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EFFECT OF BREWER'S YEAST (*SACCHAROMYCES CEREVISIAE VAR. ELLIPSOIDEUS)*, BAKER'S YEAST (*SACCHAROMYCES CEREVISIAE*) AND DUAL CULTURE (*SACCHAROMYCES CEREVISIAE VAR. ELLIPSOIDEUS* AND *SACCHAROMYCES CEREVISIAE)* ON THE FERMENTATION OF PINEAPPLE JUICE INTO WINE

BY

PATRICK ADONIS AIDOO

SEPTEMBER, 2011

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A THESIS SUBMITTED TO THE SCHOOL OF RESEARCH AND GRADUATE STUDIES, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE (POSTHARVEST TECHNOLOGY) DEGREE

BY

PATRICK ADONIS AIDOO

SEPTEMBER, 2011

DECLARATION

I hereby declare that, except for specific references which have been duly acknowledged, this project is the result of my own research and it has not been submitted either in part or whole for any other degree elsewhere.

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DEDICATION

This work is dedicated to my dear wife, Mrs. Esther Boatemaa Agyapong Aidoo and my dear parents, Mr. and Mrs. Aidoo

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I would like to sincerely express my gratitude to all who have supported me through guidance, prayers and encouragement to successfully complete a work of this kind. I am particularly thankful to the Lord God Almighty for His mercy and grace upon my life.

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ABSTRACT

A study was conducted at the chemistry laboratory of Teshie Presbyterian Senior High School in the Greater Accra Region of Ghana to produce wine from pineapple juice using bakers' and brewer's yeasts. A 3 x 3 factorial in Complete Randomised Design was used and replicated three times. Parameters studied included sugar content (glucose, sucrose, fructose and total sugar), alcohol content, pH, titratable acidity and sensory analysis. The results revealed that as sugar content decreases, alcohol content increases; as pH decreases acid content increases. The total sugar content of the must containing 20ml (1.0×10^6 cfu/g) of yeast decreased from 17.3^{o}Brix to 5.0^{o}Brix as alcohol increased from 0% to 8.5% 10days, after incubation, and then decreased to 7.6% 14days after incubation. In the must containing 10ml (6.0×10^6 cfu/g) of yeast, total sugar decreased from 17.3^{o}Brix to 5.3^{o}Brix as alcohol content increased from 0% to 8.2% 10days after incubation and then decreased to 7.45% 14days after incubation. The control (must without yeast) also showed a gradual decrease in sugar content from 17.3^{o}Brix to 9.3^{o}Brix as alcohol content consistently increased from 0% to 5.3% 14days after incubation. Significant differences were observed between the yeast concentrations and the control ($P<0.05$). The study concluded that yeast strains and concentration were highly efficient in utilizing the pineapple sugar to produce wine with substantial alcohol content, maintaining the keeping qualities of pineapple juice wine, thus reducing wastage, and was highly accepted. The sensory analysis showed that the wine produced was in the category of standard wines (score of 13-16). The process of ageing for three months and racking at two weeks interval produced standard wine with neither outstanding characteristics nor defects.

