STUDYING A COST EFFECTIVE METHOD FOR REMOVING SEDIMENTS FORMED IN COCONUT VINEGAR

BY

H. M. S. N. HERATH

(02/AS/A/033)

A Research Report Submitted In Partial Fulfilment of the Requirement for the Special Degree of Bachelor of Science

In

Food Science and Technology

Department of Food Science and Technology

Faculty of Applied Sciences

Sabaragamuwa University of Sri Lanka

Buttala - 91100

October 2007

DECLARATION

The research work described in this thesis was carried out by me at C.D.De Fonseka and Sons (Pvt) Limited, Panadura under the supervision of Mr. G.I.M.S. Rangana and Dr. K. B. Palipane. A report on this has not been submitted earlier or concurrently to another university for another degree.

26.10.2007 .....

Date

.....

H.M.S.N.Herath

Certified by,

External supervisor

Mr. G.I.M.S.Rangana
Quality Control Manager
CDDEF Group of Companies
Eluwila
Panadura

.....

G.I.M.S.Rangana

Date: 26|10|2007

Quality Control Manager
CDDEF Group of Compan
Eluwila
Panadura.

Internal supervisor

Dr. K. B. Palipane
Head / Department of Food Science and Technology
Faculty of Applied sciences
Sabaragamuwa University of Sri Lanka
Buttala

.....

K.B.Palipane

Date: 12-11-2007

Head/Dept. of Food Sciences & Technology
Faculty of Applied Sciences
Sabaragamuwa University of Sri Lanka
BUTTALA

AFFECTIONATELY DEDICATED TO MY BELOVED PARENTS AND TEACHERS

ACKNOWLEDGEMENTS

First, I wish to express my deepest gratitude to my internal supervisor, Dr. K.B. Palipane, Head / Department of Food Science and Science Technology, Faculty of Applied Sciences, Sabaragamuwa University of Sri Lanka for his guidance and invaluable assistance given me to make this study a success.

I express my sincere gratitude to my external supervisor Mr. G.I.M.S.Rangana, Quality Control Manager of CDDEF Group of Companies, for his guidance, encouragement and kind assistance given throughout my study.

I extend my sincere thank to Mr. Rohantha de Fonseka, chairman /CEO and Mr. Summit Peiris, General Manager and all the staff members of the CDDEF Group of Companies for the assistance and generous help given me throughout this period.

I am very grateful to Mr. E. G. Somapala, government analyst, for his guidance and assistance given me to make this study a success.

I wish to extend my thanks to all the staff members of the Faculty of Applied Sciences, Sabaragamuwa University of Sri Lanka and Mr. Jagath Wansapala, lecturer, Faculty of Applied Sciences, Jayawardanapura University of Sri Lanka.

Finally, I also wish to express my sense of gratitude to all those who have helped me during this project for their endless encouragement and heartiest assistance.

ABSTRACT

Vinegar is a preservative most commonly used in food preparations such as in pickling and salad dressings. Although acetic acid is the key ingredient in vinegar, acetic acid cannot be called as vinegar. Naturally fermented vinegar contains lot of constituents other than acetic acid which imparts its distinct flavor. Therefore, the natural fermented vinegar has a high demand in the market.

Coconut vinegar produced by the company C.D.De Fonseka and Sons (Pvt) Ltd has a market share of over 75% in the Sri Lankan vinegar market. But from the very inception they have been facing a problem of sedimentation in vinegar. The sediments form and appear at the bottom of the bottle when kept in a place after bottling. Therefore, this study has been focused to determine a cost effective method for removing sediments formed in coconut vinegar. To achieve this main objective, a set of several specific objectives were planned. These specific objectives include identification of biological and chemical components in the sediments, identification of the causes of sedimentation in vinegar and identification of the effect of sediment on quality parameters.

Analysis of a sample of sediment was carried out by the Industrial Technology Institute to identify the microbiological and chemical components of it. Other experiments were designed to identify the effect of yeast level, filtration, pasteurization temperature and filling temperature on sedimentation and to identify a suitable clarification agent which would result good clarity in vinegar.

Results showed the presence of bacteria, yeast and iron and copper ions in the sediment. It is clear that there is an effect of yeast level and pasteurization temperature on sedimentation. Acidity of vinegar is increased during storage due to the presence of acetic acid bacteria.

Use of yeast at a level of 0.0875% and pasteurization at 70°C for 20 minutes help in reducing the degree of sedimentation. Casein at a level of 0.5% can be used to clarify vinegar, with a less settling period, and thereby increase the quality of coconut vinegar by removing sediments.

TABLE OF CONTENT

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

SLSI	Sri Lanka Standard Institute
W	Weight
V	Volume
min	minimum
max	maximum
ITI	Industrial Technology Institute
P.O.V.	Permanganate Oxidation Value
A.O.V.	Alkaline Oxidation Value
I.V	Iodine Value
RT	Room Temperature
H	Hot
F	Filter
NF	Not Filter

CHAPTER 1
INTRODUCTION

1.1 Introduction

Vinegar is used as a food preservative. It is commonly used in food preparation, particularly in pickling processes, vinaigrettes and other salad dressings. It is an ingredient in sauces such as mustard, ketchup, mayonnaise and chutneys.

Vinegar was a part of the human story even before we began writing down what we were doing. Since it is the natural result of the refining of alcoholic beverage, we can safely say that it has been around as long as beer, wine and other spirits. Like most other important discoveries, it was probably the result of an accident (Lawrence, 1989).

Almost any material containing sugars (or starches which can be converted enzymically to sugars) and small amounts of nitrogenous substances can be made into vinegar (Ranken and Rill, 1993). The most popular types of vinegar in a region are usually reflected from the local alcoholic beverages, for example apple or cider vinegar in the United States, wine vinegar in Europe and malt vinegar in England. Traditionally in Sri Lanka, the vinegar is made from the fermented sap of coconut or coconut toddy. Other sources include fermenting coconut water with added sugar and spirits obtained from the subsequent fermentation of molasses mainly.

Today, it is agreed that vinegar is essentially acetic acid, but acetic acid is not vinegar. How ever, acetic acid is the principal flavouring ingredient in vinegar. In fact, from a chemical view point, vinegar is essentially nothing more than a weak solution of acetic acid in water with small amounts of various soluble extractive and mineral salts obtained from the raw materials used with by-products from the life processes of the organisms associated with vinegar manufacture. These extractives, salts and by-products give the vinegar its distinctive flavour and determine its quality. Because of that it can be found many chemicals in naturally fermented vinegar, not found in acetic acid.

Coconut vinegar made of coconut toddy or coconut water is the widely used vinegar type in Sri Lanka. In this naturally fermented vinegar, sedimentation takes place at the bottom of the bottle after bottling. It is due to the dead microbial cells, coagulated proteins, excess amounts of some cations and anions such as ferric, cuprous and phosphates and colloidal particles which may cause the turbidity in vinegar. Unless those substances are removed from the vinegar prior to filling to bottles, they settle down to the bottom of the bottle during the storage and results vinegar in good clarity with the time. But the sediments appear at the bottom of the bottles creates receiving of customer claims, because vinegar should be clear and free from sediments according to the SLSI specifications.

1.2 Objective

- Increment of the quality of coconut vinegar by preventing the sediment formation

Specific objectives

- Identification of components in the sediments formed in coconut vinegar
- Identification of the causes for sedimentation in vinegar
- Identification of the effects of sediment on quality parameters

CHAPTER 2
REVIEW OF LITERATURE

2.1 Vinegar

Vinegar is the liquid produced from a suitable raw material containing starch or sugar or starch and sugar by the process of double fermentation, alcoholic and acetous, and which contains at least 4% w/v acetic acid (Krick and Sawyer, 1991).

2.1.1 Origin and History

Vinegar has been known and appreciated as an important food adjunct for as long as man has been able to practice the arts of brewing and wine making (Hickey and Underkofler, 1954). Then the use of vinegar was discovered thousands years ago. So what probably happened was that some wine turned sour and vinegar was born.

There are references to the healing and soothing properties of vinegar in Bible. Roman Legionnaires used vinegar as a beverage. The Romans used vinegar extensively in their cooking and at their meals. It was used as a dip for various foods. They often sweetened it to improve the taste. In Babylonian times it was used as a herb flavoured condiment. Pliny relates that Cleopatra, to gain a wager, dissolved pearls in vinegar that she then drank. Hippocrates used vinegar as a medicine. An account of the methods used in making vinegar was given in 1616 by Oliver de Serres (Pederson, 1971).

Vinegar was produced only for local consumption until the middle ages. The first industrially manufactured vinegars were produced in flat open vats. These were slow processes, in which a film of bacteria floated on the surface of the wine. In the nineteenth century, surface fermentations were developed into more rapid processes. Beginnings in 1949 submerged processes were developed. However the older methods are still being used, because of the better flavour of the product (Crueger, 1982).

During the later half of the 1800's, chemists learned how to make acetic acid and with that, commercial acetic acid was produced on a large scale, primarily from calcium acetate. The calcium acetate was obtained from the destructive distillation of wood.

Acetic acid produced in this way was very pure, strong and comparatively cheap. It was known as glacial acetic acid and it was virtually 100 percent pure acetic acid. The strength is reduced to 4%-5% by adding water and that imitation vinegar is sold labelling as diluted acetic acid (Lawrence, 1989).

2.1.2 Varieties of vinegar

Since vinegar can be made from anything with sugar, too many different types of vinegar are made throughout the world. Each country may use starting materials native to their area and tailored to the specific tastes of the region.

Vinegar can be classified into two groups mainly.

1) Natural vinegar
2) Artificial vinegar

2.1.2.1 Natural vinegar

Natural vinegar is made by microbial fermentation, which is containing much fermentation by products other than acetic acid in water, such as fruit acids, esters, inorganic salts, vitamins and etc. varying according to the origin.

The following varieties of vinegar are classified by the United States Food and Drug Administration (FDA) compliance policy guide for labelling purposes according to their starting material.

2.1.2.1.1 Malt vinegar

Malt vinegar is the product made by the alcoholic and subsequent acetous fermentations, without distillation, of an infusion of barley malt or cereals whose starch has been converted by malt. It contains, in 100 ml (20°C), not less than 4 g of acetic acids (Jacobs, 1975).

2.1.2.1.2 Cider vinegar

Cider vinegar is the product made by the alcoholic and subsequent acetous fermentations of the juice of apples. It contains, in 100 ml (20°C), not less than 4 g of acetic acid (Jacobs, 1975).

2.1.2.1.3 Wine vinegar

Wine vinegar is the product made by the alcoholic and subsequent acetous fermentations of the juice of the grapes. It contains, in 100 ml (20°C), not less than 4 g of acetic acid (Jacobs, 1975). It is the most commonly used vinegar in Mediterranean countries and central Europe.

2.1.2.1.4 Spirit vinegar

It is the product made by the acetous fermentation of dilute distilled alcohol. It contains not less than 4% w/v and not more than 15% w/v acetic acid. It is water white and has a strong acid flavour (Kill and Ranken, 1993).

2.1.2.1.5 Sugar vinegar

Sugar vinegar is the product made by the alcoholic and subsequent acetous fermentation of sugar syrup, molasses or refiners syrup. It contains, in 100 ml (20°C), not less than 4 g of acetic acid (Jacobs, 1975).

2.1.2.1.6 Glucose vinegar

Glucose vinegar is the product made by the alcoholic and subsequent acetous fermentations of a solution of glucose. It is dextrorotatory and contains, in 100 ml (20°C), not less than 4 g of acetic acid (Jacobs, 1975).

Apart from the above classification, other types of vinegar are also available such as Fruit vinegar, Balsamic vinegar, Rice vinegar, Coconut vinegar, Beer vinegar, Flavoured vinegars etc.

