos (*Dioscorea hispida)*

Kayos (*Dioscorea hispida)* is a wild variety of ube that can be found in the forest of Badiangan. Its leaves were heart-shaped like that of its common cousin, the ube. Kayos, although proven to contain poisonous substance, has long been an "alternative meal" for most tribal folks in South Cotabato and the neighboring areas. During the 1998 drought, Glungga residents said an entire family died in their village after eating an ill-prepared kayos meal. Preparation of kayos takes at least three days, which includes soaking the grated tubers for a day or two in a river or any running water "to naturally wash away the poison." Known in the scientific community as Dioscorea hispida, kayos is classified as a bitter yam whose "tubers are poisonous unless specially prepared." In the Philippines, kayos is also called nami, bagai, gagos, kalut, karat, karoti, kalot, korot, kulot, mamo and orkot. According to an article published late last year by the Los Baños-based Philippine Council for Agriculture, Forestry and Natural Resources Research and Development (Pcarrd), the tubers of kayos contain a poisonous alkaloid called dioscorine that could paralyze the nervous system. (http://www.sunstar.com.ph/static/gen/2005/06/05/feat/charcoal.making.kayos.spell.survival.for.s..cotabato.farmers.html)

B.3 Baong (*Dioscorea sp.*)

Baong is a wild variety of ube that can be found in the forest of Badiangan. Its stem and roots grow thorns. Baong tubers can grow at an average of thirty (30) centimeters in length and weigh at an average of 0.5 kg. The skin is rough, with thorns and small roots. The color of the skin is light brown. The inside of the tuber or the substance, called the "meat" is white in color.

B.4 Banayan (*Dioscorea luzonensis*)

Banayan is a wild variety of ube that can be found in the forest of Badiangan. In Luzon, it is called Camangeg. Banayan tubers can grow at an average of seven (7) feet in length and weigh at an average of 0.5 kg. The skin is rough with small roots. The color of the skin is light brown. The inside of the tuber or the substance, called the "meat" is white in color.

Its discovery puts an added value on this wild root after boiling or as ginataan. The tubers with their fibrous roots are harvested from August to October when a high supply is observed. One vine usually produces one elongated tuber. Banayan is rich in carbohydrates, protein and vitamins. The tubers are also known as a good source of high quality flour needed in pharmaceutics and has its own particular color, taste and aroma that differ greatly from other yam species. (Pablico S., Ph.D., 2007)

C. Procedure

C.1 Starch Hydrolysis

Starch is the commonest storage carbohydrate in plants. It is used by the plants themselves, by microbes and by higher organisms so there is a great diversity of enzymes able to catalyze its hydrolysis. Starch from all plant sources occurs in the form of granules which differ markedly in size and physical characteristics from species to species. Acid hydrolysis of starch has had widespread use in the past. It is now largely replaced by enzymatic processes, as it required the use of corrosion resistant materials, gave rise to high color and starch content (after neutralization), needed more energy for heating and was relatively difficult to control.

Starch hydrolysis is the conversion of starch into simple sugars or dextrose. These simple sugars are then fermented and distilled into ethanol.

C.2 Fermentation

Ethanol fermentation is a form of anaerobic respiration used primarily by yeasts when oxygen is not present in sufficient quantity for normal cellular respiration, the cellular energy-producing system, to continue.

Fermentation is a biochemical process carried out by microscopic organisms called yeast. Yeast is commonly known as a major component in making bread. Bakers use the yeasts ability to make carbon dioxide gas to make the bread rise, making it thicker. Yeast is anaerobic, meaning it can live and eat without needing oxygen. When oxygen is limited, yeast consumes simple sugars, but is unable to absorb all of the available energy in sugar. During the partial absorption process while digesting the sugar, it is converted into ethanol and carbon dioxide gas. While ethanol can also be made from the sugar found in most kitchens, it takes a lot of sugar to fill the tank of your car with ethanol. Some countries, such as Brazil, that grow a lot of sugar use it to make ethanol for cars. Brazil has been producing ethanol fuels for decades. The United States does not have enough sugar cane plants to do this, so the U.S. has focused on making ethanol from corn. (http://e85.whipnet.net/)

Ethanol respiration is the form of fermentation used to make alcohol and bread. Yeast cells in the dough of a bread will be cut off from their sources of oxygen, and will generate alcohol (which is boiled away due to the heat) and carbon dioxide (which form bubbles that cause bread to rise). It is also used to mass-produce alcoholic beverages. The yeasts, cut off from oxygen, will ferment a starchy grain or vegetable (such as wheat, corn, potatoes, rye).

Fermentation is a biochemical process in which micro-organisms such as bacteria, yeasts, moulds or enzymes are used to break down an organic compound, usually a carbohydrate, in the absence of oxygen, the conversion of sugar into alcohol.

In alcoholic fermentation, such as occurs in brewer's yeast and some bacteria, the production of lactic acid is bypassed, and the glucose molecule is degraded to two molecules of the two-carbon alcohol, ethanol, and to two molecules of carbon dioxide.