TABLE OF CONTENTS

TITLE PAGE ii

DECLARATION iii

DEDICATION iv

ACKNOWLEDGEMENTS iv

ABSTRACT vi

TABLE OF CONTENTS vii

LIST OF FIGURES xi

1.0 INTRODUCTION 1

2.0 LITERATURE REVIEW 4

2.1 PINEAPPLE 4

2.1.1 Origin and Distribution of Pineapple 4

2.1.2 Morphology of Pineapple 5

2.1.3 Climatic Requirement of Pineapple 5

2.1.4 Variety of Pineapple Cultivated 6

2.2 FERMENTATION 7

2.2.1 Definition of Fermentation 7

2.2.2 History of Fermentation 8

2.2.3 Fermentation in Fruit Juice 10

2.2.4 Fruit Fermented into Wine 12

2.3 FACTORS INFLUENCING FERMENTATION 15

2.3.1 Effect of Temperature on Fermentation 15

2.3.2 Effect of pH on Fermentation and Wine Quality 18

2.3.3 Effects of Sugar Content on Fermentation 20

2.3.4 Effect of Micro Organisms on Fermentation 21

2.3.5 Effect of Acid on Fermentation and Wine Quality 24

2.4 RELATIOSHIP BETWEEN SUGAR CONTENT AND ALCOHOL PRODUCTION 26
2.5 CONTAINERS USED IN FERMENTATION 27

3.0 MATERIALS AND METHODS 30
3.1 LOCATION OF EXPERIMENT 30
3.2 YEAST STRAINS AND MEDIA 30
3.3 PRODUCTION OF PINEAPPLE JUICE 31
3.4 FERMENTATION OF PINEAPPLE JUICE 32
3.5 EXPERIMENTAL DESIGN 34
3.6 TREATMENTS USED 34
3.7 PARAMETERS SUDIED 35
3.7.1 Ambient and Must Temperatures (^{o}C) 35
3.7.2 Sugar Content (^{o} Brix) 35
3.7.3 Alcoholic Content 35
3.7.4 pH of Must 35
3.7.5 Acid Levels 35
3.7.6 Sensory Analysis 36
3.8 ANALYSIS OF DATA 37

4.0 RESULTS 38
4.1 NUTRITIONAL COMPOSITION OF PINEAPPLE JUICE AND WINE 38
4.2 MICROORGANISMS PRESENT IN PINEAPPLE JUICE AND WINE 39
4.3 AMBIENT AND MUST TEMPERATURE 40
4.4 GLUCOSE CONTENT OF PINEAPPLE WINE 41
4.5 SUCROSE CONTENT OF PINEAPPLE WINE 43
4.6 FRUCTOSE CONTENT OF PINEAPPLE WINE 45
4.7 TOTAL SUGAR CONTENT OF PINEAPPLE WINE 47
4.8 ALCOHOL CONTENT OF PINEAPPLE WINE 49

4.9 pH OF PINEAPPLE WINE 52
4.10 TITRATABLE ACIDITY OF PINEAPPLE WINE 54
4.11 CONSUMERS ACCEPTABILITY OF PINEAPPLE WINE 56

5.0 DISCUSSIONS 57
5.1 NUTRITIONAL COMPOSITION OF PINEAPPLE JUICE AND WINE 57
5.2 MICROORGANISMS PRESENT IN PINEAPPLE JUICE AND WINE 57
5.3 AMBIENT AND MUST TEMPERATURE 59
5.4 EFFECT OF YEAST ON SUGAR CONTENT OF THE WINE 59
5.5 ALCOHOL CONTENT OF PINEAPPLE WINE 60
5.6 pH OF PINEAPPLE WINE 62
5.7 TITRATABLE ACIDITY OF PINEAPPLE WINE 62
5.8 CONSUMERS ACCEPTABILITY OF PINEAPPLE WINE 63

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS 65
6.1 SUMMARY 65
6.2 CONCLUSION 67
6.3 RECOMMENDATION 69

REFERENCES **70**
APPENDICE 79

LIST OF TABLES

Table 3.1: Score card for sensory analysis..... 37
Table 4.1: Nutritional composition of pineapple juice and wines……..... 38
Table 4.2: Micro organisms present in raw pineapple juice and wine……... 39
Table 4.3: Effect of yeast and concentration on glucose content of must…….. 43
Table 4.4: Effect of yeast and concentration on sucrose content of must…….. 45
Table 4.5: Effect of yeast and concentration on fructose content of must……. 47
Table 4.6: Effect of yeast and concentration on total sugar content of must……. 549
Table 4.7: Effect of yeast and concentration on alcohol content of must…….. 51
Table 4.8: Effect of yeast and concentration on pH of must…….. 53
Table 4.9: Effect of yeast and concentration on total titratable acidity of must……. 55
Table 4.10: Consumers acceptability of pineapple wine…… 56

