PRODUCTION OF WINE FROM THE YELLOW PULP OF LOCUST BEAN

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DECLARATION

I hereby declare that this research project was carried out by me in the department of chemical engineering under the supervision of **Engr. D.O. Agbajelola**. And that it has not been presented nor published before any panel which I am aware of.

____________________ ____________________

Abel Williams Gin Date

CERTIFICATION

I hereby certify that I supervised, read and approved this project work presented by **Abel Williams Gin (2000/10625EH)**, for the fulfillment of the award of bachelor degree in chemical engineering (B. Eng) in the department of chemical engineering..

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External examiner **Date**

Dedication

This project is dedicated to God Almighty and my parents.

Acknowledgement

I am grateful to **God Almighty** for seeing me through my studies and for his abundant love and favour. I am aware of the hands that have one-way or the other brought to the success of this research work. First and foremost, I wish to express my profound gratitude to my project supervisor, **Engr. D.O. Agbajelola** whose constructive criticism and useful instruction has made this project a success. I wish him brighter days of Gods unfettered blessings.

I must not fail to acknowledge the support of my good friend, **Mr. David Ganga** who provided some of the useful experimental materials and information needed for this work. A research project of this nature would have not been possible without a reference to the work of others- thanks to all authors from whom I have drawn references. I am aware that that time, space and human memory could fail one trying to fully acknowledge all other persons or institutions who have contributed towards the success of this study.

My appreciation goes to my dear parents, **Mr and Mrs Gin** for all the total and invaluable support they gave me. I am eternally grateful to them. I remain indebted to my friends- **Infaenyi Akwuelu, Micah Yusuf, Joshua Gana, Akinola Areo, Clement Yari** for making my stay in the university a pleasant one. I will like to specially thank my special friend **Wale Banigbe** for giving me unlimited access to his computer with which I typed this project. He has been a continuing influence on my thinking. May God bless him.

ABSTRACT

The pulp of African locust bean fruit, Parkia Biglobosa was used to produce table wine using brewer's yeast (Sachromyces Cerevisiae) on must samples with and with additives. Must sample A (7.4% Brix) contained no additive while sample B (3.3% Brix) and sample C (5.7 Brix) were ameliorated with sugar and honey respectively. The pH of the must showed a marked decrease (A: 3.80-3.40; B: 3.80-3.00; C: 3.74-3.44) as well as total sugar (A:8.5-7.4; B:14.0-3.3;C:18.8-5.7 % Brix) with concomitant increase in titratable acidity (A:0.59-0.86;B:0.50-0.59, C:0.43-0.50 % tartaric acid) with increasing period of fermentation. Chemical analysis of the aged (10 days at 0-4°C) wine showed that the alcohol content (%V/V) was 3.5, 10.5 and 10.9 for samples A, B, and C respectively. The results obtained compared favourable with imported wines and is of significance in that "elonuwan" (a portable wine) produced through an indigenous local technology by certain Nupe women in Bida emirate of Niger state could be upgraded to a viable commercial venture.

TABLE OF CONTENT

CHAPTER THREE

CHAPTER FOUR

CHAPTER FIVE

CHAPTER ONE

1.0 INTRODUCTION

Wine refers to product made by alcoholic fermentation of fruit juices, honey etc. by direct conversion of their sugars: glucose, fructose and/or saccharose through enzyme induced chemical alteration process to ethanol and carbondioxide. Though wine is generally produced from carbohydrates materials, the nature of such materials used, and the way they are produced largely determine the characteristics of the wine produced.

The yellow pulp of Africa locust beans is very sweet and rich in carbohydrates. It is used by many to sweeten 'ogi' (cereal porridge) or may be drunk when dissolved in water. It is believed to be a useful aid in the treatment of stomachache. It is used in stews and its fermented seeds are used to prepare a condiment rich in protein called soucribala or mustard seed or dadawa. Some women in Bida emirate council of Niger state, Nigeria, use this edible pulp to make a traditional alcoholic beverage. The art of its production is restricted to some families (as it is believed to be hereditary) and hence its production is usually on a small scale.

In most part of Nigeria, this yellow pulp is treated as waste, as it is usually washed into a body of water during processing of locust bean fruit for dadawa fermentation. The composition of this material has made it suitable for production of wine.

1.1 Background of Study

In principle, wine can be made out of everything even out of water. Most wines however are made from grapes, blackberry, and apples, red berries. The production of wine uses a biotechnology known as fermentation in which desirable microorganism are used in the production of value added products of commercial importance under controlled conditions. The main microorganism involved in alcoholic fermentation is yeast (saccharomyces cerevisiae). The process of fermentation is basically the feeding of sugars and nutrients in solution to yeast, which returns the favour by producing carbondioxide gas and alcohol. This process goes on until all the sugar is gone or yeast cannot tolerate the alcohol percentage of the wine beverage.

1.2 Justification of Study

Several reasons were responsible for the research interest of utilizing the fruit pulp of locust bean in wine production.

1. The middle belt climate of Nigeria favors the growth and survival of the fruit pulp. Also the tropical climate prevailing in Nigeria is ideal for growth and multiplication of micro organisms (yeast).
2. This nutritious yellow fruit pulp (which contains 19% reducing sugar, 9% non-reducing sugar and 36% of other forms of carbohydrates- Oyenuga, 1968) is under-utilizable. Elsewhere, this yellow pulp is washed into the body of water during processing of locust bean for dadawa fermentation.
3. The cheapness of the raw material
4. This study is of significance in that 'elonuwan' (a portable wine) hitherto produced through an indigenous local technology by certain Nupe women in Bida emirate of Niger state could be upgraded to a viable commercial venture.
5. Commercial scale production of this wine may save Nigeria some foreign exchange presently expended on wine importation

1.3 Aim and Objectives

The aim of this study is to study the feasibility of producing fruit wine from the yellow pulp of locust beans in the laboratory using controlled processing methods.

The objectives of this study are the following:

1. To prepare different samples of yellow pulp must with or without additives.
2. To undertake the fermentation of locust bean pulp solution using wine yeast (saccharomyces cerevisiae) under prevailing laboratory conditions.
3. To characterize the products obtained and establish their suitability as wine.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Wine

Wine has been produced and utilized unknowingly as far back as 400years ago by the Pharaoh's in Egypt as beverage. An indication of the antiquity of the knowledge of the effect of wine has been traced to Noah who built for himself a vineyard and grew grapes which he fermented into a sort of alcoholic drink (beverage) on which he became drunk with unfortunate result to his respectability.

Historically, wine is the product of fermentation of grapes species vitis vinifera. The high sugar content of most v.vinifera varieties at maturity is a major factor for their selection for use in most of the world's wine production. Their natural sugar content provides the necessary material for fermentation. It is sufficiently to produce a wine with alcohol content of 10% or higher. Wines containing less alcohol are unstable because of their insensitivity to bacterial spoilage. The grapes moderate acidity when ripe is also favourable to wine making.

