STUDY ON QUALITY IMPROVEMENT OF COCONUT WATER VINEGAR.

By

W.L.I. Wijesekara.

Thesis submitted in partial fulfillment of the requirements for the degree of
Bachelor of Science
In
Food Science and Technology,
Faculty of Applied Sciences,
Sabaragamuwa University of Sri Lanka,
Buttala,
Sri Lanka.

February 2002

Faculty of Applied Sciences,
Sabaragamuwa University of Sri Lanka,
Buttala,
Sri Lanka.

DECLARATION.

The work described in this thesis was carried out by me at the Food Research Unit, Department of Agriculture, Peradeniya and the faculty of Applied Sciences, Sabaragamuwa University of Sri Lanka, Buttala; Under supervision of Mr. T.D.W. Siriwardena and Mr. M.A.J. Wansapala. A report on this has not been submitted to any other university for another degree.

....

W.L.I. Wijesekara.

Date. 04. 04. 2002

Certified by,

Mr. T.D.W. Siriwardena,
External Supervisor,
Head,
Food Research Unit,
Department of Agriculture,
Peradeniya,
Sri Lanka.

....

Date. 05.04.2002

Mr. M.A.J. Wansapala,
Internal Supervisor,
Course Coordinator,
Degree program in Food Science & Technology,
Department of Natural Resources,
Faculty of Applied Sciences,
Sabaragamuwa University of Sri Lanka,
Buttala,
Sri Lanka.

....

Date. 06/04/2002

AFFECTIONATELY DEDICATED TO

MY EVERLOVING

PARENTS

&

BROTHERS.

ACKNOWLEDGEMENT.

I wish to express my sincere thanks to my internal supervisor Mr. M.A. Jagath Wansapala, Course Coodinator, B.Sc. degree progaram in Food Science and Technology, Department of Natural Resources, Faculty of Applied Sciences, Sabaragamuwa University of Sri Lanka, Buttala, Sri Lanka, for given me the facilities to carry out this research study successfully.

I would like to express my heartiest thanks to Mr. T.D.W. Siriwardena, my external supervisor, Head, Food Research Unit, Department of Agriculture, Gannoruwa, Peradeniya, Sri Lanka, for his valuable guidance, encouragement and supervision given me to complete this study.

Also I would like to thanks Dr. K.K.D.S. Ranaweera, Dean, Faculty of Applied Sciences, Sabaragamuwa University of Sri Lanka and Dr. D.B.M. Wickramarathne, Head, Department of Physical Sciences, Faculty of Applied Sciences, Sabaragamuwa University of Sri Lanka, for their numerous advises.

I also extend my thanks to all Academic staff and all Laboratory staff members for their incorporation throughout my research study.

At last but not least I wish to forward my thanks to my dearest parents, brothers and colleagues for helping me in numerous ways to make this study successfully.

ABSTRACT.

A study was conducted to produce high quality and low turbidity coconut water vinegar. A reliable method for differentiates coconut water vinegar from artificial or imitation vinegar has been studied.

In this experiment, traditional vat method was used to produce coconut water vinegar and it was aged for two months after pasteurization and before bottling. Brix value of coconut water was adjusted up to 15° brix by adding sugar (sucrose) before fermentation. During fermentation pH value and total acidity were measured. Total acidity was measured by titration method and a Hanna HI 8519 pH meter was used to measure pH value. Oxidation Value and Formol Value of coconut water vinegar were also measured. Formol Value was 0.833 and Oxidation Value was 1614.66. This Oxidation Value specific for coconut water vinegar and can be used to differentiate it from artificial vinegar, which contain Oxidation Value 0-20.

CONTENTS.

	PAGE No.
ABSTRACT.	i
ACKNOWLEDGEMENT.	ii
LIST OF FIGURES.	vi
LIST OF TABLES.	vii
CONTENTS.	
01. INTRODUCTION.	1
02. LITERATURE REVIEW.	3
2.1 DEFINITIONS AND ORIGIN OF VINEGAR.	3
2.2 TYPES OF VINEGAR.	4
2.2.1 NATURAL OR BREWED VINEGAR.	4
2.2.2 SPECIALTY VINEGARS.	5
2.3 USES OF VINEGAR.	5
2.4 MAIN STEPS OF VINEGAR PRODUCTION.	6
2.4.1 FERMENTATION I (ALCOHOLIC FERMENTATION).	7
2.4.2 FERMENTATION II (ACETIC ACID FERMENTATION).	7
2.5 METHODS OF VINEGAR PRODUCTION.	8
2.5.1 SLOW PROCESS.	8
2.5.2 ORLEAN'S SLOW PROCESS.	9
2.5.3 QUICK VINEGAR PROCESS.	9
2.6 POST PRODUCTION PROCESS OF VINEGAR.	10
2.6.1 AGEING OF VINEGAR.	10
2.6.2 CLARIFICATION OF VINEGAR.	10
2.6.3 PASTEURIZATION OF VINEGAR.	10
2.6.4 COLORING OF VINEGAR.	11
2.7 CAUSES FOR SPOILAGE OF VINEGAR.	11
2.7.1 VINEGAR FLIES.	11
2.7.2 VINEGAR EELS.	11
2.7.3 LACTIC ACID BACTERIA.	11

2.7.4 WINE FLOWERS. 12
2.7.5 VINEGAR LOUSE. 12
2.7.6 VINEGAR MITES. 12
2.8 COCONUT WATER. 12
2.8.1 COMPOSITION OF COCONUT WATER. 13
2.8.2 COCONUT WATER AS A WASTE PRODUCT. 13
2.9 PRODUCTION OF VINEGAR FROM COCONUT WATER. 15
2.10 COMPOSITION OF COCONUT WATER VINEGAR. 15

03. MATERIALS AND METHODS. 17
3.1 MATERIALS. 17
3.1.1 COCONUT WATER. 17
3.1.2 FERMENTATION JARS. 17
3.1.3 pH METER. 17
3.1.4 REFRACTOMETER. 17
3.1.5 GLASSWARE. 17
3.1.6 YEAST STARTER. 18
3.1.7 AUTOCLAVE. 18
3.1.8 AERATOR. 18
3.1.9 COMMERCIAL VINEGARS. 18
3.1.10 OTHER MATERIALS. 18
3.2 METHODOLOGY. 18
3.2.1 PREPARATION OF COCONUT WATER VINEGAR. 19
3.2.2 DETERMINATION OF TOTAL ACIDITY. 21
3 2.3 DETERMINATION OF FORMOL VALUE. 21
3.2.4 DETERMINATION OF OXIDATION VALUE. 22
3.2.5 TEST FOR MINERAL ACIDS. 23
3.2.6 TEST FOR VINEGAR EELS. 23
3.2.7 DETERMINATION OF pH VALUE. 24

04. RESULTS AND DISCUSSION. 25
4.1 CHANGES IN TOTAL ACIDITY AND pH VALUE DURING FERMENTATION OF SUGARED COCONUT WATER. 25
4.2 FORMOL VALUE. 27

4.3 OXIDATION VALUE. 28
4.4 MINERAL ACIDS AND VINEGAR EELS. 28
4.5 ALCOHOLS AND ACETIC ACID YIELD. 29

05. CONCLUSIONS. 30

REFERENCES. 31

APPENDIX. 32

LIST OF FIGURES.