LIST OF FIGURES

Figure 3.1: Flow Chart for Pineapple Juice Extraction 31
Figure 3.2: A Flow diagram for the production of pineapple wine by controlled fermentation 33
Figure 4.1: Effect of yeast on must temperature 40
Figure 4.2: Effect of yeast concentration on must temperature 41
Figure 4.3: Effect of Yeast on Glucose Content of Pineapple Must 42
Figure 4.4: Effect of yeast concentration on glucose content of pineapple must 42
Figure 4.5: Effect of yeast on sucrose content of pineapple must 44
Figure 4.6: Effect of yeast concentration on sucrose content of pineapple must 44
Figure 4.7: Effect of yeast concentration on fructose content of pineapple must 46
Figure 4.8: Effect of yeast concentration on fructose content of pineapple must 46
Figure 4.9: Effect of yeast on total sugar content of pineapple must 48
Figure 4.10: Effect of yeast concentration on total sugar content of pineapple must 48
Figure 4.11: Effect of yeast on alcohol content of pineapple must 50
Figure 4.12: Effect of yeast concentration on alcohol content of pineapple must 51
Figure 4.13: Effect of yeast on pH of pineapple must 52
Figure 4.14: Effect of yeast concentration on pH of pineapple must 53
Figure 4.15: Effect of yeast on total titratable acidity of pineapple must 54
Figure 4.16: Effect of yeast concentration on total titratable acidity of pineapple must 55

CHAPTER ONE

1.0 INTRODUCTION

Pineapple (*Ananas comosus*) belonging to the Bromeliaceae family is grown in different parts of Ghana either for export or for the local market. Pineapples are sliced and eaten fresh in the homes, or processed into fruit juices for consumption or concentrates for future use. Pineapple as a fruit crop has a lot of economic, nutritional, medicinal, and industrial importance **(**Sarah *et al*., 1997). According to Dull (1971), pineapple as food for human consumption contains about 81.2-86.2% moisture, 13-19% total solids of which sucrose, glucose, and fructose are the main components, 2-3% fibre and rich source of vitamin C. Lipids and nitrogenous compounds constitute 0.1% of which 25-30% of the nitrogenous compounds are true proteins. The fruit is also rich in calcium (Ca) which has proteolytic activity due to the enzyme bromelin. According to the author, consumption of pineapple enhances the detoxification of the human body and prevents blood clotting. It also prevents kidney problems, protects the heart and regulates stomach acidity and help prevents constipation (Dull, 1971).

The pineapple industry in the country is faced with a lot of challenges ranging from fruit rejections from export markets to inadequate storage and processing facilities coupled with unavailable markets. Also poor handling and transportation of the fruits contribute significantly to high post harvest losses. According to Appert (1987), losses in pineapple fruits harvest could be as high as 50%. Research Institutions and other private companies are trying hard to reduce these losses by processing the fruits into drinks and fresh cut fruits for the local market. Little work however is done on processing the fruits into other product such as wine which are currently not available on the Ghanaian market.

According to Adams and Moss (1995) maize and cassava dough are fermented and maize, millet, and other cereal grains are brewed into local drinks such as 'pito' and 'toosi' in Ghana and in other Sub-Saharan African countries. Very little however is known about fermentation of pineapple, cashew, mango, and oranges juices into wine (Au Du, 2010).

Wine, an alcoholic beverage, made from a variety of fruit juices by the fermentative action of selected yeast, is adapted to an ageing process handed over from generation to generation. Traditionally, wine is produced from apple, pear, grape and berry which are available in Europe, Middle East, America or North and South Africa (Kunkee and Vilas, 1994). Most of the wines available on the market today are all imported.

FAO (2002) and 'Comisión Veracruzana de Comercialización Agropecuaria' (COVECA, 2002) reported that there are several pineapple varieties commonly grown in Africa and for that matter Ghana that have favourable pH (4.5-6.5) and sufficient sugar levels for fermentation to occur. Keller (2010) also noted that pineapple juice could be converted into wine in the presence of yeast. Thus, through fermentation, the highly perishable pineapple fruit could be converted into a highly nutritious wine which can be made available all year round. Marketing of this product will ensure security for pineapple growers as wine and other alcoholic beverages are in high demand through out the country and the world as a whole. Thus ensuring a secure source of income for both the farmers and the investors. Wine production from other sources other than the traditional fruits has been successfully done on carrot (Izuagbe, 1982), banana (Akhimien *et al*., 1987) and cashew (Au Du, 2010).

