THE USE OF *DIALIUM GUINEENSIS* FOR THE MANUFACTURING OF WINE

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DEDICATION

THIS WORK IS DEDICATED TO MY BELOVED PARENTS WHO ALWAYS ENCOURAGE ME NOT TO BURY MY THOUGHTS, BUT TO PUT MY DREAMS TO REALITIES.

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I like to express my gratitude to the entire laboratory staff at the Microbiology, Chemistry and Biochemistry Units, of the Federal University of Technology, Minna. I especially thank Mr. S. Oyeowo who took this work personal.

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Lastly, I would like to appreciate the unquantifiable guidance of my supervisor, Dr. (Mrs.) N. Dee, a very interesting woman, the merits of this work are a personal tribute to her.

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DECLARATION

I hereby delcare that this work is original and has not been carried out elsewhere. All literature cited have been duely listed in the references.

CERTIFICATION

This thesis entitled 'The use of *Dialium guineensis* for the manufacturing of wine' was examined and found to meet the regulations governing the award of degree of Master of Technology (M. Tech.) of the Federal University of Technology Minna and is approved for its contribution to knowledge and literary presentation.

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ABSTRACT

In Nigeria, wine is a popular beverage that has been consumed for a long time. The climatic condition in Nigeria is not favourable for the cultivation of grape and other fruits which are conventionally used for manufacturing wine. The consequence of this is that almost all the commercially available wines consumed in Nigeria are imported from other countries. Scientists in different parts of the country are trying to work on the possibility of using our local fruits for manufacturing wine instead of totally depending on expensive imported wines. The present work explored the possibility of using the fruit of *Dialium guieneensis (Icheku)* as a substrate for making wine. The results point towards the possibility of using Icheku fruit to make wine in a large scale in Nigeria and in a form that can compare favourably with imported commercially available wines. Icheku fruit can be preserved and stored without much deterioration for a long time. This gives it a major advantage over all other locally available fruits previously investigated for wine making. These fruits include cashew, orange, and pineapple.

The important characteristics of Icheku wine include Alcoholic content, 6.92 - 13.60% (V/V), pH, 3.81-4.04, reducing sugar, 0.40 - 1.80% Protein 0.60 - 0.67%; Total titratable acidity (TTA), 0.64 - 0.83%; Volatile acidity, 0.027 - 0.035, Ascorbic acid content, 69.44 - 79.36mg/100ml and Glycerol content 0.27 - 0.30%. These results are comparable to those reported by some workers for

cashew, orange and pineapple wines and to values found in this work for some commercially available wines in Nigeria. The results are discussed along the line of self reliance through biotechnological ventures with special bias to the use of locally available raw materials for the commercial production of wine.

CHAPTER ONE

INTRODUCTION

Dialium guineensis is a tree that grows abundantly in the Eastern part of Nigeria but scantly in the West and Middle belt regions. It grows wild. The fruit of *Dialium guineensis* is an indehiscent achene that measures about 1.3m^2 in area. It has a black fibrous exocarp (husk) which covers a pink mesocarp that encloses one or two seeds. The fruit has the following traditional names `Ohiome' (Bini), `Akin' (Ijaw), `Awin' (Yoruba); Tsamiyan Kurmi (Hausa), and `Icheku' (Igbo)(Olorode, . 1984). The fruit will be called the Igbo name, *Icheku* in this work.

Icheku matures between the months of March and May and the annual yield may run into thousands of tonnes. Some of this is consumed by people because of its sweet taste and a substantial quantity wastes in the bush owing to ignorance of its potentials.

Wine is generally regarded as the product made by the normal alcoholic fermentation of the juice of sound grape modified by the usual cellar treatment. A number of local fruits have been investigated for use in making wine. They include cashew, (Falade, 1981; Macleod and Traconis, 1982; Oritz, et *al*, 1982; Ogunmoyela, 1983; Aderiye et *al*, 1991) orange (Van. Bure 1970; Mitchell, 1982) and pineapple (Singleton and Gotner 1965; Felton, 1971). These fruits have short shelf life and have not been successfully preserved. During storage, they deteriorate, producing musts that have high acidity content; increased peroxide and saponification values, and are characterised by the presence of carbonyl

compounds. The must tends to produce off flavour and hence not good for making wine, (Nagy and Nordy, 1970).

Plate 1. A branch of the tree with some fruits.

Key (a) *Icheku* Fruit

(b) The leaf

(c) The branch

This storage problem is a major hinderance in the commercialization of the products of the studies carried out on these local fruits for purposes of manufacturing wine. *Icheku* has a major advantage over the forenamed fruits. It has a very low water content and from my experience the fruits can be preserved by completely drying it under the sun followed by occasional exposure to the heat of the sun. The fruits used in this work were stockpiled in a cool dry place (the laboratory cabinet) for 11 months without any deterioration.

Industrially, wine is produced from fruits like Apple, Rosehip, red, white, and black Currants, Strawberry, Raspberry, Plum berry, Black berry, Blue berry and Goose berry (Gallander, 1976). However, grape is the most important fruit used all over the world (Rose, 1977). This is because it contains an ideal balance of sugars, acids and tannins; and yeasts naturally grow on the surface of the grape skin (Gallander, 1976). A wine made from a fruit is named after the fruit, for instance, `Apple wine'. Wine produced from honey is called Mead (Jarczyk and Wzorek, 1977).

In West Africa, palmwine is a naturally fermented drink that is tapped from palm trees. It has a distinctive taste, aroma, flavour, alcoholic content and is very popular (Okafor, 1978). Generally, the climatic condition of West Africa is not favourable for the cultivation of grapes and other fruits mentioned earlier for industrial wine production. The consequence of this is that nearly all commercially available wines consumed in this region

particularly in Nigeria are either imported as bottled drinks or imported in large containers; blended and bottled in Nigeria.

The term fermentation is derived from the Latin verb, (*Fervere*: to boil) thus describing the appearance of yeast on extracts of fruits or melted grains. The `boiling' appearance is due to the production of carbon dioxide bubbles caused by the anaerobic catabolism of the sugars present in the extract. The application of yeasts for preparing alcoholic beverages dates back far beyond the advent of recorded history. It is known that beer was produced by the Sumerians more than 7000 years B.C. and wine by the Assyrians 3500 years B.C. The techniques of malting and brewing were already highly developed among the Babylonians without any conscious knowledge of the underlying biochemistry, the existence of enzymes or of yeast, and the role these agents play in the malting or fermentation process (Stewart, 1974). In the old Mesopotamia, a part of the employee's salary was paid in the form of wine or beer. In Egypt of the Pharaohs, wine was regarded as a holy gift from *Osiris*, the god of the dead. In Isreal and Rome of the old testament, the possession of vineyards was considered to be an enviable sign of wealth. Wine was reputed to have certain therapeutic values and was used as a drug because it was believed to be a good remedy against a number of diseases, including *Angina pectoris*, Sour stomach and kidney stones (Stewart, 1974).

Although fermentation has been known to the ancient world, the first true large scale fermentation with even some form of process control dates back to the 1700s when wooden vats of up to 1,500

barrels capacity were introduced (Corran, 1975). In recent years, there have been radical developments occurring in the fermentation techniques employed in wine making and other yeast utilizing industries such as the solid state fermentation proceses. The basic biochemical and microbiological research approach has been very aggressive and the result is a clearer understanding of the scientific principles underlying wine making. Today, wine is made all over the world. However, a large proportion of the world's wines are made in the countries located near the Mediterranean Sea. This is due to the presence of favourable environmental conditions for the cultivation of grapes. France leads the world in wine making. Italy, Spain, Algeria, Portugal, Rumania, Argentina, Russia, Hungary, Yugoslavia, USA, Chile, Greece, Bulgaria, South Africa, Germany and other countries also make wine industrially.

According to Rose, (1977), grape wine can be classified as either Natural wines, Fortified wines, or Dry wines.

A. **NATURAL WINES:** These have sugar left or added after fermentation. They include:

(i) Sparkling Wines: Made effervescent by secondary fermentation or by artificial carbonation.

(ii) Still wines: Do not contain carbondioxide and include

(a) Appetizer wines which contain about 20% alcohol (V/V)

(b) Desert wines that contain between 12-15% alcohol (V/V), and

(c) Table wines which do not have more than 14% alcohol (V/V).

Table wines are further divided into:

White table wines which are fermented from white variety of grapes or must of black grape, Rose table wines where the skin is removed before all the pigments are extracted and Red table wines got from black variety of grapes with pigments leached from skin by alcohol produced during fermentation.

B. **FORTIFIED WINES:** Here, distilled spirit is added after fermentation. This increases both the volume of wine and percentage by volume of alcohol.