FIGURE 1. PROCESS FLOW OF COCONUT WATER VINEGAR PRODUCTION. 20

FIGURE 2. DISTILLATION UNIT FOR DISTILL VINEGAR. 22

FIGURE 3. CHANGES IN TOTAL ACIDITY AND PH VALUE DURING FERMENTATION. 26

LIST OF TABLES.

TABLE 2.1 COMPOSITION OF COCONUT WATER. 13

TABLE 2.2 COMPOSITION OF EFFLUENT WATER FROM A DESICCATOR. 14

TABLE 2.3 COMPOSITION OF COCONUT WATER VINEGAR. 16

TABLE 4.1 CHANGES IN TOTAL ACIDITY AND pH VALUE DURING FERMENTATION. 24

TABLE 4.2 FORMOL VALUES OF DIFFERENT VINEGAR SAMPLES. 26

TABLE 4.3 OXIDATION VALUES OF DIFFERENT VINEGAR SAMPLES. 27

CHAPTER – 01

INTRODUCTION

Vinegar has been known and appreciated as an important food adjunct (condiment and preservative) for as long as man has been able to practice the arts of brewing and wine making. Vinegar is one of the keys to good cooking. Because of its sourness, which is able to bring out the sweetness, saltiness and glutamate-like taste, it deepens the taste of the entire dish. The aroma of vinegar is another important element in cooking. It harmonizes well with and enhances the aromas of other cooking materials. Vinegar also preserves the color and shape of some foods, thereby preventing them from going bad or losing their appeal, one of the shortcomings of vinegar is its sharp and irritating sourness and odor, which many people find unpleasant. For this reason the production of a well balanced, mild and aromatic coconut water vinegar is of utmost importance when putting together cooking materials to make delicious, aromatic dishes.

Coconut (*Cocos nucifera*) belongs to Family Palmaceae and is a monocotyledonous tree, which bears fruits throughout the year. Coconut is commonly grown in Sri Lanka, the cultivation covers about 439,000 hectares and had produced about 2,808 million nuts in 1999 (Annual Report, Central Bank of Sri Lanka).

Coconut water is the liquid endosperm of the coconut fruit and it is a refreshing and a safe drink due to its sterility. But in industries, where kernel is the product, coconut water is being wasted in tons. A typical desiccated coconut mill in Sri Lanka, discards about 8 Metric Tons of coconut water daily (Priyananda, 1997). Therefore a large number of coconut water is disposed daily. This disposal creates many problems, including environmental pollution. It may get infiltrated into drinking water wells making the water unsuitable to drink. It causes rice plants to grow tall, producing low amounts of grain when added to paddy fields. This waste coconut water, could provide additional income to the farmers, if it is converted into marketable product like coconut water vinegar.

The coconut water vinegar cannot usually compete with commercial ones due to formation of turbid substances and precipitates during storage. The improvement of quality of coconut water vinegar, particularly its clarity, may therefore increase its sale ability. This will consequently benefit coconut water vinegar producers as well as augment the income of small coconut farmers. Commercially, sparkling clear vinegar is obtained by passing the solution through a multi-plate filter at high pressure with diatomaceous earth as the filtering aid (United States Industrial Chemicals Company, 1969). For white distilled vinegar, distilled denatured alcohol is used as substrate, for acetic acid fermentation by bacteria (Nickol, 1979). However, both processes seem inappropriate for small-scale operation due to the cost of required equipment in the former and of nutrients added to the fermenter to sustain the growth of bacteria in the latter.

Artificial or imitation vinegar contains acetic acid, not wholly derived from alcoholic and subsequent acetous fermentation. Commercial acetic acid is available in Sri Lanka (being used in the rubber industry as a latex coagulant). The main problem in the vinegar industry is its adulteration with diluted commercial acetic acid.

Objectives of this study are,

(i) Determination of quality control parameters of coconut water vinegar.
(ii) Identify the main industrial problems in coconut water vinegar production.
(iii) Identify a reliable method to differentiate between coconut water vinegar and artificial vinegar.

CHAPTER – 02

LITERATURE REVIEW.

2.1 DEINITION AND ORIGIN OF VINEGAR.

Vinegar has been used for thousands of years both as a preservative and to give an acid flavor to foods. For flavoring it is used to marinate foods, in dressings for salads and vegetables and it is also used as a condiment at the table.

It is intriguing to think that in today's computerized, sophisticated world, we're still using one product, which was discovered – quite by chance – more than 10,000 years ago.

The word vinegar comes from the French *vinaigre,* meaning sour wine. That is its origin, the discovery that a cask of wine gone past its time had turned to a wonderful new product. The first vinegars developed were from wine that had soured naturally.

Vinegar is believed to be the oldest fermented product known to man. Its origin is associated with the first time an alcoholic drink went sour. The ancients were quick to find the remarkable versatility of vinegar. The Babylonians used it as a preservative and as a condiment and it was they who began flavoring it with herbs. Roman legionnaires used it as a beverage. Cleopatra demonstrated its solvent property by dissolving precious pearls in it to win a wager that she could consume a fortune in a single meal. Hippocrates extolled its medicinal qualities and, indeed, it was probably one of our earliest remedies. Biblical references show how it was much used for its soothing and healing properties. And when Hannibal crossed the Alps, it was vinegar, which helped pave the way. Obstructive boulders were heated and doused with vinegar, which cracked and crumbled them.

The first large scale production of vinegar occurred in France during the 16th century for use by the French as well as for export to the British Isles and to various European countries. The first major quantities of vinegar were reportedly produced in England by processing soured beer and ale.

2.2 TYPES OF VINEGAR.

Mainly vinegars are of two types,

(I) Natural or brewed vinegar.

(II) Artificial or non-brewed vinegar.

2.2.1 NATURAL OR BREWED VINEGAR.

By definition, the United States Food and Drug Administration recognizes six types of vinegars distinguished by their sources as,

(I) Cider vinegar.