2.1.2.2 Artificial vinegar

Artificial vinegar is made by adding water to acetic acid to reduce its strength to 4%-5% and treated with harmless flavours and colours. Therefore microbial fermentation is not involved. In such cases it must be stated in the label that it is 'diluted acetic acid'.

2.1.3 Properties of vinegar

2.1.3.1 pH value

The pH value of the vinegar is typically in the range of 2 to 3.5, depending on the concentration of acetic acid. Commercially available vinegar usually has a pH of about 2.4.

2.1.3.2 Relative density

It depends on the acidity of the vinegar. Vinegar containing 5% acetic acid should have a relative density of about 1.019 (Kirk and Sawyer, 1991).

Table 2.1 - Relative density of some vinegar types

Vinegar type	Relative density
Coconut vinegar	1.007 – 1.013
Malt vinegar	1.013 – 1.022
Cider vinegar	1.013 – 1.024
Wine vinegar	1.013 – 1.021
Spirit vinegar (concentrated)	1.015 – 1.020

Source: Kirk and Sawyer, 1991

2.1.3.3 Shelf life

The shelf life of vinegar is almost indefinite. Because of vinegar's acid nature it is self-preserving and does not need refrigeration. White vinegar will remain virtually unchanged over an extended period of time. Some changes can be observed in other types of vinegar as colour changes or the development of haze or sediment. This is however, only an aesthetic change. The product can still be used with confidence.

2.1.4 Composition of vinegar

Although acetic acid is the primary constituent of vinegar aside from water, vinegar contains vitamins such as riboflavin, nicotinic acid, vitamin B_1 and mineral salts and esters which impart the taste of the vinegar.

Table 2.2 - Composition of vinegar

Constituent	Average value
Total acid, as acetic acid	4.94%
Total solids	2.54%
Non-sugar solids	1.90%
Reducing sugars in solids	19.6%
Total ash	0.367%
Soluble phosphoric acid	17.3 mg
Insoluble phosphoric acid	12.0 mg

Source: Jacobs, 1975

2.2 Production of vinegar

2.2.1 Raw materials

Vinegar may be produced from a wide variety of raw materials, the main requirements being a satisfactory, economic source of alcohol and accessory flavouring constituents. In this study it is focused that the production of vinegar is from coconut water.

2.2.1.1 Coconut water

Coconut water is being wasted in copra and desiccated coconut industry. So it can be put into use with the production of vinegar from matured coconut water.

The water of coconut, technically the liquid endosperm, is the most nutritious wholesome beverage that the nature has provided for people. It has caloric value of 17.4 per 100g. The major chemical constituents of coconut water are sugars and minerals and minor ones are fat and nitrogenous substances.

Sugars

Sugars in the forms of glucose, fructose and sucrose form an important constituent of the coconut water. The concentration of sugars in the water steadily increases from about 1.5% to about 5% in the early months of maturation and then slowly falls reaching about 2% at the stage of the full maturity of the nut. In the early stages of

maturity sugars are in the form of glucose and fructose and sucrose appears only in the later stages which increases with the maturity while the reducing sugars falls. Approximately 90% of the total sugars are sucrose in the fully mature nut.

Minerals

Coconut water contains most of the minerals such as potassium, sodium, calcium, phosphorous, iron, copper, sulphur and chlorides. Among the minerals more than half is potassium the concentration of which is markedly influenced by potash manuring.

Protein

Coconut water contains small amounts of protein. The percentage of arginine, alanine, glutamic acid, aspartic acid and tyrosine are higher.

Vitamins

Coconut water contains both ascorbic acid and vitamins of B group such as nicotinic acid, pantothenic acid, biotin, riboflavin etc.

Table 2.3 - Composition of mature coconut water

Constituent	Value
Total solids	5.4%
Reducing sugars	0.2%
Minerals	0.5%
Protein	0.1%
Fat	0.1%
Potassium	247.0 mg %
Sodium	48.0 mg %
Calcium	40.0 mg %
Magnesium	15.0 mg %
Phosphorous	6.3 mg %
Iron	79.0 mg %
Copper	26.0 mg %

Source: Thampan, 1993

2.2.1.2 Sugar

Sugar is added to the coconut water to increase its content up to 10%, because its concentration is not sufficient for the alcoholic fermentation with yeasts to get the required alcohol content.

2.2.1.3 Yeasts

The alcoholic fermentation to form ethanol from sugars depends on the activity of yeast which consequently makes essential for vinegar production. The yeast has a great effect on vinegar quality because of the effect of its metabolic by products.

Yeast are unicellular fungi of large size compared to bacteria of oval, elongate, elliptical or spherical cell shape having length in the range of 20 μm and diameter of about 6 μm to 7 μm. these organisms produce pigments of many colours; with red and black pigment producers being common. Most common yeasts grow best in the presence of plenty of moisture.

Yeast is unusual in that they can live and grow with or without oxygen. Yeast can live without oxygen by the process that is referred to as fermentation. The yeast cells metabolize on simple sugars and produce carbon dioxide and alcohol, as waste products. In vinegar production, this process step is carried out by *Saccharomyces cerevisiae* var. *ellipsoideus*.

2.2.1.4 Acetic acid bacteria

The acetic acid fermentation is carried out by the acetic acid bacteria presence in the air. They oxidize ethanol to acetic acid and water.

These bacterial cells are ellipsoidal to rod shaped, straight or slightly curved, 0.6-0.8 μm by 1.0-4.0 μm, occurring singly, in pairs or in chains. Involution forms frequent in some strains; may be spherical, elongated, swollen, club shaped, curved or filamentous. They are gram-negative, catalase-positive, oxidase-negative and obligately aerobic, non spore forming bacteria. They are as pale colonies; most strains produce no pigments. A minority of strains produce brown water-soluble pigments or show pink colonies due to porphyrins (Krieg et al., 1984).

The optimum temperature for the activity of the acetic acid bacteria ranges between 25°C-30°C. No acid is produced by the bacteria below 15°C and their reproduction ceases at about 10°C. The optimum pH for growth is 5.4-6.3 (Nathanael, 1954).

Several distinct forms of bacteria have been applied in vinegar manufacture, and they may be found distributed throughout the liquid in such numbers that the 'gyle' has a silky streamlined appearance. In liquids of low alcohol concentration, the bacteria usually cohere in gelatinous masses to form the slimy, tough and almost transparent zoologeal mat known as 'vinegar flowers' or 'mother of vinegar'. Four well known varieties *Acetobacter aceti*, *Acetobacter pasteurianum, Acetobacter rutzingianum*, and *Acetobacter xylinum* have been found in the vinegar industry (Nathanael, 1954).

2.2.2 Production process

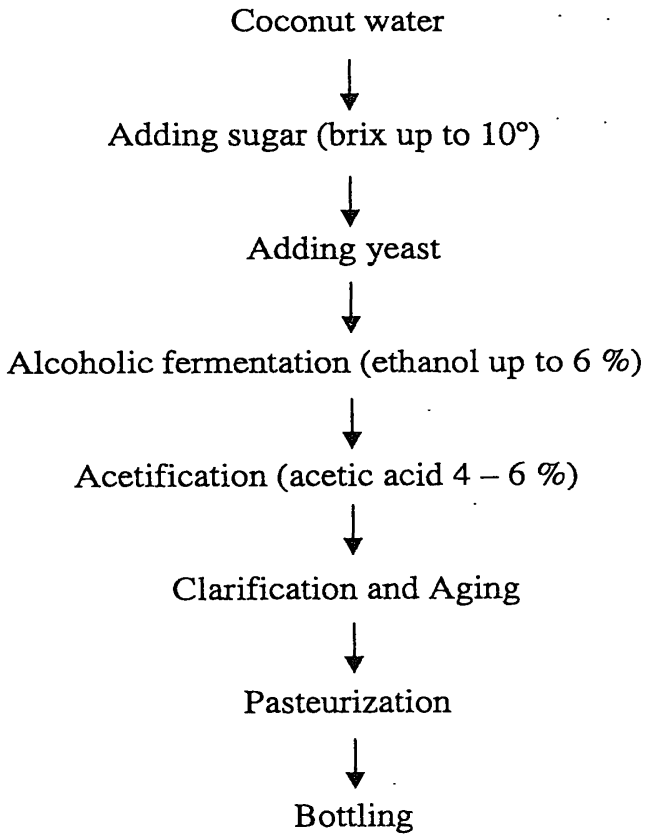

Figure 2.1 - Flow chart for the production process of vinegar

2.2.2.1 Collecting coconut water

Coconut water, a waste product in copra and desiccated coconut industry is collected to plastic barrels and transferred to the vinegar processing plant. Then it is pumped to the fibre vats for preparing for the fermentation process.

2.2.2.2 Addition of sugar

The coconut water, consisting of about 2% sugar content is concentrated to 10% level, by fortifying with sugar.

2.2.2.3 Addition of yeast

The fortified coconut water is then inoculated with yeast, *Saccharomyces cerevisiae*, to obtain the required alcohol content for the subsequent acetous fermentation. It is about 0.1%.

2.2.2.4 Fermentation

Fermentation involves the use of microbial metabolic process, in which carbohydrates and other nutrients are oxidized partially to a variety of breakdown products such as alcohols, acids, amino acids, other metabolites and antibiotics and a small amount of energy (Sivasankar, 2003).

The manufacture of vinegar requires two fermentation processes. The first transforms the sugar into alcohol by yeasts. The second changes the alcohol into acetic acid and is brought about by vinegar bacteria. The first must be completed before the second begins.

2.2.2.4.1 Alcoholic fermentation

In the alcoholic fermentation, sugar is breakdown into Ethyl alcohol and Carbon dioxide by alcohol producing yeasts (*Saccharomyces* species). The change occurs is usually described in the following equation.

$$\underset{180\ g}{C_6H_{12}O_6} \longrightarrow \underset{92\ g}{2\ C_2H_5OH} + \underset{88\ g}{2\ CO_2}$$

Theoretical yield:

100 parts of sugar ⟶ 51 parts of ethyl alcohol + 49 parts of Carbon dioxide

The hexose (6-carbon) sugars being fermented are isomerised if necessary and phosphorylated to fructose-1, 6-diphosphate which is split into two triose units. The triose units are converted to pyruvic acid and it is decarboxylated to acetaldehyde. Then the acetaldehyde is reduced to ethanol by accepting Hydrogen from reduced nicotinamide adenine dinucleotide (NADH). If acetaldehyde is not available glycerol is produced instead of ethanol (Anerine et al., 1980).

In the presence of a high concentration of sulphur dioxide in acid solution, acetaldehyde, carbon dioxide and glycerol are the primary products and alcohol is a by-product. If the sulphite solution is alkaline, acetaldehyde, glycerol, alcohol and carbon dioxide are all produced (Anerine et al., 1980).

Favourable pH range for the growth of *Saccharomyces cerevisiae* is between 2.4 to 5.0. A favourable temperature of 23°C to 26°C should be maintained during fermentation. At a temperature of near 37°C the fermentation becomes abnormal, and ceases at about 40°C (Desrosier, 1998).

With the exception of aeration at the start, air is not necessary during alcoholic fermentation. It is objectionable in the later stages because it may results in growth of undesirable bacteria with a subsequent loss in alcohol. So the alcoholic fermentation should be conducted in containers in which the juice is not unduly exposed to air. The container must not be sealed airtight as it may bursts due to the pressure from the gas released (Desrosier, 1998).

The juice is allowed to ferment until practically all the sugar is converted into alcohol and carbon dioxide. When fermentation is completed, gas is no longer evolved and the juice tastes 'dry' or free of sugar. After the alcoholic fermentation, the juice should be free from yeast, pulp and sediment by settling and racking or by filtering before beginning the acetic fermentation.

If acetic acid is produced in any quantity, the fermentation may cease before all the sugar has been converted. A concentration of 0.5% acetic acid markedly diminishes the activity of the yeast and higher concentration will completely inhibit the alcoholic fermentation (Nathanael, 1958).