Factors affecting the fermentation process are the temperature of fermentation and pH. The correct fermenting temperature ranges from 30-35°C. When fermenting temperature exceeds 35°C, the yeast will die. The correct pH must be 7. When pH is below or above 7, the yeast will die. (Kooll Company, Inc., Negros Occidental)

C.3 Distillation

Distillation, a process of heating a liquid until its more volatile constituents pass into the vapor phase, and then cooling the vapor to recover such constituents in liquid form by condensation. The main purpose of distillation is to separate a mixture of several components by taking advantage of their different volatilities, or the separation of volatile materials from nonvolatile materials. In evaporation and in drying, the purpose usually is to obtain the less volatile constituent; the more volatile constituent, in most cases water, is discarded. In distillation, on the other hand, the principal object of the operation is to obtain the more volatile constituent in pure form. The removal of water from glycerin by vaporizing the water, for example, is called evaporation, but the removal of water from alcohol by vaporizing the alcohol is called distillation, although similar apparatus is used in both cases. (Microsoft Encarta Premium 2007)

The functions of distillation are three fold: (1) to recover all the low boiling point solvents in the fermented mash; (2) to fractionate and separate them into reasonable stat of purity and quantity; and (3) to rectify and concentrate the products within economical limits. These operations are made with minimum loss of heat.

Alcohol losses in distillation are mainly through leakage, air vents and spent wash. The first can be eliminated entirely, the second reduced to the minimum by making provisions of air vents of incondensable gases to the feed tank acting as condenser, and the third reduced to practically nil by proper feeding and temperature control. (Kooll Company, Inc., Negros Occidental)

D. Related studies

Though the majority of ethanol produced in the U.S. is made from corn, new technology has been developed to make ethanol from a wider variety of "cellulosic" sources. These cellulosic sources for ethanol include corn stover (the stalks and residue left over after harvest), grain straw, switchgrass, quick-growing tree varieties (such as poplar or willow), or even municipal waste.This technology does exist today, and work continues to bring this technology to commercial scale.

At the end of 2004, Thailand had ethanol production capacity of some 4.36 million liters per day, divided among 24 producers. The Thai Oil plant could thus increase capacity by some 23%–46%.Piti Yimprasert, Thai Oil president, said the company had opted to produce ethanol from cassavas because it viewed there would be enough supply of the crop for the large ethanol plant. Sugarcane and molasses are other feedstocks for Thai ethanol.

Cassava is a hardy, starchy crop that grows with minimal inputs and delivers a reasonable yield even on infertile land where the cultivation of other crops is difficult. For processing into ethanol, the cassava starch is initially converted to glucose by the enzyme or acid process, similar to the process used for cereal grains and other starchy feedstocks.

Cassava ethanol projects are also in development in Papua New Guinea (PNG) and Nigeria. (Greencar Congress).

Keawsompong S. conducted a study on the Ethanol Production from Cassava Chips: Simultaneous Saccharification and Fermentation Process. They evaluated that three sources of carbohydrate feed stocks namely sugar cane, sugar molasses and cassava root have the potentiality on this purpose. The results suggest that sugar cane is not a suitable resource due to inadequate cane productivity (60 million tons/ year) comparing to sugar mill capacity (75 million tons/ year) and the complication of sugar cane and sugar legislation. Molasses is also limited by sugar cane production. With the productivity of 60 kg/ ton sugar cane, around 3 million tons of molasses are produced annually. Around 30-40% of molasses produced is for export and the rest used for local distillery, animal feed meal and food industry. Only the consumption of molasses used for export (around 1 million tons) can be reasonably employed for ethanol production (800,000 liters ethanol/day) with annexed distillery to sugar mill process. In contrast, the current production of cassava roots is about 20 million tons and around 80-90% roots are consumed by two major industries, namely starch and chip/pellet industries, providing root surplus for ethanol production at 2 million liters/day. Cassava roots as readily transformed to dried chips are the most suitable raw material for ethanol production with the production cost and time can be minimized via the Simultaneous Saccharification and Fermentation process as already implemented in bioethanol production of cereal grains. In this study, cassava chips (starch content ≈ 75% dry basis) after liquefied by α- amylase were simultaneously saccharified and fermented to ethanol by using the mixture of Rhizozyme™ (Alltech) and *Saccharomyces cerevisiae*. Rhizozyme™ was used for the saccharification of liquefied cassava starch and other fibrous materials while *Saccharomyces cerevisiae* converted the soluble sugars to ethanol. The yield of bioconversion and ethanol concentration was approximately 7.5g ethanol/ 25g cassava chips and 9.5 %, respectively, depending on the composition of cassava chips. By the simultaneous process, the production time was 25% faster than the conventional process, i.e. sequential saccharification and fermentation. (http://knowledge.biotec.or.th/doc_upload/2004123151846.doc)

Gunasekaran P. (1999) conducted a study on the Ethanol Production Bacteria vs. Yeast. The yeast *Saccharomyces cerevisiae* and facultative bacterium *Zymomonas mobilis* are better candidates for industrial alcohol production. *Zymomonas mobilis* possesses advantages over *Saccharomyces cerevisiae* with respect to ethanol productivity and tolerance. But the bottlenecks in *Z. mobilis* are:

i) its inability to convert complex carbohydrate polymers like cellulose, hemicellulose, and starch to ethanol,

ii) its resulting in byproducts such as sorbitol, acetoin, glycerol and acetic acid,

iii) formation of extracellular levan polymer.