Cider vinegar made from apple juice by fermentation is called " apple cider vinegar " or simply " cider vinegar ". It should contain at least 1.6g of apple solids per 100ml of which more than 50% are reducing sugars and at least 4% of acetic acid as total acidity. Cider vinegar is particularly well known in the United States, Switzerland and Austria because of its desirable aroma.

(II) Wine vinegar.

Wine or grape wine vinegar made from grapes by acetic fermentation. It should contain at least 1g of grape solids per 100ml, 0.13 g of grape ash and at least 4% acetic acid as total acidity. Wine or grape vinegar is well popular in France.

(III) Malt vinegar.

Malt vinegar is the product made by the alcoholic and subsequent acetous fermentation, without distillation, of an infusion of barley malt or cereals whose starch has been converted by the malt. It is well known in England and South Africa. Malt vinegar contains not less than 4% of acetic acid as total acidity.

(IV) Sugar vinegar.

Sugar vinegar is made by the alcoholic and subsequent acetic acid fermentation of sugar syrup, molasses or refiner's syrup.

(V) Glucose vinegar.

Glucose vinegar is made by the alcoholic and subsequent acetous fermentation of glucose solutions.

(VI) Spirit vinegar.

This type of vinegar is made by the acetic fermentation of dilute ethyl alcohol. It should contain at least 4% of acetic acid as total acidity. It may be colored with caramel. This vinegar is also called "distilled vinegar ".

2.2.2 SPECIALTY VINEGARS.

Specialty vinegars make up a category of vinegar products that are formulated or flavored to provide a special or unusual taste when added to foods. Specialty vinegars are favorites in the gourmet market.

Herbal vinegars: Wine or white distilled vinegars are sometimes flavored with the addition of herbs, spices or other seasonings. Popular flavorings are garlic, basil and tarragon but cinnamon, clove and nutmeg flavored vinegars can be a tasty and aromatic addition to dressings.

Fruit vinegars: Fruit or fruit juice can also be infused with wine or white vinegar. Raspberry flavored vinegars, for example, create sweetened vinegar with a sweet-sour taste.

2.3 USES OF VINEGAR.

Vinegar is a common condiment in every household. It is used for seasoning meat, fish and vegetables. In the other food field, it is used in the manufacture of pickles, catsup, mayonnaise, mustard dressings, sauces and additives in manufactured foods to enhance their flavor. It contains essential amino acids and helpful vitamins, minerals and enzymes.

Throughout history vinegar has been ascribed a medicinal value. Hippocrates, the ancient Greek physician, known as the father of medicine, is believed to have prescribed it to his patients. Hippocrates was born around 460 B.C. Nowadays vinegar is found to be almost a universal preservative and a cure all. It also can kill infections, soothe coughs, ease the pain of throat, calm nausea, relieve varicose veins, ease arthritis, fade headache away, treat burns, soothe aching feet, cool sun burn, reduce itch of welts and hives, stop hiccups, treat bee sting, remover corn and calluses, protects the skin from ravages of the sun, fade age spots, minimize memory loss etc.

2.4 MAIN STEPS OF VINEGAR PRODUCTION.

Two distinct types of biochemical processes make vinegar, both the result of the action of microorganisms. The first process is brought about by the action of yeasts (*Saccharomyces cervisiae*), which change natural sugars to alcohol under controlled conditions. This is called the alcoholic fermentation. The second process results from the action of a group of bacteria (*Acetobacter aceti*) upon the alcohol portion, converting it to acetic acid. This is the acetic or acid fermentation that forms vinegar.

Proper bacterial cultures are important; timing is important; and fermentation should be carefully controlled.

The chemical reactions involved in these two processes can be represented as follows;

$$(I)\ C_6H_{12}O_6 \longrightarrow 2\,C_2H_5OH + 2\,CO_2$$

$$(II)\ 2\,C_2H_5OH + 2\,O_2 \longrightarrow 2\,CH_3COOH + H_2O$$

2.4.1 FERMENTATION – I (ALCOHOLIC FERMENTATION).

The first fermentation is anaerobic. *Saccharomyces cervisiae* is used to produce alcohol in this fermentation. If any wild *Acetobacter* is present and produce more than 0.5% acetic acid, the growth of *S. cervisiae* will be inhibited. For this reason, 125 ppm sulphur dioxide or an equivalent amount of bisulphate is added to the sugar solution to prevent the growth of mold, wild yeast, *Acetobacter* and lactic acid bacteria.

Fruit juices or sugar solutions of low concentration ferment of their own accord owing to wild yeasts normally present in fruits and in the atmosphere, but this is not desirable, because different yeasts produce different kinds of decomposition products. In order to get vinegar of good quality, it is, therefore, essential to destroy all these naturally occurring yeasts and other microorganisms by pasteurization, and then to inoculate the sterile solution thus obtained with pure yeasts.

Acidity conditions favor the production of ethanol by yeasts; pH range for the growth of *Saccharomyces cervisiae* is between 2.4 to 5 and the optimum temperature is 20 to 30C.

During alcoholic fermentation, anaerobic conditions are created, releasing carbon dioxide, which dissolve in the solution and generate carbonic acid. Because of this pH drops further and the ethanol concentration rise.

At the end of the yeast fermentation, all the sugars have been consumed.

$$C_6H_{12}O_6 \longrightarrow 2\,C_2H_5OH + 2\,CO_2$$

2.4.2 FERMENTATION – II (ACETIC ACID FERMENTATION).

Acetic acid fermentation is brought about by acetic acid bacteria *Acetobacter aceti*. These are strongly aerobic. Their activity is greatly reduced or inhibited by direct sunrays. Even diffused day light checks their growth. Acetic acid fermentation should therefore, be carried out in dark rooms fitted with orange or red glass panes.

Acetic acid bacteria require for their growth; nutrients, which are generally present in the alcoholic liquor made from fruit juices or sugary substances. If, however, distilled alcohol is used, addition of food for the bacteria becomes essential. Usually malt sprouts, phosphoric acid, potassium carbonate, trisodium phosphate and ammonium hydroxide are used. For acetic fermentation, the alcohol content of the fermented liquid is adjusted to 7 to 8 percent alcohol, because acetic acid bacteria do not function properly at higher strengths. Mother vinegar containing acetic acid bacteria is then added to it in order to check the growth of undesirable microorganisms and to hasten the process. It is generally added at the rate of one part to three parts of the fermented juice.

The range of pH for acetic acid bacteria is 4.5 to 7.5 and temperature is 5 to 42 C.
Buchanan and Gibbons (1974), listed only three species of acetic acid bacteria, namely, *Acetobacter aceti, A. pasteuriensis* and *A. peroxydens.* These three organisms consume alcohol and produce acetic acid, and as a result the pH goes down.