From the stoichiometry of the equation it can be calculated that 1 *l* of ethanol should yield 1.036 kg of acetic acid and 0.313 kg of water. This leads to the approximate relationship that 1% v/v ethanol will give 1% w/v acetic acid, and it can be used to calculate the fermentation efficiency. It implies that, in the absence of over-oxidation, evaporation losses and conversion to biomass, the sum of the concentration of ethanol (% v/v) and the concentration of acetic acid (% w/v) known as the total concentration should remain constant throughout acetification (Adams and Moss, 1999).

Since the conversion of ethanol to acetic acid is primarily an oxidation process, or a dehydrogenation in which atmospheric oxygen acts as the hydrogen acceptor, the success of the fermentation will depend to a great extent on the ready availability of large quantities of oxygen. The rate of conversion of alcohol to acetic acid also depends on the activity of the organism, the amount of alcohol present and the temperature (Nathanial, 1958).

2.2.2.4.2.1 Methods of acetification

There are number of techniques for acetification which differ in the means by which three interacting components, ethanol, bacteria and oxygen brought together. Vinegar may be made either by slow acetification of vinegar stock in barrels or by rapid oxidation in generators.

a) Slow acetification

The slow acetification process commonly used for the production of commercial quantities of vinegar is known as the French or Orleans process. This method is the oldest and is conceded to be the best process for the production of the finest quality vinegar (Hickey and Underkofler, 1958).

In the Orleans process, vinegar stock in partially filled casks drilled with air holes is left to acetify until the acidity reaches the appropriate level. At this point, a proportion typically one-third to two-third, is drawn off through the tap, replaced with fresh stock and the process restarted. The vinegar stock is usually added via a pipe passing through the top of the barrel and resting on the bottom. In this way the surface film of bacteria is not disturbed and the delays and losses that result from having to reform

the film are avoided. Usually the time taken to complete one acetification cycle is of the order of 14 days (Adams and Moss, 1999).

Orleans vinegars have a relatively high concentration of ethyl acetate, detected by its characteristic odour. Only a small proportion of the world's vinegar is produced by this method, although it is claimed to produce the finest quality vinegar.

b) Rapid acetification

The quick vinegar process derives its name from the faster rates of acetification achieved by increasing the area of active bacterial film and improving oxygen transfer to the acetifying stock. The acetic acid bacteria grow as a surface film on an inert support material packed into a false-bottomed vat. The acetifying stock is sprayed on to the surface of the packing material and trickles down against a counter-current of air which is either pumped through the bed or drawn up by the heat of reaction within it. The packing material normally consists of some lignocellulosic material such as brich twigs, vine twigs, ratton, wood wool or sugarcane bagasse. The vinegar stock is collected in a sump at the bottom of the vat and recirculated until the desired level of acidity is reached (Adams and Moss, 1999).

The fastest rates of acetification are achieved using submerged acetification in which acetic acid bacteria grow suspended in a medium which is oxygenated by sparging with air. The most commercially successful technique to have been developed is the Frings Acetator which uses a patented self-priming aerator to achieve very efficient oxygen transfer (Adams and Moss, 1999). Submerged culture is very efficient and rapid, a semi-continuous run normally takes 24 – 48 hours. However it requires more careful control than simpler processes.

2.2.2.4.3 Factors influencing fermentation

Energy sources

Certain sugars, particularly glucose, fructose, sucrose and maltose are the normal substrates for yeasts, but they do not ferment lactose, pentose, dextrin or starch. They also can grow on a variety of other carbon sources especially aerobically (Anerine et al., 1980).

Above about 25% sugar, it retards fermentation and at even higher levels (about 70%) most yeast will not ferment (Anerine et al., 1980).

Carbon dioxide and pressure

The effect of carbon dioxide in alcoholic fermentation is too often neglected. A carbon dioxide content of 15 g per litre (about 7.2 atm) essentially stopped yeast growth. The carbon dioxide effect on yeast growth did not prevent alcoholic fermentation. A much higher carbon dioxide pressure up to 30 atm was necessary to half the alcoholic fermentation.

Alcohol

Alcohol itself has an inhibiting effect on fermentation which increases with temperature.

Acids

Organic acids have an inhibitory effect on alcoholic fermentation.

Nitrogen

Yeast can synthesize their own needed amino acids from ammonium ions or certain other simple nitrogen sources and sugar carbon. Although they have no absolute requirements for amino acids, nitrogen sources stimulate the rate of yeast growth (Anerine et al., 1999).

Growth factors

Yeasts may respond to accessory growth factors. It is found that biotin, inositol, nicotinic acid, pantothenic acid, p-aminobenzoic acid, pyridoxine and thiamine are necessary with certain yeasts. Biotin is an absolute requirement of most strains of *Saccharomyces*.

Minerals

Alcoholic fermentation requires Magnesium, Potassium, Zinc, Cobalt, Iodine, Iron, Calcium, Copper and anions of Phosphorous and Sulphur.

2.2.2.5 Clarification and aging

2.2.2.5.1 Clarification

According to the specifications for vinegar, it should be clear and free from sediments. Therefore, it is necessary to clarify it. Fining may be desirable for the clarification.

Ordinary fining agents which may be used for the clarification of vinegar include egg albumen, casein, gelatine, isinglass and bentonite. These clarification agents cause coagulation and settling of the colloidal particles in the cloudy vinegar (Hickey and Underkofler, 1954).

Principles of fining

Fining is the addition of a reactive or adsorptive substance to remove or reduce the concentration of one or more undesirable constituents.

The mechanisms of action of fining agents may be electrical (charge) interaction, bond formation, and/or absorption and adsorption. In the case of electrical interaction, particles of opposite charge to the fining agent are induced to coalesce with the agent, forming larger particles. Due to its greater density, the complex eventually settles from solution (Fugelsang et al., 1997).

Turbidity in vinegar may be due to tissues of the raw material, yeast, bacteria, colloids derived from the raw material or from changes occurring during aging or storage. These particles may be in the form of proteins, pectins and gums, metallocolloids and degradation products of polyphenols. Absorption / adsorption of colloidally suspended material by fining agents may enhance filterability. Effectiveness of fining agents is dependent on the agent, method of preparation and addition, concentration, pH, metal content and temperature (Fugelsang et al., 1997).

Fining is surface reaction and therefore the method of hydration and addition is important.

2.2.2.5.1.1 Bentonite

Bentonite, volcanic clay like material, is a complex hydrated aluminium silicate with exchangeable cationic components ($Al_2O_3.4SiO_2.H_2O$). Bentonites are mined from several areas of the world and come in different levels of purity, particle size, adsorption and swelling capacity. It is available in calcium, sodium or magnesium forms. Bentonite is applicable in removal of proteins and enzymes which may catalyze the oxidation and browning in juice. The mechanism of removal is by adsorptive interaction between the flat negatively charged surfaces of the bentonite platelets and the positively charged proteins. Bentonite may indirectly bind phenols that have complexed with proteins (Fugelsang et al., 1997).

Bentonite requires only minutes to react with and precipitate protein. 75% of the total protein removal by bentonite occurs in the first minute after contact (McLaren et al., 1958). Since, adsorbed protein may "slough off" bentonite platelets upon standing, prolonged contact may result in less efficient removal of protein. Therefore, in-line centrifugation or filtration to remove bentonite rather than traditional gravity clarification may be desired (Fugelsang et al., 1997). Bentonite fining is known to indirectly prevent or impede formation of copper and possibly iron casse in vinegar, where metal levels may be a problem.

2.2.2.5.1.2 Gelatine

Gelatine is prepared from collagen, the major structural protein in skin and bones. The iso-electric point of gelatine is pH 4.7. Therefore, it occurs in the vinegar as a positively charged entity capable of reaction with negatively charged molecules via hydrogen bond formation (Fugelsang et al., 1997).

It is applied to reduce harshness and improve clarity in the vinegar.

2.2.2.5.1.3 Casein

Casein occurs in solution as a positively charged macromolecule with a molecular weight of approximately 375,000. So, it is effective in removing negatively charged molecules and thereby reduces the turbidity in vinegar. Casein reduces the concentration of both copper (by up to 45%) and iron (by 60%). Use of concentrations of at least 25 g/l appears to improve the clarification (Fugelsang et al., 1997).

2.2.2.5.2 Aging

Freshly made vinegar usually harsh in flavour and odour when compared with the same vinegar after storing for sometime in barrels or tanks. Therefore when the vinegar has reached its maximum strength it must be aged before bottling (Nathanael, 1958).

During the period of aging, the harsh flavour and aroma disappear and vinegar becomes mild, with an agreeable and pleasing flavour and odour. It is also helpful to settle the dead microbial cells and particles which may cause the turbidity in vinegar and form sediment at the bottom of the bottles after bottling. Therefore during the period of aging which could range between 3-6 months, a certain amount of sedimentation takes place and improves the appearance and clarity of the vinegar.

The chemical changes which occur during aging are thought to be similar in many respects to those which occur when wine is stored. Esterification is one of the known changes and may be illustrated by the following equation.

$$CH_3COOH + C_2H_5OH \longrightarrow CH_3COOC_2H + H_2O$$

The vinegar should be placed in well-filled wooden barrels or tanks for aging. Unless the vinegar is stored in air tight containers, some of the acetic acid may be oxidized by the vinegar bacteria. *Acetobacter aceti*, found in most generators and the cellulose forming *Acetobacter xylinum* are species representative of the "over-oxidizing" vinegar bacteria. These bacteria may be active, if supplied with enough air, cause the oxidation of the acetic acid which they have already produced. It can be represented by the following equation (Hickey and Underkofler, 1954).

$$CH_3COOH + O_2 \xrightarrow{\text{Acetobacter enzymes}} 2C\,O_2 + 2H_2O$$

2.2.2.6 Pasteurization

The vinegar should be pasteurized at 150°F-160°F for 20 minutes before bottling. It kills vinegar bacteria and prevents the formation of "mother" which could lead to spoilage. Therefore maintaining the correct time temperature combination during the pasteurization is very important. Pasteurized vinegar keeps indefinitely when tightly

capped and stored at room temperature. Temperatures above 160°F cause a loss of acidity, flavour and aroma (Hickey and Underkofler, 1954).

2.2.2.7 Bottling

Vinegar is filled into plastic bottles after pasteurizing and sealed properly with the caps. They should be clean and food grade quality which doesn't affect the quality of the product. Glass containers are also used for high quality vinegars.

2.3 SLSI specifications for coconut vinegar

According to the SLSI requirement vinegar shall be manufactured and packed under hygienic conditions. It should have characteristic aroma and taste and should be free from any objectionable odours and foreign matters. Vinegar should not contain any added colouring matter other than caramel and any acid other than acetic acid and acids produced during the fermentation process. Vinegar should be free of sediments, turbidity and vinegar eels (SLS 168:1996).

The vinegar should conform to the requirements in the following table.

Table 2.4 - Requirements for coconut vinegar

Characteristic	Requirement
Total acidity, as acetic acid, g / ml, min	04
Total solids, g / ml, min	01
Permanganate oxidation value, min	750
Alkaline oxidation value, min	80
Iodine value, min	160
Residual ethyl alcohol, % by volume, max	01

Source: SLS 168:1996

2.4 Sedimentation of vinegar

The sedimentation takes place at the bottom of the vats during fermentation process as well as in the storage. Mainly, dead microbial cells and colloidal particles which may cause the turbidity in vinegar settle at the bottom of the vats with the time. When

these particles are settled at the bottom of the vat the vinegar should be siphoned off without disturbing to the sediment. When the sedimentation takes place it improves the appearance and clarity of vinegar. However, if vinegar is turbid when bottled, it starts to form and appear sediment at the bottom of the bottle when kept in a place after bottling.

2.4.1 Biological sediments

Both fungi and bacteria are involved in the fermentation process during manufacturing of vinegar.