As reported in batch fermentation, sugar concentrations as high as 223 g/l could be fermented to 105 g/L ethanol in 70 hours. The percentage theoretical yield was 92%. Whereas in a continuous fermentation using mixed cultures of *Zymomonas mobilis* and *Saccharomyces cerevisiae*, production of 54.3 g/L of ethanol was observed within 3 days. A high ethanol productivity of 70.7 g/L/hr was obtained with a final ethanol concentration of 49.5 g/L and yield of 0.5 g/g. this amount to 98% of the theoretical yield and 99% substrate conversion.
(http://www.andrew.cmu.edu/user/jitkangl/Fermentation%20of%20Ethanol/Fermentation%20of%20Ethanol.htm)

Ethanol has been used by humans for thousands of years, in part because it is easy to make. Ethanol can be produced from any biological feedstock's that contain appreciable amounts of sugar or materials that can be converted into sugar such as starch or cellulose. Sugar beets and sugar cane are examples of feedstock's that contain sugar. Corn contains starch that can relatively easily be converted into sugar. (http://e85.whipnet.net/)

In the Philippines, one source of ethanol is molasses. The fermentation of molasses requires exceedingly careful control of yeast, pH and temperature to ensure the best results. (Kooll Company, Inc., Negros Occidental)

CHAPTER III

METHODOLOGY

A. Extraction

Materials needed:

a) weighing scale
b) sack
c) knife
d) chopping board
e) blender
f) cloth
g) basin
h) plastic containers

Gathering of Plants:

The ube (*Dioscorea alata*), kayos (*Dioscorea hispida*), baong (*Dioscorea sp.*) and banayan (*Dioscorea luzonensis*) was gathered and collected from the forest of Badiangan. This town is located in the province of Iloilo. The subject for extraction was the tuber because it contains starch. The ube, kayos, baong and banayan was checked thoroughly for any rot or abnormalities in the appearance of the tuber. The ube, kayos, baong and banayan was stored inside a sack at normal room temperature.

Procedure:

The tuber was peeled, sliced into small pieces and ground thoroughly using a blender. After grinding, the ground tuber was placed in a clean cloth to act as filter, squeezed of its juice and with a basin ready to catch its juice as it was being squeezed. The juice was let alone to allow the starch to settle at the bottom of the basin and when it had settled, the water part was decanted, careful that the starch was not disturbed. The starch that still contains small amount of water was exposed under the sun for drying. After having dried up, the starch was gathered and stored inside a container at normal room temperature. (Province of Iloilo, Agriculture Department)

The process stated above was applied to ube (*Dioscorea alata*), kayos (*Dioscorea hispida*), baong (*Dioscorea sp.*) and banayan (*Dioscorea luzonensis*) in extracting starch. Extracted starch was transported to Kooll Company, Inc., Negros Occidental.

B. Starch Hydrolysis

Materials and Reagents Needed:

a) water
b) alpha-amylase
c) sodium hydroxide
d) calcium chloride
e) cookers
f) gluco-amylase

Procedure:

The starch powder of each material (ube, kayos, baong and banayan) was placed in three (3) packs for replicates with each pack weighing one hundred (100) grams.

Each of the 100g of starch powder was mixed with 400mL of distilled water. Sodium hydroxide was added gradually until a pH of 6.5 was achieved. Then 0.1g of alpha-amylase was added to the solution. Thirty-two (32) mg of calcium chloride was added to the slurry. The slurry was mixed for ten (10) minutes. The slurry was placed in a boiling (100°C) water bath for ten (10) minutes or until the starch was liquified. Then allow the slurry to cool. After cooling, 1g of alpha-amylase and 32mg of calcium chloride was added. The slurry was incubated for two (2) hours at 70°C water bath. After incubating, the slurry was placed in a boiling (100°C) water bath for thirty (30) minutes.

The slurry was cooled. 3.2g of gluco-amylase was added to convert starch molecules to fermentable sugars (dextrose). The slurry was placed in a 60°C water bath for twenty-four (24) hours. The mixture was heated to 80°C and cooled.

C. Activation of Yeast

Agar-agar slant culture

The starting point in the preparation of the fresh batch of yeast for fermentation was the selection of a single yeast-cell from a master culture of yeast that was well acclimatized. This master culture of yeast came from U.P. Biotech Laboratory. This was accomplished by dipping a sterilized platinum needle into the Erlenmeyer flask and brushing it over the surface for a sterile medium of agar, hardened in a Petri dish. After several streaks were made with the process aforementioned using sterilized platinum needle, the point was reached where only single cells were dislodged on the medium. Following incubation for about 48 hours at 75 to 80°F, colonies of cells become visible to the naked eye.

After macroscopic examination through the naked eye, four or five of the best colonies were selected from amongst the colonies which did not touch adjacent groups, so that it was certain that each of the colonies grew from a single cell. The selected colonies were used to inoculate fresh media in test tubes. After incubation from one to two days, the best tube was selected and used to inoculate fresh tubes to be use as the starting point in the production of pure culture yeast.

The yeast selected was placed in a broth, the Peptone Yeast Glucose Extract. PYGE consists of 2% Peptone, 1% Yeast and 5% Glucose for every 100mL water. The solution was agitated for 24 hours or until the yeast had grown.