2.1.1 Types of fruit wine

Information on studies on the production of fruit wine from the pulp of African locust bean (Parkia Biglobosa) is rare. However, some information is available on related products. Ojeh produced dry table wine (koko wine) from cocoa juice pulp by fermentation using bakers yeast (S Cerevisiae) and found it to be acceptable and comparable to other commercial one (Ojeh, 1981). Also, Steinkraus reported the production of a mexican pulque-wine from the juice of agave atrivirens the century plant. Grapes are the most common fruit used as raw material for alcoholic fermentation. They are used in distilled liquor to make brandy. Other fruits can be used to produce wine. When fruits other than grapes are used, the name of the fruit is included, as in papaya or pineapple wine. Apples and citrus with sufficient fermentable sugars are crushed, and the fermentable juices are either pressed out for fermentation or the entire mass is fermented. Tropical fruits such as guava, mango, pineapple, pawpaw, ripe banana, ripe plantain, tangerine and cashew fruit also contain fermentable sugars with levels varying from 10 to 20%. Overripe plantain pulp was reported to contain 16-17% fermentable sugar, with skin containing as much as 30%.

2.2 Traditional Method of Production of Fruit Wine

In the traditional process of producing fruit wine from the yellow pulp of locust bean, the pulp is pounded with pulverized millet stalks until a smooth consistency is obtained. This is the slurried in water and boiled for 2-4 hours before expressing the juice. The slurry is allowed to flow through an ingeniously locally made sieve comprising of a perforated metal pot containing a bed of dry pieces of sorghum stalks (1.5-3.0 cm^3) overlaid on several layers of dried pericarps (fruit cover) of Parkia Biglobosa (locust bean fruit) to obtain a sweet clear yellowish fluid which is then allowed to undergo natural fermentation in earthen vessel for 7 days to traditional alcoholic beverage.

Fermentation occurs in nature in any sugar containing mash from fruits, berries, honey or sap tapped from palms. If left exposed in a warm atmosphere, air-borne yeast act on the sugar to convert it to alcohol and carbondioxide.

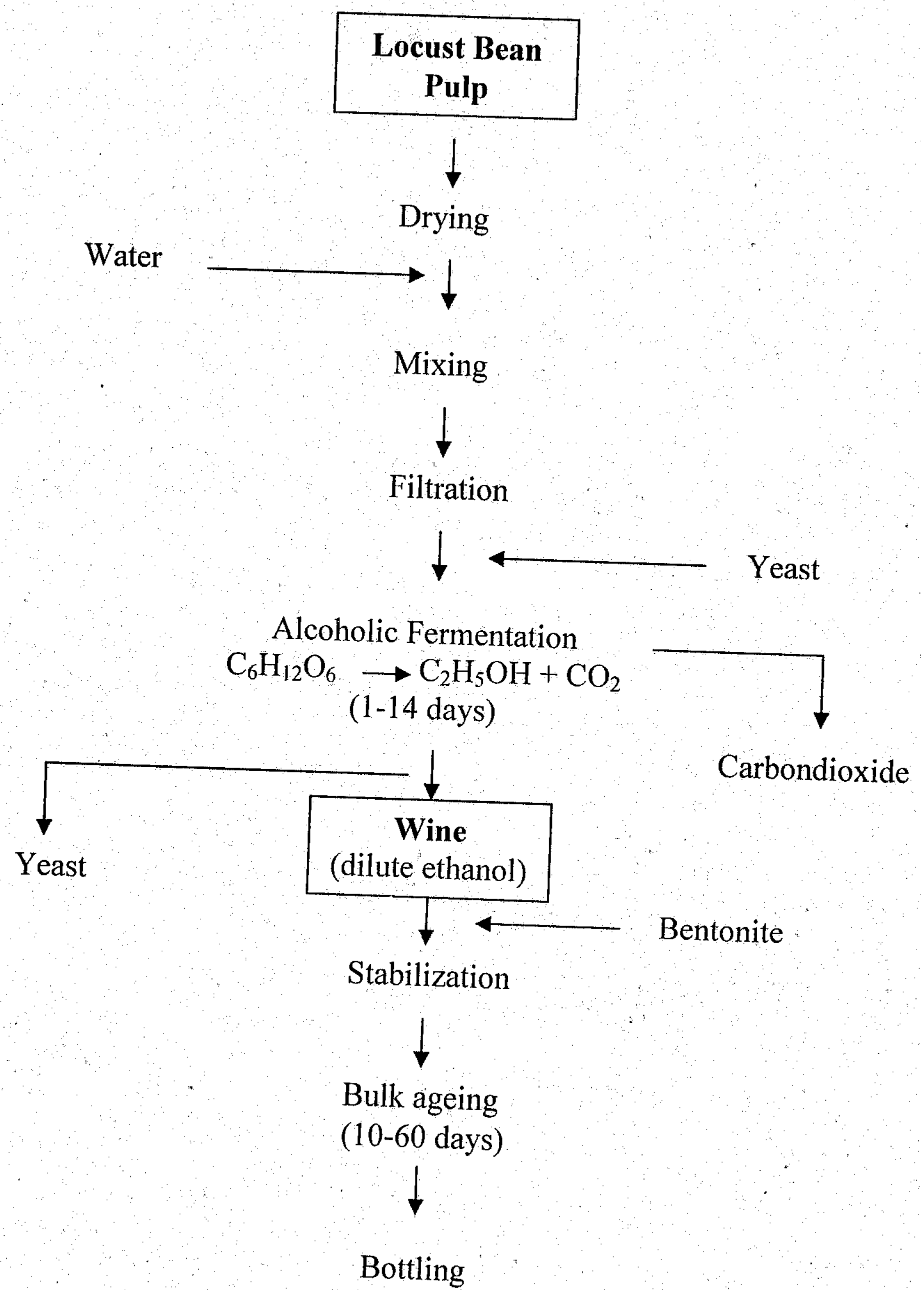

Fig.1 Flow Chart for the Production of Wine from Yellow Pulp of Locust Bean

2.3 The African Locust Bean

The African locust bean is of various varieties among which are Parkia Biglobosa and Parkia Clappertonia. The African locust bean is commonly found in the savannah regions and its harvesting season is usually around the month of March in the year (Oyenuga, 1968). The average composition of the whole fruit of African locust bean (on dry basis) is crude protein (12.71), fat (6.75), total ash (6.21), crude fiber (25.03), and carbonhydrate (49.30) (Oyenuga, 1968). The variety used for the proximate analysis above was Parkia folicoidea (now being called Parkia Biglobosa). The pod is usually used as an animal feed because its composition can readily meet the maintenance rations of the animals (Oyenuga, 1968). Inside each pod are a number of seeds, which are normally embedded, in a yellow dry powdery pulp. The pulp is usually made into a local food called dorowa, particularly among the Hausa speaking community.

The mature pod of the African locust bean occurs in large bunches. Each pod may vary between 12 and 30 cm in length. The mature pod contains yellow dry powdery pulp. Dorowa has a high fermentable carbohydrate content (19% reducing sugars, 9% non reducing sugar, and 36% other carbohydrates). Presumably, this pulp supplies available sugars that stimulate the rapid growth of microorganism involved in fermentation. The sweetness of the pulp can be attributed to the presence of the reducing sugar particularly the fructose.