2.4.1.1 Fungi sedimentation

Unicellular fungi, yeasts convert the simple sugars into ethanol in the alcoholic fermentation. Yeasts appear as a film, fine haze, and/or precipitate (Fugelsang et al., 1997). With the beginning of the acetification, yeast cells are dead and start to settle the dead cells into the bottom.

2.4.1.2 Bacterial sedimentation

Bacteria in the species of *Acetobacter* and *Gluconobacter* are responsible for the oxidation of ethanol to acetic acid. Acetic acid bacteria appear as graphite colored film or precipitate. If vinegar is contaminated with lactic acid bacteria it appears as an amorphous sediment or silky haze, sometimes as a streaming cloud (Fugelsang et al., 1997). Acetic acid bacteria are killed when vinegar is pasteurized at 150°F-160°F for 20 minutes. When those dead cells are not removed, it begins to settle at the bottom of the bottle

If *Acetobacter xylinum*, harmful vinegar bacteria which destroy acetic acid present in finished vinegars form slimy sediment at the bottom of the bottle. *Acetobacter aceti* and other species also decompose acetic acid rapidly under favourable conditions and cause turbidity and form sediment in finished vinegars if air is available (Hickey and Underkofler, 1954).

2.4.2 Chemical sediments

2.4.2.1 Organic sediments

Due to the complexes of protein and polysaccharides haze and precipitates are formed. These colloidal substances are settled down at the bottom of the bottle, forming sediment.

2.4.2.2 Inorganic sediments

Sedimentation can take place due to the complexes of some cations and anions presence in vinegar. It is well known that iron contamination may if concentrated enough, cause darkening or clouding of vinegar; copper and tin may cause clouding; zinc will produce poisonous zinc acetate; and all of the metallic ions will, in solution adversely affect the taste of vinegar. Copper probably is the most serious offender, although in most cases copper and iron are found as simultaneous contaminants of the vinegar (Hickey and Underkofler, 1954).

When accessory substances are enough it is produced the copper complex which causes the turbidity called "copper casse". Initially as a white haze and later as a reddish-brown amorphous precipitate, may develop upon storage of bottled vinegar with excess copper. The precipitate "casse" develops only under the strongly reducing conditions. Reoxidation by exposure to air or the addition of a strong oxidizing agent causes the precipitate to disappear. The sediments from copper instability can be with colloids due to copper-protein and amino acid-copper complexes. Protein levels act as the limiting factor in cloud formation. Removal or reduction of protein may help prevent copper casse. Heat and light are known to accelerate casse formation. Maintaining the copper levels at less than 0.3 mg/l is necessary to prevent the casse formation in vinegar (Hickey and Underkofler, 1954).

Iron, at higher than trace levels participating in the formation of complexes with phosphates resulting in instabilities called "white casse". It is reported to occur only within the pH range of 2.9 to 3.6. Maintaining the iron levels at less than 5 mg/l is necessary for inhibiting the casse formation (Hickey and Underkofler, 1954).
However, with iron also present as a contaminant, complete clearing of the vinegar will never occur because of the simultaneous formation of the iron phosphate complex. In the reduced form (ferrous), iron will not react with phosphates. However,

once the iron is oxidized to the ferric state, it will produce the defects described above.

2.4.3 Physical sediments

The debris coming with raw materials, small particles and dust settle down at the bottom of the containers of vinegar during the storage. So, certain amount of sediments is formed due to those external things.

2.5 Analytical techniques of sediment

2.5.1 Isolation

The sediment can be isolated by either centrifugation, filtration or aspiration directly from the floor of the vessel. Tanner and Vetsch (1956) recommended washing the collected material in 5 ml of 95% ethanol and recentrifuging (Fugelsang et al., 1997).

2.5.2 Weight measuring

The sediment can be quantified by measuring the weight of the isolated sample using an electronic balance.

2.5.3 Microbiological analysis

Micro organisms live in mixed populations in nature and most of them are able to multiply under a large variety of conditions. To study an organism we must be able to grow it in a medium. The growth medium should contain the substances that can be utilized by the micro organisms as their food. Maintaining the necessary temperature, pH and aerobic / anaerobic conditions according to the type of organism is important in the microbiological analysis.

2.5.3.1 Aerobic plate count media

A solid medium used for counting the colonies growing aerobically at 30°C under the conditions specified (SLS, 1991).

2.5.3.2 Yeast / mould media

Yeast extract-Dextrose-Chloramphenicol agar medium is used for the cultivation and maintenance of yeast and moulds (SLS, 1991). They form colonies at 25°C under the

conditions specified while the other organisms do not grow readily on this selective medium.

2.5.3.3 Acetobacter media

This is used for the cultivation and maintenance of *Acetobacter* and *Gluconobacter* species (Atlas, 1996).

Acetic acid bacteria produce small, wet looking colonies which may have a strong smell of acetic acid while the other bacteria do not grow readily on this medium (Kaltenberg, 1996).

2.5.3.4 Cell and colony morphology

Bacterial cells vary with different species. Therefore it is used to detect / isolate different kinds of species. Cells differ in characters such as size and shape.
Colonies also differ with variety of species or genus or family. The shape or colour of the colonies can be used for species / genus confirmation together with other characters (Work and Griffiths, 1968).

2.5.3.5 Gram-stain test

The Gram stain test is a particularly important staining technique and when used in conjunction with visible light microscopy, shows the shapes and arrangements of bacterial cells as well as their Gram reaction (Garbutt, 1997).

Gram staining involves a double-stain technique using the dyes methyl violet and safranine. Gram- positive organisms may be identified as those which retain the methyl violet stain under the conditions of the test, and therefore appear intense black/blue in colour. Gram-negative organisms do not retain this dye but take up the contrasting pink colour of the safranine. Gram staining should only be carried out on organisms after growth for 24 hours or less (Kaltenberg, 1996).

2.5.4 Bio-chemical analysis

2.5.4.1 Catalase-test

Catalase readily breaks down hydrogen peroxide and release oxygen. Some bacteria are positive (*Acetobacter*) and some are negative (*Lactobacillus*) for this test. This is an enzyme reaction of bacteria (Ley, 1984).

2.5.5 Chemical analysis

Chemical components of the sediment such as cations and anions can be identified qualitatively as well as quantitatively by using the analytical methods.

2.5.5.1 Qualitative analysis

2.5.5.1.1 Determination of copper ions

Preliminary identification of suspect sample using 10% Hydrochloric acid is useful in separation of metal-containing complexes from complexes of protein and phenolics. When 0.5% Potassium ferrocyanide is added to acidified vinegar, the formation of red coloration is a positive presumptive test for copper and its complexes (Fugelsang et al., 1997).

2.5.5.1.2 Determination of iron ions

When 0.5% Potassium ferrocyanide is added to acidified vinegar, the formation of blue coloration is a positive presumptive test for iron (Fugelsang et al., 1997).

2.5.5.2 Quantitative analysis

2.5.5.2.1 Spectrometric analysis for copper using diethyldicarbamate

Copper will react with diethyldicarbamate to form a colored complex measured spectrometrically at 450 nm. Extraction of this complex into an amyl acetate-methanol solvent mixture isolates it from other colored compounds in the sample (Fugelsang et al., 1997).

2.5.5.2.2 Spectrophotometric analysis for iron using thiocyanate

It depends on the quantitative reaction of thiocyanate anion (SCN^-) with Fe^{3+} and subsequent spectrophotometric measurement of the colour formed. The colorimetric reaction depends on concentration of iron present, the concentration of thiocyanate and the acidity of the solution. The method permits determination of both Fe^{2+} and

Fe^{3+} via hydrogen peroxide-induced oxidation of the iron II to iron III (Fugelsang et al., 1997).

CHAPTER 3
MATERIALS AND METHODOLOGY

3.1 Location

The experiments were carried out at the vinegar laboratory of C.D.De Fonseka and Sons (Pvt) Limited at Panadura.

3.2 Materials and methodology

3.2.1 Identification of the microbiological components in the sediment

A sample of sediment in vinegar was tested for the presence of bacteria and yeast by the ITI. Aerobic plate count was carried out by them using the test method SLS 516-1-1991 and yeast count by using the method SLS 516-2-1991.

3.2.2 Identification of the chemical components in the sediment

A sample of freshly bottled vinegar was tested for the iron and copper ions according to the test method of AOAC 999.11-2000 and phosphorous ions according to the test method of AOAC 38.032 by the ITI.

3.2.3 Identification of the causes of sedimentation in vinegar

3.2.3.1 Materials

Coconut water
Yeast
Sugar
Filtering cloth
Plastic containers
Transparent plastic bottles (350 ml)
Thermometer (range -10°C to 110°C)
Burner
Electric balance (0.01g-120g)

3.2.3.2 Methodology

To study the effect of yeast level, filtration, pasteurisation temperature and temperature of filling for the sediment, it was carried out the experiment as shown in the Figure 3.1.

Coconut water with the brix value of 2° was filtered through a filter cloth. Then sugar was added up to brix 12° and mixed well. Two samples were prepared with the addition of 0.1% and 0.0875% of yeast level. Those samples were kept closely for the alcoholic fermentation for about 6 days. The brix value was checked using the refractometer and alcohol content was checked using the ebulliometer daily.

When they were reached maximum alcohol strength, kept for acetic acid fermentation in the presence of air until the acetic acid level was reached more than 4%. Acidity of those samples was checked from the beginning to the end of the process by the titration of sample with standardized sodium hydroxide solution.

Half of the sample 1 was filtered using a filter cloth and the other half was not filtered. It was divided the filtered A sample into three and heated them to 60°C, 65°C and 70°C for 20 minutes. Then they were filled into transparent plastic bottles when it was hot and when cooled to room temperature, to see the effect of filling temperature for sediments. The same was done for the non-filtered part of the sample 1. Sample 2 was also subjected to the same procedure done for the sample 1.

The bottles filled with vinegar was tightly capped and kept in a place without disturbing. After one month of period, the sediments of the bottle were separated by centrifuging. After discarding of the supernatant wet weight of the sediment was measured using the electric balance. Like wise, weight of the sediment of each bottle was taken. Then the data were statistically analysed using ANOVA to see the effect of each treatment.

