FEASIBILITY OF PRODUCTION OF ETHANOL FROM YAM BEANS (*Pachyrizus erosus*) USING CRUDE METHODS

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ABSTRACT

Ethanol is a volatile, colorless, odorless liquid which can be produced from starch crops. Yam beans are an underutilized starch crop. This study determined the feasibility of producing ethanol from yam beans using crude methods. This study aimed to characterize the ethanol which can be produced from yam beans. The yam beans were broken down by grating. They were then hydrolyzed, saccharified, fermented for 7 days using *S. cerevisiae* and then distilled. Total recovered volume was 5 ml, which is a 0.08% to 0.25% recovery. The recovered liquid had 6.5 to 7 pH, a clear appearance, no turbidity, low amount of sediments and mass density of 0.83 to 0.88.

Key Words: Yam Beans, Ethanol, Fermentation, Bioethanol, Biofuels

APPROVAL SHEET

This research paper herein entitled:

FEASIBILITY OF PRODUCTION OF ETHANOL FROM *Pachyrizus erosus* USING CRUDE METHODS

Prepared and submitted by Freilich Ezekiel Q. Areola in partial fulfillment of the requirements in Science Research 2, has been examined and recommended for acceptance and approval.

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CHAPTER 1
INTRODUCTION

A. Background

Ethanol, also known as alcohol is a volatile, flammable, colorless liquid. It has a chemical formula of C_5H_5OH and a boiling point of 78.4^{O}C.

Ethanol has been used by humans for at least 9000 years. It was first prepared synthetically in 1826 and was used as lamp fuel in the United States as early as 1840. In 1908, the Ford Model T could be adapted to run on pure ethanol. During the Prohibition era, ethanol became associated with moonshiners and it fell into disuse until the late 20th century.

In recent years, due largely to the uncertainties posed by fuel supplies and efforts to lessen carbon dioxide, bioethanol (along with biodiesel) has become one of the most promising biofuels today and is considered as the only feasible short-to-medium term alternative to fossil transport fuels in Europe and elsewhere (EREC).

In Brazil and the United States, there is a large presence of industrial-grade ethanol plants, with dry-milling machines, large fermentation vats and multi-column distillation plants.

There have been many types of feedstock used in the production of ethanol, such as potatoes (Liimatainen and others, 2004), corn (Mojovic and others, 2006), sweet sorghum (Mamma and others, 1996), bagasse (Sendelius, 2005), barley (Palmarola – Adrados and others, 2005), cassava (Sriroth and others, 2003), wheat (Kaparaju and others, 2009) and sugar beet (Halleux and others, 2008).

Yam bean (*Pachyrizus erosus)* colloquially known as *singkamas*, is a plant grown primarily for its large taproot. It is a tropical tuber legume easily grown and holds great potential as a new source of starch (Melo et al).

B. Statement of the Problem

This study determined the feasibility of producing ethanol using crude methods from yam beans.

C. Objectives of the Study

General Objective: This study aimed to characterize the ethanol which can be produced from yam beans using crude methods.

Specific Objective: Purposely, this study aimed to:

1. Measure the amount of ethanol produced from yam beans and find the percent recovered.
2. Determine the ethanol's, a) pH b) mass density c) appearance.
3. Compare the values with standards.

D. Significance of the Study

Bioethanol is seen as a good fuel alternative because the source crops can be grown renewably and can be found in most climates around the world. In addition, the use of bioethanol is generally CO_2 neutral, which refers to achieving net zero carbon emissions by balancing a measured amount of carbon released with an equivalent amount sequestered or offset. This is achieved because in the growing phase of the source crop, CO_2 is absorbed by the plant and oxygen is released in the same volume that CO_2 is produced in the combustion of the fuel. This creates an obvious advantage over fossil fuels which only emit CO_2 as well as other poisonous emissions.

Global energy demand is projected to rise rapidly in this century due to population growth, increasing standards of living, and the energy intensity of developing economies. Biologically derived fuels (biofuels) can be renewable, sustainable, and expandable to meet the growing demand.

This study can provide knowledge to the shared mind of science by collecting data regarding yam beans. Also, if successful, the production of ethanol would provide an alternate revenue source for yam bean farmers around the world.

E. Scope and Delimitation

This study aimed to ferment and distill ethanol from yam beans, measure the amount and calculate the percent recovered and to determine the ethanol pH, mass density and appearance of ethanol which were produced from yam beans.