D. Fermentation

Materials and Reagents needed:

a) 1L Erlenmeyer Flask

b) Agitator

c) Urea

d) Graduated cylinder

e) Brix Hydrometer

Procedure:

The brix of ube, kayos, baong and banayan was measured. A hydrolyzed starch was taken from each replicate from each source and the brix was measured. Eight brix was achieved in the first phase of the fermentation. Distilled water was added to lower the brix if it exceeded 8 brix while more of the hydrolyzed starch was added if the brix is below 8. Then this was placed inside the Erlenmeyer flask together with the PYGE broth and the urea, which served as food for the yeast. Place the Erlenmeyer flasks in the agitator for 12 hours of fermentation.

After the first 12 hours of fermentation, the brix was adjusted to 10 by adding more hydrolyzed starch. Then it was left to ferment for another 12 hours without agitating. It was placed in a steady table at room temperature.

E. Distillation

Materials needed:

a) Burner
b) Still pot
c) Still head
d) Thermometer
e) Condenser
f) Still receiver
g) Rubber tubing

Procedure:

The fermented ube, kayos, banayan and baong were decanted separately and then placed in separate containers.

The decanted liquid was placed inside the still pot and then on top of the burner. The liquid was now heated until a temperature of 80°C was achieved and kept constant until distillation was over. Only half was distilled and the distilled ethanol was cooled to not less than 27°C and was ready for testing.

F. Measuring of Parameters

Determination of Purity or Proof

Materials needed:

a) German thermohydrometer (0-10% alcohol by volume)

b) Plain hydrometer glass cylinder

c) Alcoholometer table

Procedure:

The hydrometer cylinder was filled with the alcohol sample. The alcohol sample was cooled to not less than 27°C. The alcohol sample was immersed in the thermohydrometer slowly. The thermohydrometer was allowed to stand for a few more minutes. The proofing cylinder was brought to the temperature of the alcoholic liquid in the cylinder until the mercury of the inserted thermometer was not moving. The temperature was read immediately after the hydrometer reading. The alcoholometer table was used to determine the proof of alcohol sample corresponding to the reading. The proof was divided by two(2) to get the alcohol content (expressed as a percentage).

Alcohol Content= Proof / Two (2)

CHAPTER IV

RESULTS AND DISCUSSION

This study aimed to measure and compare the alcohol content of ube (*Dioscorea* a
baong (*Dioscorea sp.*), kayos (*Dioscorea hispida*) and banayan (*Dioscorea luzonensis*).

A. Results

Table 1 shows the starch extracted percentage from the different plant sources. been observed that the starch content differed from each of the different plant sources. (*Dioscorea hispida*) produced the highest percent of starch content with 8.8% whil (*Dioscorea alata*) produced the least percentage of starch with 3.18%.

Table 1. Starch extracted from ube (*Dioscorea alata*), baong (*Dioscorea sp.*), kayos (*Dios hispida*) and banayan (*Dioscorea luzonensis*).

Sources	Starch Extracted Percentage
Ube (*Dioscorea alata*)	3.18
Baong (*Dioscorea sp.*)	5.18

However, Table 2 presents the mean alcohol content percentage from the different sources. Ube (*Dioscorea alata*) has the highest alcohol content which has 4.15% while Banayan (*Dioscorea luzonensis*) has the lowest alcohol content which has 3.9%.

Table 2. Mean alcohol content from different sources

Sources	Mean alcohol content percentage
Ube (*Dioscorea alata*)	4.15
Baong (*Dioscorea sp.*)	4.10
Kayos (*Dioscorea hispida*)	4.10
Banayan (*Dioscorea luzonensis*)	3.90

To test if there was a significant difference in the alcohol content of the plant sources, one-way ANOVA was used. Table 3 shows that there is no significant difference in the alcohol content from the different plant sources.

Table 3. Statistical Analysis of Data

	Sum of squares	Df	Mean square	F	Sig.	Interpretation

B. Discussion

The alcohol content was measure using a hydrometer. The hydrometer is just an approximation based on the temperature and the brix reading. The hydrometer measures the density of alcohol. It may be more accurate if gas chromatography was used. When measuring amounts of liquids in a solution gas chromatography is more efficient because it uses a computer that computes accurately (Wikipedia.com). In order for the gas chromatography to work, hydrogen gas will be needed and large volumes of alcohol are tested.

The brix of the molasses and corn is too large compared to the brix of ube (*Dioscorea alata*), baong (*Dioscorea sp.*), kayos (*Dioscorea hispida*) and banayan (*Dioscorea luzonensis*). Brix is the amount of fermentable sugars in a plant source. Corn has 45 brix while molasses has 80-90 brix. This means that molasses have more fermentable sugars compared to the plant sources in the study. Having a high amount of fermentable sugar means high alcohol yield. Therefore the alcohol content of ube (*Dioscorea alata*), baong (*Dioscorea sp.*), kayos (*Dioscorea hispida*) and banayan (*Dioscorea luzonensis*) are not comparable to alcohol yield of corn and molasses.

The Philippines by nature is rich in its land resources. Dioscorea species are usually wild and do not need meticulous tending. They could grow anywhere all year long. Currently there are ten ube farms in Alaminos City, Pangasinan, kayos is a staple food in South Cotabato and Banayan is known in Luzon. The researchers have found that these plant sources are also abundant in the mountainous regions of the municipalities in Central Panay. Therefore they are quite abundant and may serve alternative sources of alcohol in times of short supply of molasses and cassava.

CHAPTER V
SUMMARY, CONCLUSION AND RECOMMENDATIONS

This study aimed to determine and compare alcohol content of extracts of ube (*Dioscorea alata*), kayos (*Dioscorea hispida*), baong (*Dioscorea sp.*) and banayan (*Dioscorea luzonensis*).