Acetic acid fermentation is the oxidation of ethyl alcohol by acetic bacteria, which produce acetic acid and water. The chemical process can be represented as follows,

$$C_2H_5OH + 2O_2 \longrightarrow CH_3COOH + H_2O$$

Theoretical conversion of glucose into acetic acid,

$$1g \text{ of glucose} \longrightarrow 0.51g \text{ of } C_2H_5OH \longrightarrow 0.67g \text{ of } CH_3COOH$$

2.5 METHODS OF VINEGAR PRODUCTION.

2.5.1 SLOW PROCESS.

This process takes a long time. The juice of sugary solution is filled into barrels and allowed to undergo alcoholic and acetic acid fermentation slowly. To screen off dust and flies, the bunghole of the barrel is covered with a piece of cloth and the barrel is placed in a wet warm place. In about five to six months, the sugar solution turns to vinegar.

Disadvantages of the slow process are,

- Alcoholic fermentation is often incomplete.

- Acetic acid fermentation is very slow.
- Quality of the vinegar is inferior.
- The yield is low.

2.5.2 ORLEANS SLOW PROCESS.

The origin of this method is Orleans region of France. In this process, about three-fourth of the barrel is filled with the juice. After inoculating with mother vinegar, the barrel is placed on its side. Two holes are made on either side of the barrel just above the level of the juice in addition to the bunghole. These three holes are covered with wire gauze or cheesecloth to exclude insects, dust and flies.

The barrels are kept in a warm place at 21 C to 27 C. It is allowed to proceed till the acid level reaches to its maximum strength. The process usually takes about three months for the complete conversion of the liquid into vinegar. About three-fourth of the vinegar is then withdrawn, and an equal quantity of fermented alcoholic solution is added for the further vinegar fermentation. The process can be repeated once in every three to four months. Care should, however, be taken to see that the vinegar bacteria on the surface of the fermenting juice is not disturbed, otherwise the broken film will sink to the bottom, and in the absence of air exhaust the nutrients without producing any vinegar. In order to avoid the breaking and sinking of the film, a perforated support may be placed in the barrel, about 2.5 cm below the surface of the fermenting liquid, to support the film, when the vinegar is withdrawn. Vinegar produced by the Orleans slow process ages during the process of fermentation, and is clear and of superior quality.

2.5.3 QUICK VINEGAR PROCESS.

This process is called the "generator" or the "German" process. Additional supply of oxygen is made available for the bacteria by increasing the surface for the action of the bacteria culture. This increases the rate of fermentation. The equipment used in this process is known as "upright generator". It has a false bottom and head, vent holes and spurge for discharging the liquor.

2.6 POST PRODUCTION PROCESS OF VINEGAR.

2.6.1 AGEING OF VINEGAR.

To improve its flavor, vinegar is kept in plain oak barrels for about six months. During this period, its harsh flavor changes to a more pleasant aroma and bouquet. This may be owing to the oxidation of vinegar brought about by air entering through the pores in the wood. Acetic acid may also react with alcohol to form ethyl acetate, which has a fruity flavor.

2.6.2 CLARIFICATION OF VINEGAR.

Before bottling, vinegar must be made sparkling clear. During ageing, most of the suspended materials settle down leaving a major portion of the liquid clear. The clear liquid can be siphoned out for further clarification. This can be accomplished either by using finings such as Spanish clay, bentonite, isinglass, casein, gelatin, or by filtering through pulp filters or aluminum plate and frame presses. If finings are used, the vinegar has to be stored for about a month to render it clear.

Marques and Van Den (1987) have stated that the egg albumin was the most effective agent in clarifying coconut water vinegar. Well-beaten egg albumin (preferably 7-9 g egg white/liter vinegar or two egg whites per 10 liters of vinegar) was added to raw vinegar. The solution was then stirred and heated to coagulate the albumin. It was allowed to stand for a day, decanted and filtered. The clarified vinegar was aged for 3-4 weeks for further sedimentation before bottling and pasteurization. This vinegar was very close to commercial clear vinegars in clarity.

2.6.3 PASTEURIZATION OF VINEGAR.

The vinegar, after ageing and clarification, is pasteurized to check any spoilage. It is heated in an open vessel to about 66 C and then cooled to room temperature. It can also be flash-pasteurized by passing it through aluminum pipes surrounded by hot water or steam at 66 C. Bottled vinegar is pasteurized by immersing the bottles in hot water till the vinegar inside attains a temperature of 60 C.

Pasteurization with silver: - In this process, the vinegar is either filtered through silver bearing sand, or is electrolyzed with silver electrodes at a very low current. The vinegar picks up sufficient concentration of silver ions, example; about 2 ppm to render it sterile.

2.6.4 COLORING OF VINEGAR.

Caramel color, which is the only permitted color in the case of vinegar, is employed for coloring vinegar.

2.7 CAUSES FOR SPOILAGE OF VINEGAR.

2.7.1 VINEGAR FLIES.

Known as *Drosophila cellaris*, vinegar flies are small flies, which propagate in piles of fermenting pomace or rotten fruits. Although they do not propagate by themselves in any way affect the quality of the vinegar, they hinder work. They can, however be kept away by screening the premises and by ensuring sanitary conditions.

2.7.2 VINEGAR EELS.

One of the most troublesome natural contaminants of vinegar, during manufacture is the vinegar eel or eelworm. This is a free living nematode, *Anguillula aceti*, from 1-2 mm in length and about 0.04 mm in breadth, which lives its life cycle in vinegar. It requires very little oxygen and tolerates temperatures of 0-37 C. Its pH tolerance is between pH 1.6 and 11.0. It is harmless to man, but they destroy the acid in vinegar.

They can, however, be destroyed by heating the vinegar to 60 C or by filtration. They do not grow if the container is filled to the brim.

2.7.3 LACTIC ACID BACTERIA.

Lactic acid bacteria are generally found in fermented juices. They cause cloudiness and produce disagreeable mousy flavors in the fermented juices, besides producing lactic acid and other acids. The bacteria interfere with acetic acid fermentation and lower the quality of the vinegar. In alcoholic fermentation, they may be avoided by using a starter of pure yeast, and by adding, during fermentation 20 to 25% of unpasteurized vinegar to the fermented alcoholic juice.

2.7.4 WINE FLOWERS.

This is a kind of film yeast. If the fermented juice is unnecessary exposed to air, wine flowers grow on the surface of the liquid. They destroy the alcohol and also cause cloudiness. Their growth can, however, be checked by, spreading a neutral oil like liquid paraffin over the surface of the fermented liquid or adding 20% to 25% of unpasteurized vinegar or filling the barrels to the brim.