Fortified wines include, Artificially flavoured wines and Port, Madeira and Sherry which owe their flavour to natural fermentation products. Sherry is characterised by a nutty flavour and contains between 0-7% sugar, Port is sweet, dark red and strong and Madeira has a mild mouthfeel.

C. **DRY WINES:** These wines contain no sugar or relatively small amounts which are difficult to detect by the taste perception.

The group of microorganism, known as `yeasts' are used in wine making. Yeasts belong to fungi in which the unicellular form is predominant (Lodder, *et al*, 1952). Vegetative reproduction is usually, but not always by budding. A Dutchman, Atonie Van Leeuwenhoek, is credited with being the first man to have observed yeast microscopically. However, it was not until 1837 that Cagniards and Latour demonstrated that beer contains spherical

bodies that are able to multiply and which belong to the vegetative kingdom. It was Schwain who termed yeast `Zuckerpily' or 'sugar fungus' from which the name 'Saccharomyces' originated (Lodder *et al.*, 1952). Pasteur finally proved that fermentation is due mainly to living cells. It can be seen therefore that the involvement of yeast in wine making is an old and well established one and that in fact wine making can be said to be born out of the yeast technology. A variety of yeasts have been used for wine production. These include *Saccharomyces cerevisiae* and *Saccharomyces bayanans* (Heinzel and Truper, 1976; Goswell, 1986) *Saccharomyces uvarum* (Dott, *et al.*, 1976), and *Botrytis cinera* (Veldhuis, 1972). The selection of a yeast is one of the major factors that must be supervised with respect to wine making.

The fundamentals of wine making are similar in all principal wine making regions but methods vary to some extent owing to the varieties of fruits used, the climate and other factors. The process generally involves the following:-

1. LOCATING THE SUITABLE SUBSTRATE

A suitable substrate is screened, and then extracted by a suitable method. The extract (must) so obtained is subjected to a prefermentation treatment to make it conducive for yeast fermentation. These treatments include dilution and acidity adjustment, nutrient level supplementation and sterilization.

2. SELECTING THE YEAST

This involves the isolation of a pure culture of the yeast and the building up of the culture to provide a starter culture which should comprise about 6-10% of the entire fermentation medium (Amerine et al., 1980). Here, a large supply of oxygen is essential for the rapid multiplication of yeast cells and sterille portions of the extract are used. It is very crucial that the culture is a pure one as any contaminating microorganisms may produce undesirable products which may cause serious problems in the final product.

3. THE FERMENTATION

Here, the starter culture is added to the extract contained in the fermentation medium. The fermentation is carried out at carefully controlled temperatures. The optimum is normally 26-29^{0}C (Amerine and Joslyn, 1970); temperature above 35^{0}C is unsuitable as it favours the development of spoilage bacteria which impair wine quality and tends to evaporate the alcohol produced. However, too low a temperature makes the whole process too slow to be practical. The end of the fermentation depends on the desired strength of the wine but is generally taken to be when carbon dioxide bubbling has stopped or at a sugar level of less than 0.7%.(Amerine and Joslyn, 1970).

4. **SECONDARY FERMENTATION**

If desired, the wine is taken through secondary fermentation where the free run wine is placed in bottles or containers, supplemented with sucrose, then closed. This process lasts for 7-11 days and produce wines that effervesce (Amerine, et al., 1980).

5. **RACKING**

By `racking' is meant the drawing off of the wine from the lees or sediment. Wine is racked to facilitate its clearing and to prevent undesirable flavours from being extracted from the old yeast.

6. **STORAGE AND AGING**

Two important changes take place during storage and aging: clearing of the wine and development of flavour. Substances including tartates, certain proteins and other matters are present in new wine and if not removed will produce a sediment and probaly cloudiness (Amerine et al., 1980). During aging, flavour which is due to a combination of taste and odour is developed in wine as a result of oxidative changes and ester formation.

7. **CLARIFICATION**

Wine may clear naturally over a period of time; however for economy of production, resort is frequently made to the use of fining agents followed by filtration, heating, refrigeration or a

combination of the processes. Fining (clarifying) agents include Bentonite, Isinglass, Gelatin, and white of egg. They are normally used carefully as directed by the manufacturer or after laboratory tests have been carried out with small portions. When not properly used, they may become a source of cloudiness in the wine.

8. PACKAGING

The clarified wine is blended, pasteurised and corked. The bottle may be labelled for commercial purposes.

The aroma, taste and bouquet of wine are determined by the variety of fruits used, their chemical composition, nature of the fermentation process and changes which are made to occur or occur naturally during the post fermentation period, (Formica, 1981). Wine is a complex mixture of organic and inorganic compounds and contains Esters, Sugars, Aldehydes, Diacetyls, Tannins, Fusel oils, fatty acids, fixed acids, and Alcohols (Amerine, et al., 1980). The development of aroma is inhibted by the presence of large quantities of Carbon dioxide, Sulphur dioxide and by exlusion of air. The nature of the ester present is of much more importance than the quantity. The esters of Acetic acid contribute much to the flavour and bouquet of wine. Aroma and flavour are best developed at low temperatures (Ough and Amerine, 1966).

Wine defects refer to the undesirable changes observed in wine. These include browning or other colour deterioration, haziness, cloudiness, deposits, and undesirable taste and colour. Defects may result from various causes. When caused by microbial

infection, either by aerobic or anaerobic microoganisms, they are called *wine diseases* (Amerine, *et al.*, 1980).

DEFECTS BY AEROBIC MICROORGANISMS

The most implicated organisms are the Mycodermas and Acetic acid bacteria. They grow well in the presence of oxygen but not when the wine is well supervised and kept under anaerobic conditions. *Candida mycoderma* (wine flower) forms a film over the surface of wine and attacks the extracts, the alcohol and occasionally the organic acids. Acetic acid bacteria produce vineger from wine (Amerine and Joslyn, 1970).

DEFECTS BY FACULTATIVE AND OBLIGATE ANAEROBIC

These organisms cause the disease known as `Tourne' which is considered to be the most serious and common disease in wines. The responsible organisms are long slender rods which can grow in wines with alcoholic content up to 20% or more but survives better if the alcoholic content is not too high. `Tourne' is indicated by increasing volatile acidity (0.12-0.14%), decreasing fixed acidity, a `silky' type of cloudiness and in extreme cases, an odour and taste that is termed `mousey' (Kunkee and Goswell, 1977). *Lactobacillus hilgardi*, a non motile, non sporulating rod cause `silky' cloudiness in wines (Kunkee and Goswell, 1977). Mannitol forming bacteria produce volatile acids, lactic acid and mannitol from glucose. Some cocci cause cloudiness, butyric acid bacteria produce bitter-taste and lactic acid bacteria may produce sour

wine. All the microbial defects may be detected by visual observation followed by the microscopical examination of the sediment obtained by centrifuging a sample of the wine. Infection is prevented at low temperatures, and low content of sugar and other nutrients. It is inhibited slightly by Tannin but very strongly by Sulphur dioxide and Metabisulphites, (Kunkee and Goswell, 1977). Pasteurization and high degree of cleanliness will lower or remove the incidence of microbial diseases.

DEFECTS NOT CAUSED BY MICROORGANISMS

Iron and steel may cause cloudiness of wine forming the so called `ferric casse'. The tin and copper dissolved from bronze by the juice if sufficient in quantity may cause flavour and colour losses during the aging processes. The ferric casse may be treated by oxidising with Oxygen; Tannin (0.05%) is added followed by clarification with Casein and Bentonite. In another method, the iron is oxidised, Tannin added and then Gelatin; settling is permitted which is followed by racking, filtration and acidification with Citric acid. In the third method, Tartaric acid is added and the wine refrigerated. Cream of tartar and iron salts are precipitated. The use of stainless steel, nickle or inconel in the construction of fermentation vessels prevents the defects (Frazier and Westhoof, 1978).

There are two types of stabilization processes: Microbiological (malo-lactic fermentation) and Chemical (clarification). The malo-lactic fermentation is carried out by

ethanol and acid tolerant facultative anerobes, hence their survival in wines (Pilone *et al.*, 1966). The malo-lactic bacteria include *Lactobacillus buchneri, Lactobacillus casei, Lactobacellus delbrueckii, Lactobacillus pastonanus, Leuconostoc citrovorum, Leuconostoc mesenteroides* and *Pediococcus cerevisiae*. When inoculated into wine, they carry out a sort of secondary fermentation, fermenting malic and citric acid. Sugars are converted to water and carbon dioxide. They use up ions like potassium, magnesium, sodium, mangenese and calcium during the fermentation (Stammer *et al.*, 1964). Malo-lactic fermentation does not occur in fortified wines (alcohol content of 19.5% or more) and is inhibited by temperature of 5-10^{0}C and reduced acidity. (Kunkee and Goswell, 1977). After this, wine becomes biologically stable due to the removal of fermentable substrates and Malic acid. Generally microbial spoilage is prevented by low pH and temperatures, low sugar content (0.2% or less), absence of air and growth substances. Alcoholic content of 14-15% inhibits acetic acid bacteria; deacidifying cocci is inhibited at 17% alcohol, *Leuconostoc* species at 14%, heterofermentative lactobacilli at 18% and homofermentative lactobacilli at 10% (Frazier and Westhoof, 1978).