It was hypothesized that there is no significant difference in the alcohol content of extracted ethanol obtained from ube (*Dioscorea alata*), kayos (*Dioscorea hispida*), baong (*Dioscorea sp.*) and banayan (*Dioscorea luzonensis*).

A. Summary of significant findings

In this study the researchers decide to use various different sources of ube namely ube (*Dioscorea alata*), kayos (*Dioscorea hispida*), baong (*Dioscorea sp.*) and banayan (*Dioscorea luzonensis*) as sources of starch for processing into alcohol.

The researchers have found out among the four plant sources, kayos (*Dioscorea hispida*) yielded the highest amount of starch while ube (*Dioscorea alata*) produced the least starch.

In the study the researchers have found out alcohol yielded from ube (*Dioscorea alata*) has the highest alcohol content while banayan (*Dioscorea luzonensis*) has the lowest alcohol content.

In the study the researchers used SPSS (Statistical Package for Social Sciences) and determined that there was no significant difference among the alcohol content of ube (*Dioscorea alata*), kayos (*Dioscorea hispida*), baong (*Dioscorea sp.*) and banayan (*Dioscorea luzonensis*).

B. Conclusion

The ethyl alcohol content of extracts from ube (*Dioscorea alata*), kayos, (*Dioscorea hispida*), baong (*Dioscorea sp.*) and banayan (*Dioscorea luzonensis*) ranges from 3.90% to 4.15% and statistically they are the same.

C. Recommendations for further studies

1. It is recommended that succeeding researches will use different kinds of yeast to know if it will yield the same amount of alcohol from the same sources.

2. The researchers further recommended to use other different species of Genus Dioscorea as sources of alcohol production.

3. Furthermore, it is recommended to use gas chromatography and pycnometer to measure the alcohol content.

4. Lastly, the researchers recommend that future researches will test the extracted alcohol with other parameters.

LITERATURE CITED

Anonymous. Ethanol Science & Technology. Available from: <http://www.nwicc.cc.is. uslpquo/continuing/business/ethanol/module.htm.> Accessed 2007 January 22.

Alexander M. Alternative Energy. Ethanol. Available at: <http://www.google.com.> 2006.

Anonymous. Available at: <http://en.wikipedia.org/wik/Ethanol#As_a_fuel.>

Anonymous. Available at: <http://www.journeytoforever.com.> Accessed 2006 November 20.

Anonymous. Renewable Fuels Association. Industry Statistics. Available at: <http://www.ethanolrfa.org/industry/statistics/#E>. Accessed 2007 April 22. 2005.

Anonymous. Available at: <http://www.da.gov.ph/BAS_WebDocs/agri_stats.html>. Accessed 2007 March 15.

Anonymous. E85. Ethanol Gasoline Blend. Available at: <http://e85.whipnet.net/>. Accessed 2007 March 15.

Anonymous. Kooll Company, Inc., Negros Occidental.

Anonymous. Available at: <http://www.da.gov.ph/tips/ubi.pdf>. Accessed 2007 April 23.

Brunot K, Haugh C & Ingold D. Understanding Ethanol Fuel Production and Use. Available at: <http://sleekfresk.9th.cx:81/3wdev/VITAHTML/SUBLEV/ENI/E7>. Accessed 2007 February 5.

Estabillo A. Charcoal-making, kayos spell survival for S. Cotabato farmers. Available at: <http://www.sunstar.com.ph/static/gen/2005/06/05/feat/charcoal.making.kayos.spell.survival.for.s..cotabato.farmers.html>. 2005 June 5. Accessed 2007 May 6.

Kang J. Fermentation of Ethanol. Available at: <http://www.anovew.com.edu/user/jitkng/fermentation>. Accessed 2006 December 14.

Keawsompong S, Piyachomkwan K, Walapatit S, Rodjanaridpiched C, & Sriroth K. "Ethanol Production from Cassava Chips: Simultaneous Saccharification and Fermentation Process. Available at: <http://knowledge.biotec.or.th/doc_upload/2004123151846.doc>. Accessed 2007 April 24.

Najaf pour G. Evaluation and Isolation of Ethanol Producer Strain SMP-6. Available at: <http://www.andrew.cmu.edu/user/jitkangl/Fermentation%20of%20Ethanol/Fermentation%20of%20Ethanol.htm>. Accessed 2007 April 26.

Pablico S. Camangeg, Excellent for Making Haleya. Manila Bulletin. 2007 March.

Reed G. Production of Fermentation Alcohol as a Fuel Source. 835-859.

Wang N.S. Experiment No. 5. Starch Hydrolysis by Amylase.