2.4 Microorganism Involved in Alcoholic Fermentation

Yeast, the main microorganism involved in alcoholic fermentation, is found through out the world. More than 8000 strains of this vegetative microorganism have been classified. About 9-10 pure strains, with their sub classification are used for fermentation of grain mashes. They belong to the type Saccharomyces Cerevisiae. Each strain has its own characteristic and impacts it special properties to a distillate when used in fermentation. A limited number of yeast in the classification Saccharomyces Ellipsoids is used in the fermentation of wines from which brandy is distilled. Different yeast produces different results, and has different tolerance levels.

2.5 Fermentation of Sugars

Fermentation has been used in the manufacturing of alcoholic beverages. Since the ancient times, people who lived along the Nile River in North Africa brewed beer around 300 BC. It was not until the AD 1880's that scientist particularly the French scientist Pasteur discovered microbes' fermentation in beer, wine and milk. In 1990's other fermentation types were developed. Since 1914, the most important application of fermentation has been in the production of anti-biotics. (Giovanelli, 1996)

The fermentation process involved in bread making, wine making and brewing are among the oldest chemical arts. For many years it is believed that the transformation of sugar by yeast into ethanol and CO_2 was inseparably connected with life processes of the yeast cell. This view was abandoned when Edward Buchner (Nobel Laureate, 1907) demonstrated that yeast juice would bring about alcoholic fermentation in the absence of any living yeast cells. He proposed that the fermenting activity of yeast was due to the presence of remarkably active catalyst of biochemical origin, now called enzymes. It was later shown that enzymes are complex but non-living poly peptides, that in some instances can be synthesized in the laboratory. It is now recognized that enzymes bring about most of the chemical transformation that go on living cells of plants and animals. The overall equation for fermentation of sucrose is typical.

$$C_{12}H_{22}O_{11} + H_2O \longrightarrow C_6H_{12}O_6 + C_6H_{12}O_6 \longrightarrow 4CH_3CH_2OH + 4CO_2 \ldots 1$$

Sucrose glucose fructose ethanol carbohydrate

The first step in the fermentation of disaccharides, such as sucrose or maltose, is simple hydrolysis to monosaccharide: hexoses like glucose and fructose. Theses are then coverted to their 6- phosphate esters, then to 1,6-diphosphate, and to phosphate esters of tioses dihydoxy acetone (a ketotriose) and glyceraladehyde (an aldotriose). The dihydroxyacetone and glyceraldehydes are readily interconverted by enolization. The next step involves phosphorylation of the phosphogylceric acid, which lead to pyruvic acid. The pyruvic acid looses carbondoixide to for acetaldehyde, and the aldehyde is reduced to ethanol.

Enzymes show an extra ordinary selectivity- each step of the fermentation requires a particular enzyme as catalyst. Inorganic salts are also important > Pasteur found that salts such as magnesium sulfate and various phosphates were needed to promote yeast growth and fermentation. After fermentation has been completed, fractional distillation produces an ethanol-water azeotrope (b.pt.= 78.150) containing 95.6% alcohol by wt., (97.2%) by volume.

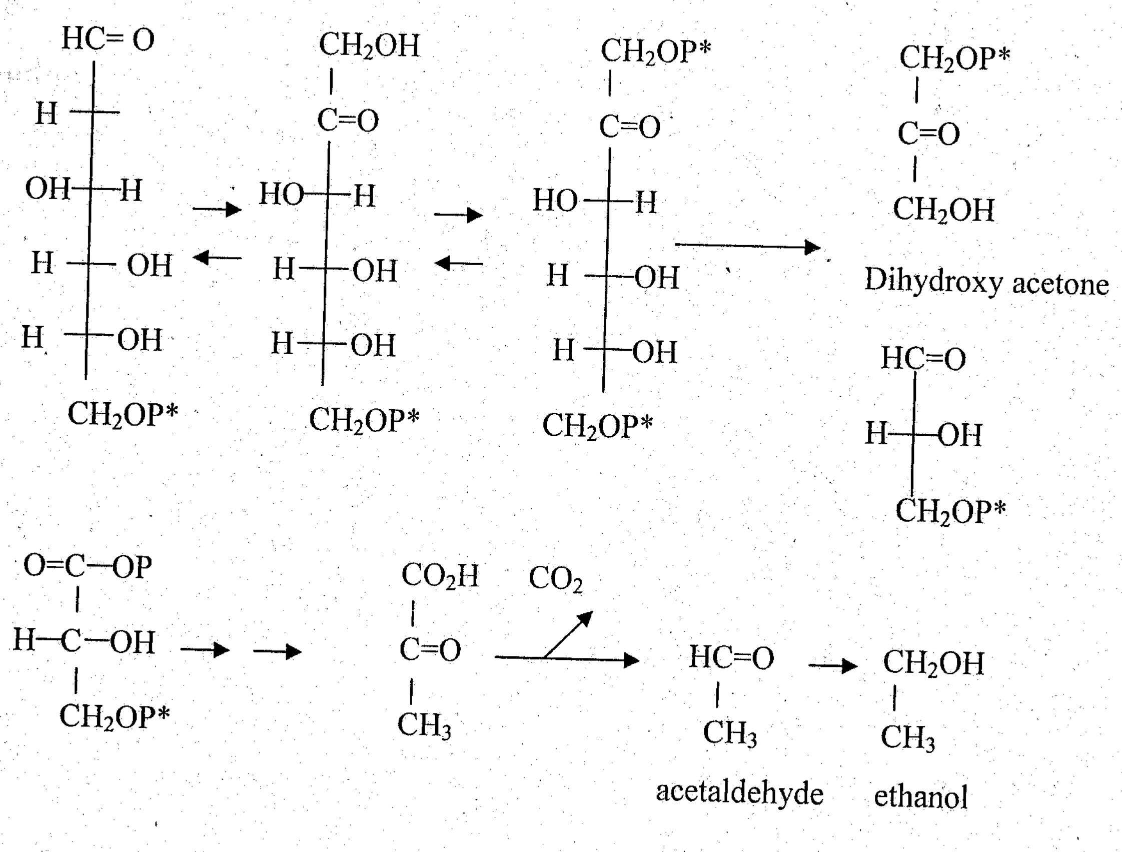

Fig2.1. General Outline for Steps in Alcoholic Fermentation (P*= phosphate ester). (Giovanelli, 1976)

⇩
Glucose 6 phosphate

⇩

Fructose 6 phosphate

⇩

Fructose 1,6 diphophate

⇩

↙ ↘

Glycerol aldehydes 3,-phosphate (G3-P) ⇄ Dihydroxy acetone phosphate

NAD^+

⇩ $NADH + H^+$ Dehydrogenase enzyme

1,3-diphosphoglyceric acid

⇩ ADP
ATP kinase

3-phosphogyceric acid

⇩ mutase

2-phosphoglyceric acid

⇩ enolase Mg^{2+} cofactor

phosphenol pyruvic acid

⇩ kinase

PYRUVIC ACID

decarboxylase
⇩ CO2
Mg^{2+}

Acetaldehyde

⇩

Alcohol dehydrogenase / Zn^{2+} cofactor

⇩

Ethanol alcohol

Fig 2.2. The Breakdown of Sugar Through Gylcolytic Path Way to Give Alcohol and CO_2 (i.e Embeden- Meyerhoff- Parners or Emp Pathways) (Embeden,1979)

2.6 Wine Quality and Characteristics

Taste is the only reliable index of wine quality despite the fact that consumers make their purchases on the basis of external appearance. Numerous internal characteristics influence wine palability. At the onset, it must be recognized that wine quality is influenced by many other factors other than production practices and other complex interrelationship exist among other factors. Applying a particular cultural practice may result in the combination of desirable and undesirable effects on wine quality. Taking full advantage of the relationship between cultural practices and quality, we must know the specific quality factors preferred.