The manufacture of wine is a biological process, so monitoring the biochemical conditions is very crucial for a successful fermentation (Amerine and Joslyn, 1970). Monitoring of these conditions or factors aid the maintenance of constant conditions

and may also provide information on the progress of fermentation (Stanbury and Whitetaker, 1984).

In the quality control of wine production, all the Chemical, Physical, Biological, Biochemical and Organoleptic methods that are available are employed in activities directed towards the maintenance and improvement of the quality of the product taking into account all the factors that may positively or negatively influence the quality. The aim is to produce safe and wholesome wines which are compartible with the market for which they are desiged in terms of its consistent value for money. There are two phases of [illegible] wine quality control.

CONTROL POINTS FOR PHASE 1

These include proper fruit maturity, visual inspection for rotting, fruit flies etc., cleaning and sanitization of extraction materials or equipment, use of pure yeast culture, control of fermentation temperature; prompt racking to avoid the development of off flavours, browning and instability etc. During aging the visual and taste inspection of wine is important.

CONTROL POINTS FOR PHASE II

These include sterilization of equipment in the bottling room, and personal cleanliness of workers. Finished products should be inspected for correct labelling and corking.

However, there is no information in the literature concerning the use of *icheku* for wine production. The aim of this research therefore is to propose *icheku* as a substrate for making wine. It is a response to the Governments call or `backward integration' and self reliance.

CHAPTER TWO

MATERIALS AND METHODS

2.0 COLLECTION AND PRESERVATION OF THE FRUITS

Healthy mature fruits were bought from Amalla market in isi Uzo Local Government Area of Enugu State. The fruits were preserved by drying completely under the sun. They were stored under dry conditions in the laboratory cabinet from where they were exposed to the sun after every four months. A control set of fruits were not completely to dried.

2.1 PREPARATION OF THE EXTRACT

About 200g of the fruit (without the exocarp) was soaked in approximately 150ml of sterile distilled water for 5 minutes. The fruits were hand pressed to further release the mesocarp before removing the seeds. The syrup was then filtered through a muslin cloth into a sterile container. The muslin cloth was washed with 200ml of distilled water to further enhance the extraction.

2.2 ASSAY FOR MICROBIAL PRESENCE IN THE EXTRACT

A serial dilution was carried out asceptically using 1ml of extract. The 10^{-1} and 10^{-3} dilutions were used to assay for microbial presence using Potato dextrose agar and Nutrient agar. The lncubation period was 24hrs.

2.3 ANALYSIS OF THE EXTRACT

i) QUALITATIVE TEST FOR REDUCING SUGAR

Benedict's qualitative reagent was put in a test tube containing five drops of the extract and boiled for five minutes (Plummer, 1971). The composition of the Benedict's qualitative reagent is as follows: $CuSo_4 . 5H_2O$ 8.6$_g$, $NaCo_3$ anhydrous, 50.0g., Tri sodium citrate, 86.4g and distilled water to 500ml (Cheesebrough, 1987).

ii) TOTAL SOLUBLE SUGARS

This was determined using a refractometer (Belingham and Stanley model). A drop of the must (well stirred) was placed on the measuring prism and covered. The light aperture was opened and the instrument turned towards the light source. The amount of sugar present was read off from the scale.

iii) QUALITATIVE ESTIMATION FOR SUGARS BY ASCENDING CHROMATOGRAPHY

The method was described by Smith and Fienber (1965). A baseline of 16cm was drawn from one end of the Whatman No. 1 filter paper. 1% standard solutions of Glucose, Fructose, Lactose, Maltose and Sucrose were prepared in distilled water. A developing solvent of n-butanol/acetic acid/water (4:1:5) was prepared in a separating funnel, allowed to stand for 10 minutes and the supernatant collected and used as the solvent. Six spots were made on the paper, the first 5 for the standard sugars and the last one for the extract. Ascending chromatography was run overnight to

ensure adequate separation of the sugars. The paper was dried in a fume chamber and sprayed with a locating agent made up of m-phenyl diamine (4_g), Autophosphoric acid (H_3PO_4) (4ml) and Acetone or Ethanol (200ml); prepared fresh. The paper was dried again in the fume chamber and placed in the oven at 110°C for 10 minutes for colour development.

iv) <u>TOTAL TITRATABLE ACIDITY (TTA)</u>

Five ml of extract was added to 100ml of distilled water in a conical flask and 5 drops of 1% phenolphatalein solution added. The mixture was titrated against 0.1N Sodium hydroxide solution. The end point was indicated by a persistent blue colouration. The TTA was expressed as Tartaric acid per 100ml and calculated by multiplying volume of Sodium hydroxide used by 0.150 (Heinzel and Truper, 1976).

(v) <u>P^H</u>

This was determined using a digital pH meter (Crison, micro pH 2000 model). The probe (electrode) was dipped into the extract and the pH value read off the screen.

vi) <u>SPECIFIC GRAVITY</u>

A hydrometer (Zeal, 1.00 - 1.500) was used. Some extract was poured into the hydrometer jar and the hydrometer spinned to get rid of any air bubbles clinging to its sides. The reading was taken when the hydrometer became still and with the eye at surface

level. The specific gravity was read at 15°C as directed by the manufacturer.

vii) PROTEIN CONTENT

The Biuret method was used as described by Cooper (1977). A calibration curve(Appendix) was obtained using various concentrations of Bovine Serum Albumin (BSA) (0,2,4 - 10mg/ml). Biuret reagent (4ml) was added to 1ml of the various concentrations of BSA contained in respective test tubes and incubated for 20 minutes, at 37°C. A 10^{-1} dilution of the extract was treated similarly. The optical density of the various solutions was obtained using a spectrophotometer (WPN S101 model) at 540nm against a reagent blank. The Biuret reagent was composed of Potassium sodium tartate (9g), Potassium iodide (5g), 6N Sodium hydroxide (100ml) and distilled water (to 1 litre), (Cheesebrough, 1987).

viii) ASH CONTENT

A crucible (ashing plate) was weighed, 10ml of extract put into it and heated over a bunsen flame to a constant weight. Water and volatile components evaporated as vapour, the organic compounds were burnt off as carbondioxide and oxides of nitrogen and the non volatile components remained as residue, (weight of plate + residue) - (weight of plate) = weight of residue. Amount of residue per litre of extract was then calculated.

ix) ASCORBIC ACID

Three grams of the *Icheku* fruit was ground with 75.0ml of 0.5% oxalic acid and filtered through a Whatman No. 1 filter paper. The filtrate (20 ml) was shaken with one teaspoonful of activated charcoal and filtered again. The filtrate (0.5ml) was placed into a test tube and made up to 4ml with 4.0% oxalic acid previously treated with activated charcoal. 0.3 ml of 0.5% Dichlorophenol indophenol was added to the mixture and the tube vortexed at low speed. The tube was incubated at room temperature for 10 minutes and 1ml of 4% Thiourea was added. 1.2ml of 2,4 Dinitrophenyl hydrazine (2% in 75% H_2SO_4) was added dropwise with mixing to the tubes and vortexed thoroughly. The tube was then incubated at 50°C for 2 hrs. and allowed to cool to room temperature. The optical density was measured with a spectrophotometer (WPA S101 model) at 510nm against a reagent blank. A calibration curve of vitamin C (0.05 - 0.08 mg per ml) (Appendix) was obtained using the described procedure. The amount of ascorbic acid present in mg/100g of fruit was calculated from:

$$mg/100g = \frac{(conc. \times 0.02 \times 0.5 \times 99.2)}{3} \times 100 \times 100$$

(Temple *et al.*, 1990).

2.4 PRIMARY FERMENTATION

0.025% (W/V) of Sodium metabisulphite was used to sterilise the fruit extract. The inoculum used was a pure culture of *S. cerevisiae* (commercial baker's yeast), 5g of the dry yeast was

added to about 25ml sterile portion of the extract and left at 37°C for 15 minutes (Aderiye, *et al.*, 1991). This activated yeast was added to the extract at a 6% (V/V) concentration, 4hrs after sterilizing the extract. The fermentation vessel was a sterile 500ml flat bottom flask fitted with a rubber bung carrying a `U' tubing which allowed the exit of carbon dioxide. The `U' tube contained Sodium metabisulphite solution (0.1%) to prevent contamination. Three different flasks were prepared as follows:

Flask (i) contained 500ml of extract.