APPENDIX A

RAW DATA

Table 1. Starch Extraction

Sources	Amount (kg)	Starch Extracted (g)
Ube	11	350
Baong	10	580
Kayos	5	440
Banayan	10	370

Table 2. Brix

Source	Brix
Ube	21.54
Banayan	19.44
Baong	18.24
Kayos	16.86

Table 3. Extracted Alcohol

Source	Sample (mL)	Extracted (mL)	Alcohol Content (%)
Ube	600	300	4.15
Banayan	600	300	4.00
Kayos	600	300	4.00
Baong	600	300	3.90

APPENDIX B

ONE-WAY ANOVA

ANOVA

VAR00002

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9.623E-02	3	3.208E-02	1.634	.257
Within Groups	.157	8	1.963E-02		
Total	.253	11			

APPENDIX C

LIST OF PLATES

Plate 1. Ube

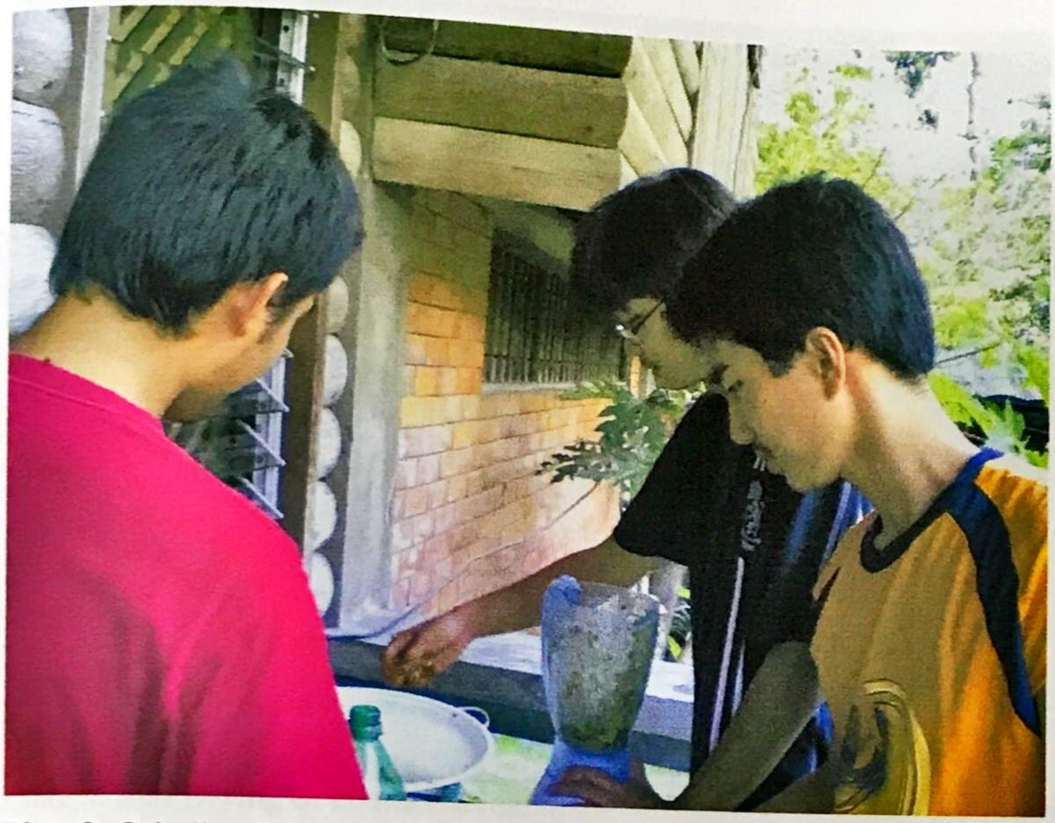

Plate 2. Grinding Process of Plant Sources

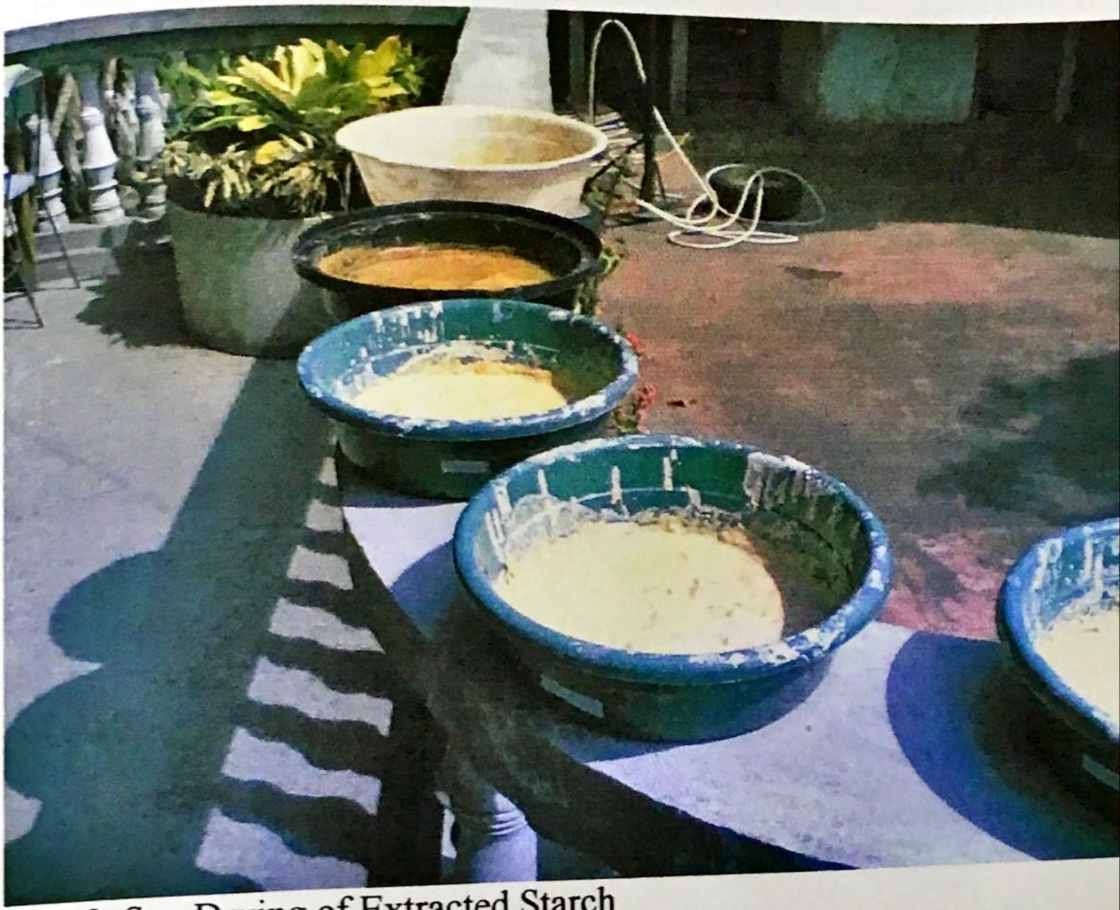

Plate 3. Sun Drying of Extracted Starch