According to FAO (1998) fermentation is one of the most ancient and most important food processing technologies which had been neglected by scientists and policy makers

especially in traditional fermented products from developing countries. Fermentation is a relatively efficient, low energy preservation process which increases the shelf life and decreases the need for refrigeration or other form of food preservation technology. It is therefore a highly appropriate technique for use in developing countries and remote areas where access to sophisticated equipment is limited. Fermented fruits wines are popular throughout the world, and in some regions it makes a significant contribution to the diet of millions of individuals. The possibility and the use of pineapple for the production of wine will create employment, income generation for farmers and address the post harvest losses associated with glut on the local market in Ghana. The findings of this study will inform decisions with regards to the preservation and storage of pineapple in particular and fruits in general.

The main objective of this research therefore is to assess the performance of baker and brewer yeast on alcohol production in the form of wine from pineapple juice through fermentation. Specific objectives of the research are therefore:

- to determine the efficiency of production of alcohol in the form of wine from pineapple juice using brewer‘s and baker’s yeast
- to determine the effect of dual yeast (mixture of brewer ‘s yeast and baker’s yeast) culture on alcohol production from pineapple juice
- to compare the performance of the Baker’s yeast, Brewer’s yeast, and the dual culture in the production of alcohol during the fermentation process.
- to determine consumer acceptability of alcoholic beverage produced from pineapple juice.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 PINEAPPLE

2.1.1 Origin and Distribution of Pineapple

Pineapple is the second harvest of importance after bananas, contributing to over 20 % of the world production of tropical fruits (COVECA, 2002). Nearly over 70% of the pineapple is consumed as fresh fruit in producing countries especially in Africa and for that matter Ghana. Its origin has been traced to Brazil and Paraguay in the Amazonic basin where the fruit was domesticated. It has been defined as the most probable area of origin. The zone comprised of upper Panama and Brazil, Paraguay and Argentina, including the northern Amazonian forest and the semi-arid regions of Brazil, Venezuela and Guyanas (Medina, *et al*., 2005; Collins, 1949, 1960).

Worldwide distribution and production started by 1500 when pineapple was propagated in Europe and the tropical regions of the world (Medina, *et al*., 2005). The most wide spread variety is *Cayena lisa* (smooth cayenne) which was first introduced in Europe from French Guyana. It was until late 14th century when canned pineapple was produced commercially in Hawaii (FAO, 2004).

Thailand, Philippines, Brazil and China are the main pineapple producers in the world supplying nearly 50 % of the total output (FAO, 2004). Other important producers include India, Nigeria, Kenya, Indonesia, México and Costa Rica and these countries provide most of the remaining fruit available (50%) (Medina, *et al*., 2005)

2.1.2 Morphology of Pineapple

The pineapple plant is a short herbaceous perennial with 30-80cm trough-shaped and pointed leaves 30-100 cm long, surrounding a thick stem. This shape of the plant has to drive water onto the stem. This water might be absorbed by axils. The early inflorescences has about 100-200 flowers (Sarah *et al*., 1997; COVECA, 2002). Flowers of pineapple are spirally placed and each is supported by bracteas. Each flower consists of 3 calyxes, 3 bluish corollas, 6 filaments and a carpel with three parts of stigma. Inflorescence goes to bloom about 3 weeks and it blooms from down to up. Pineapples are auto sterile and fruits developed are parthenocarpic. (Elfic, 2004).