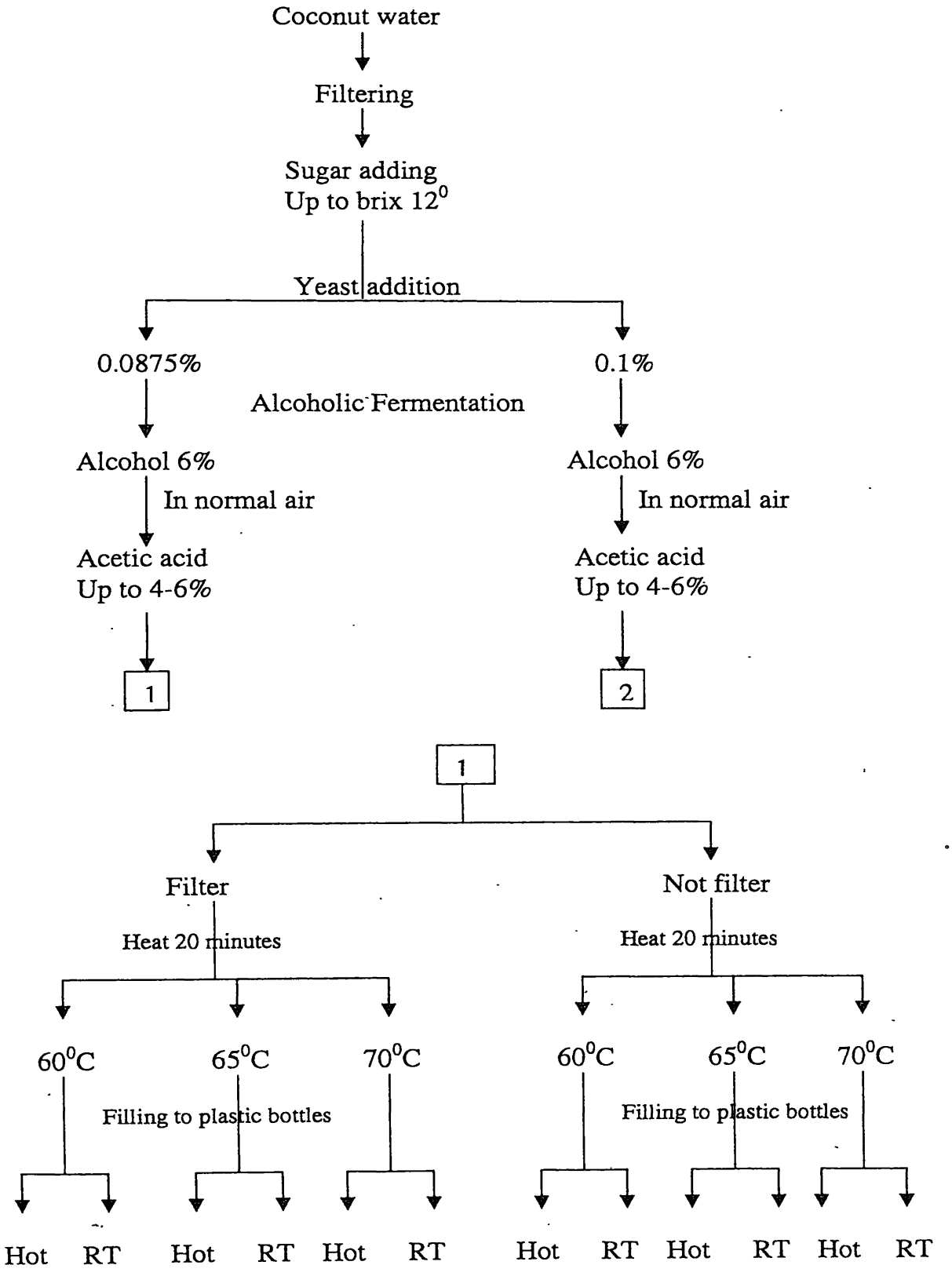

Figure 3.1 - Experiment for the identification of the causes of sedimentation in vinegar

3.2.4 Determination of brix value

3.2.4.1 Materials

Erma-hand refractometer (range 0-50)

Distilled water

3.2.4.2 Methodology

Few drops of sample were kept on the glass of refractometer and value on the scale was read.

3.2.5 Determination of alcohol content

3.2.5.1 Materials

Dujardin-Salleron ebulliometer

Distilled water

3.2.5.2 Methodology

50 ml of distilled water was put into the boiler of the ebulliometer and the boiling point was determined. Zero of the sliding scale was set to the boiling point of water. After that the boiling point of vinegar sample was determined. According to that value, it can read the alcohol content of the sample on sliding scale.

3.2.6 Determination of total acidity

3.2.6.1 Materials

Burette - 50 ml

Pipette - 10 ml

Stand

Titration flask

Standardized sodium hydroxide solution

Phenolphthalein

3.2.6.2 Methodology

10 ml of vinegar sample was taken into a conical flask. Then it was titrated with standardized sodium hydroxide solution using phenolphthalein as the indicator until a faint pink colour was obtained. Three titrations were done for each sample. The total

acid content in fermenting solution was calculated as follows. (Preparation of standardized sodium hydroxide solution was attached in Appendix 1)

Total acidity, as acetic acid, g per 100 ml = CV

Where,

C is the concentration of the sodium hydroxide solution

V is the volume, in ml, of sodium hydroxide solution required for the titration

3.2.7 Use of clarification agents for the removal of sediment

3.2.7.1 Materials

Coconut vinegar

Bentonite

Casein

Gelatine

Transparent plastic bottles (375 ml) and caps

Beakers – 500 ml

Electric balance

Spoon (stainless steel)

Thermometer (range -10°C to 110°C)

Burner

3.2.7.2 Methodology

3.2.7.2.1 Use of bentonite

Bentonite was added to vinegar samples containing 300 ml of volume in each as shown in the table 3.1

Table 3.1 - Addition of Bentonite

Sample	1	2	3	4	5
Quantity (g)	0.5	1.0	1.5	2.0	2.5
Percentage (%)	0.17	0.33	0.5	0.67	0.83

Each sample were mixed well to dissolve the agent and put into transparent plastic bottles and closed with plastic caps. They were kept in a place without disturbing to settle down.

Effect of temperature

Another set of samples were prepared by adding the same quantity of bentonite (Table 3.1) to vinegar heated at 60°C for 20 minutes. They were kept to settle down after mixing well and filling to transparent plastic bottles.

Method of addition

The quantity of bentonite was added in three portions once a two days as shown in the Table 3.2

Table 3.2 - Addition of bentonite several times

Sample	1			2			3			4		
Quantity(g)	1.0			1.5			2.0			2.5		
Portions(g)	0.34	0.33	0.33	0.5	0.5	0.5	0.68	0.66	0.66	0.84	0.83	0.83

It was mixed well at each addition and kept to settle.

3.2.7.2.2 Use of casein

Casein was added as shown in the Table 3.3 to 300 ml of vinegar at room temperature and mixed well to dissolve.

Table 3.3 - Addition of casein

Sample	1	2	3
Quantity (g)	3	15	30
Percentage (%)	1	5	10

Each sample were filled into transparent plastic bottles, closed with caps and allowed to settle. Clarity of the vinegar with the time is observed.

Effect of temperature

Casein, as shown in the Table 3.3 was added to vinegar heated at 60°C for 20 minutes. After filling to bottles they were kept to settle down without disturbing.

Method of addition

The quantity of casein was added as three portions once a two days as shown in the Table 3.4. It was mixed well at each addition and kept to settle.

Table 3.4 - Addition of casein several times

Sample	1			2			3		
Quantity (g)	3			15			30		
Portions (g)	1	1	1	5	5	5	10	10	10

3.2.7.2.3 Use of gelatine

Gelatine was added as shown in the Table 3.5 to 300 ml of vinegar at room temperature and mixed well to dissolve.

Table 3.5 - Addition of gelatine

Sample	1	2	3
Quantity (g)	3	15	30
Percentage (%)	1	5	10

Each were filled into transparent plastic bottles, closed with caps and allowed to settle. Clarity of the vinegar with the time was observed.

3.2.7.2.4 Combined effect of casein and bentonite

Casein and bentonite were added to vinegar at room temperature according to the proportions given in the Table 3.6.

Table 3.6 - Addition of casein and bentonite together

Sample	Casein		Bentonite	
	Quantity (g)	Percentage	Quantity (g)	Percentage
1	0.25	10	2.25	90
2	0.5	20	2.0	80
3	0.75	30	1.75	70
4	1.0	40	1.5	60
5	1.25	50	1.25	50

After mixing well they were filled into bottles and kept to settle. The clarity of each sample with the time was observed.

3.2.8 Identification of the effect of sediment for the quality parameters of vinegar

Freshly bottled vinegar samples in different batches were tested for permanganate oxidation value, alkaline oxidation value, iodine value and total acidity. After one month of storage period they were tested for above parameters again. Then the data were statistically analysed using paired-t-test to see the effect of sediment for those parameters.

3.2.8.1 Preparation of sample for analysis

3.2.8.1.1 Materials

Distillation assemble

Burner

Measuring cylinders – 50 ml, 100 ml

Distilled water

Coconut vinegar

3.2.8.1.2 Methodology

60 ml of the sample was distilled from a 350 ml flask fitted with a small tap funnel. When 45 ml of distillate has come over, 15 ml of distilled water was added to the flask via the funnel and further 15 ml was distilled to give a total volume of 60 ml distillate (Kirk and Sawyer, 1991).

3.2.8.2 Determination of Permanganate oxidation value

3.2.8.2.1 Materials

Pipettes – 5 ml, 10 ml

Burette – 50 ml

Glass-stoppered bottles – 250 ml

25% Sulphuric acid

0.02M Potassium permanganate

10% Potassium iodide

0.02M Sodium thiosulphate

Starch solution

3.2.8.2.2 Methodology

5 ml of distillate was added to a 250 ml-glass-stoppered bottle. 10 ml of 25% sulphuric acid was added to it. Then exactly 15 ml of 0.02M potassium permanganate was added and allowed to stand for 30 minutes. 5 ml of 10% potassium iodide solution was added to it. Then the liberated iodine was titrated with 0.02M sodium thiosulphate using starch near the end point. It was carried out a blank at the same time (Kirk and Sawyer, 1991). (Solution preparations were attached in the Appendix 1)

Permanganate oxidation value = 40 (b-a)

Where,

a is the volume, in ml, of sodium thiosulphate solution used in the titration of sample and

b is the volume, in ml, of sodium thiosulphate solution used in the titration of blank

3.2.8.3 Determination of Alkaline oxidation value

3.2.8.3.1 Materials

Pipettes – 2ml, 10 ml

Measuring cylinder – 100 ml

Burette – 50 ml

Glass-stoppered bottles - 250 ml

Electric balance

Distilled water

10% Sodium hydroxide

0.02M Potassium permanganate

25% Sulphuric acid

Potassium iodide

0.02M Sodium thiosulphate

Starch solution

3.2.8.3.2 Methodology

2 ml of distillate was added to a 250 ml-glass- stoppered bottle. 100 ml of distilled water was added into it. 10 ml of 10% sodium hydroxide and exactly 10 ml of 0.02M potassium permanganate were added. It was allowed to stand for 30 minutes and then acidified with 10 ml of dilute sulphuric acid. 0.5 g of potassium iodide was added into it and liberated iodine was titrated with 0.02M sodium thiosulphate solution using starch near the end point. A blank was carried out at the same time (Kirk and Sawyer, 1991). (Solution preparations were attached in the Appendix 1)

Alkaline oxidation value = 8 (b-a)

Where,

a is the volume, in ml, of sodium thiosulphate solution used in the titration of sample and

b is the volume, in ml, of sodium thiosulphate solution used in the titration of blank

3.2.8.4 Determination of Iodine value

3.2.8.4.1 Materials

Pipettes – 5ml, 10 ml

Burette – 50 ml

Glass-stoppered bottles - 250 ml

Litmus papers

10M Potassium hydroxide

1M Potassium hydroxide

0.1M Iodine

25% Sulphuric acid

0.02M Sodium thiosulphate

Starch solution

3.2.8.4.2 Methodology

5 ml of distillate was added into a 250 ml-glass-stoppered bottle. It was made just neutral to litmus with 10M potassium hydroxide. 10 ml of 1M potassium hydroxide and exactly 10 ml of 0.1M iodine were added. It was allowed to stand in the dark for 15 minutes. Then 10 ml of 25% sulphuric acid was added. Liberated iodine was titrated with 0.02M sodium thiosulphate using starch near the end point. A blank was carried out at the same time (Kirk and Sawyer, 1991). (Solution preparations were attached in the Appendix 1)

Iodine value = 40 (b-a)

Where,

a is the volume, in ml, of sodium thiosulphate solution used in the titration of sample and

b is the volume, in ml, of sodium thiosulphate solution used in the titration of blank

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Microbiological components in the sediment

Results of the microbiological analysis of the sample of sediment are given in Table 4.1 (Test report is attached in Appendix 2).