Plate 4. Starch Hydrolysis of Extracted Starch with Enzymes

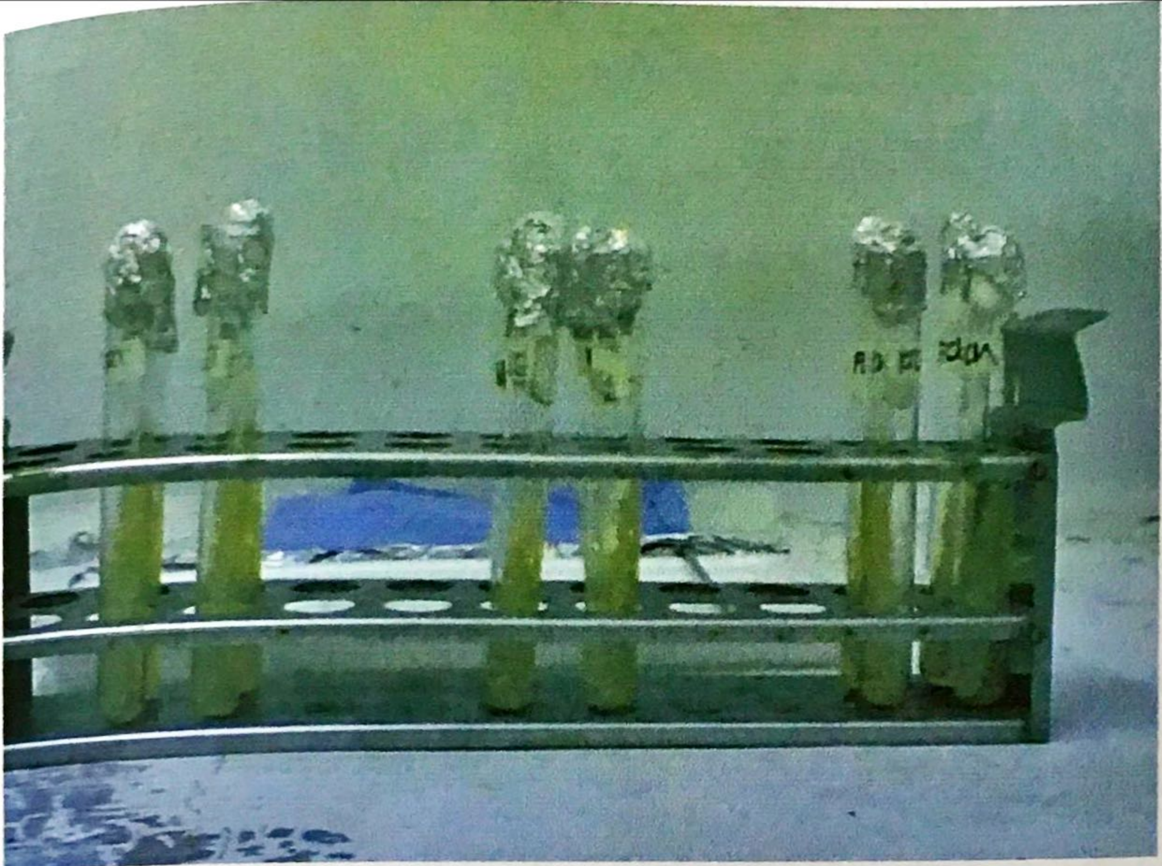

Plate 5. Agar-Agar Slant Culture of *Saccharomyces cerevisiae*

Plate 6. PYGE Broth

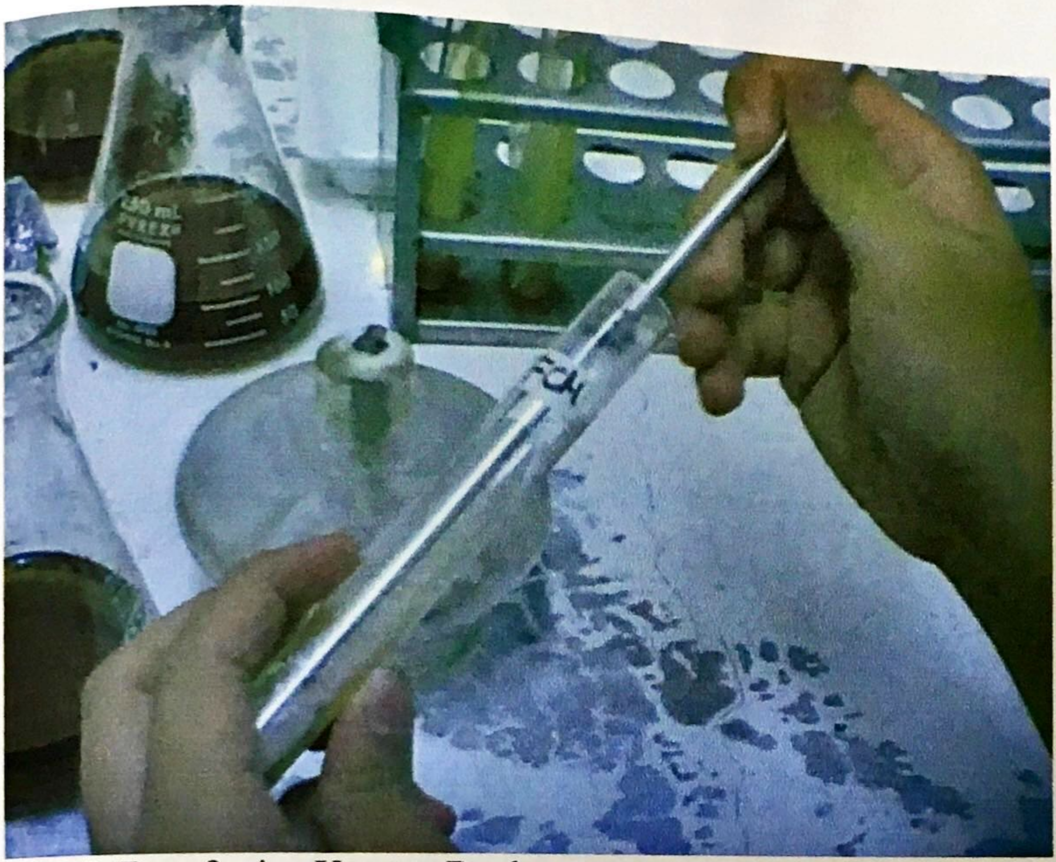

Plate 7. Transferring Yeast to Broth

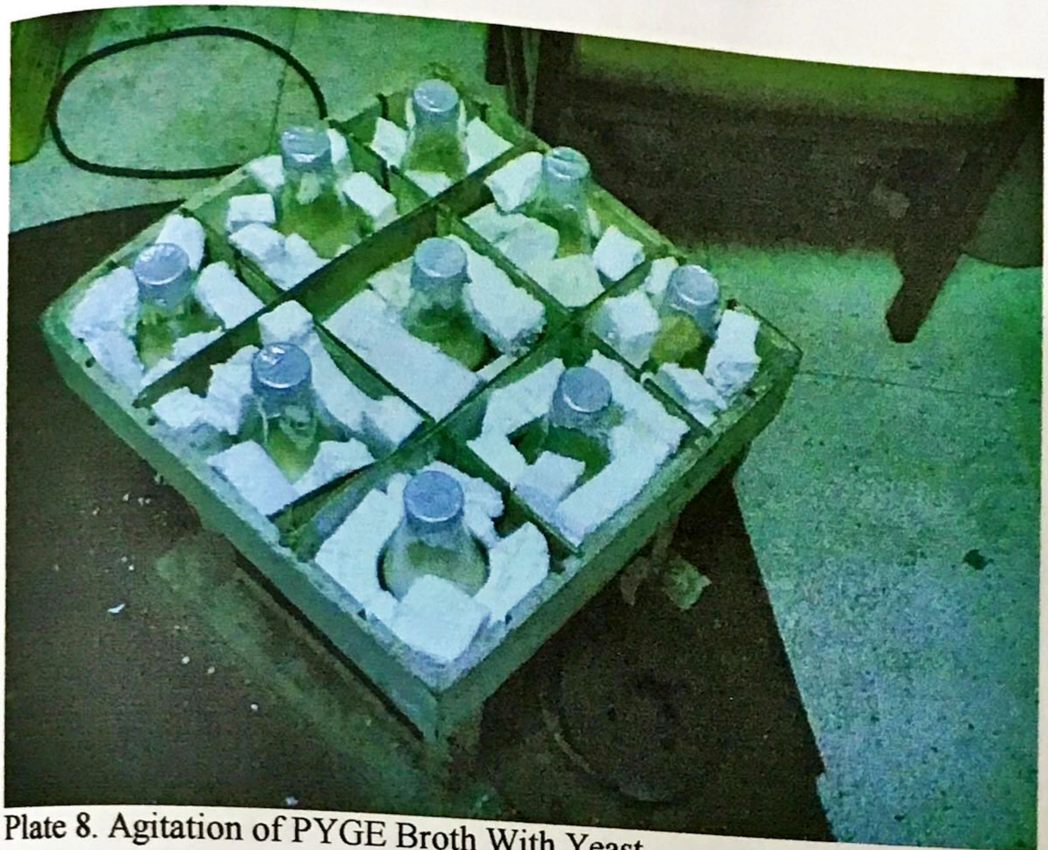

Plate 8. Agitation of PYGE Broth With Yeast

Plate 9. Fermentation

Plate 10. Distillation