The study was conducted in the Philippine Science High School - Western Visayas Research Laboratory and the duration of this study did not exceed six weeks.

F. Definition of Terms

Beer - Fermented beverage (McGraw-Hill Dictionary of Scientific Terms); A semi dilute ethanol solution produced after fermentation; composed of ethanol, solids from original feedstock, yeast and water.

Bioethanol - Any alcohol fuel produced from, but not limited to, agricultural feedstocks.

Mass density - mass per unit volume (www.hyperphysics.com).

Mash - Fermentable starchy mixture from which alcohol or spirits can be distilled (McGraw-Hill Dictionary of Scientific Terms). This is the meal mixed with water and alpha-amylase, which is then cooked in a steam cooker.

Meal - Substance produced by grinding (McGraw-Hill Dictionary of Scientific Terms). In this study, this would be the yam beans which have been pulverized.

Percent recovery - mass of purified substance / mass of impure substance x100.

pH - A number used in expressing acidity or alkalinity (Merriam-Webster).

Yam beans - a starchy taproot native to Mexico; this is the raw material for the study.

CHAPTER 2

REVIEW OF RELATED LITERATURE

A. Bioethanol

Ethanol is any clear, colorless alcohol-based alternative fuel produced by fermenting and concentrating starch crops that have been converted into simple sugars.

Alcohol (ethanol) is employed in nearly all industries. In addition, it is the raw material for making hundreds of chemicals, such as acetaldehyde, ethyl acetate, acetic acid, ethylene dibromide, glycols, ethyl chloride, all ethyl esters. The largest single use of ethanol is as a motor fuel and fuel additive. It is also used in the manufacture of varnishes and perfumes, in many medicines and drugs, and as a preservative of biological specimens.

B. Production of Bioethanol

B.1 Processes

B.1.1 Milling

In the study conducted by Liimatainen et al, the raw material, in their case, potatoes, were mashed to a particle size of about 5 mm using a grinder.

Pre-treatment can be carried out in different ways such as mechanical combination (Cadoche and Lopez, 1989), steam explosion (Gregg and Saddler, 1996), ammonia fiber explosion (Kim et al., 2003), acid or alkaline pretreatment (Damaso et al., 2004; Kuhad et al., 1997) and biological treatment (Keller, et al., 2003)

B.1.2 Hydrolysis

After pre-treatment there are two types of processes to hydrolyze the feed stocks into monomeric sugar constituents required for fermentation into ethanol. The hydrolysis methods most commonly used are acid (dilute and concentrated) and enzymatic.

There are two types of acid hydrolysis process commonly used - dilute and concentrated acid hydrolysis. The dilute acid process is conducted under high temperature and pressure and has reaction time in the range of seconds or minutes. The concentrated acid process uses relatively mild temperatures, but at high concentration of sulfuric acid and a minimum pressure involved, which only creates by pumping the materials from vessel to vessel. Reaction times are typically much longer than for dilute acid process.

The acid, alkaline or fungal pretreated lignocellulosics can be saccharified enzymatically to get fermentable sugars (Ghose and Bisaria, 1979; Kuhad et al., 1997; Itoh et al., 2003; Tucker et al., 2003)

B.1.3 Fermentation

The glucose syrup obtained after hydrolysis is used for ethanol fermentation. Ethanol production from sugars derived from starch and sucrose has been commercially dominated by the yeast *S. cereviseae* (Lin and Tanaka, 2006). Thermotolerant yeast could be more suitable for ethanol production at industrial level. In high temperature process energy savings can be achieved through a reduction in cooling costs. Considering this approach, Sree et al. (1999) developed solid state fermentation system for ethanol production from sweet sorghum and potato employing a thermotolerant *S. cereviseae* strain (VS3).

B.1.4 Distillation

Distillation is a separation process that involves heating a liquid to its boiling point, transferring the vapor to a different portion of the apparatus, then condensing the vapor and collecting the condensate in another container. This technique is one of the most useful for separating a mixture of liquids when the components have different boiling points. Industrially, distillation is the basis for the separation of crude oil into the various, more useful hydrocarbon fractions. Chemically, distillation is the principal method for purifying liquids (e.g. samples, or solvents for performing reactions). Successful distillation depends on several factors, including the difference in boiling points of the materials in the mixture, and therefore the difference in their vapour pressures, the type of apparatus used, and the care exercised by the experimentalist.