2.1.3 Climatic Requirement of Pineapple

A temperature range between 23 to 24°C is optimal for growing pineapple (FAO, 2002). When ambient temperature drops to 10-16°C, fruit growth is constrained. Plants may stand sub-freezing temperatures for very short periods. Conversely, exposure to temperatures well over 30°C results in heat damage due to increased respiration rate and metabolism and impaired nutrient absorption (Bartholomew and Kadziman, 1977).

Pineapple production regions are usually confined to altitudes below 800m above sea level, although Kenya reports production fields located between 1400 and 1800m, and Malaysia orchards as high as 2400m (Davies, 1994). When pineapple is grown at altitudes greater than 1000m above sea level, smaller fruits are produced; the pulp has less attractive colour, flavour and tartness are elevated. Plant growth occurs within a temperature range of between 21°C and 35°C and an annual rainfall of about up to 1100mm per annum and it

should be evenly distributed. The optimal pH for growth is between 5.5 and 6.2 (Sarah *et al*, 1997; COVECA, 2002).

2.1.4 Variety of Pineapple Cultivated

There are several cultivars with different sugar brix. According to COVECA (2002), *Cayena lisa* contains 19% sugar brix, Spanish from Singapore (10%-12% sugar brix), Green Selacia (10%-12% sugar brix), Queen (14%-18% sugar brix), Red Spanish (12% sugar brix), Perola (13%-16% sugar brix), Perolera (12% sugar brix). However, several new varieties have been introduced to improve the quality of the fruit for the international markets such as MD2 (Golden ripe, Extra sweet and Maya gold). These varieties are hybrids that were developed in Hawaii from *Cayena lisa* with an average weight ranging from 1.3 to 2.5 kg. It has an intense orange to yellow-orange colour and a high sugar content of 15 to17° Brix. The fruit are sweet, compact and fibrous. Main differences found with respect to the *Cayena lisa* variety are: better resistance to internal darkening, lesser ascorbic acid content more prone to rotting and sensitive to *Phytophthora* (COVECA, 2002; FAO, 2002).

The *La Josefina* variety was released in 1996 for the fresh fruit market (FAO, 2002). It is a hybrid developed from other two clones. Its production cycle is annual with a generation of 2 to 3 suckers per plant. Average fruit weight is 1.1 to 1.3 kg and contains an elevated sugar concentration (17 to 22°Brix). Differences with respect to the *Cayena lisa* variety are: longer shelf life, greater sugar content and resistance to black heart disorder and shorter production cycles. Finally, variety RL41 is a hybrid obtained from cultivars *Cayena lisa* and "*Manzana*" with an average weight of 1.4 to 2 kg and a high sugar content, 15 to 18° Brix. Compared to *Cayena lisa*, this variety has a greater ascorbic acid content and shorter

production cycles, as well as lesser resistance to rotting but more resistant to flower induction (FAO, 2002).

2.2 FERMENTATION

2.2.1 Definition of Fermentation

According to Garrison (1993), the process of fermenting is basically feeding sugars and nutrients in solution to yeast, which return the favour by producing carbon dioxide gas and alcohol. This process goes on until either all the sugar is gone or the yeast can no longer tolerate the alcoholic percentage of the beverage. Different yeasts produce different results, and have different tolerance levels (Anon, 2005).

Fermentation is a process of deriving energy from the oxidation of organic compounds, such as carbohydrates, and using an endogenous electron acceptor, which is usually an organic compound (Klien *et al.*, 2005), as opposed to respiration where electrons are donated to an exogenous electron acceptor, such as oxygen, via an electron transport chain**.**

The risk of stuck fermentation and the development of several wine faults can also occur during this stage which can last from 5 to 14 days for primary fermentation and potentially another 5 to 10 days for a secondary fermentation. Fermentation may be done in stainless steel tanks, which is common with many white wines like Riesling, in an open wooden vat, inside a wine barrel and inside the wine bottle itself as in the production of many sparkling wines (Wikipedia, 2010; Robinson, 2006; Kunze, 2004).