Flask (ii) contained 500ml of extract supplemented with 0.1% (W/V) Ammoniumsulphate, (Maldonado, *et al.*, 1975).

Flask (iii): as in Flask (ii) but supplemented with 10% (W/V) sucrose. All fermentations were carried out in two sets of flasks after adjusting the pH to 4.6. The fermentation was considered completed when gas bubbling stopped and sugar analysis indicated a level of not more than 0.7% (Amerine et al., 1980).

2.5 1ST RACKING

The fermented broth was allowed some time (about 20 minutes) for the sediments to settle. The supernatant was then racked off the sediments, using a sterile rubber tube into sterile containers.

2.6 SECONDARY FERMENTATION

400ml of the fermented broth from one of flasks iii was decanted into a sterile fermentation vessel and supplemented with

6% (W/V) sucrose and kept at ambient temperature for 7 days (Frazier and Westhoof, 1978).

2.7 2ND RACKING

This was done at the end of the secondary fermentation following the same procedue used for the first racking.

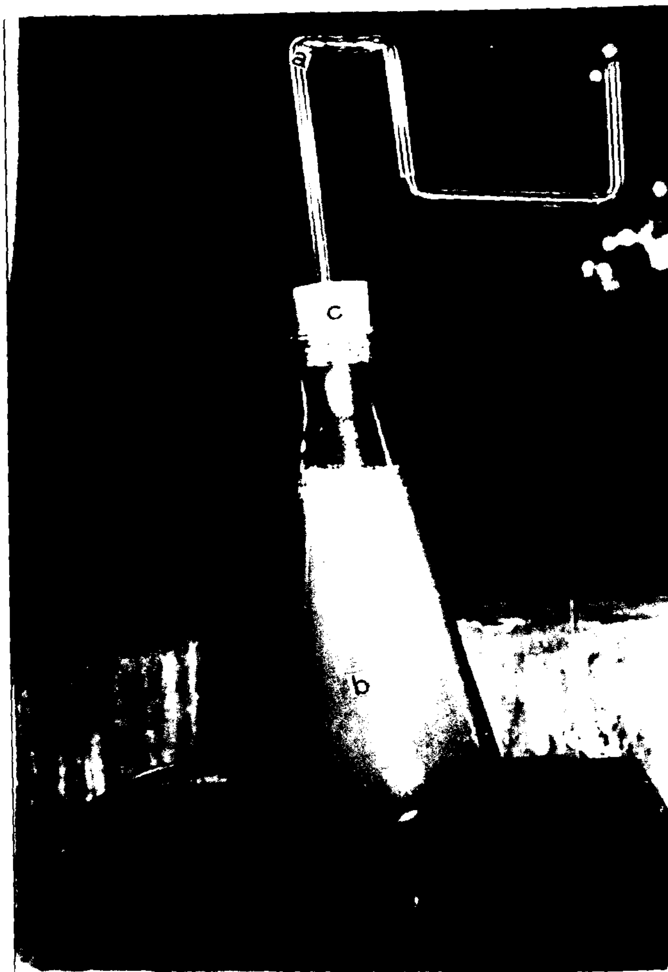

Plate 2 The Fermentation Apparatus

Key: (a) `U' tubing

(b) fermentation flask

(c) Rubber bung

2.8 MICROSCOPIC EXAMINATION FOR CONTAMINATING MICROORGANISMS

The fermenting extract was screened for microbial contamination at intervals of 24hrs throughout the duration of the fermentation process. 1ml of the fermenting extract was withdrawn (after shaking the flask properly) with a sterile pipette. This was centrifuged at highspeed to obtain a sediment from which several smears were prepared, gram stained and observed under the microscope, first under x40 objective then under the oil immersion objective.

2.9 P^H AND TEMPERATURE MONITORING

These were checked every 24hrs to detect any pH and temperature changes that may be favourable to the growth of spoilage microoganisms.

2.10 OTHER PARAMETERS

The Sugar content, Total titratable acidity, and specific gravity were also checked every 24 hours.

2.11 STORAGE AND AGING

The racked fermented broth was stored at refrigirator temperature (4^{0}C) for 2 weeks.

2.12 CLARIFICATION

Bentonite, Gelatin, and Gelatin with Tannic acid were tested as clarifying agents using the method described by Mitchell, (1982). 10ml of fermented broth was put into 15 test tubes. The test tubes were divided into 3 sets, A,B,C of 5 test tubes each. 1% solution of bentonite, 1% solution of Gelatin and 1% solution each of Gelatin and tannic acid were added in varying volumes (0.1,0.2,0.3,0.4 and 0.5ml) to the tubes of the three sets (A,B,C) respectively. The tubes were plugged with cotton wool and kept at 4°C until fermented broth became clear. A control was provided by a test tube containing 10ml of fermented broth without the addition of any clarifying agent.

2.13 3RD RACKING AND PASTEURIZATION

After clarification, the fermented broth was filtered (using a filter paper) into a sterile 200ml bottles, capped and pasteurised by heating in a waterbath at 65°C for 30 minutes (Amerine and Joslyn, 1970).

2.14 STABILIZATION TEST

The pasteurised clear fermented broth (CFB) was kept at room temperature for 7 days to check for any unsightly developments before putting it back into the refrigerator (Toukis, 1976).

2.15 ANALYSIS OF THE CLEAR FERMENTED BROTH (CFB)

i) The CFB was analysed for pH, Total titratable acidity, Volatile acidity**, Protein, Ash Ascorbic acid content (2ml of CFB sample was added to 38ml of 0.5% oxalic acid) and qualitative presence of reducing sugars by methods already described for the extract analysis.

*Analysis was done for the respective CFBs designated as

Type i - from Flask i,

Type ii - from Flask ii,

Type iii - from Flask iii,

Type iv obtained from secondary fermentation.

Seven commercially available wines were also analysed for some properties (Table 6).

** The Volatile acidity is expressed as acetic acid per 100ml of CFB and calculated by multiplying volume of Sodium hydroxide used with 0.0064.

ii) Alcoholic Content

This was determined by fractional distillation (Okafor, 1978).

iii) Reducing Sugars

A calibration curve of glucose (Appendix) was made follwoing the underlined procedure:

1ml of various concentrations of glucose (0.1,0.2,0.3 - 1.2ml/ml) were put in test tubes. 2ml of DNS reagent was added to each tube, heated in a water bath, cooled under tap water and 10ml

of distilled water added into each tube. The optical density was read off a spectrophotometer (WPA S101 model) at 540nm against a reagent blank. The absorbance values were plotted against corresponding glucose concentration.

1ml of the CFB was given a double dilution and treated similarly as the various glucose concentrations and the amount of reducing sugar present determined from the standard curve (Miller, 1959). DNS reagent was prepared by dissolving 10g of 3'5' dinitro salysalic acid in 200ml of 2N Sodium hydroxide solution and made up to 500ml with distilled water. 300g of potassium Sodium tartate was dissolved in the resulting solution and made up to 1 litre with distilled water.

iv) <u>Glycerol Estimation</u>

100ml of CFB was concentrated to about 10ml and 5g of fine sand and 4ml of milk of lime were added. The mixture was evaporated to dryness and to the residue obtained, 50ml of ethyl alcohol was added. After the alcohol treatment, the paste was heated on a waterbath and then filtered through a Whatman No. 1 filter paper. The filtrate was evaporated to dryness and 20ml of absolute alcohol and 100ml of anhydrous ether were added to it. The resulting solution was then filtered again and the filtrate washed with 2 parts of absolute alcohol and 2 parts of anhydrous ether, then dried at 100°C. The dried mass was weighed, ignited and reweighed. Loss in weight on ignition gave glycerol content (AOAC, 1984).

CHAPTER THREE

RESULTS

3.1 No deterioration was observed in the fruits that were completely dried under the sun. 30% of the fruits not completely dried (control) was attacked by mildew.

3.2 EXTRACT

About 400ml of extract was collected from 200g of fruit.

3.3 No microbial growth was observed on Nutrient Agar and with the 10^{-3} dilution on PDA. However, with 10^{-1} dilution on PDA one colony of a fungus was observed. The colony was initially white but turned black due to the production of sporangia. It grew fast covering the petri dish of 9 cm diameter within 2 days. Under the x 40 objective of the microscope, hyphae was coenocytic and sporangiophores arose from the rhizoids. it is probably a *Rhizopus* species.