The most common methods of distillation are simple distillation and fractional distillation. Simple distillation can be used when the liquids to be separated have boiling points that are quite different. Fractional distillation should be used when the boiling points are similar.

B.2 Potential Sources

It can be made not only from corn(Pimentel et al, 2005), barley (Linko et al, 1983), and wheat (Nigam, 2001), but also from cellulose feedstock like corn stalks(Fengwei et al, 2002), rice straw (Karimi et al, 2006), sugar cane bagasse,(Van Zyl et al, 1988), switchgrass(Pimentel et al, 2005), and municipal solid waste that have considerable amounts of sugar and other material that can be converted into starch or cellulose.

C. Quality Testing of Ethanol

C.1 pH

The pH is a measure of the acid strength of a fuel ethanol and is a predictor of the fuel ethanol's corrosion potential. Low pH (less than 6) fuel ethanol can cause corrosive wear in fuel injectors and engine cylinders. High pH(more than 9) fuel ethanol can cause fuel pump plastic parts to fail.

C.2 Acidity

Low molecular weight organic acids such as acetic acid are corrosive to many metals. It is necessary to limit the concentration of these acids in the fuel ethanol to minimize damage to metal engine components.

C.3 Relative density

Specific Gravity (now more commonly called Relative Density) is required for the conversion of measured volumes to volumes at 15 degrees C (the standard temperature). The specific gravity of an ethanol product may be an indicator of contamination.

C.4 Visual appearance

To ascertain the quality of ethanol, it is observed visually. The ethanol should be colorless, clear and bright at ambient temperatures. Presence of other colors such as yellow would indicate contamination. Presence of sediments along with presence of turbidity will be noted.

D. Yam Beans

D.1 General Characteristics

Yam bean (*Pachyrizus erosus)* colloquially known as *singkamas*, is a plant grown primarily for its large taproot. It belongs to the family Leguminosae, subfamily Papilionoidea. This leguminous plant has species native from the Amazon region and from Mexico semiarid region (Sorensen, 1996), which grow well in tropical and sub-tropical regions, in acid and sodic soils and has high potential in nitrogen fixation (Stamford et al., 2002). Yam beans as potatoes (Solanum tuberosum), yams (Dioscorea rotundata) and cassava (Manihot utilissima) yield tuberous roots (60 t/ha) and starch is the main component. The starch from the yam bean is similar to other tuber starches and contains about 23% amylase (Melo et al., 1994).

The yam bean (*Pachyrhizus spp*) is one of the legume root crops. Unlike its close relatives the soybean and the *Phaseolus* beans, the yam bean is exclusively used for its tuberous roots (Sørensen, 1996; Sørensen et al., 1997). The name yam bean is used to designate the species within the genus *Pachyrhizus*, in particular the three cultivated species; *P. erosus*, from the semiarid tropics of Central America; *P. tuberosus* from the tropical lowlands of both slopes of the Andean mountain range (Sørensen, 1996) and *P. ahipa* from Andean highland (Sørensen et al., 1996). Moreover, *P. erosus* is cultivated in many South East Asian countries. The yam bean is attractive for agronomy and plant breeding. As a root crop it might provide high yields as well as high yield stability and as a legume it will produce protein rich food and improve sustainability in cropping systems.

D.2 Uses

The young tubers are eaten raw in salads, or cooked as a vegetable, or in pickles and chutney. They are popular among the lower income groups in parts of Latin America and the Caribbean. In the USA they are becoming increasingly used, both for eating in their own right and as a substitute for Chinese water chestnut. As the roots mature their starch content increases and older roots are sometimes used as a source of starch or for animal feeding. In China, the dried roots are reported to be used as a cooling food for people with fever.

CHAPTER 3
METHODOLOGY

A. Acquiring of raw materials

Bacterial alpha-amylase and gluco-amylase were acquired from the Biotech Department of the University of the Philippines – Los Banos campus. Brewer's yeast, also known as baker's yeast, and 5 kg of yam beans were acquired from SM Supermarket.

B. Preparation of yam beans.

The yam beans were cut into one-eighths and then grated. They were then collected into a large plastic container. Cold water was then added. From the large plastic container, they were then separated into 3 1L beakers.

C. Liquefaction

The hotplate was heated to a temperature of 100°C. The slurry was added to the 1L beaker slowly. It was then cooked for one hour. The meal was then cooled to 77°C. 50 mL of alpha-amylase enzyme was then added after cooling.