Table 4.1 - Microbiological test results

Test	Result
Aerobic plate count per ml	<10
Yeast count per ml (at 30°C / 72 hrs)	<10

According to that, count numbers are less than 10 in both mediums. Yeast count results show the presence of yeast in the sediment. Both acetic acid bacteria and yeasts can grow on aerobic plate count. If any other aerobic bacteria present such as lactic acid bacteria they also grow on that medium. By doing the confirmation tests it can be ensured the species of bacteria present in the vinegar. According to the results it is clear that bacteria and yeast cause for the sedimentation in vinegar. Those microbial cells multiply with the time. Then the amount of sediment increase during storage as the dead cells of those organisms are settled to the bottom of the bottle. This happens due to the insufficient time-temperature combination in pasteurization of vinegar before bottling. Hickey and Underkofler said that vinegar should be pasteurized at 150°F-160°F for 20 minutes before bottling for the destruction of acetic acid bacteria. After pasteurization vinegar should not be exposed to air. If air is entered, vinegar is contaminated and microbial growth can take place again. So it is necessary to seal the cap properly after bottling to prevent the contamination. Otherwise destruction of acetic acid also can happen.

4.2 Chemical components in the sediment

The levels of the analysed ions in the vinegar sample are given in the Table 4.2 (Test report is attached in Appendix 3).

Table 4.2 - Chemical analysis results

Test	Result
Iron, mg/l	13.0
Copper, mg/l	0.3
Phosphorous, mg/l	40.0

This Colman's vinegar was a freshly bottled one and didn't contain any amount of sediment. But the vinegar has turbidity and so it is not clear. Iron at higher than trace levels participating in the formation of complexes with phosphates resulting in instabilities called 'white casse'. Iron levels at less than 5 mg/l is necessary for inhibiting the casse formation (Fugelsang et al., 1997).

According to the test results, freshly bottled vinegar contains iron levels of 13 mg/l. So, it is higher than the content need for inhibiting the casse formation. Then iron can form precipitates with accessory substances such as phosphates. Due to the high phosphorous level according to results, it can be said that ferric phosphate cause in the sedimentation of vinegar.

Precipitate may also develop upon storage of vinegar with excess copper. The precipitated 'casse' develops only under the strongly reducing conditions. Maintaining the copper levels at less than 0.3 mg/l is necessary to prevent the casse formation (Fugelsang et al., 1997). Test results indicate that it was 0.3 mg/l in freshly bottled vinegar. Then, there is a possibility of having precipitates of copper complexes in the sediment. Ribereau-Gayon (1933) proposed that casse existed largely in combination with colloids resulting turbidity in the vinegar.

Coconut water also contain high amount of calcium according to the Table 2.3. Then there is a possibility of resulting a precipitate of calcium phosphate during the storage. Complexes of protein and polysaccharides may also be present in the sediment as they form haze and precipitates. Dickinoson and Miller said that formation of colloidal particles and ion complexes cause for the turbidity in vinegar. So, those are the main contributors for the chemical components of the sediment.

Metal levels have been reduced in vinegar than in coconut water as the yeasts are effective in reducing them during the fermentation. The yeast cell surface has a net negative charge that can rapidly and reversibly bind with exogenous divalent cations such as iron or copper. Thoukis and Amerine (1956) reported that 45% - 70% of the iron was removed during fermentation by adsorption onto the yeast cell membrane (Fugelsang et al., 1997).

4.3 Identification of the causes of sedimentation in vinegar

Following graphs show the changing of brix value, alcohol content and acidity of the fermenting samples according to the data in Appendix 4. It has taken 6 days for the alcoholic fermentation. There wasn't a significance difference in the alcoholic fermentation with the yeast level as it was slightly differed the level of yeast in two samples. But the sample which contains high percentage of yeast (0.1%) had produced more alcohol content. After the brix value had dropped to 5 it was constant in all the samples. At that stage samples had reached to its maximum alcohol strength. With the beginning of acetification alcohol content was gradually reduced and reached to zero at 22nd day in the sample 1. In the 2nd sample it has taken only 19 days.

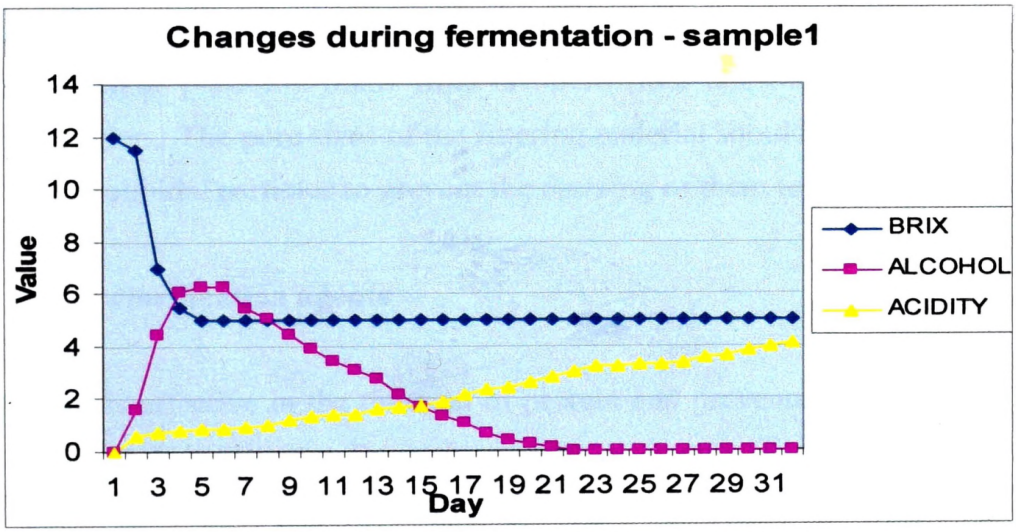

Figure 4.1 - Changes during the fermentation in sample-1

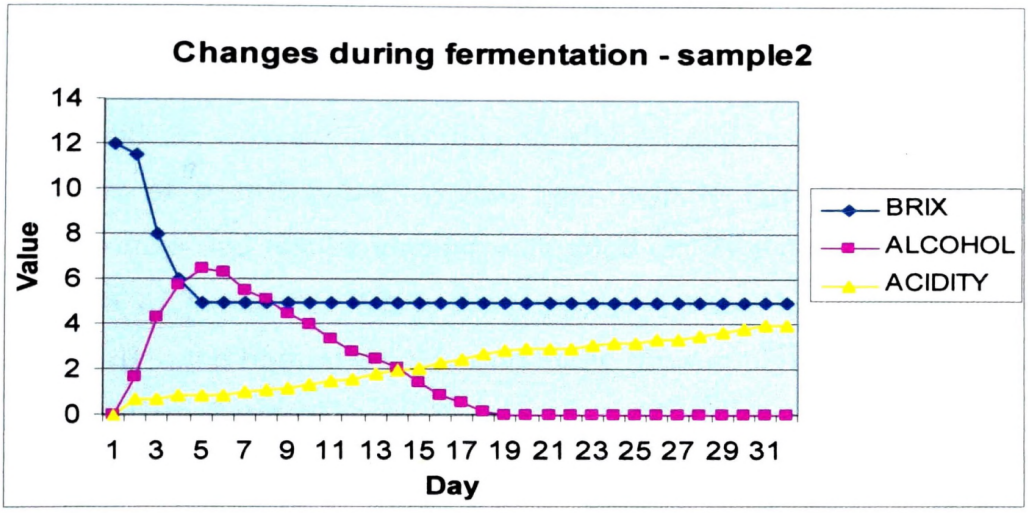

Figure 4.2 - Changes during the fermentation in sample- 2

The weight of the sediments collected by centrifuging after a month of period (see Appendix 5) were statistically analysed using ANOVA. According to the results it is 95% sure that there is an effect of yeast level and pasteurization temperature for the sedimentation in vinegar. But there is no effect of filtration and filling temperature for the sedimentation (Appendix 6). Use of yeast at a level of 0.0875% and pasteurization at 70℃ for 20 minutes help in reducing the degree of sedimentation. Here, filtering cloth was used for filtering the samples. As their pore sizes are not small enough for good filtration, there is no effect of it for the sedimentation. But, if a good filtration system such as plate and frame filter or micro filter is used, it helps in reducing the sedimentation. The pore sizes of the filtering material should small than the microbial cells and colloidal particles to prevent the entering of them to vinegar when bottling.

4.4 Use of clarification agents

Bentonite

Bentonite is effective in the removal of protein and prevents the formation of copper and iron casse in vinegar. Bentonite occurs as negatively charged molecule in the solution and binds with positively charged molecules and thereby results clarity in vinegar.

Addition of bentonite according to the Table 3.1 didn't result a large difference in clarity of the sample 3, 4, and 5 within a five days of storage. But after the 7th day,

clarity is high in 5th sample. Quantity in both sample 1 and 2 were not enough to settle down the colloidal particles in vinegar. So addition of 0.83% of bentonite as in the sample 5 is enough to reduce the turbidity in vinegar and results good clarity in it (Figure 4.3). Use of centrifugation system may help to speed up the settling of particles to the bottom and results vinegar with good clarity without a storage period, after the addition of bentonite. When centrifuging is used, high molecular weight particles are settled to the bottom quickly due to the force applying on them other than the separation with gravitational force.

Figure 4.3 - Vinegar with 0.83% bentonite

When bentonite was added to heated vinegar samples, settling of colloidal particles and agent were high in there than in the samples at room temperature. Then the time taken to clarify the vinegar is less. Proteins are coagulated when it heats and facilitates the settling of particles.

When the bentonite quantity was added separately in 3 times as shown in the Table 3.2, it was taken much time to settle particles as each was shaken after the new addition of agent.

Casein

As casein occurs positively charged macromolecules in the solution it is effective in removing of negatively charged molecules in vinegar and there by reduce the turbidity in vinegar. According to Fugelsang et al. (1997) casein reduces the concentration of both copper (by up to 45%) and iron (by 60%). Casein flocculates, and the resulting precipitate adsorbs and mechanically removes suspended material as it settles.

When casein was added to vinegar samples as in the Table 3.3, it resulted good clearance with less time than in the use of bentonite. Sample with casein of 5% resulted good clearance after five days (Figure 4.4) while the sample with 1% take more time. But its clearance is also in a good position (Figure 4.5). With the 10% of casein it was resulted the highest clarity but a brown colour was there.

Figure 4.4 - Vinegar with 5% casein

Figure 4.5 - Vinegar with 1% casein

Settling of the colloidal particles in the heated samples was also high. But the addition of the agent in several times need much time to settle the particles.

Gelatine

When gelatine was added as in the Table 3.5, it resulted a considerable clarity after a month of storage period.

When using casein and bentonite together it is needed to increase the casein proportion than in the Table 3.6 to get a good clarity with less storage period.

According to these results in selecting a clarification agent, use of casein is suitable when compared with the control sample. When vinegar is clarified with 5% level of casein it didn't result any amount of sediment at the bottom of the bottle after a month of storage period. Casein can be reused to clarify vinegar by adding small amount of casein to it.

Historically, Germans found that excess iron and copper could be removed by the addition of ferrocyanide, also known as 'blue fining'. Peynaud said that 1 mg iron requires 5.65 mg of potassium ferrocyanide. Because the major decomposition product of the reaction is cyanide the practice is not permitted by many countries. That reaction is slow and requiring up to 7 days.

4.5 Effect of sediment on the quality parameters of vinegar

Table 4.3 shows the acidity, permanganate oxidation value, alkaline oxidation value and iodine value of several vinegar samples in different batches before and after one month of storage period.

Table 4.3 - Results of quality parameters

Sample	Acidity		P.O.V		A.O.V		I.V	
	Before	After	Before	After	Before	After	Before	After
1	4.46	4.65	1920	2116	197	75	876	194
2	4.32	4.33	2400	2278	212	157	698	644
3	4.19	4.22	2446	2320	147	156	716	556
4	4.32	4.39	2096	2222	139	251	500	1020
5	4.25	4.30	1904	2136	164	124	480	382
6	4.25	4.18	2220	2316	157	248	426	708
7	4.22	4.33	2426	1896	142	147	730	554
8	4.11	4.18	2050	1924	173	243	604	1444
9	4.12	4.51	2222	2100	186	211	428	1552
10	4.34	4.36	2228	1944	153	236	164	1564

When the data are statistically analysed using paired-t-test, p value<0.05 for acidity. So it provides enough evidence to say that the acidity of samples after the sedimentation is higher than acidity of samples before the sedimentation take place (Appendix 6). Acidity can be increased during the storage when the acetic acid bacteria and residual alcohol are present in vinegar. So it is clear that the acetic acid bacteria present in vinegar after bottling due to the improper pasteurization and cause for the sedimentation during the storage.