3.4 ANALYSIS OF THE EXTRACT

(a) A dirty brown precipitate indicated presence of reducing sugar.

(b) Table 1 INITIAL COMPOSITION OF THE EXTRACT.

COMPONENT	MEAN VALUE
Total sugars (%)	9.80
Total titratable acidity (% tataric (acid)	0.58
pH	3.19
Specific gravity	1.10
*Protein (%)	3.17
Ash (%)	0.56
*Ascorbic acid (mg/100ml)	112.43

*The standard curve for Protein and Ascorbic acid are shown in the Appendix respectively.

(c) Ascending chromatograpy indicated the presence of Glucose Fructose, Sucrose and Maltose in the extract (plate 3).

3.5 PRIMARY FERMENTATION:

Gas bubbling in the fermentation flask was observed within 4 hrs after inoculating the extract with the starter culture. Fermentation period was eight days in flasks 1 and 11 and eleven days in flask 111. The fermented broth was milky in colour and cloudy in all the flasks.

3.6 CHANGES IN THE COMPOSITION OF THE EXTRACT DURING PRIMARY FERMENTATION

A general decline in the values of total sugars, and specific gravity was observed. (Fig. 1) The decline was more pronounced in the first four days. There was an increase in Titratable acidity and a decrease in pH value. (Fig. 2) .

Table 2: Changes in composition of extract during primary fermentation

PARAMETER	TIME (days)				
	1	2	4	6	8
*Total sugars	9.8	7.8	4.2	4.0	3.0
Specific gravity	1.10	1.08	0.96	0.91	0.89
pH	4.6	4.48	4.24	4.18	4.10
*TTA	0.58	0.59	0.62	0.63	0.64

*Values are expressed in g/100ml

3.7 MICROSCOPY

No contaminating microorganism was detected under the miscroscope.

3.8 TEMPERATURE

The temperature ranged between 27 - 29°C.

3.9 SECONDARY FERMENTATION

The secondary fementation lasted for seven days. During this period, there was a slight decrease in the pH value while Total titratable acidity increased (Fig.3)

Table 3: P^H and TTA changes during secondary fermentation

PARAMETER	TIME (DAYS)						
	1	2	3	4	5	6	7
pH	3.91	3.87	3.85	3.83	3.83	3.82	3.81
TTA (%)	0.68	0.74	0.75	0.90	0.81	0.82	0.84

3.10 CLARIFICATION

The results are shown in table 4 below

Table 4: Results of Clarification Tests

Concentration Used (g/l)	Clarifying time (days)			
	Bentonite	Gelatin	Gelatin & Tannic Acid	*Control
1	5	8	Not clarified	
2	4	4	"	
3	3	4	"	
4	3	3	"	
5	6	2	"	

*The control clarified in eleven days.

3.11 CLEAR FERMENTED BROTH (CFB)

310ml of the clear fermented broth was collected after clarification. It had an amber colour and a good aroma.

3.12 PASTEURIZATION AND STABILIZATION

The amber colour deepened after pasteurization. No sediments, undesirable odour or colouration was observed when the CFB was left at room temperature for 7 days.

3.13 ANALYSIS OF THE CFBS

Table 5: Chemical analysis of the CFBs

Component	CFB			
	I	II	III	IV
Alcohol %(V/V)	6.92	6.82	10.20	13.60
pH	4.03	4.04	3.91	3.80
TTA (% tataric acid)	0.64	0.64	0.68	0.83
Volatile acidity (% acetic acid)	0.023	0.027	0.029	0.035
Glycerol (%)	0.290	0.270	0.300	0.280
Ash (%)	0.660	0.680	0.650	0.630
Protein (%)	0.620	0.670	0.630	0.600
Ascorbic acid (mg/100ml)	79.360	79.360	79.360	69.440

3.14 Ascending chromatograpy indicated the presence of fructose and glucose in CFB III and fructose, Glucose and Sucrose in CFB IV.

Plate 3. Ascending paper chromatography

Key

(G) Glucose (M) Maltose (iii) CFB Type iii

(F) Fructose (L) Lactose (iv) CFB Type iv

(S) Sucrose (E) Extract

Plate 4   Pasteurised CFB Type iv.

3.15 Table 6: Chemical properties of some commercially available wines and Icheku wine Type IV

COMPONENT	WINE							
	YAGO	*DC	*RB	BACCHUS	CAPEL	MALIBU	CALYPSO	ICHEKU
Protein	0.59	0.70	0.64	-	0.62	0.55	0.54	0.60
Reducing sugar %	1.20	1.30	1.20	2.40	1.60	1.90	1.70	1.80
Ascorbic acid mg/100ml	9.28	89.28	89.28	36.37	52.91	49.60	49.60	69.44
Alcohol % V/V	7.00	7.10	6.50	17.00	9.30	24.00	24.00	13.60

*DC = Day Country; RB = Royal Banquette.

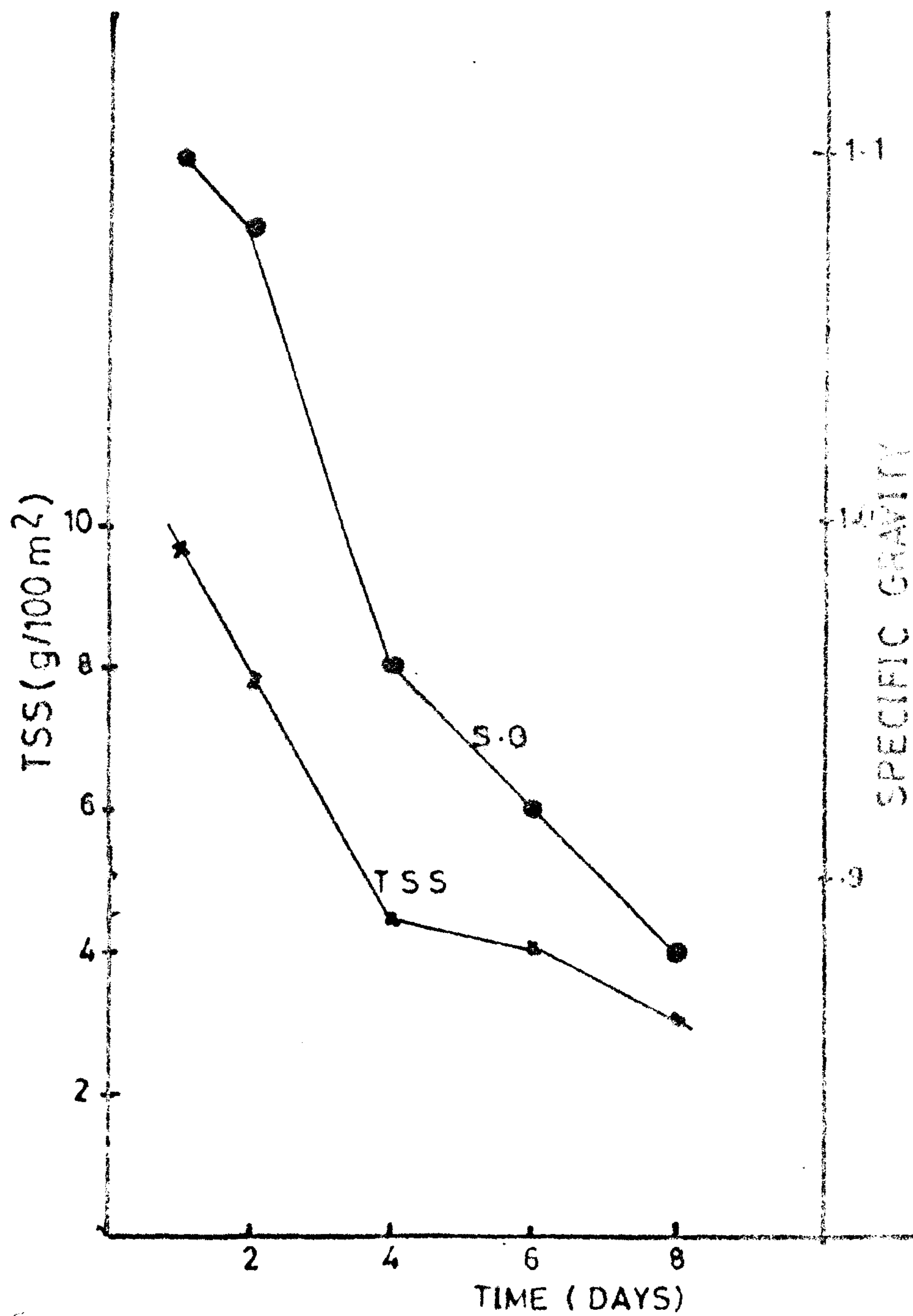

Fig. 2

CHANGES IN TOTAL SOLUBLE SUGARS AND SPECIFIC GRAVITY DURING PRIMARY FERMENTATION.