D. Saccharification

The temperature was then to 100 °C raised after 15 minutes. It was kept at 100°C for 30 minutes. It was then mixed for 30 minutes. After this, 8 teaspoons of glucoamylase enzymes were added.

E. Fermentation

Each 1L beaker had 4 ounces of yeast mixed in. It was then stirred for 15 minutes before having its top covered by 3 layers of cling wrap. The fermentation lasted for 7 days. The average temperature was 27 °C. It was then mixed regularly everyday for 5 minutes.

F. Filtration of ethanol solution from beer

The ethanol solution was separated from the beer using a 20-µm cheesecloth. The solution was collected into 3 1L beakers.

G. Distillation

200 ml was collected from the ethanol solution in 1L beaker. It was filtered into a distilling flask. It was distilled using the distillation set-up at 78 ± 3 degrees Celsius. The distillation occurred for 3 hours. The distillates were then collected from the Erlenmayer flask. All the distillates were filtered into 3 screwcap test tubes, one each for all the distillate from each 1L.

H. Analysis of distillate

H.1 Appearance

The distillate was evaluated in the test tube. Its color, the presence of turbidity and the presence of sediments were evaluated and noted.

H.2 Relative density

Each screwtop test tube was opened individually. The 10 ml graduated cylinder was tared on the analytical balance after the value remained constant.

After this, the ethanol was filtered into the graduated cylinder. The volume was noted. It was then put into the balance and the mass was noted. The density was calculated by dividing the mass by the volume at the time of weighing.

H.3 pH

Each test tube was opened individually. A strip of pH paper was inserted and then wet with the ethanol. The color change was then compared with the provided guide to find the ethanol's pH.

H.4 Percent Recovery

The volume inside each 1L beaker was noted. This is the "impure substance" volume. After the distillate was collected, the volume of all the distillate from the contents of each beaker was measured. This is the "pure substance" volume. The formula is [(volume of pure substance\volume of impure substance) x 100].

I. Disposal

The remaining solid mash was put into plastic bags and then buried on the grounds of Philippine Science High School Western Visayas. The distillate was put into waste bottles.

CHAPTER 4

RESULTS AND DISCUSSIONS

This study was conducted to determine the characteristics of ethanol recovered from yam beans, *Pachyrizus erosus*. It aimed to compare the appearance, color and pH from standards and % recovery and density from other studies.

A. Results

The amount of distillate recovered was low. Table 1 shows the physical characteristics of the liquid recovered after distillation of fermented yam beans.

Table 1. Physical characteristics of recovered liquid

	% recovery	Appearance	Presence of sediments	Color	pH	Mass density
Distillate	From 0.08% to 0.25%	Clear	Low to none	colorless	6.5-7	From 0.83-0.88
Standard*	-	Clear	None	Colorless	6-9	0.78

*(taken from the ASTM International Standards 2004)

B. Discussion

Yam beans are a starchy root crop. Once its starch is hydrolyzed with enzymes, glucose is released from the starch. The glucose can then be fermented by yeast to produce ethanol, which can then be recovered by distillation.

A recovery percentage of 0.08% to 0.25% was obtained. This value is relatively low, especially when compared with other studies. Liimatainen and others' study (2004), which used waste potatoes as a feedstock, was able to recover around 6.5% to 9.5% of ethanol. This was 99 to 105% of the theoretical yield from almost all of their cultivars, except for one cultivar which had an 87% yield of ethanol from the theoretical yield. This was explained by the evaporation of water during hydrolysis. The theoretical yield was calculated from the starch content alone. Abouzied and others' study (1986) using potato starch was able to recover around 0.17% to 1.33%. This figure was greater than 96% of the theoretical yield. The low recovery percentage was explained by the fact that in their study, they used unhydrolyzed potato starch which was simultaneously fermented by *A. niger* and *S. cerevisiae*. The 1.33% was considered by them to be an efficient yield. In comparison, corn ethanol facilities are able to achieve over 10% recovery. This can be explained by the fact that yam beans are not very starchy, with yam beans having less than 10% starch.(Sorensen, 1996)

The studies of Liimatainen and others (2004) and Abouzied and others (1986) were able to verify that they produced ethanol using gas chromatography. However, in this study, it is not immediately possible to conclude that the recovered distillate is ethanol. This is because certain parameters were not accomplished due to the small amount of distillate or lack of equipment to conduct the experiment. For instance, the method to test for the ethanol content using gas chromatography was not used due to the lack of the gas chromatograph. Another parameter which was not accomplished is the specific gravity. It wasn't possible to test its specific gravity because the volume of the distillate was not enough to test by hydrometer.