When the data are statistically analysed using paired-t-test, p value>0.05 for permanganate oxidation value, alkaline oxidation value and iodine value. So it shows that the means of those parameters are equal before and after the sedimentation (Appendix 6). Then it is 95% sure that there is no effect of sediment for those quality parameters.

CHAPTER 5
CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

- It is evident that yeast and bacteria are present in the sediment.
- Excess levels of iron and copper ions contribute to sedimentation in vinegar.
- Acidity of vinegar is increased during storage with the sedimentation due to the presence of acetic acid bacteria.
- There is an effect of yeast level and pasteurization temperature on the degree of sedimentation. Use of yeast at a level of 0.0875% and pasteurization at 70℃ for 20 minutes help in reducing the sedimentation.
- Casein at a level of 5% can be used to reduce the turbidity and thereby increase the clarity of vinegar within 5 days.

5.2 Recommendations

- Confirmation tests should be done to identify the species of bacteria present in the sediment.
- Further studies should be done to reduce the level of casein used for the removal of sediments because 1% also results good clarity as well as 5% with the time.
- Further clarification should be achieved considering the total solids of vinegar.
- Applying a centrifugation system to speed up the settling of particles after the addition of clarification agent may help to reduce the need of settling period.

REFERENCES

Adams, M.R. Moss, M.O. (1999), Food microbiology, published by the Royal society of chemistry, Cambridge, 687pp

Atlas, R. M. (1996), Microbiological media, 2nd edition, CRC press, USA, 1580pp

Banwart, G. J. (1998), Basic food microbiology, 2nd edition, CBS publishers and distributors, New-Delhi, 749pp

Barshad, Mc Laren, Peterson, A.D. (1958), The adsorption and reactions of enzymes and protein on clay minerals, Proceedings of soil science society of America, 395pp

Berner, F.S. Davis, K.H. (2005), Handbook of industrial chemistry, 1st edition, CBS publishers and distributors, New-Delhi, 832pp

Berry, D. R. Russell, I. Stewart, G. G. Yeast Biotechnology, CBS publishers and distributors, New-Delhi, 680pp

Crueger, W. Crueger, A. (1982), Biotechnology: a text book of industrial microbiology, Science Tech, Inc, Madison, 297pp

Cruess, W. V. (1958), Commercial fruit and vegetable products, 4th edition, Mc-Graw Hill publishing company, New York, 653pp

Desrosier, N. W. Desrosier, J. N. (1998), The technology of food preservation, 4th edition, CBS publishers and distributors, New-Delhi, 542pp

Dickinoson, E. Miller, R. (2001), Food colloids-fundamentals of formulation, Published by the Royal Society of Chemistry, Cambridge, 412pp

Diggs, L. J. (1987), Vinegar-the user friendly standard text: Reference and guide to appreciating, making, and enjoying vinegar, Published by Guiet Storm publication, USA, 297pp

Dunn's and Prescott (1987), Industrial Microbiology, 4th edition, CBS publishers and distributors, New-Delhi, 859pp

Fugelsang, K.C. (1997), Wine microbiology, International Thomson publishing, New York, 222pp

Fugelsang, K.C. Gump, B.H. Nury, F.S. Zoecklein, B.W. (1997), 1st edition, CBS Publishers and distributors, New-Delhi, 608pp

Gales, P. W. (1990), Spices and other condiments, AOAC Official Methods of Analysis, 7th edition, Anheuser-Busch, Inc, 1007-1009p

Garbutt, J. (1997), Essential of food microbiology, A member of the Hodder Headline Group, London, 567pp

Hickey, R.J. Underkofler, L.A. (1954), Industrial fermentation, volume 1, Chemical publishing Co, Inc, New York, 535pp

http://en.wikipedia.org/wiki/vinegar accessed on 4th April 2007

http://en.wikipedia.org/wiki/yeast accessed on 7th April 2007

http://www.makewine.com/makewine/fining.html accessed on 20th April 2007

http://www.versatilevinegar.org/ accessed on 4th April 2007

Jacobs, M.B. (1975), The chemical analysis of foods and food products, 3rd edition, CBS Publishers and distributors, New-Delhi, 942pp

James, C. S. (1995), Analytical chemistry of foods, 1st edition, Chapman and Hall, London, 856pp

Kaltenberg, S. (1996), Kaltenberg laboratory manual, Beer von koniglicher, Hoheit, 570pp

Kill, R.C. Ranken, M. D. (1993), Food industries manual, 23rd edition, Published by Blackie Academic and Professional, Glasgow, 581pp

Kirk, R. S. Sawyer, R. (1991), Pearson's composition and analysis of foods, 9th edition, Published by John Wiley and sons, Inc, New York, 687pp

Kunze, W. (1996), Technology Brewing and Malting, 7th edition, VLB Berlin, Verlagsabteilung, 703pp

Ley, J. D. Swings, J. Gossele, F. (1936) Genus *Acetobacter*, Krieg, N.R. and Holt, J.G. (1984), Bergey's Manual of Systematic Bacteriology, Vol. I, Copyright, Williams & Wilkins Company, Baltimore, 569 - 575 p

Microbiological test methods part 1-General guidance for enumeration of microorganisms colony count techniques at 30°C, SLS 516:part1:1991, 1st revision, Sri Lanka Standards Institution, Sri Lanka, 13pp

Microbiological test methods part 2-Enumeration of yeasts and moulds, SLS 516:part2:1991, 1st revision, Sri Lanka Standards Institution, Sri Lanka, 9pp

Morrie, R. D. (1994), Simple detection of wild yeast and yeast stability, Brewing Techniques, London, 785pp

Morris, E.O. (1962), Effect of environment on microorganisms, Recent Advances in Food Science, Vol 1, Butterorths, London, 24-36p

Nathanael, W. R. N. (1954), acetic acid fermentation and the generator process for the manufacture of coconut toddy vinegar, Published by Coconut Research Institute, Sri Lanka, 1-12p

Pederson, C.S. (1971), Microbiology of food fermentations, The AVI publishing Company, Inc, Westport, Connecticut, 276pp

Raymond, E. Riegel, (1960), Industrial chemistry, 5th edition, Reinhold publishing corporation, New York, 1000pp

Roberts, T.A. Skinner, F.A. (1983), Food microbiology: Advance and prospects, published by Academic Press, Inc, Florida, 380pp

Specification for coconut toddy vinegar, SLS 168:1996, 1st revision, Sri Lanka Standards Institution, Sri Lanka, 12pp

Thampan, P. K. (1993), Hand book of Coconut Palm, 3rd edition, Oxford and IBH publishing company limited, USA, 497pp

Work, E. Griffiths, H. (1968), Morphology and chemistry of cell walls of micrococcus, Journal of Bacteriology, USA, 641pp

APPENDIX 1

Preparation of standardized Sodium Hydroxide solution

- 500 g of sodium hydroxide was dissolved in 1000 ml of distilled water (solution A)
- It was taken 100 ml from that solution
- Then distilled water was added up to 1000 ml(solution B)
- 1.26 g of Oxalic acid was dissolved in small amount of distilled water
- It was titrated with solution B
- Then the calculations were done as follows

Oxalic acid moles = 1.26 / 126.07

= 0.0999 moles

Strength of sodium hydroxide = $\frac{0.0999 / 2 \times 1000}{V}$ $moll^{-1}$

Where , V = volume of required sodium hydroxide for the titration

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 shaken thoughraly until dissolving was completed
- Distilled water was added up to the mark carefully

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 shaken thoughraly until dissolving was completed
- Distilled water was added up to the mark carefully

Preparation method of 0.1M Iodine

- 12.69 g of Iodine and 18 g of Potassium Iodide 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 shaken thoughraly until dissolving was completed
- Distilled water was added up to the mark carefully

Preparation method of 10M Potassium Hydroxide

- 561.1 g was measured by using the electronic balance
- It was placed in a standardized stoppered 1000 ml flask and amount of distilled water was added
- Then it was shaken thoughraly until dissolving completed
- Distilled water was added up to the point carefully

Preparation method of 10% Potassium Iodide solution

- 10 g of Potassium Iodide was placed in a 100 ml volumetric flask
- Small amount of distilled water was added and dissolved
- Then distilled water was added up to 100 ml

Preparation of 10% Sodium Hydroxide

- 10 g of sodium hydroxide was placed in 100 ml volumetric flask
- Small amount of distilled water was added and dissolved
- Then distilled water was added up to 100 ml

Preparation of 25% Sulphuric acid

- 100 ml of concentrated sulphuric acid was taken into a beaker
- Then distilled water was added up to 400 ml

Preparation of 10% Potassium Hydroxide

- 10 g of Potassium Hydroxide was placed in a 100 ml volumetric flask
- Small amount of distilled water was added and dissolved
- Then distilled water was added up to 100 ml

APPENDIX 2

... Continuation Sheet

TEST REPORT

Report No. SS 5204

Customer: C. D. De Fonseka & Sons (Pvt) Ltd Eluwila, Panadura.	**Test Item:** **VINEGAR** **Service Requested:** Customer's request dated 22nd May 2007
Description: Test item of Vinegar (approximately 350 ml) in a plastic bottle closed with a sealed cap .	**Identification of Test Item:** **Label:** **CO/L** **3152** **Date of Receipt of Test Item:** 22nd May 2007
Test Dates: 05th June 2007 to 08th June 2007	

TEST RESULTS

Tests/ unit	Method	Result
Aerobic plate count per ml	SLS 516-1-1991	<10
Yeast count per ml (at 30°C/72 hrs)	SLS 516-2-1991	<10

SLS 516-1-1991– Microbiological test methods General guidance for enumeration of microorganisms colony count technique

SLS 516 -2-1991 – Microbiological test methods for enumeration of Yeast & Moulds

C. Piyd

Mrs. C. Piyathilake
Technical Officer.

.....
Authorized Signatory

Mrs. S. Wickramaratne
Head
Chemical & Microbiological Laboratory

2007/06/18
/msd

APPENDIX 3

... Continuation Sheet

TEST REPORT

Report No. SS 5427

Customer:	Test Item:
C.D. De Fonseka & Sons.. Eluwila, Panadura.	VINEGAR Service Requested: Parameters requested by the customer in letter dated 2007/06/07
Description: 1. Approximately 340ml of Colman's vinegar in a sealed plastic bottle. 2. One test item (Approximately 340mlx2) of Vinegar in sealed plastic bottles.	Identification of Test Item 1.Colman's Natural Coconut Toddy Vinegar 340ml EXP 06 NOV 2006 Batch No. 253g RECKITT BENCKISER (Lanka) Limited P.O. Box 16, Mount Lavinia 2. 3498 CO/L 3430 CO/L Date of Receipt of Test Item: 2007/06/07
Test Dates: 2007/06/15 to 2007/06/22	

TEST RESULTS:

Test /Unit	Method	Result	
		Colman' s Vinegar	Vinegar
Iron, mg/L	AOAC 999.11-2000.	13	6
Copper, mg/L		0.3	0.6
Phosphorous, mg/L	AOAC 33.032	40.0	40.0

Analysis was carried out by Mrs. R. M. S. Rathnayake & Mrs. M. Herath, Technical Officer

.....