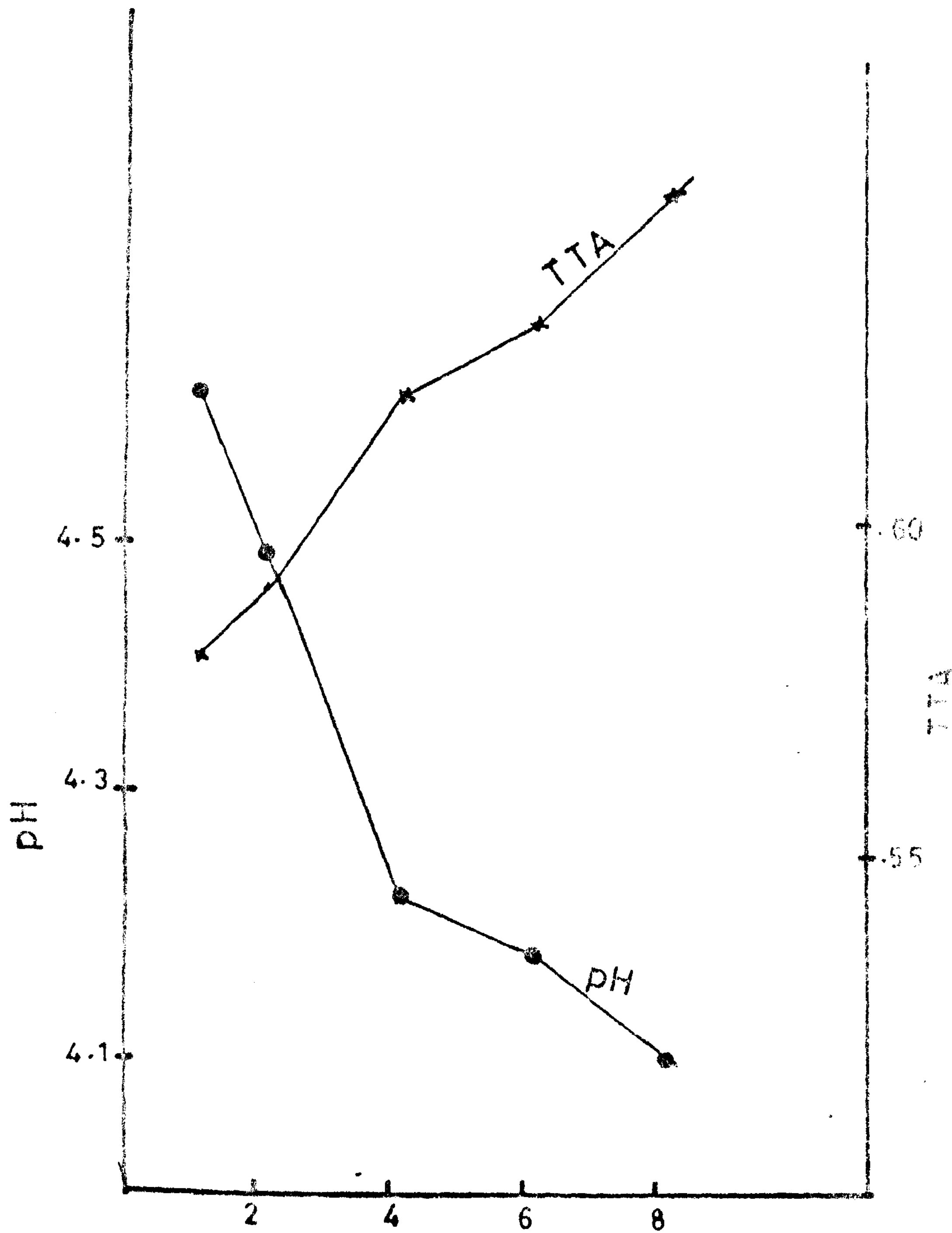

Figure 1. pH AND TTA CHANGES DURING PRIMARY FERMENTATION.

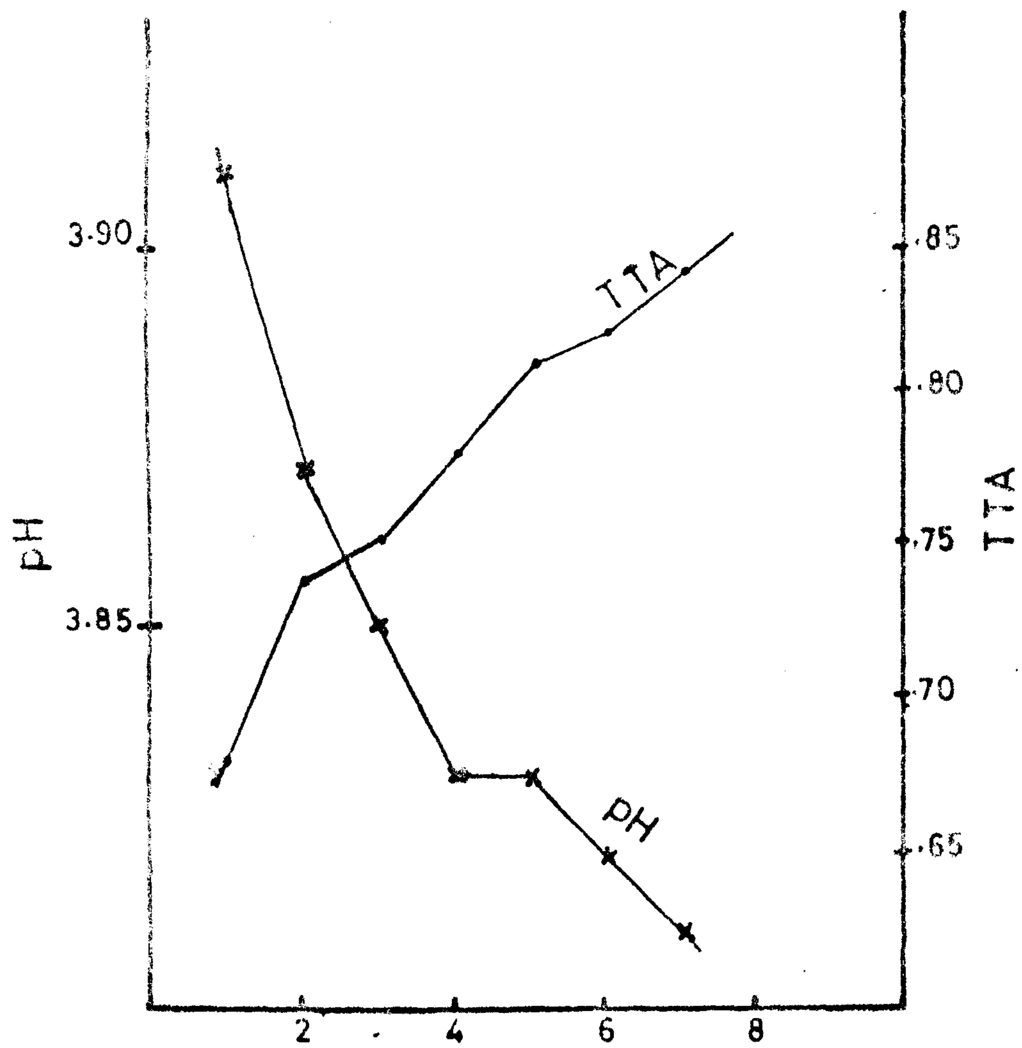

Fig. 3. pH AND TTA CHANGES DURING SECONDARY FERMENTATION.

CHAPTER FOUR

DISCUSSION

The preservation and storage potentials of Icheku fruit endows it with a major advantage over all other local fruits previously investigated for the purpose of manufacturing wine. The *Rhizopus* sp. isolated from the extract at 10^{-1} dilution is a weak parasite that commonly grow on fruits and other detached plant parts causing disease in their transit and storage, (Alexopolus and Mims, 1979). The scanty growth may be attributed to the unfavourable pH value (3.19) of the extract.