Fermentation is a cheap and energy efficient means of preserving perishable raw materials such as pineapple juice (FAO, 2002). Harvested fruits may undergo rapid deterioration if proper processing and storage facilities are not provided, especially in the humid tropics

where the prevailing environmental conditions accelerate the process of decomposition (FAO, 2010). Although there are several options for preserving fresh fruits, which may include drying, freezing, canning and pickling, many of these are inappropriate for the produce and for use on small-scale in developing countries. For instance the canning of fruits at the small-scale has serious food safety implications and contamination especially botulism (FAO, 1998).

Freezing of fruits and vegetables is not economically viable at the small-scale. Fermentation requires very little sophisticated equipment, either to carry out the fermentation or for subsequent storage of the fermented product. It is a technique that has been employed for generations to preserve fruits in the form of drinks and other food for consumption at a later date and to improve food security. Basically most fruits can be fermented; if not all provided they are well prepared (Garrison, 1993).

2.2.2 History of Fermentation

Fermentation is one of the oldest forms of food preservation technologies in the world. Indigenous fermented foods such as bread, cheese and wine, have been prepared and consumed for thousands of years and are strongly linked to culture and tradition, especially in rural households and village communities. The development of fermentation technologies is lost in the midst of history (Yokotsuka, 1985). Anthropologists have postulated that it was the production of alcohol that motivated primitive people to settle down and become agriculturists. Some even think the consumption of fermented food is pre-human (Stanton, 1985).

The first fermented foods consumed probably were fermented fruits. Hunter-gatherers would have consumed fresh fruits but in times of scarcity would have eaten rotten and fermented fruits. Repeated consumption would have led to the development of the taste for fermented fruits. There is reliable information that fermented drinks were being produced over 7,000 years ago in Babylon (now Iraq), 5,000 years ago in Egypt, 4,000 years ago in Mexico and 3,500 years ago in Sudan (Dirar, 1993; Pedersen, 1979).

There is also evidence of fermented meat products being produced for King Nebuchadnezer of Babylon. China is thought to be the birth-place of fermented vegetables and the use of Aspergillus and Rhizopus moulds to make food. The book called "Shu-Ching" written in the Chou dynasty in China (1121-256 BC) refers to the use of "chu" a fermented grain product (Yokotsuka, 1985).

Knowledge about traditional fermentation technologies has been handed down from parent to child, for centuries. These fermented products have been adapted over generations; some products and practices no doubt fell by the wayside. Those that remain today have not only survived the test of time but also more importantly are appropriate to the technical, social and economic conditions of the region (FAO, 1998). In Ghana, corn dough and cassava dough are fermented and cereal grains are locally brewed into local drinks such as ‘pito,’ and ‘toosi’.

According to Robinson (2006), natural occurrence of fermentation means it was probably first observed long ago by humans. The earliest uses of the word "Fermentation" in relation to winemaking was in reference to the apparent "boiling" within the must that came from

the anaerobic reaction of the yeast to the sugars in the grape juice and the release of carbon dioxide. The Latin 'fervere' literally means to boil. In the mid-19th century, Louis Pasteur noted the connection between yeast and the process of the fermentation in which the yeast act as catalyst through a series of a reaction that convert sugar into alcohol. The discovery of the Embden–Meyerhof–Parnas pathway by Gustav Embden, Otto Fritz Meyerhof and Jakub Karol Parnas in the early 20th century contributed more to the understanding of the complex chemical processes involved the conversion of sugar to alcohol (FAO, 2010).

2.2.3 Fermentation in Fruit Juice

According to Robinson (2006), the process of fermentation in wine is the catalyst function that turns fruit (grape) juice into an alcoholic beverage. To Walker (1988), this organic process is the "slow decomposition process of organic substances induced by micro-organisms, or by complex nitrogenous substances (enzymes) of plant or animal origin. During fermentation yeast interacts with sugars in the juice to create ethanol, commonly known as ethyl alcohol, and carbon dioxide (as a by-product). In winemaking the temperature and speed of fermentation is an important consideration as well as the levels of oxygen present in the must at the start of the fermentation (Keller, 2010; Van Rooyen and Tromp, 1982). Fermentation does not necessarily have to be carried out in an anaerobic environment. For example, even in the presence of abundant oxygen, yeast cells greatly prefer fermentation to oxidative phosphorylation, as long as sugars are readily available for consumption (Dickinson, 1999).