The crude methods used in this study are not an efficient way of producing ethanol. Therefore, it is not a viable source of ethanol because the cost of producing ethanol from yam beans was much too expensive to produce. The revenue would not have been able to recoup the expenses of making ethanol.

However, based on the recovered liquid's characteristics, we are able to find that it matches the characteristics of ethanol.

CHAPTER 5
SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary:

This study is aimed to characterize the ethanol which can be produced from yam beans using crude methods. Specifically, it aimed to:

1. Measure the amount of ethanol produced from yam beans *(Pachyrizus erosus)* and calculate the percent recovered.
2. Measure the ethanol's specific gravity, pH, appearance and compare them with standard values

Ethanol was successfully produced from yam beans, with a yield of 5 mL.

Conclusions:

Ethanol can be produced from the fermentation of yam beans using crude methods. However, the percent recovery is low.

Recommendations:

It is recommended for further studies to:

- Increase the number of parameters to be used to better describe the distillate, specifically the use of gas chromatography to verify the ethanol content.
- Modification of the method, specifically the fermentation process.

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APPENDIX A. Raw Data

Calculating the percent recovery

	Beaker 1	Beaker 2	Beaker 3
Initial volume	680 mL	670 mL	500 mL
Recovered distillate	1.7 mL	1.4 mL	0.4 mL
Percent recovery	0.25%	0.18%	0.08%

Calculating the density:

	Beaker 1	Beaker 2	Beaker 3
Initial Volume	1.5 mL	1.2 mL	0.33 mL
Mass	1.33 g	1.15 mL	0.275 g
Calculated density	0.881 g/mL	0.885 g/mL	0.833 g/mL