2.7.5 VINEGAR LOUSE.

These rarely become a serious pest. The louse is a small form of aphid and develops only around generators under certain conditions.

2.7.6 VINEGAR MITES.

These mites are the enemies of acetic acid bacteria. They multiply rapidly and interfere with the oxidation of alcohol. When they die, their bodies settle to the liquid and begin putrefy. The putrefactive bacteria so produced sooner or later overpower the acetic acid bacteria.

2.8 COCONUT WATER.

Coconut water is the liquid endosperm of the seed of the coconut (Cocos *nucifera*) that nourishes an immature embryo, which later produce a spongy mass of cotyledony tissue that eventually fills the central cavity of the seed (Serrano *et al*, 1970). According to Banzon *et al*

(1990), a fully matured nut contains about 250 g of water. The less matured contains considerably more.

2.8.1 COMPOSITION OF COCONUT WATER.

The composition changes with ripening and germination of the coconut fruit. It also varies with the nutritional availability of the soil, variety of coconut etc. Concentration of total solids at early stages is about 2.5 g per 100 ml of coconut water (Child, 1974). This amount increases gradually as the nut ripens, reaches a maximum of about 6 g per 100 ml at about seventh month, (just before the kernel begins to form), and again declines. During the germination, water finally disappears and just before this, contains only about 2 g per 100 ml of total solids (Child, 1974).

The general composition of coconut water is given in table 2.1.

Constituents	*Amount (%)*
Total solids	4.71
Total sugars	2.56
Protein	0.55
Oil	0.74
Ash	0.62
Chlorides	0.17

Table 2.1 Composition of coconut water, (Joson, 1989).

2.8.2 COCONUT WATER AS A WASTE PRODUCT.

In making desiccated coconut and various other products, coconut water is being discarded in large volumes. Composition of effluent water from a desiccator is given in table 2.5.

This wastewater includes coconut water and the wash water used in the industry to wash coconuts (Banzon and Velasco, 1982).

Component	*Amount*
Acidity	759
Chlorides	772 mg/l
Nitrite	0.12 mg/l
Phosphate	42.50 mg/l
Phenols	4.8 mg/l
Sulfates	133 mg/l
BOD	5800
Suspended solids	184
Turbidity	220 Jackson Units
Grease & oil	76 ppm
PH	6.3
Volatile suspended solids	180

Table 2.2 Composition of effluent water from a desiccator, (Banzon and Velasco, 1982).

Although the water from immature nuts is very popular, water from mature nuts is becoming an industrial nuisance. The problem mainly arises from the large volume and high BOD. Usually, disposal of such effluent is done by, discharging it directly into a river or a stream. The result is an odorous decomposition and formation of black deposits, making it unsuitable to be used, unless the river flows very fast. It also may get accumulated in wells making the water unsuitable to drink. This also can be disposed into paddy fields as irrigation water. This makes plants to grow very tall, producing low amount of grains. It can be used to irrigate coconut plantations, but transport is difficult (Banzon *et al*, 1982).

The tendency to pollute the environment is enhanced by the relatively high sugar content present in coconut water, making it a suitable medium of microbial proliferation. Banzon *et al* (1982) have also stated that this effluent can be treated with standard methods of water (sewage) treatment using aerobic digestion. Some have tried to reduce the BOD by anaerobic digestion. But all these procedures are non-economical. Therefore the newest trends are to utilize the coconut water in various industries, mostly in vinegar industry.

2.9 PRODUCTION OF VINEGAR FROM COCONUT WATER.

The coconut water is strained through a filter cloth. Sugar content is adjusted to 15 degrees Brix (162 g/l) and pasteurized by heating to boiling point. The boiled mixture is cooled to lukewarm (37-40 C) and then inoculated with the active dry yeast (0.5 g/l). The mixture is allowed to undergo alcoholic fermentation for 5 to 7 days.

Another container preferably with faucet near the bottom is filled with mother vinegar or starter culture up to ¼ of its volume. The alcoholic coconut water is transferred to this container by siphoning to fill it up to ¾ of the capacity of the container. The remaining ¼ of the container is provided as headspace for effective acetic acid fermentation. The mixture is stirred thoroughly then covered with clean cloth. The mixture is allowed to undergo acidification for 7 days.

The vinegar is harvested by opening the faucet or by siphoning if no faucet is provided. The amount harvested is equivalent to the amount of the alcoholic coconut water added. The remaining vinegar will serve as the starter for the next batch of alcoholic coconut water. The procedure is repeated to have a semi-continuous process.

2.10 COMPOSITION OF COCONUT WATER VINEGAR.

The composition of vinegar depends largely on the nature and characteristics of the raw materials used. Good vinegar contains a minimum of 4% acetic acid content.

Coconut water vinegar as analyzed by the Food and Nutrition Research Institute in Philippines,

Substance	*Quantity/%*
Moisture	98.0
Total carbohydrates	1.4
Ash	0.3
Fat	0.1
Phosphorous	34 mg%

Calcium	24 mg%
Iron	0.1 mg%
Riboflavin	0.01 mg%
Protein, Thiamin & Niacin	Traces

Table 2.3 Composition of coconut water vinegar.

CHAPTER - 03

MATERIALS AND METHODS.

3.1 MATERIALS.

3.1.1 COCONUT WATER.

Coconut water was collected from nuts used in a project at the Food Research Unit, Gannoruwa.

3.1.2 FERMENTATION JARS.

Sugared coconut water was stored in glass bottles for fermentation. 750 ml glass bottles and 4 L large glass jars were used.

3.1.3 pH METER.

pH Meter (Hanna, HI 8519 pH meter) was used to measure the pH value of coconut water and pH values in each day during fermentation of sugared coconut water.

3.1.4 REFRACTOMETER.

Refractometer (Erma Hand refractometer, range 0 – 32°) was used for measure the Total Soluble Solids or the Brix value of coconut water.

3.1.5 GLASSWARE.

50 ml vensil burettes were used for all titration. Borosil petridishes, test tubes, boiling tubes and conical flasks were used as glassware. 1 ml, 5 ml and 10 ml Borosil pipettes were also used.

3.1.6 YEAST STARTER.

Activated dry yeast (Red Star) was used.

3.1.7 AUTOCLAVE.

3.1.8 AERATOR.

Fish tank aeration type aerator was used.

3.1.9 COMMERCIAL VINEGARS.

Following commercial vinegars were taken from "Food City", Kandy.
Colmon's Natural Coconut Toddy Vinegar, 775 ml bottles.
Heinz Apple Cider Vinegar, 473 ml bottles.
Garlic Wine Vinegar (Premium Red Wine), 375 ml bottles.