Sugars are the most common substrate of fermentation, and typical examples of fermentation products are ethanol, lactic acid, and hydrogen (Garrison, 1993). However,

more exotic compounds can be produced by fermentation, such as butyric acid and acetone (Au Du, 2010). Yeast carries out fermentation in the production of ethanol in beers, wines and other alcoholic drinks, along with the production of large quantities of carbon dioxide (Keller, 2010). Fermentation products contain chemical energy (they are not fully oxidized), but are considered waste products, since they cannot be metabolised further without the use of oxygen or other more highly-oxidized electron acceptors. The consequence is that the production of adenosine triphosphate (ATP) by fermentation is less efficient than oxidative phosphorylation, whereby pyruvate is fully oxidized to carbon dioxide. Juice temperature must be warm for fermentation. However, yeast cells will die if temperature is too hot (Robinson, 2006).

Ethanol fermentation performed by yeast and some types of bacteria breaks the pyruvate down into ethanol and carbon dioxide. It is important in bread-making, brewing, and wine-making. Usually only one of the products is desired; in bread-making, the alcohol is baked out, and in alcohol production, the carbon dioxide is released into the atmosphere or used for carbonating the beverage. When the ferment has a high concentration of pectin, minute quantities of methanol can be produced (Au Du, 2010).

Hydrogen gas can be produced in many types of fermentation (mixed acid fermentation, butyric acid fermentation, caproate fermentation, butanol fermentation, glyoxylate fermentation), as a way to regenerate NAD+ from NADH. Electrons are transferred to ferredoxin, which in turn is oxidized by hydrogenase, producing H_2. Hydrogen gas is a substrate for methanogens and sulphate reducers, which keep the concentration of hydrogen sufficiently low to allow the production of such an energy-rich compound (Madigan and

Martinko, 2005). However, in the case of some fruit juice a risk factor involved with fermentation is the development of chemical residue and spoilage, which can be corrected with the addition of sulphur dioxide (SO_2), although excess SO_2 can lead to a wine fault (Robinson, 2006).

2.2.4 Fruit Fermented into Wine

There are many fermented drinks made from fruit in Africa, Asia and Latin America. These include drinks made from bananas, grapes and other fruit. Grape wine is perhaps the most economically important fruit juice alcohol (FAO, 2010). It is of major economic importance in Chile, Argentina, South Africa, Georgia, Morocco and Algeria. Because of the commercialisation of the product for industry, the process has received most research attention and is documented in detail. Banana beer is probably the most wide spread alcoholic fruit drink in Africa and is of cultural importance in certain areas. Alcoholic fruit drinks are made from many other fruits including dates in North Africa, pineapples in Latin America and jack fruits in Asia (FAO, 2010).

White grape wine is an alcoholic fruit drink of between 10 and 14% alcoholic strength. This prepared from the fruit of the grape plant (*Vitis vinifera*), and is pale yellow in colour (Ranken, *et al*., 1997). There are many varieties used including Airen, Chardonnay, Palomino, Sauvignon Blanc and Ugni Blanc. The main difference between red and white wines is the early removal of grape skins in white wine production. The distinctive flavour of grape wine originates from the grapes as raw material and subsequent processing operations. The grapes contribute trace elements of many volatile substances (mainly terpenes) which give the final product the distinctive fruity character.

In the case of cashew, the apples are cut into slices to ensure a rapid rate of juice extraction when crushed in a juice press. The fruit juice is sterilised in stainless steel pans at a temperature of 85^{o}C in order to eliminate wild yeast (Wimalsiri *et al*., 1971). The juice is filtered and treated with either sodium or potassium metabisulphite to destroy or inhibit the growth of any undesirable types of micro-organisms - acetic acid bacteria, wild yeasts and moulds. Wine yeast (*Saccharomyees cerevisiae var ellipsoideus*) is added. Once the yeast is added, the contents are stirred well and allowed to ferment for about two weeks (Au Du, 2010).