APPENDIX B. PLATES

Plate 1. 1L beaker after grinding, hydrolysis and adding of yeast

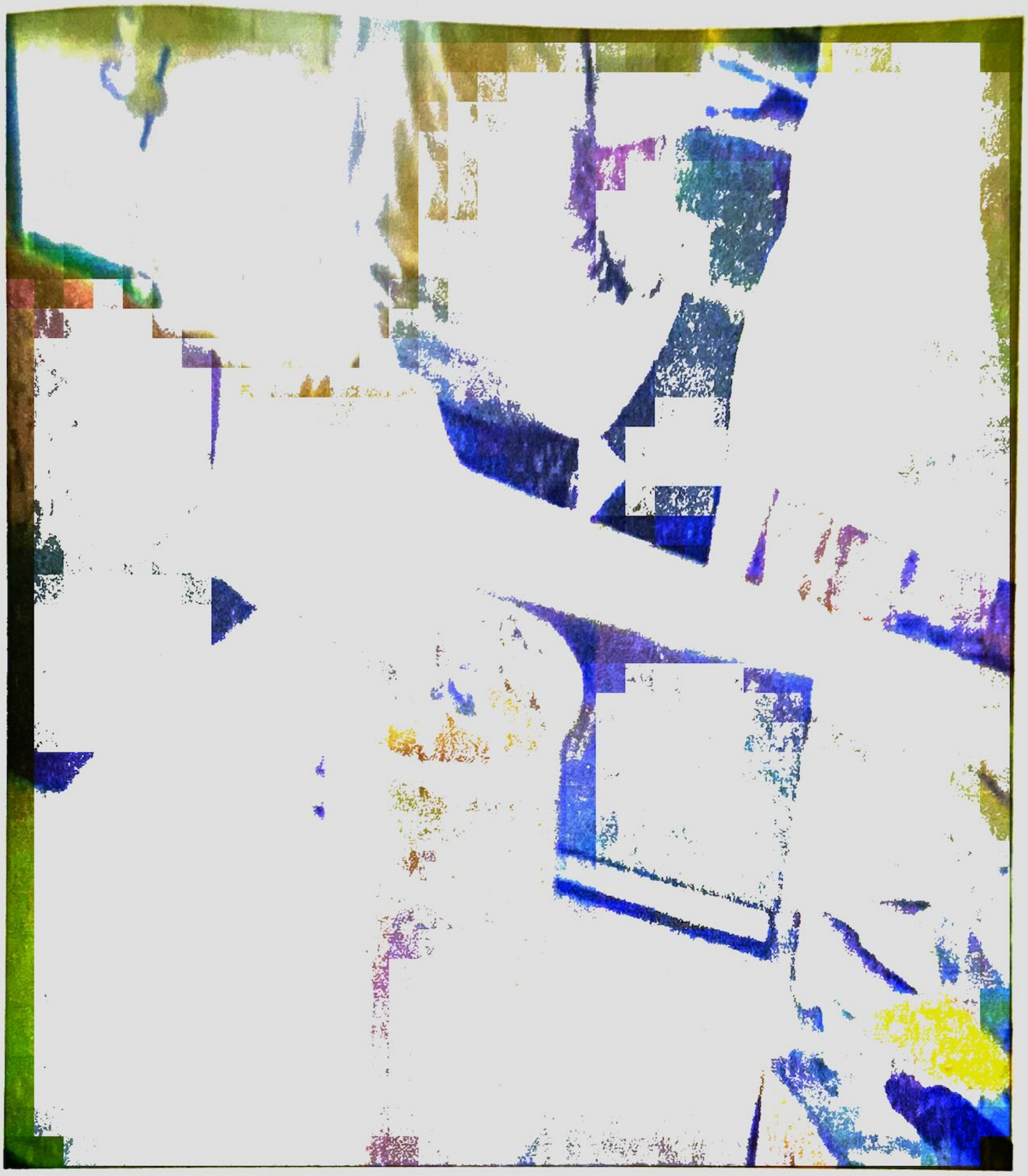

Plate 2.Regular stirring of the mash during fermentation