The chemical composition of the Icheku extract is summarized in Table 1. The 9.8% (W/V) total sugar in the extract is comparable to the 4 - 12% (W/V) obtained for banana, pineapple, orange and cashew (Felton, 1971; Gallender, 1976; Falade, 1981). The sugar level in *Icheku* extract is lower than the range of 12-27% (W/V) reported for grape (Rose, 1977). However, the 9.8% (W/V) total sugar in the extract is conducive for the growth of the wine yeast. It was reported that high sugar levels (up to 25% (W/V)) exert osmotic pressure causing most of the alcohol produced to remain intracellular and become toxic to the yeast cells (Amerine and Joslyn, 1970; Amerine *et al.*, 1980; Panchal and Stewart, 1980a). Ascending chromatography indicated glucose, fructose, maltose and sucrose to be the reducing sugars present in the Icheku extract (Plate 3). Except for the presence of maltose, this result agrees with literature reports for cashew, orange and pineapple

(Singleton and Gotner, 1965; Haq et al, 1975; Gallander, 1976). All the reducing sugars detected are however the normal substrates for wine yeast (Barnnet, 1976). The protein and ash contents of the Icheku extract are comparable to those found for grape and cashew (Ochigbo, 1963; Rose, 1977; Aderiye, et al; 1991).

The Ascorbic acid value of 112.43(mg/100ml) found in the Icheku extract contrasts with the range of 34.56-97.11(mg/100ml) reported for pineapple, grape, orange, lemon, tangerine, lime and cashew juices (Temple, et al., 1990). Ascorbic acid is a very essential co-factor for wine yeasts. It was reported that it enhances enzymatic hydroxylation and accelerates glucose uptake as well as the excretion of ethanol when present at a concentration of 1-1.5mg/ml in the fermenting must (Lehninger, 1975;). Ascorbic acid is however inhibitory in the excess of 15mg/ml (Panchal and Stewart, 1980a).

The 0.58% TTA level and the pH value of 3.19 in the extract is comparable to reported values for cashew, pineapple and orange juices. The TTA level in these fruit juices ranged from 0.5-1.5% and the pH value from 3.0 - 5.6 (Ochigbo, 1963; Felton, 1971; Gallander, 1976; Falade, 1981). The low pH value is desirable. It may be responsible for the near sterility observed in the Icheku fruit. In large scale alcoholic fermentation, the manufacturer prefers a low pH of the must to achieve the desired sterility since it is impracticable and costly to sterilise the bulk process (Amerine and Joslyn, 1970).

Most workers report the specific gravity of orange, pineapple, cashew and grape juice to be in the range of 1.035 - 1.090. This observation contrasts with the value of 1.100 observed for *Icheku* extract. The disparity in value may be attributed to a higher level of isoluble solids in *Icheku* extract.

The 0.025% (W/V) of Sodium metabisulphite used to sterilise the Icheku extract is small when compared with the 0.1% (W/V) used for sterilizing cashew juice (Aderiye et al, 1991). This disparity is expalained by the low level of contaminating microorganism in the Icheku extract. The concentration maintained the desired sterility throughout the duration of the fermentation. The use of Sodium metabisulphite to sterilise must is extensively reported in literature (Dott *et al*, 1976). It is preferred to autoclaving which results in precipitation of sugars, release of pectins, destruction of pectinase and loss of bouquet and flavour conferred by volatile esters (Slatter, 1972). Furthermore, it has little or no effect on wine yeast; is 80% effective against bacterial population and clears the entire mould population (Davis et al, 1982). This may be attributed to a physioloical adaptation since wine yeasts produce sulphites (Heinzel and Truper, 1976).

The baker's yeast used in this work can preferably utilise Glucose, Fructose,Sucrose and Maltose as carbon source. Brewer's yeast can also be used after appropriate treatment to free them from bittering substances and other contaminating materials (Toukis, 1976).

Generally, during alcoholic fermentation, the production of carbon dioxide bubbles agitate the contents of the vessel. It was shown that a carbon dioxide pressure of 7.2atm. stopped yeast growth but not fermentation and when up to 30 atm. stopped both yeast growth and fermentation (Ough and Amerine, 1966). In order to avoid the build up of excess carbon dioxide pressure, a `U' tube was used in this work.

Previous reports (Jarczyk and Wzorek, 1977; Kunkee and Goswell, 1977; Mitchell, 1982) agree with the general delcine in the values of sugars and specific gravity observed in this work as fermentation progressed. The decline is due to utilization of sugars by yeasts. The more pronounced decline observed in the first four days may be attributed to the period of exponential phase of growth of the yeast cells. The increase in TTA of the fermenting broth agrees with the observation of Ough and Amerine (1966); this is because during fermentation, organic acids like Pyruvic, Succinic and Acetic acids are produced (Rankine, 1965). Low pH had been shown to contribute to flavour and aroma and to inhibit the growth of spoilage bacteria (Amerine et al,1980).

Most workers refer to bentonite and gelatin as clarifying agents of choice (Miller, 1959; Amerine et al,1980). This observation is in agreement with the present findings (Table 4). Bentonite does not impair taste and ensures protein stability (Dam et al, 1931). Gelatin removes tannin from wine. Mitchel (1992) [illegible], recommended that Gelatin be used together with an equal amount of Tannic acid. Gelatin with tannic acid could not clear

the fermented *Icheku* broth (Table 4). However, tannin may not be present in the fermented broth since it is generally associated with skin of fruits, leaves, barks and the woody stem (Maldonando *et al*., 1975; Ihekoronye and Ngoddy, 1985).

The chemical composition of the clear fermented broths (CFBs) is summarised in table 5. The alcohol content of CFBs I and II (6.92 and 6.8% V/V) are relatively low. This may be attributed to the absence of sucrose supplements in Flasks I and II. Mansoirs et al., (1978), observed that wines obtained from the fermentation of only the sugars naturally contained in the must have about 50% less fermentation by-products (esters, fusel oils, aldehydes, fatty acids etc.) and worse organoleptic features as compared with wines obtained with full additional sweetening. However, the volume of alcohol present in CFBs I and II are comparable to that of Royal Banquette; a popular sparkling wine that is commercially available in Nigeria (Table 6). The alcoholic content of CFBs III and IV are also comparable to that obtained for some commercially available wines in Nigeria (Table 6); some fruit and honey wines, and for some wines produced in Iraq. The values ranged from 7.9-14.6% (V/V): (Jarczyk and Wzorek, 1977; Sachde *et al*., 1980). Gay Lussacs theoretical yield of 51% ethanol by weight of fermented glucose is unattainable because enzymatic systems in the wine yeast convert sugar into amino acids, nucleosides and other building blocks (Amerine *et al*., 1980).

The range of sugar content in some commercially available wines in Nigeria (Table 6) and some Iraqi wines (Sachde et al.,

1980) was 0.12 - 10.05%. This is in agreement with the values obtained for the CFB's 9.0.4-1.8%). Ascending chromatography indicated Glucose and Fructose to be present in CFBs III and Glucose, Fructose and Sucrose to be present in CFBs IV, (plate 3). This agrees with literature reports for Cashew, Orange and Pineapple wines (Singleton and Gotner, 1965; Haq *et al.*, 1975; Formica, 1981). An additional sugar, raffinose was detected in palwine (Ayernor and Mattews, 1972).

The TTA and pH range (0.64 - 0.83% and 3.81 - 4.04 respectively) of the CFBs are comparable to that obtained for Pineapple, Orange, Cashew and some Iraqi wines. The range for these wines was, TTA, 0.52 - 0.92% and pH, 2.8-4.2 (Felton, 1971; Gallander, 1976; Riberau-Gayon, 1978; Sachde *et al.*, 1980; Falude, 1981). Wines are known to be the most acidic of all fermented drinks (Riberau-Gayon, 1978). The percentage volatile acidity in the CFBs ranged between 0.027 - 0.035. This is in agreement with a range of 0.023 - 0.036% reported for grape and pineapple wines (Singleton and Gotner, 1965; Amerine and Joslyn, 1970). Increasing volatile acidity (up to 0.12) is indicative of spoilage in wines ((Amerine, et al., 1980). The protein (0.60 - 0.67%) and ash (0.63-0.68%) contents the CFBs are comparable to those found by Ochigbo, (1963) for cashew wine. The glycerol content (0.27 - 0.30%) of the CFBs is in agreement with the range of 0.29 - 1.0% reported in some wines (Swejcar and Papcum, 1976). Glycerol and other volatiles are very important in the development of flavour and aroma (Rodin et

al., 1966; Flath and Forrey, 1970; Macleod and Traconis, 1982; Pino, 1982).

The Ascorbic acid content of the CFBs (69.44-79.36mg/100ml) indicate about 40% reduction in the original value of 112.43mg/100ml as contained in the *Icheku* fruit. This may be attributed to the fact that yeasts use Ascorbic acid for enzymatic hydroxylation (Lehninger, 1975). It was shown that high temperature (greater than 27^{0}C) and oxidation affect ascorbic acid degradation (Dulkin and Friedeman, 1956; Smooth and Nagy, 1980; Kranner *et al.*, 1982) and that the intense colour formation such as yellow or red pigments in juices is due to the destruction of ascorbic acid (Hayashi *et al.*, 1983; Lee and Nagy, 1988). The increase in the browning index of the CFBs after pasteurization may thus be attributed to ascorbic acid degradation. The ascorbic acid level found in the CFBs is comparable with the level in some commercially available wines in Nigeria (Table 6).