3.1.10 OTHER MATERIALS.

Sugar, Distilled water, Stainless steel spoons, Muslin clothes, Rubber bands and Light Microscope.

3.2 METHODOLOGY.

3.2.1 PREPARATION OF COCONUT WATER VINEGAR.

Coconut water was strained through a piece of muslin cloth and adjusted the Brix value up to 15° Brix by adding sugar. Then heating the solution to boiling point pasteurized the sugared coconut water. Then it was cooled to 35°C and inoculated with active dry yeast. This was allowed to ferment for one week in glass bottles fitted tightly with plastic covers.

After the one week the clear liquid was siphoned to wide mouth large glass jars to separate the sediments and dead yeast cells. Vinegar starter or mother vinegar produced at the Food Research Unit was used to inoculate this alcohol liquid. The inoculated alcoholic coconut water was covered with a clan muslin cloth by the aid of a rubber band and allowed to ferment for another week. An aerator was used to aerate the solution.

The vinegar was harvested after a week and pasteurized in the autoclave under 100°C and 15 lb.psi pressure during 10 minutes. Then it was bottled.

This coconut water vinegar was used to quality control tests.

Another batch was prepared in similar way and after the pasteurization, it was allowed to ageing before bottling.

The process flow for coconut water vinegar production is shown in Fig. 1.

COCONUT WATER

↓

FILTER

↓

ADD SUGAR

↓

BOIL

↓

COOL TO 37°C

↓

ADD YEAST STARTER

↓

MIX

↓

ALCOHOLIC FERMENTATION

↓

SIPHON

↓

ADD MOTHER VINEGAR STARTER TO THE CLEAR LIQUID

↓

ACETIC ACID FERMENTATION

↓

FILTER

↓

PASTEURIZE, 100°C 10 minutes

↓

AGEING

↓

BOTTLING

Figure 1. Process flow of coconut water vinegar production.

3.2.2 DETERMINATION OF TOTAL ACIDITY.

Reagents,

0.1M NaOH solution.

Phenolphthalein indicator solution.

Procedure,

10 ml of vinegar sample was taken into a conical flask and few drops of phenolphthalein indicator were added. Then it was titrated carefully to a faint pink color end-point with 0.1M NaOH solution.

Repeated three times for each sample.

This procedure was also done for the commercial vinegar samples.

Total Acidity was tested every day except holidays during the conversion of coconut water into acetic acid. Here mean value for each week was taken for plot the graph.

Calculation,

The Total Acidity as acetic acid content of the original vinegar sample,

% Acetic acid (m/v) = T * 0.6

Where T = mean titre (in ml) of 0.1M NaOH solution required to neutralize the acidity in 10 ml of the vinegar sample.

3.2.3 DETERMINATION OF FORMOL VALUE.

Reagents,

0.1M NaOH solution.

40% Formaldehyde solution.

Phenolphthalein indicator solution.

Procedure,

10 ml of vinegar sample was taken into a stoppered conical flask by using a 10 ml pipette. Few drops of phenolphthalein were added and then it was titrated with 0.1M NaOH to rose pink. 5 ml of 40% formaldehyde was also neutralized to the same tint with 0.1M NaOH solution and phenolphthalein. Then the two solutions were mixed in the stoppered conical flask and allowed to stand for 5-10 minutes.

When pink color had disappeared, titrated back with 0.1M NaOH and phenolphthalein, to the same tint. The amount of 0.1M NaOH solution required for the final titration was only recorded. Same procedure was repeated three times for each vinegar sample.
This procedure was also done for the commercial vinegar samples.

Calculation,

Formol value = amount of 0.1M NaOH solution (in ml), required for the final titration only.

3.2.4 DETERMINATION OF OXIDATION VALUE.

Reagents,

0.02M Sodium thiosulphate solution.
0.02M Potassium permanganate solution.
10% Potassium iodide solution.
Dilute Sulphuric acid.
Starch indicator solution.

Procedure,

Distillation: - 60 ml of vinegar sample was distilled from a distillation unit fitted with a small tap funnel. After 45 ml of distillate had came over, 15 ml of distilled water was added to the flask down the tap funnel and distillate a further 15 ml to give a total volume of distillate of 60 ml.

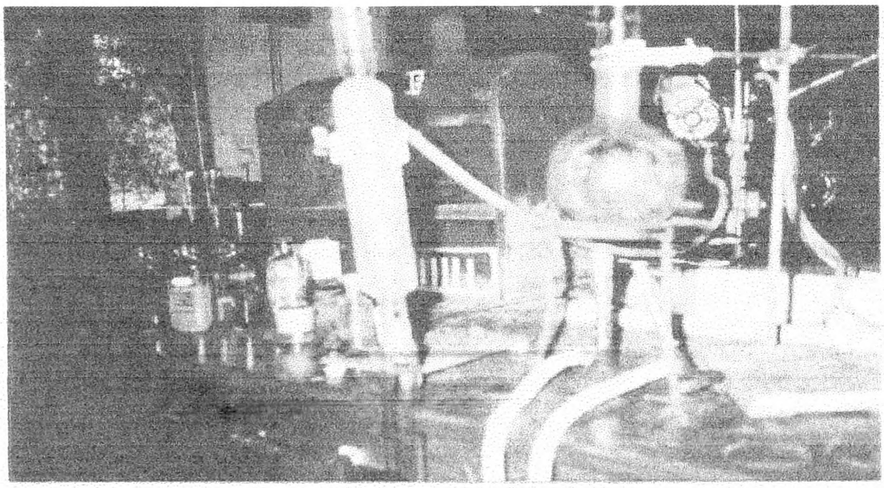

Figure 2. Distillation unit for distill vinegar.

5 ml of the distillate was added to a 250 ml glass stoppered bottle by using a 5 ml pipette. 10 ml of dilute sulphuric acid (1+3) and exactly 15 ml of 0.02M potassium permanganate solution were also added to the above flask. Then it was allowed to stand for 30 minutes. After that 5 ml of potassium iodide solution was added. Then liberated iodine was titrated with 0.02M sodium thiosulphate solution, using starch near the end-point. A blank titration was carried out at the same time using 5 ml of distilled water in place of vinegar.

The amounts of 0.02M sodium thiosulphate required for vinegar sample (a ml) and for blank titration (b ml) were recorded.

Procedure was repeated three times.

Same procedure was also done for the commercial vinegar samples.

Calculation,

For 5 ml of distillate,

Oxidation Value = 40(b – a)

3.2.5 TEST FOR MINERAL ACIDS.

Reagents,

Ethyl Alcohol.