The wine quality components that contribute to flavour (taste) are pH, titratable acidity (TA), and total solids (TS). They are of vital concern in consumer acceptance and preference and are basis for considering wine quality. Knowledge of the changes in these internal composition that take place in wine is based on mainly upon the studies of Collision (Collision, 1913), (Harding et al, 1940) and (Harding and Lewis, 1941).

2.6.1 pH

pH strongly affects several important wine properties including colour, biological and chemical stability etc. pH is the measure of the number of hydrogen ions present in a solution. Although pH depends on the total acid content, other factors like potassium content can influence pH, and because of these factors pH is not directly related to titratable acid. Nevertheless wine pH is a fundamental parameter. It also has profound influence on the biological and chemical effectiveness of sulphur dioxide in wine. pH value reflects the quantity of acids present, the strength of acids and the effect of minerals and other materials in the wine. Many different factors are involved:

1. Total amount of acid present in wine:

Wine acids produce hydrogen ions, and pH is the measure of the number of hydrogen ions present in a solution. Overall, wine pH will be lower when titratable acid is higher. However, high titratable acid does not always produce low pH values. The presence of potassium and other factors alter wine pH. Malic acid is weaker than tartaric acid, so wines unusually high in malic acid can have high TA and a high pH. High acid, high pH wine requires special treatment using an ion exchange technique, which is very expensive.

2. The ratio of malic acid to tartaric acid in wine

Tartaric acid produces almost three times more hydrogen ions than malic acid, so gram for gram tartaric acid produces a much lower pH than malic acid. Therefore, when the total acid content is fixed, pH depends on the relative amount of tartaric and malic acid in the juice or wine.

3. The quantity of potassium present in wine

Potassium (K) is essential for vine growth and fruit production. Potassium is a mineral, and vines obtain potassium through their roots. The roots remove potassium from the soil, and the potassium is distributed to all parts of the vine. Early in season, when growth rate is high, much of the potassium accumulates in the leaves. Then the potassium ions are moved from the leaves into the berries later in the season when fruits start to ripen. Potassium carries a positive electric charge just like hydrogen ions. Under certain conditions, potassium ions can change places with hydrogen ions at the extreme ends of the tartaric acid molecules. These are the hydrogen that ionizes easily in water solution.

2.6.2 Advantages of Low pH

A variety of chemical reactions take place in wine, and many of these reactions are affected by the total no of hydrogen ions present. For example, wine pH has a direct influence on the hot stability of wine. Under warm storage conditions, protein precipitates out of white and blush wine and serious haze and sediment problems occur when protein precipitates after wine is bottled. Consequently, white and blush wine is always treated with bentonite to remove excess protein.

Wine yeast is quite tolerant of pH, yeast growth does not change significantly over normal range of wine pH values, and overall fermentation characteristics are little affected by pH. On the other hand, wine bacteria do not tolerate low pH and wine pH strongly influences both bacteria growth rate and bacterial fermentation characteristics. This is why malolactic fermentation is not likely to occur in wines with pH lower than 3.3. Bacterial activity is reduced in low pH wines, and many bacterial problems become insignificant when wine pH is low.

Wines with low pH values generally have better visual qualities. At low pH values, red wines show more colour, and the colour is better. Colour intensity increases, and the red colour become purpler at low pH values. Both red and white wines have better colour stability when pH is low. Some important polymeric reactions are accelerated at low pH values, and much of the unstable colour pigments precipitate out of the wine early in the wine making process. After the unstable pigments are gone, wine colours are more stable.

Table 3: Relationship Between Wine Characteristics and the pH
(Eisenman L. http://home . att.net/lumeisenman/chapter 6:html)

Wine characteristics	Low pH (3.0-3.4)	High pH (3.6-4.0)
Oxidation	Less	More
Amount of colour	More	Less
Kind of colour	Ruby	Brown
Yeast fermentation	Unaffected	Unaffected
Protein stability	More stable	Less stable
Bacterial growth	Less	More
So2 effectiveness	More	Less

2.6.2 Titratable Acidity (TA)

Quantitative determinations of acidity play an important role in ensuring food product quality and stability. Information obtained on acid levels can help in detecting cases of food adulteration, monitoring fermentation processes, and evaluating the organoleptic properties of fermented foods.

Titratable acid is the measure of the quantity of all acids in wine. In other words, it is the measure of the acid titratable or capable of being titrated. Titratable acidity is primarily responsible for the tart taste of table wines. Acids have generally been long associated with sour taste. Tartaric is the strongest of all wine acids. It has two hydrogen atoms that can ionize. Malic acid is another wine acid, which is weaker than tartaric acid.

COOH
|
CHOH
|
CHOH
|
COOH
(a) Tartaric acid

COOH
|
CHOH
|
CH2
|
COOH
(b) Malic acid

CH3
|
COOH
(c)Acetic acid

Fig 2.3 Wine Acids

The total concentration of acid in a solution can be determined by titration. The titration process is performed by placing in a flask a known volume of acid solution whose concentration is to be determined. To the flask, a few drops of indicator e.g. phenolpthalien, which is colourless in acid solution and pink in acid solutions- is introduced. A base solution of known concentration is gradually added until the acid is completely neutralized. This point is indicated when the solution permanently changes colour. The concentration of acid can then be calculated from the volume of base used. The value obtained called titratable acid, is an estimate of the total acid in the solution. It accounts for both the free hydroniums ions present in the equilibrium mixture and the hydrogen ions released from undissociated acid molecules.

2.6.3 Total soluble sugar

This is the sugar content of a wine. There are two methods for determining total soluble sugar, TS: measuring the specific gravity with hydrometer, or the refractive index with a hydrometer. The former is the official method in most, if not all, parts of the world. A hydrometer calibrated in percentage total sugar TS (or Brix) is placed in a cylinder containing about 500ml of strained juice, is allowed to float for a few minutes, and then read. Temperature of the juice is determined at the same time so that the hydrometer reading can be calibrated to a standard temperature usually 20°C. The alternative, non-official method is to determine the TS with an Abbe or hand refractometer, both of which have a juice scale at either 20°C or 25°C or rarely 28°C. This measurement is must faster and utilizes only one drop of juices and ideal for filed use. Values, however tend to be lighter than those obtained with hydrometer.