Authorized Signatory

Mr. J. A. G. Jayasinghe
Senior Research Officer
Chemical & Microbiological - Laboratory
2007/06/25
/dpc

Page 03 of 03 pages

APPENDIX 4

Changes of brix value, alcohol and acidity of the samples during fermentation

Day	Sample 1			Sample 2		
	brix	alcohol	acidity	brix	alcohol	acidity
1	12	0	0	12	0	0
2	11.5	1.60	0.59	11.5	1.70	0.68
3	7	4.45	0.73	8	4.35	0.73
4	5.5	6.10	0.82	6	5.80	0.88
5	5	6.30	0.85	5	6.50	0.91
6	5	6.30	0.88	5	6.30	0.91
7	5	5.50	0.94	5	5.50	1.00
8	5	5.10	1.00	5	5.10	1.09
9	5	4.45	1.22	5	4.45	1.18
10	5	3.95	1.37	5	4.00	1.34
11	5	3.45	1.40	5	3.35	1.55
12	5	3.10	1.43	5	2.80	1.61
13	5	2.75	1.60	5	2.50	1.82
14	5	2.15	1.66	5	2.10	1.98
15	5	1.70	1.79	5	1.45	2.11
16	5	1.35	1.92	5	0.90	2.33
17	5	1.10	2.14	5	0.60	2.50
18	5	0.70	2.36	5	0.15	2.71
19	5	0.40	2.43	5	0	2.86
20	5	0.30	2.67	5	0	2.93
21	5	0.15	2.85	5	0	2.99
22	5	0	3.03	5	0	3.00
23	5	0	3.22	5	0	3.13
24	5	0	3.27	5	0	3.19
25	5	0	3.30	5	0	3.23
26	5	0	3.30	5	0	3.35
27	5	0	3.40	5	0	3.40
28	5	0	3.56	5	0	3.55
29	5	0	3.68	5	0	3.67
30	5	0	3.85	5	0	3.85
31	5	0	3.97	5	0	3.98
32	5	0	4.13	5	0	4.03

APPENDIX 5

Weight of the sediments of vinegar after one month

Sample	Weight % (w/v)	
	W_1	W_2
1-F-60-H	0.780	0.703
1-F-60-RT	0.620	0.723
1-F-65-H	0.410	0.588
1-F-65-RT	0.401	0.562
1-F-70-H	0.358	0.488
1-F-70-RT	0.310	0.403
1-NF-60-H	0.583	0.571
1-NF-60-RT	0.428	0.531
1-NF-65-H	0.493	0.430
1-NF-65-RT	0.450	0.615
1-NF-70-H	0.430	0.387
1-NF-70-RT	0.371	0.451
2-F-60-H	0.910	0.943
2-F-60-RT	0.900	0.973
2-F-65-H	0.850	0.888
2-F-65-RT	0.840	0.786
2-F-70-H	0.750	0.985
2-F-70-RT	0.720	0.760
2-NF-60-H	0.872	0.799
2-NF-60-RT	0.944	0.885
2-NF-65-H	1.146	0.908
2-NF-65-RT	0.775	0.797
2-NF-70-H	0.665	0.569
2-NF-70-RT	0.537	0.480

APPENDIX 6

General Linear Model: WEIGHT versus YEAST, FILTRATION, ...

Factor	Type	Levels	Values
YEAST	fixed	2	1, 2
FILTRATION	fixed	2	F, NF
TEMPERATURE	fixed	3	60, 65, 70
FILLING	fixed	2	HOT, RT

Analysis of Variance for WEIGHT, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
YEAST	1	1.19354	1.19354	1.19354	126.98	0.000
FILTRATION	1	0.05077	0.05077	0.05077	5.40	0.026
TEMPERATURE	2	0.39334	0.39334	0.19667	20.92	0.000
FILLING	1	0.03366	0.03366	0.03366	3.58	0.067
YEAST*FILTRATION	1	0.00254	0.00254	0.00254	0.27	0.607
YEAST*TEMPERATURE	2	0.02261	0.02261	0.01131	1.20	0.313
FILTRATION*TEMPERATURE	2	0.05747	0.05747	0.02874	3.06	0.061
YEAST*FILLING	1	0.00651	0.00651	0.00651	0.69	0.411
FILTRATION*FILLING	1	0.00003	0.00003	0.00003	0.00	0.954
TEMPERATURE*FILLING	2	0.00690	0.00690	0.00345	0.37	0.696
Error	33	0.31019	0.31019	0.00940		
Total	47	2.07756				

S = 0.0969522 R-Sq = 85.07% R-Sq(adj) = 78.74%

Unusual Observations for WEIGHT

Obs	WEIGHT	Fit	SE Fit	Residual	St Resid
33	1.14600	0.92244	0.05420	0.22356	2.78 R
41	0.98500	0.79573	0.05420	0.18927	2.35 R

R denotes an observation with a large standardized residual.

Ho: there is no interaction effect

H_1: there is an interaction effect

Interaction effect of yeast and filtration is not significant as the p value >0.05

Interaction effect of yeast and temperature is not significant as the p value >0.05

Interaction effect of filtration and temperature is not significant as the p value >0.05

Interaction effect of yeast and filling temperature is not significant as the p value >0.05

Interaction effect of filtration and filling temperature is not significant as the p value >0.05

Interaction effect of temperature and filling temperature is not significant as the p value >0.05

One-way ANOVA: WEIGHT versus YEAST

Source	DF	SS	MS	F	P
YEAST	1	1.1935	1.1935	62.11	0.000
Error	46	0.8840	0.0192		
Total	47	2.0776			

S = 0.1386 R-Sq = 57.45% R-Sq(adj) = 56.52%

Individual 95% CIs For Mean Based on

```
                                Pooled StDev
Level    N     Mean    StDev  ---+---------+---------+---------+------
1       24   0.5036   0.1241  (----*----)
2       24   0.8190   0.1518                       (----*----)
                                ---+---------+---------+---------+------
                                 0.48      0.60      0.72      0.84

Pooled StDev = 0.1386
```

Ho: means are equal

H_1: means are not equal

P value <0.05, so Ho is rejected

It is 95% sure that there is an effect of yeast level for the sediment. Yeast level 1 is suitable as it results less mean value of the sediment

One-way ANOVA: WEIGHT versus FILTRATION

```
Source       DF      SS      MS     F      P
FILTRATION    1  0.0508  0.0508  1.15  0.289
Error        46  2.0268  0.0441
Total        47  2.0776

S = 0.2099   R-Sq = 2.44%   R-Sq(adj) = 0.32%

                                Individual 95% CIs For Mean Based on Pooled
                                StDev
Level    N     Mean    StDev    +---------+---------+---------+---------
F       24   0.6938   0.2096              (--------------*-------------)
NF      24   0.6288   0.2103    (--------------*-------------)
                                +---------+---------+---------+---------
                               0.540     0.600     0.660     0.720

Pooled StDev = 0.2099
```

Ho: means are equal

H_1: means are not equal

P value >0.05, so Ho is accepted

It is 95% sure that means of the weight of the sediments are equal. So no effect of filtration for the sedimentation

One-way ANOVA: WEIGHT versus TEMPERATURE

```
Source       DF      SS      MS     F      P
TEMPERATURE   2  0.3933  0.1967  5.25  0.009
Error        45  1.6842  0.0374
Total        47  2.0776

S = 0.1935   R-Sq = 18.93%   R-Sq(adj) = 15.33%

                                Individual 95% CIs For Mean Based on
                                Pooled StDev
Level    N     Mean    StDev  ---+---------+---------+---------+------
60      16   0.7603   0.1710                    (-------*-------)
65      16   0.6820   0.2195              (-------*-------)
70      16   0.5415   0.1866  (-------*-------)
                                ---+---------+---------+---------+------
                                 0.48      0.60      0.72      0.84
```

```
Pooled StDev = 0.1935
```

Ho: means are equal

H_1: means are not equal

P value <0.05, so Ho is rejected

It is 95% sure that there is an effect of temperature for the sediment. Temperature of 70°C is suitable for pasteurization as it results less mean value of the sediment

One-way ANOVA: WEIGHT versus FILLING

```
Source   DF      SS      MS     F      P
FILLING   1  0.0337  0.0337  0.76  0.389
Error    46  2.0439  0.0444
Total    47  2.0776

S = 0.2108   R-Sq = 1.62%   R-Sq(adj) = 0.00%

                                Individual 95% CIs For Mean Based on
                                Pooled StDev
Level   N    Mean   StDev  ---------+---------+---------+---------+
HOT    24  0.6878  0.2210           (--------------*-------------)
RT     24  0.6348  0.2001  (--------------*-------------)
                           ---------+---------+---------+---------+
                                  0.600     0.660     0.720     0.780

Pooled StDev = 0.2108
```

Ho: means are equal

H_1: means are not equal

P value >0.05, so Ho is accepted

It is 95% sure that means of the weight of the sediments are equal. So no effect of filling temperature for the sedimentation

Paired T-Test and CI: acid 1, acid 2

```
Paired T for acid 1 - acid 2

            N       Mean     StDev   SE Mean
acid 1     10    4.25800   0.10665   0.03372
acid 2     10    4.34800   0.14786   0.04676
Difference 10  -0.090000  0.124722  0.039441


95% upper bound for mean difference: -0.017701
T-Test of mean difference = 0 (vs < 0): T-Value = -2.28  P-Value = 0.024
```

H_0: $\mu_1-\mu_2 = 0$

H_1: $\mu_1-\mu_2 < 0$

P value<0.05, H_0 is rejected. So it is 95% sure that the mean difference is less than zero. As the difference is a minus value it is clear that acid 2 is greater than acid 1.

Paired T-Test and CI: POV1, POV2

```
Paired T for POV1 - POV2

                 N      Mean     StDev   SE Mean
POV1            10    2191.2     197.7      62.5
POV2            10    2125.2     161.2      51.0
Difference      10      66.0     235.0      74.3

95% CI for mean difference: (-102.1, 234.1)
T-Test of mean difference = 0 (vs not = 0): T-Value = 0.89  P-Value = 0.398
```

H_0: $\mu_1-\mu_2 = 0$
H_1: $\mu_1-\mu_2 \neq 0$
P value>0.05.So, H_0 cannot be rejected. So it is 95% sure that the mean difference is equal to zero. Then the means of before and after are equal.

Paired T-Test and CI: AOV1, AOV2

```
Paired T for AOV1 - AOV2

                 N      Mean     StDev   SE Mean
AOV1            10     167.0      24.6       7.8
AOV2            10     184.8      61.3      19.4
Difference      10     -17.8      74.3      23.5

95% CI for mean difference: (-71.0, 35.4)
T-Test of mean difference = 0 (vs not = 0): T-Value = -0.76  P-Value = 0.468
```

H_0: $\mu_1-\mu_2 = 0$
H_1: $\mu_1-\mu_2 \neq 0$
P value>0.05.So, H_0 cannot be rejected. So it is 95% sure that the mean difference is equal to zero. Then the means of before and after are equal.

Paired T-Test and CI: IV1, IV2

```
Paired T for IV1 - IV2

                 N      Mean     StDev   SE Mean
IV1             10      562       204         65
IV2             10      862       502        159
Difference      10     -300       660        209

95% CI for mean difference: (-771, 172)
T-Test of mean difference = 0 (vs not = 0): T-Value = -1.44  P-Value = 0.185
```

H_0: $\mu_1-\mu_2 = 0$
H_1: $\mu_1-\mu_2 \neq 0$
P value>0.05.So, H_0 cannot be rejected. So it is 95% sure that the mean difference is equal to zero. Then the means of before and after are equal.

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 : 2017-09-25

3. Name of Digitizing Company : Sanje (Private) Ltd, No 435/16, Kottawa Rd, Hokandara North, Arangala, Hokandara

4. Scanning Officer

Name : B.A.C. Badarawaan

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. NEIGHSOOREI
(MSSc.PGD.ASLA,BA)
Librarian
Sabaragamuwa University of Sri Lanka
P.O.Box 02 Belihuloya,Sri Lanka
Tele:+94 45 2280045
Fax:+94 45 2280045

Date : 2017-09-25

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