Methyl Orange indicator solution.

Procedure,

2 ml of vinegar sample was mixed with 2 ml of alcohol and 2 drops of methyl orange indicator solution.

3.2.6 TEST FOR VINEGAR EELS.

Procedure,

(i) 25 ml of vinegar sample was taken into a sparkling clear glass beaker and checked for vinegar eels under direct sunlight by naked eyes.

(ii) A drop of vinegar sample was placed on a slide and viewed through the light microscope. Checked for vinegar eels.

3.2.7 DETERMINATION OF pH VALUE.

pH value was tested every day during yeast and acetic fermentation of coconut water. Also done for the commercial vinegar samples.

CHAPTER – 04

RESULTS AND DISCUSSION.

4.1 CHANGES IN TOTAL ACIDITY AND pH VALUE, DURING FERMENTATION OF SUGARED COCONUT WATER.

Week	*Day*	*Total Acidity %*	*pH Value*
1	1	0.2	4.5
	2	0.2	4.5
	3	0.4	4.3
	4	0.8	4.2
	5	0.9	4.2
2	1	1.6	4.0
	2	2.3	3.9
	4	2.5	3.9
	4	2.7	3.8
	5	2.8	3.8
3	1	3.5	3.5
	2	3.9	3.5
	3	4.3	3.4
	4	4.7	3.4
	5	5.0	3.3
4	1	5.1	3.2
	2	5.2	3.19
	3	5.28	3.1
	4	5.4	3.1
	5	5.6	3.0
5	1	5.8	2.96
	2	5.88	2.94
	3	5.9	2.92
	4	5.95	2.9
	5	6.0	2.0
6	1	6.1	2.0
	2	6.14	1.98
	3	6.2	1.95
	4	6.26	1.9
	5	6.3	1.9

Table 4.1 Changes in Total Acidity and pH Value during fermentation.

- Changes in total acidity as acetic acid and changes in pH value during natural fermentation of sugared coconut water into vinegar are shown in Figure 2.

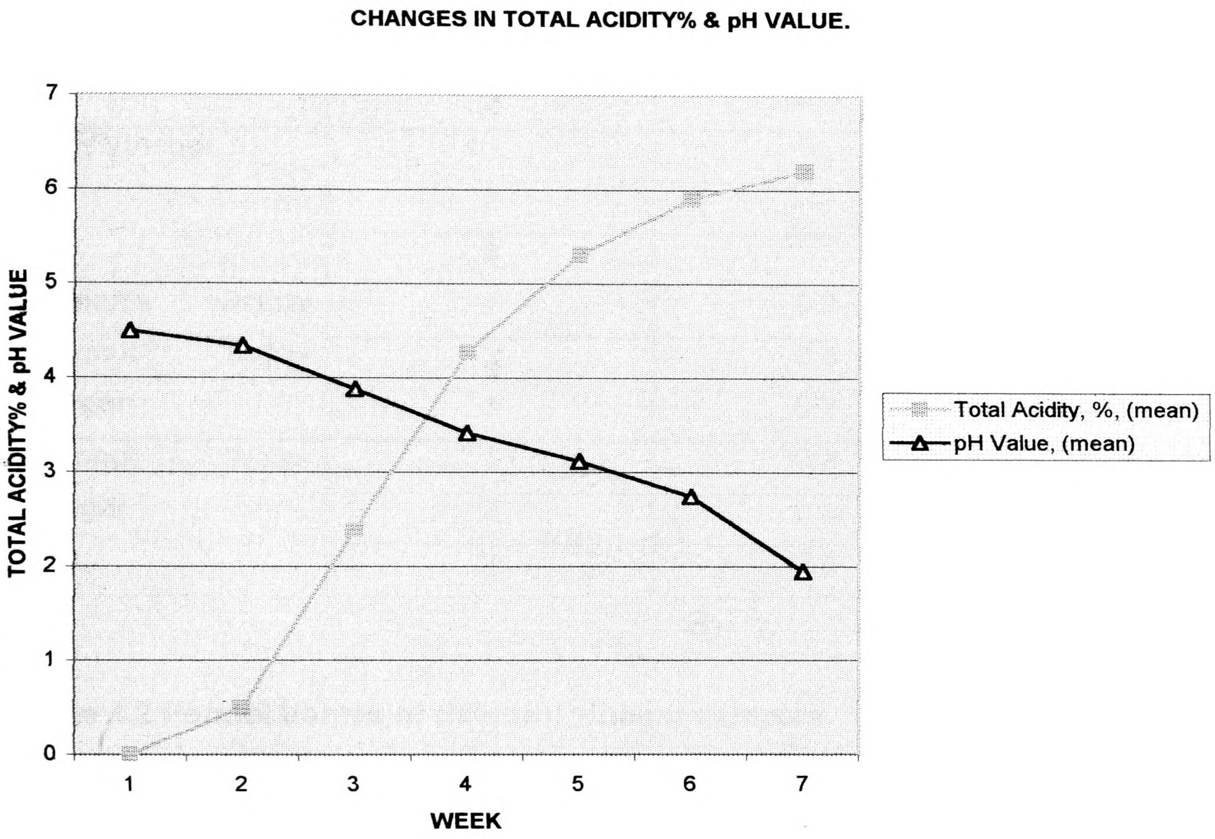

Figure 3. Changes in Total Acidity% and pH Value during fermentation.

- The pH value decreased gradually with time.
- After two weeks later, acetic acid content reached approximately 4.0% (w/v), the legal acetic acid content of vinegar.
- Afterwards, the rate of acetic acid formation declined.
- Due to the low pH value, vinegar used as a preservative especially in the pickle manufacturing.

4.2 FORMOL VALUE.

Vinegar	*Sample*	*0.1M NaOH, ml*	*Formol Value*
Grape Wine Vinegar	1	0.6	0.6
	2	0.4	0.4
	3	0.3	0.3
Cider Vinegar	1	-	-
	2	-	-
	3	-	-
Colmon's Natural Coconut Toddy Vinegar	1	0.7	0.7
	2	0.5	0.5
	3	0.7	0.7
Coconut Water Vinegar	1	0.9	0.9
	2	0.8	0.8
	3	0.8	0.8

Table 4.2 Formol Values of different vinegar samples.