CHAPTER THREE

3.0 EXPERIMENTAL

3.1 Reagents

The reagents used are listed in the Table below.

Reagents	Source
Tetraoxosulphate IV acid	Chemical Engineering Laboratory
1M sodium Hydroxide	Chemical Engineering Laboratory
Phenolphthalein indicator	Chemical Engineering Laboratory

3.2. List of Equipment/Apparatus

The list of equipment used in performing the experiment is listed below

Table 3.1 The List of Equipment Used in Performing Experiment.

Equipment /instrument	**Manufacturer**
Water bath	Pyrex, England
Refractometer	Armfield Technical Company Limited, England
Digital pH meter	Model 7020, elecronic limited
Electric oven	England
Electric balance	Gallenkamp, England
Thermometer	Chaus v/c
Thistle funnel	Zeal, England
Measuring cylinder	Clifton, England
Sample bottles	Cliftton, England
Muslin cloth	Clifton, England

Whinchester bottle Erlermeryer flask Soxhlet 4extractor assembly Pipette	Pyrex, England Pyrex England

3.3 List Materials

The lists of materials used for performing the experiment are listed below.

Table 3.2 List of Materials Used in Performing Experiment

Materials	Source
Yellow Pulp of Locust Bean	Bida town
Brewers yeast (saccharomyces cerevisiae)	International Beer and Beverages Industries (IBBI) Nigeria limited.
Potassium meta bisulphate (KMS)	International Beer and Beverages Industries (IBBI) Nigeria limited.
Ammonium sulphate	-
$Ca(OH)_2$	-
Water	-
Sugar	Minna
Honey	Minna

3.4 Experimental Procedure

Three main stages were involved in the production of wine.

1. **Preparation of the must sample (pulp solution)**

The yellow pulp from the fruit of locust bean was dried to moisture content, 14-16%. 600g of the dried pulp was mixed with 4.5l of water in a plastic bowl and stirred. The slurry was filtered through double layers of muslin cloth to obtain a sweet-yellowish must. The pH of the must was measured and adjusted to 3.80 by adding calcium hydroxide Sodium metabisulphite (0.02%) and ammonia sulphate (0.08%) were added to the must, which was divided into three portions, one (1) portion was ameliorated with sugar, and another portion with honey. These were dispensed in 2.5L Winchester bottles and sterilized at 600C for 30 min using water bath and allowed to cool overnight.

2. **Innoculum development**

Brewer's yeast already activated from the quality control laboratory of the production unit of the International Beer and Beverages Industries Nigeria Limited, Kaduna was obtained.

3. **Fermentation of must**

The cooled sterilized must was seeded with yeast inoculum previously locked in a sterilized thistle funnel and filled with aqueous solution of 5% sodium bisulphate. Proper agitation was carried out and the inoculated must left under laboratory condition for 14 days. They were siphoned in sterile Winchester bottles with fermentation locks containing 5% potassium metabisulphate and aged at 0-40C for 10 days. The titratable acidity, total solids, pH, temperature, and specific gravity were monitored every two days during the first 14 days of fermentation. The wine was then bottled and pasteurized.

4. Characterization of Developed Wine

The developed wine was characterized and the following parameters were determined

1. Determination of pH

50ml of must and wine sample were collected and the H-ion concentrations (H^+) determined using Jenway model 3070 portable automatic temperature compensation digital pH meter.

2. Determination of Specific Gravity

A hydrometer calibrated in Brix was placed in measuring cylinders containing 100mls of must and wine samples and allowed to float for a few minutes and then is read.

3. Total Soluble Sugar Determination

One drop of the wine and must samples was placed on the receptor of a hand refractormeter and sugar content read directly on the measuring scale.

4. Determination of Alcohol Level

50ml of wine and boiling chip was placed in a 250ml round bottom flask fitted with stopper. A thermometer and a soxhlet apparatus was then attached and the wine distilled into a weighed 100ml graduated cylinder. The temperature at every 5ml interval was recorded and its weight determine by the increase from the starting weight. From the weight and volume, the specific gravity was determined. The percentage yield of ethanol was calculated from the table of densities of aqueous solution of ethanol from available data books.

5. Determination of Titratable Acidity.

50ml of must and wine sample was titrated with 1M NaOH to the pHenolphthalein (indicator) end point of pH 8.3. The quantity (ml) of alkali consumed was converted to percentage anhydrous acid.