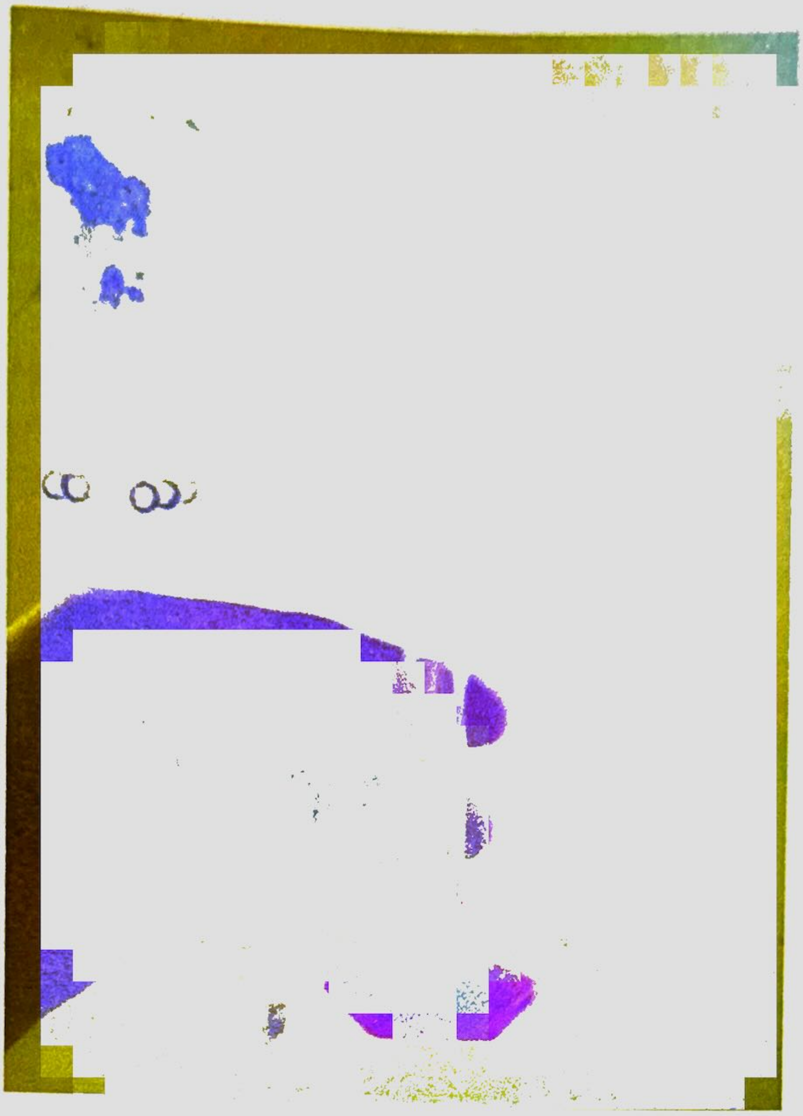

Plate 3.Amount of distillate of first replicate

Plate 4. Pouring from screwcap test tube into graduated cylinder

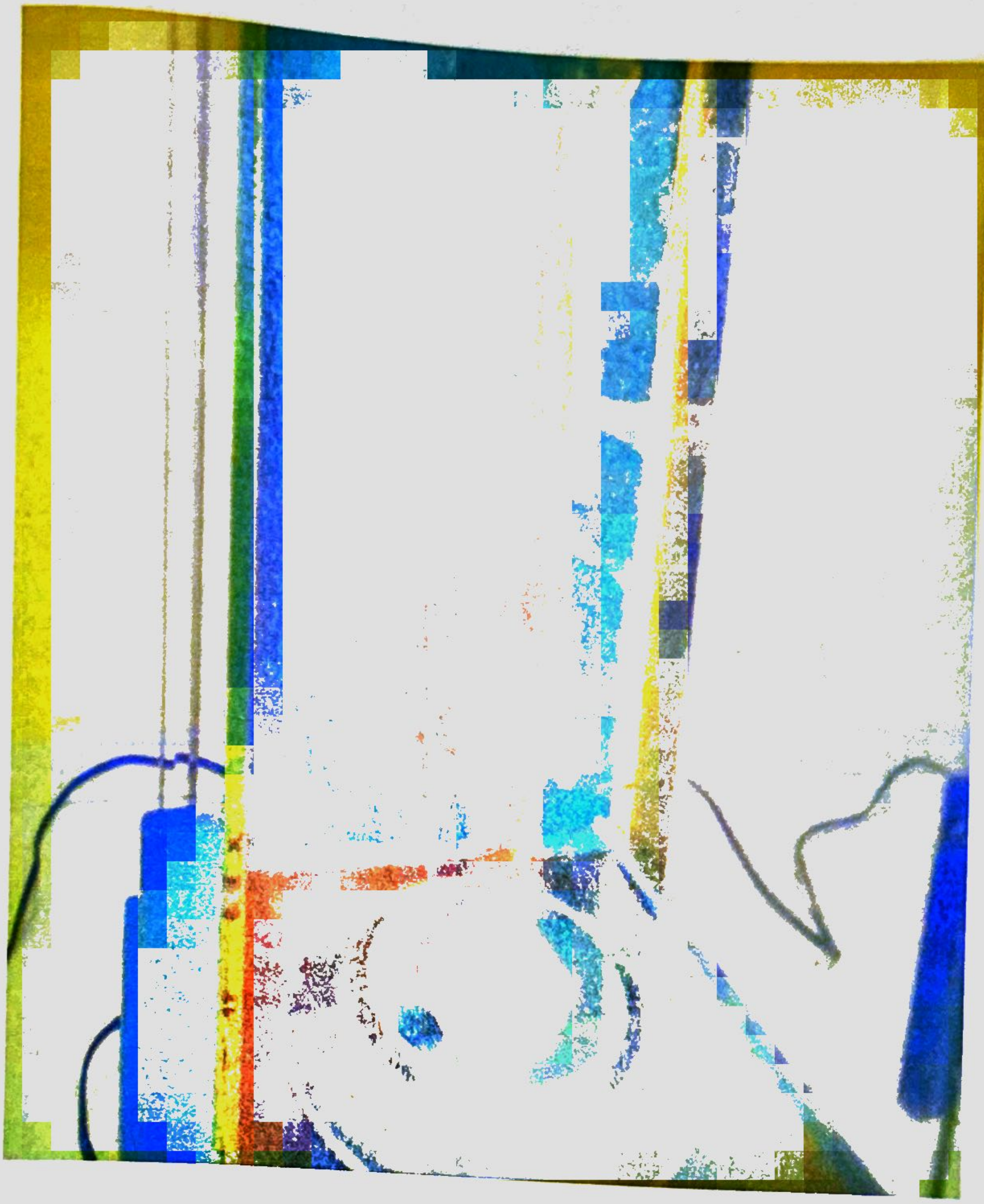

Plate 5.Weighing of distillate