- Formol value equal to the amount of 0.1M NaOH required to the final titration.
- Average formol value for Grape Wine vinegar is 0.433.
- Average formol value for Colmon's natural coconut toddy vinegar is 0.633.
- Average formol value for coconut water vinegar is 0.833.
- Cider vinegar (Heinz Company), didn't response to the formol titration, it may be produced from intermediate distillation process.
- Brewed vinegars give a definite formol value but artificial vinegars do not, therefore this titration is useful as a preliminary quick test but is not a specific test to differentiate between brewed vinegars and artificial vinegars because distilled vinegars do not give any formol value.
- With certain foods, such as vinegar it is possible to estimate the protein content rapidly by means of the formol titration. Although proteins are too week to be titrated directly with alkali, if formaldehyde is added, it reacts with the amine groups to form the methylene-amino group, and the carboxyl group is then available for titration.

4.3 OXIDATION VALUE.

Type of vinegar	*Sample*	*a ml*	*b ml*	*Oxidation Value*
Grape Wine	1	39.8	76.2	1456
	2	39.7	76.1	1456
	3	38.9	76.2	1492
Cider	1	42.5	76.1	1344
	2	42.3	76.2	1356
	3	42.4	76.2	1352
Colmon's	1	28.8	76.1	1896
	2	28.6	76.2	1900
	3	28.8	76.2	1896
Coconut Water Vinegar	1	35.9	76.2	1612
	2	35.7	76.2	1620
	3	35.9	76.2	1612

Table 4.3 Oxidation Values of different vinegar samples.

- Average oxidation value of Grape Wine vinegar is 1468.
- Average oxidation value of Cider vinegar is 1350.66.
- Average oxidation value of Colmon's natural coconut toddy vinegar is 1897.33.
- Average oxidation value of coconut water vinegar is 1614.66.
- Oxidation Value is the number of milliliters of 0.002M Potassium permanganate used by 100 ml of distillate vinegar sample in 30 minutes under stand conditions.
- Oxidation Value is specific for the type of vinegar. Therefore this can be used to differentiate between brewed vinegar and artificial vinegars. Oxidation Value of artificial vinegar is between 0-20.

4.4 MINERAL ACIDS AND VINEGAR EELS.

There were no mineral acids and vinegar eels in the tested brewed vinegar samples.

- The effect of vinegar eels and spoilage by vinegar flies can be reduced by good hygienic practices.

4.5 ALCOHOLS AND ACETIC ACID YIELD.

- Theoretically, 100 parts of (sucrose or maltose) should yield 53.8 parts of alcohol or 70.1 parts of acetic acid. In actual practice, however, even under the most favorable conditions, 44 to 47 parts of alcohol and 50 to 55 parts of acetic acid only are produced.
- The loss represents the consumption of sugar in solution by the yeast, loss of alcohol and acetic acid owing to evaporation of oxidation and also loss owing to utilization by vinegar bacteria for their growth.
- Bad vinegar has an acetaldehyde taste, smelling like thinner. This is not wanted for good and high quality vinegar products.
- The occurrence of acetaldehyde shows that the vinegar is not yet ready. It is also a reliable method to determine if the conversion from alcohol to vinegar was done.
- Avoid from the metallic equipment is essential for the minimize the turbidity of coconut water vinegar.
- Several factors are known to affect the acetification rate. These are bacterial strains, temperature and ratio of surface area to volume of the liquid and aeration. An aerator was used to better aeration.

CHAPTER – 05

CONCLUSIONS.

Following conclusions obtained from this study.

- Oxidation Value can be used to differentiate coconut water vinegar from artificial vinegar.
- Ageing two months after pasteurization and before bottling can produce clear low turbidity coconut water vinegar.

Suggestions for further studies,

- Sensory evaluation for produced low turbidity coconut water vinegar.
- Test artificial vinegar for Mercury (Hg) contamination.

REFERENCES.

1. Banzon, J. A. and Velasco, J. R., 1982. Coconut production and utilization. Philippine Coconut Research and Development Foundation, Inc., Manila. p. 351.
2. Girdhari *et al*, 1986. Vinegar. In *Preservation of Fruits and Vegetables*, 1st ed, p.270-282, McGraw Hill, Madras.
3. James, C. S., 1995. Analytical Chemistry of Foods, 1st ed, Chapman and Hall, London.
4. Nickol, G. B., 1979. Vinegar. In *Microbial Technology*, H.J. Peppler and D. Perlman (Ed.) Vol. 2. p. 531. Academic Press, New York.
5. Sanchez, C. and Cesar, L. S., 1985. Village level technology of processing coconut water vinegar. The Philippine Agriculturist. 68: 439-448.
6. Sison, B. C., 1984. Coconut water - its properties and uses. Coconut Today. 2(1): 60-64.
7. Truong, V. D. and Marquez, M. E., 1987. Handling of coconut water and clarification of coco-vinegar for small-scale production. Annals of Tropical Research. 9(1): 13-23.
8. www.versatilevinegar.com
9. www.vinegarman.com

APPENDIX.

1. PREPARATION METHOD OF 0.1M NaOH SOLUTION.

- 4 g of NaOH were measured by using the electronic balance.
- It was placed in a standardized stoppered 1000 ml flask and little amount of distilled water was added.
- Then it was shaked thoughraly until all NaOH was dissolved.
- Distilled water was added up to the point carefully.

2. PREPARATION METHOD OF 0.02M $Na_2S_2O_3$ SOLUTION.

- 4.963 g of $Na_2S_2O_3$ were measured by using the electronic balance.
- It was placed in a standardized stoppered 1000 ml flask and little amount of distilled water was added.
- Then it was shaked thoughraly until dissolving completed.
- Distilled water was added up to the point carefully.

3. PREPARATION METHOD OF 0.02M $KMnO_4$ SOLUTION.

- 3.16 g of $KMnO_4$ were measured by using the electronic balance.
- It was placed in a standardized stoppered 1000 ml flask and little amount of distilled water was added.
- Then it was shaked thoughraly until dissolving completed.
- Distilled water was added up to the point carefully.

National Digitization Project

National Science Foundation

Institute : Sabaragamuwa University of Sri Lanka

1. Place of Scanning : Sabaragamuwa University of Sri Lanka, Belihuloya
2. Date Scanned : ..2017-09-20..
3. Name of Digitizing Company : Sanje (Private) Ltd, No 435/16, Kottawa Rd, Hokandara North, Arangala, Hokandara
4. Scanning Officer

Name : ...S.A.C Sadaruwan..

Signature : ....

Certification of Scanning

I hereby certify that the scanning of this document was carried out under my supervision, according to the norms and standards of digital scanning accurately, also keeping with the originality of the original document to be accepted in a court of law.

Certifying Officer

Designation : ....Librarian..

Name : ....T. N. Neighsoorei..

Signature : ....

Mrs. T.N. NEIGHSOORE

Date : ..2017-09-20.........

"This document/publication was digitized under National Digitization Project of the National Science Foundation, Sri Lanka"