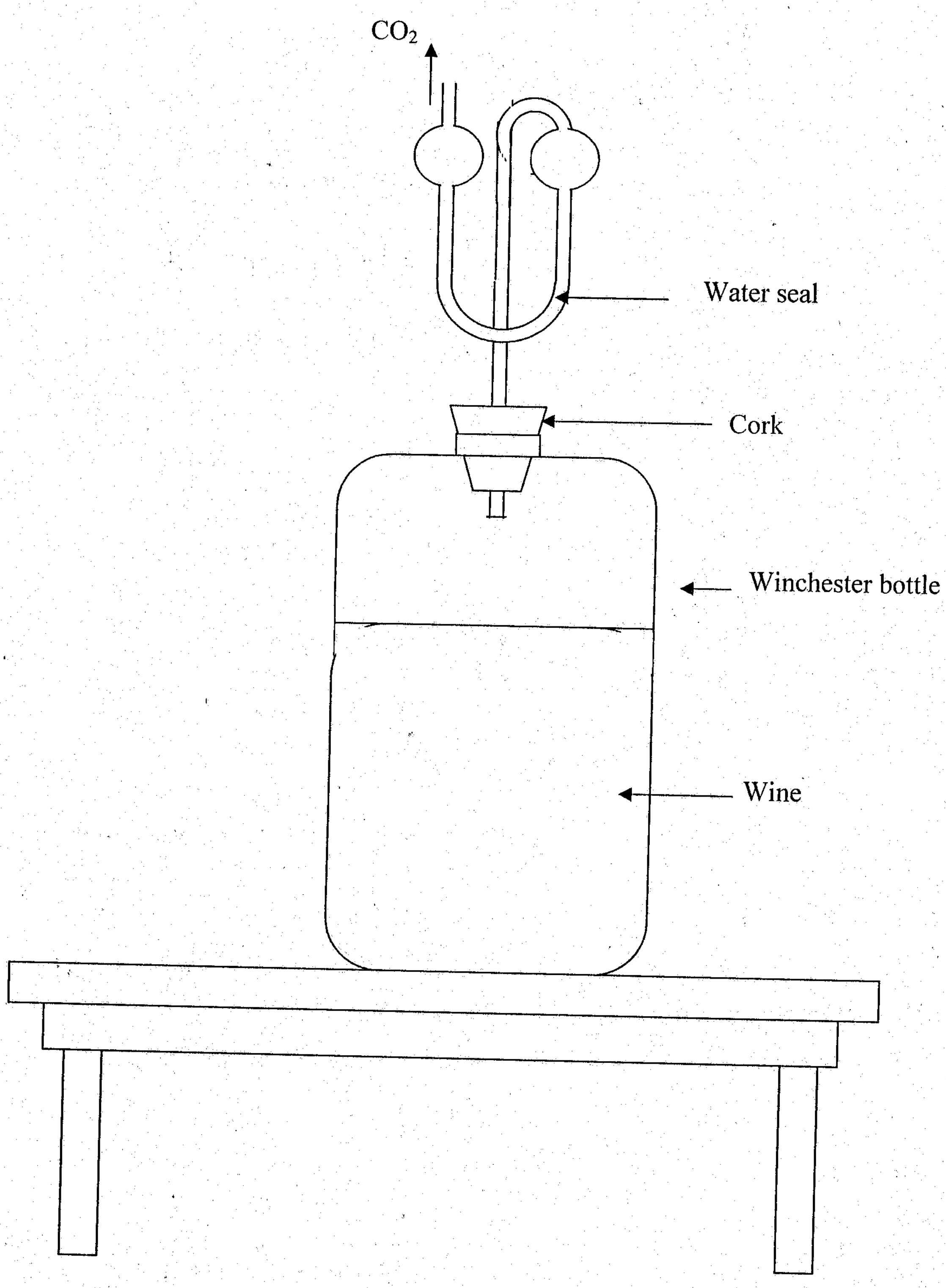

Fig.3.1 Experimental set-up

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

The results obtained from the series of analysis done on must and wine samples are presented below.

Table 4.1. Changes in Chemical Quality of Fermenting Must with Time (Unameliorated Must (A))

Fermentation Stage (Day)	Total Sugar (% Brix)	Titratable Acidity (%)	pH	Specific Gravity	Temperature ^{0}C
0	8.5	0.59	3.80	1.047	27.8
2	8.5	0.61	3.70	1.057	29.5
4	8.5	0.65	3.70	1.057	29.5
6	7.8	0.71	3.60	1.035	28.2
8	7.6	0.79	3.70	1.032	28.0
10	7.6	0.83	3.50	1.031	27.6
12	7.5	0.85	3.40	1.030	27.3
14	7.4	0.86	3.40	1.030	27.3

Table 4.2. Changes in Chemical Quality of Fermenting Must With Time (Must Ameliorated With Sugar (B))

Fermentation Stage (Day)	Total Sugar (% Brix)	Titratable Acidity (%)	pH	Specific Gravity	Temperature ^{O}C
0	14.0	0.50	3.80	1.063	29.0
2	13.2	0.50	3.60	1.050	29.3
4	11.6	0.53	3.50	1.057	27.5
6	9.2	0.55	3.50	1.043	28.1

8	7.7	0.57	3.50	1.040	28.1
10	5.5	0.58	3.54	1.037	26.0
12	3.4	0.58	3.45	1.029	26.5
14	3.3	0.59	3.00	1.028	26.6

Table 4.3. Changes in Chemical Quality of Fermenting Must with Time (Must Ameliorated with Honey (C)).

Fermentation Stage (Day)	Total Sugar (% Brix)	Titratable Acidity (%)	pH	Specific Gravity	Temperature ^{0}C
0	13.8	0.43	3.80	1.059	28.2
2	13.7	0.46	3.77	1.049	28.2
4	11.3	0.47	3.70	1.047	28.3
6	10.1	0.47	3.50	1.031	28.3
8	8.8	0.48	3.48	1.032	28.3
10	6.5	0.49	3.45	1.030	27.9
12	5.9	0.50	3.45	1.029	27.8
14	5.7	0.50	3.44	1.029	27.8

Table 4.4. Chemical Characteristics of Wine and Must

Analysis	Wine			Must		
	A	B	C	A	B	C
PH	3.40	3.30	3.44	3.80	3.80	3.76
Titratable acidity	0.86	0.59	0.50	0.59	0.50	0.43
Total solid(% brix)	7.4	3.30	5.7	8.5	14.0	13.8
Specific gravity	1.030	1.028	1.029	-	-	-
Moisture content (%)	96.5	98.0	98.2	93.8	94	95.1
Alcohol content (v|v)	3.5	10.5	10.9	-	-	-
Colour	L/Y	D/Y				

1. Each value is the mean of 3 determinations

2. A Unameliorated must; B must ameliorated with sugar; C must ameliorated with honey.

Table 4.5 Comparisms of Wine Characteristics. (Yang And Wiegand, 1949), (Obayanju And Ademokoya, 1992).

Wine type	Balling/ °Brix	Titratable Acidity g/100ml	Alcohol % v/v
Apple	4.6	0.411	12.8
Cherry	6.8	0.534	12.3
Blackberry	8.2	0.890	12.2
Raspberry	8.8	0.903	12.0
Developed wine (A)	7.4	0.86	3.5
Developed wine (B)	3.3	0.59	10.5
Developed wine (C)	5.7	0.50	10.9
Standard wine	01-15	0.1-0.5	10.2-14.2

4.2 Discussion of Results

4.2.1 Fermentation Profile of Must (Pulp Solution)

The fermentation characteristics of ameliorated and unameliorated locust bean fruit wine as presented in tables 1-3 showed a lag phase of about fourteen hours preceding the take off of active fermentation and alcohol production. From the monitoring of carbondioxide evolution, the lag phase signaled the adaptation period of the yeast cells to the medium prior to their active growth phase and sugar metabolism. Active fermentation started at day two (2) and peaked at day 14.

The chemical changes in chemical characteristics showed a steady decrease in pH of the must as shown in Figure 4.1 below.