There is no significant difference (equal mean) between CFB I and II. It may therefore, be assumed that the Icheku extracts has significant nutrient levels for the growth of wine yeasts. It had been observed that musts generally contain a high level of nutrients required by wine yeasts (Fuleki, 1965; Suomalaimen and Oura, 1971; Guirarel and Snell, 1981).

All the CFBs had good aroma after two weeks of aging and did not deteriorate after sixty days of storage.

SUMMARY

From the foregoing discussion, the following statements can be made:

i) Wine can be made from the *Icheku* fruit.

ii) Icheku fruits can be preserved and stored satsifactorily

iii) The fruit has an ideal balance of easily fermentable sugars, acids and nutrients favourable for the growth of wine yeasts and

iv) The products (CFBs) obtained from the fermentation of the Icheku fruit using *S. cerevisae* are comparable in all respects to wines cited in literature and to some commercially available wines in Nigeria.

From these observations, and because *Icheku* is cheaply available and has no competitive demand, it may be concluded that it can be used for manufacturing of wine in Nigeria instead of totally depending on expensive imported wines. The following recommendations are made towards improving on the present research findings.

(1) A pilot plant for manufacturing of wine using *Icheku* as substrate should be established. This plant should be located in the Eastern part of Nigeria considering the availability of the fruits in this region.

(2) The possibility of mechanising the extraction process should also be investigated.

(3) The use of other strains of yeasts (notably palm wine yeast) or mixed culture of yeasts may be investigated for improved wine production.

(4) The wholesomeness of the *Icheku* wine should be ascertained and

(5) Investigations may be carried out in the pilot plant to determine the actual shelf life of the *Icheku* wine.

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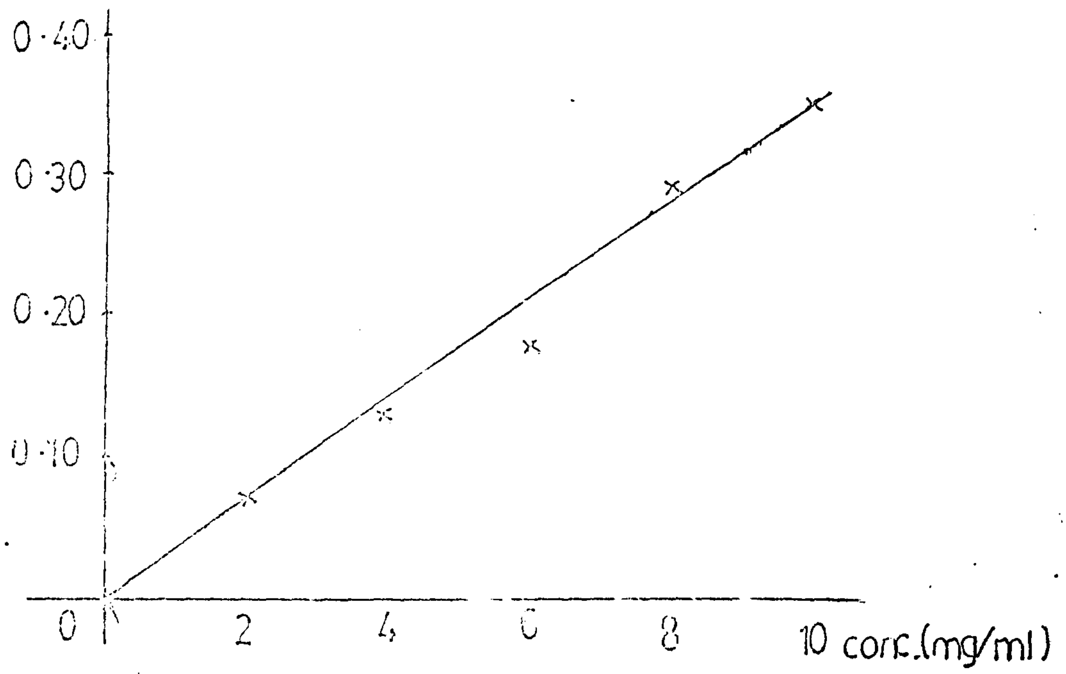
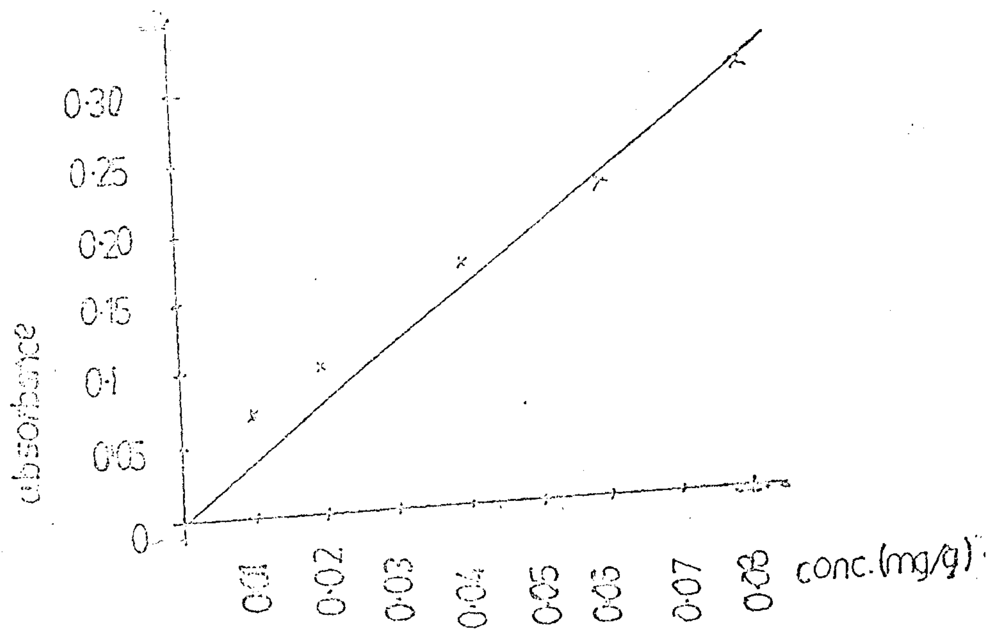


Standard curve for protein



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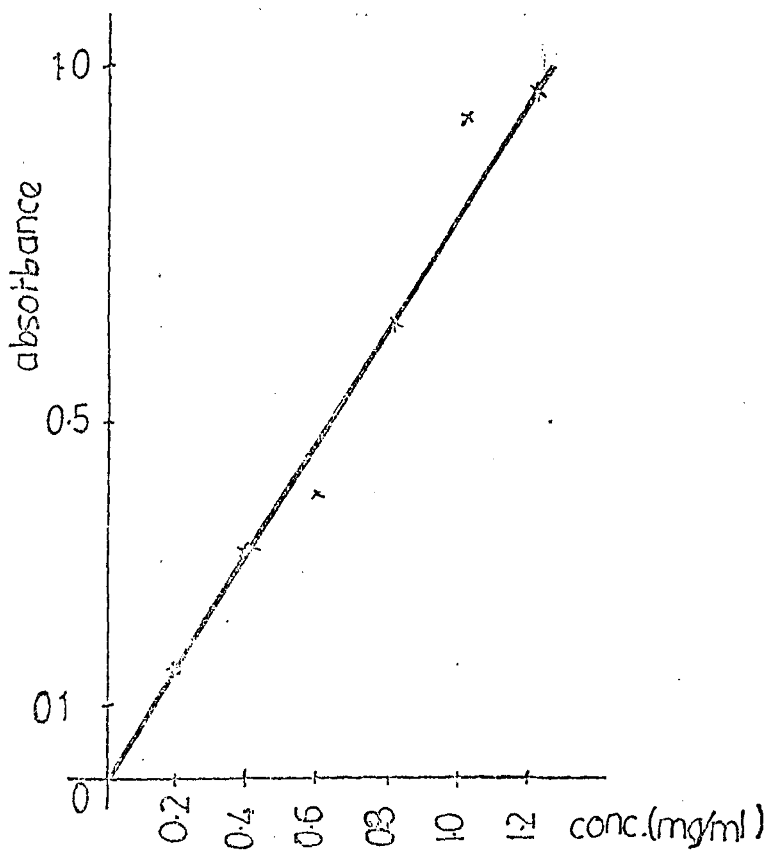

Standard curve for glucose