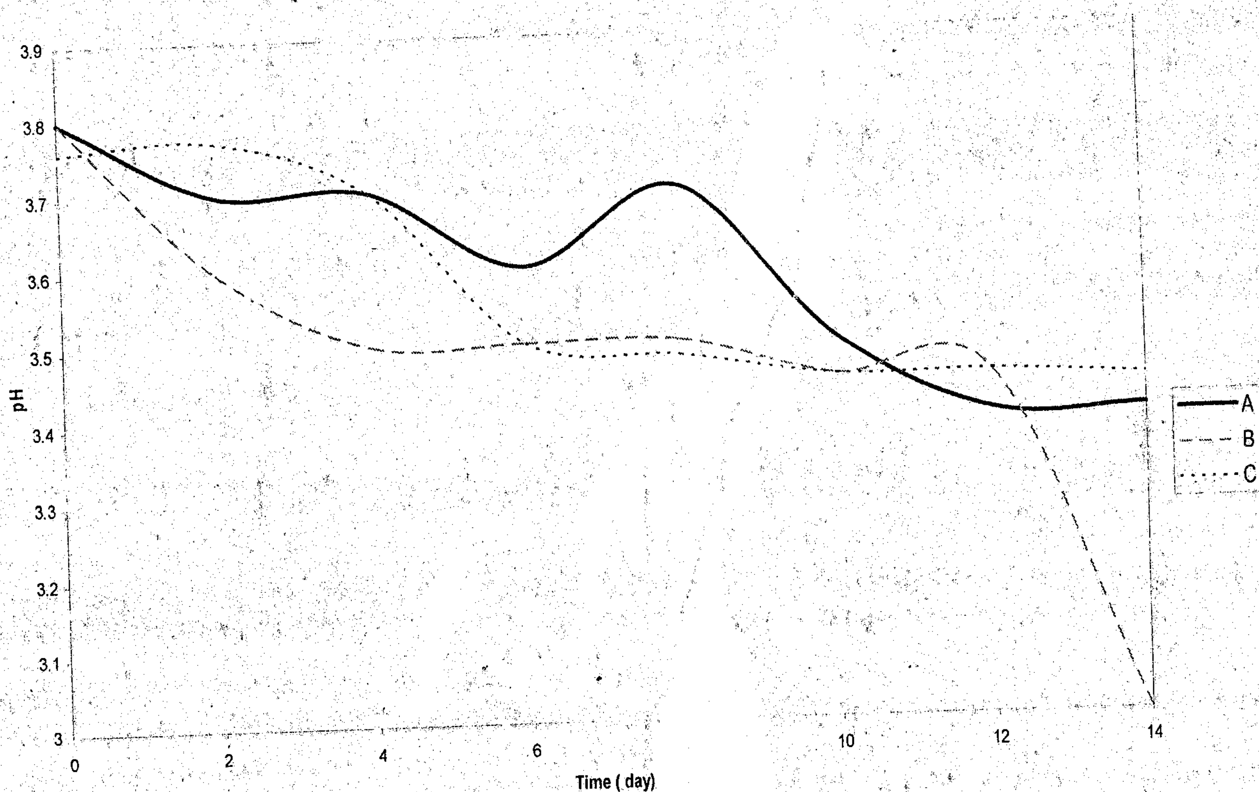

Fig 4.1: Change in pH with Time

In the must to which sugar was added as additive (sample B), the pH varied from 3.80-3.30. In the must with honey additive (sample C), the pH varied from 3.76-3.44, while the must without additive (sample A), pH varied from 3.80-3.40. So sample C has the lowest pH change. This may probably be due to the honey added in the sample. Honey, unlike the yellow pulp of locust bean has a lot of what yeast needs (acids, nutrients and mineral), but is somewhat resistant to being fermented by itself. It will speed up greatly depending on its concentration, when yeast is added.

Similarly, as shown in figure 4.2 below, the total sugar (^{0}Brix) showed a steady decrease from 8.5 to 7.4, 14.0-3.3, and 13.8-5.7 respectively indicating active fermentation by yeast. Sample A has the lowest change in total sugar. Since the yellow pulp of locust bean is composed of equal concentrations of glucose and fructose sugars. Stress can affect the yeast's ability to metabolize the last residual fructose. Sugar and honey are added in small amounts to prevent stuck fermentation. This problem sometimes occur with brewer's yeast which is more glucophilic and, therefore unable to ferment glucose.

The reduced progress of fermentation after day 10 could be attributed to deplete sugar level and decreased yeast cell population coupled with the likely inhibitory effects of high alcohol content on yeast cell metabolism. Hydrolysis of glycosides during ageing of wine could also be another probable source of alcohol beside fermentation.

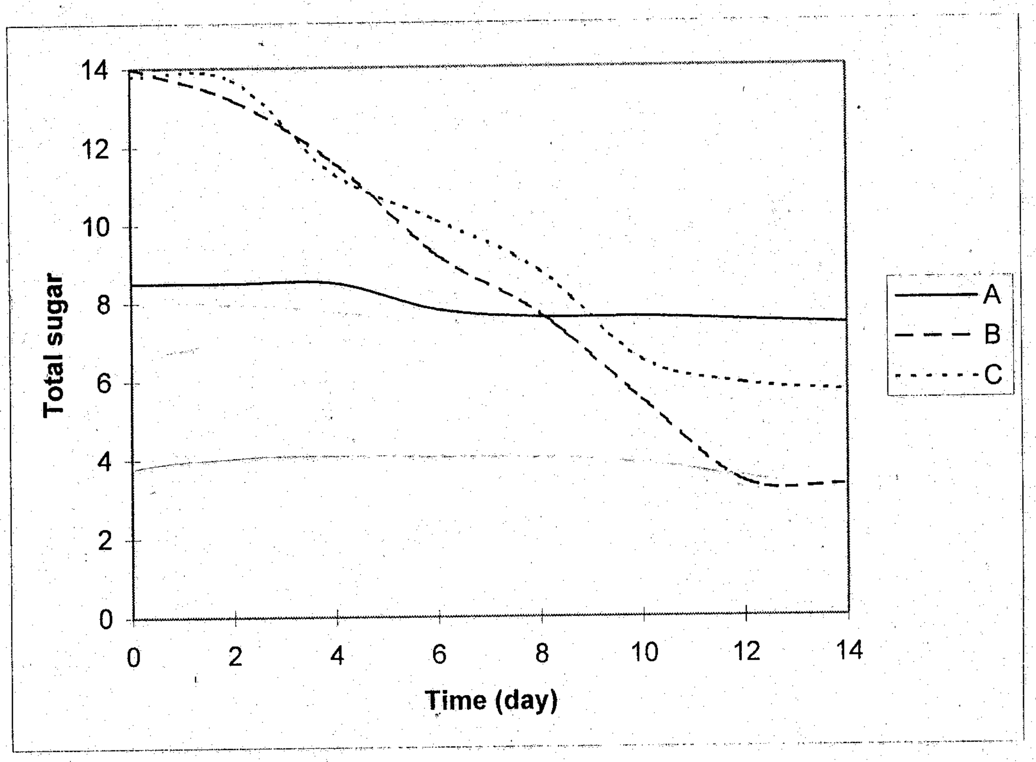

Fig 4.2 Change in Total Sugar with Time

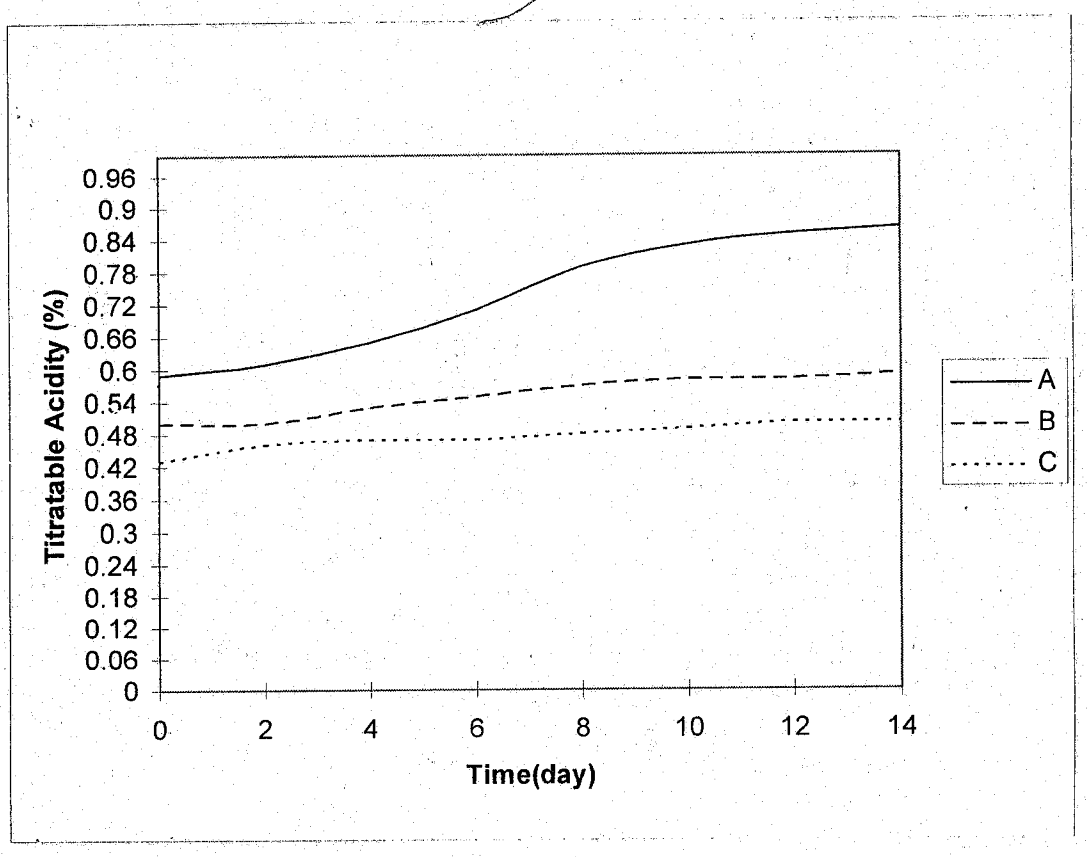

Fig 4.3 Change in Titratable Acidity with Time

The change in titratable acidity is presented in Fig.4.3. There was a contrasting increase in titratable acidity. Sample A has the highest value and range of change in titratable acidity. Tartaric acid (which is a natural component of locust bean) has two hydrogen atoms that can ionize easily. The locust bean also contain potassium (a mineral) which is got from the soil during fruit production. Potassium bitartrate is formed when potassium is exchanged for hydrogen and hydrogen becomes a free ion in the wine. Therefore as fermentation progresses, potassium bitartrate increases depending on the ratio of tartaric acid to other wine acid in sample

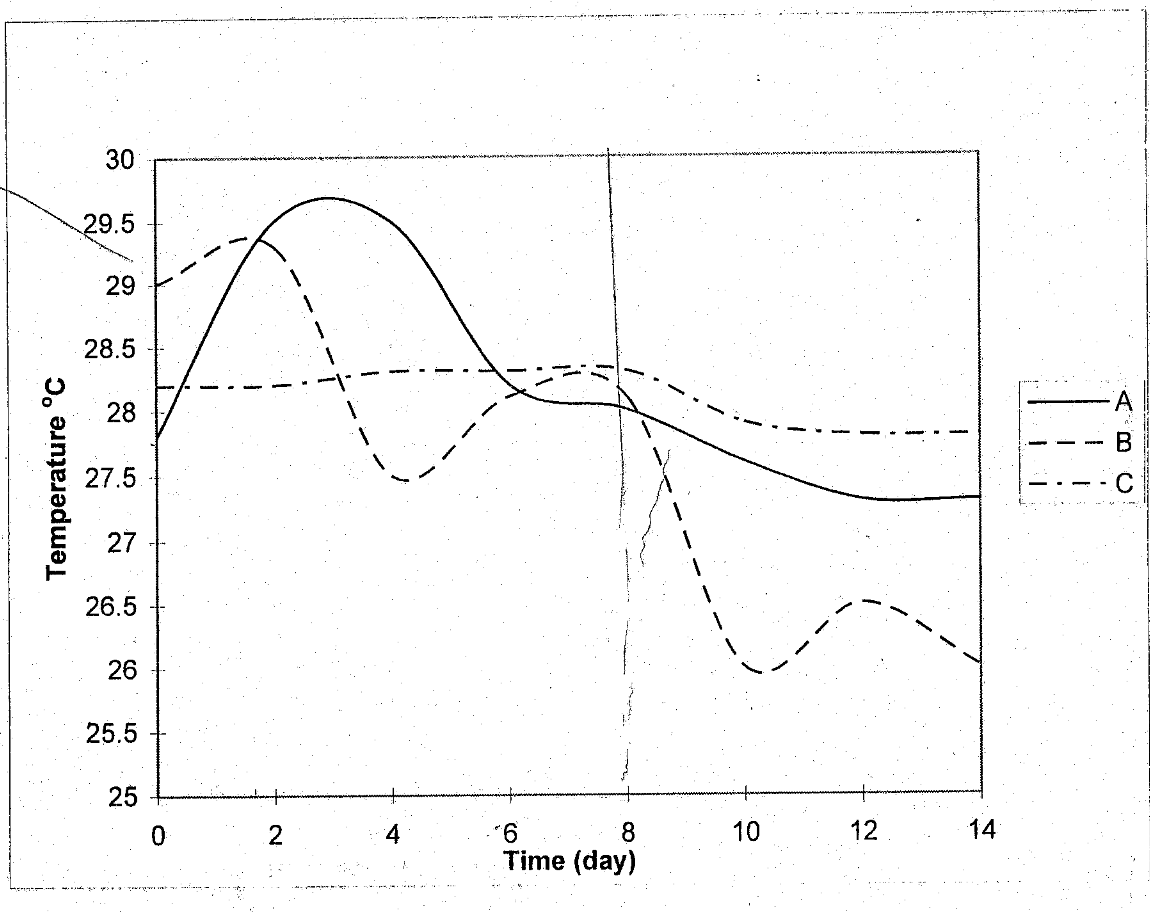

Fig 4.4 : Change in Temperature with Time

Fig. 4.4 shows that the samples had varying peaks of temperature. Sample A had the highest peak at day 3 while sample B had 3 decreasing peaks of temperature at day 2, 8, and 12. Sample C had an approximately constant temperature which is normally required for healthy fermentations. Fermentation is an exothermic reaction which results in heat production.

lower alcohol yield and high titratable acidity in the wine without additive (sample A) can be attributed to the insufficient sugar level in it. This may explain the reduced progress of fermentation observed at day 10. With only this exception, the wine produced from locust bean pulp shared similar characteristics with imported and other locally produced wines.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

This study has shown that it is feasible to produced good quality table wine from the yellow pulp of locust bean using improved traditional method. Pilot plant study on the production process could encourage the establishment of a cottage industry in Niger state.

5.2 Recommendation

From the information provided in this work in the utilization of the fruit pulp of locust bean as raw material for wine production, further research interest could be focused on improved wine production techniques that may increase the chemical and physical properties to that of standard wine. The following areas may need to be looked into:

1. The determination of the right acid and nutrient blend of must that must provide steady fermentation.
2. The determination of optimum ageing time.

These two factors above will make significant contributions to the wine quality. Commercial scale of this wine is feasible and will save Nigeria some foreign exchange presently expended on wine importation. The cheapness of the raw material, its availability all year round can drastically cut down production cost.

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