After fermentation is completed, the wine is separated from the sediment by racking. It can also be clarified further by using fining agents such as gelatine, pectin or casein which are mixed with the wine. Filtration can be carried out with filter-aids such as fuller's earth after racking. The wine is then pasteurised at 50^{o}C – 60^{o}C. Temperature should be controlled, so as not to heat it to about 70^{o}C, since its alcohol content would vaporise at a temperature of 75-78^{o}C (Au Du, 2010). It is then stored in wooden vats and subjected to ageing. At least six months should be allowed for ageing. If necessary, wine is again clarified prior to bottling. During ageing, and subsequent maturing in bottles many reactions, including oxidation, occur with the formation of traces of esters and aldehydes, which together with the tannin and acids already present enhance the taste, aroma and preservative properties of the wine (Wimalsiri *et al* , 1971).

'Colonche' is a sweet, fizzy beverage produced in Mexico by fermenting the juice of the fruits of the prickly pear cacti - mainly *Opuntia species*. The procedure for preparing

'colonche' is essentially the same as has been followed for centuries. The cactus fruits are peeled and crushed to obtain the juice, which is boiled for 2-3 hours. After cooling, the juice is allowed to ferment for a few days. Sometimes old 'colonche' or 'tibicos' may be added as a starter. 'Tibicos' are gelatinous masses of yeasts and bacteria, grown in water with brown sugar. They are also used in the preparation of 'tepache' (FAO, 2008; Dirar, 1992).

Date wines are popular in Sudan and North Africa (Dirar, 1992). They are made using a variety of methodologies. 'Dakhai' is produced by placing dates in a clean earthenware pot. For every one volume of dates between two and four volumes of boiling water are added. This is allowed to cool and is then sealed for three days. More warm water is then added and the container sealed again for seven to ten days. Many variations of date wine exist: 'El madfuna' is produced by burying the earthenware pots underground. 'Benti merse' is produced from a mixture of sorghum and dates. 'Nebit' is produced from date syrup (Dirar, 1992).

Sparkling grape wines are made in the Republic of South Africa (Van Rooyen, *et al*., 1982) Sparkling wines can be made in one of three ways. The cheapest method is to carbonate wines under pressure. Unfortunately, the sparkle of these wines quickly disappears, and the product is considered inferior to the sparkling wines produced by the traditional method of secondary fermentation. This involves adding a special strain of wine yeast (S. *cerevisiae var. ellipsoideus*) - a champagne yeast - to wine that has been artificially sweetened. Carbon dioxide produced by fermentation of the added sugar gives the wine its sparkle. In the original champagne method, which is still widely used today, this secondary

fermentation is carried out in strong bottles, capable of withstanding pressure, but early in the nineteenth century a method of fermenting the wine in closed tanks was devised, this being considerably cheaper than using bottles (Van Rooyen, *et al.,* 1982; Rose, 1961)

Jack-fruit wine is an alcoholic beverage made by ethnic groups in the eastern hilly areas of India (Steinkraus, 1996). As its name suggests, it is produced from the pulp of jack-fruit (*Artocarpus heterophyllus*). Ripe fruit is peeled and the skin discarded. The seeds are removed and the pulp soaked in water. Using bamboo baskets, the pulp is ground to extract the juice, which is collected in earthenware pots. A little water is added to the pots along with fermented wine inoculums from a previous fermentation. The pots are covered with banana leaves and allowed to ferment at 18 to 30ºC for about one week. The liquid is then decanted and drunk (Steinkraus, 1996).

2.3 FACTORS INFLUENCING FERMENTATION

2.3.1 Effect of Temperature on Fermentation

To avoid contamination and unpleasant odours in wine, everything that comes in contact with the wine must be very clean. This is especially critical when cleaning the fermenting vessel. Just as there are weeds in the garden, so there are weeds in wines. There are micro organisms that feed on alcohol and cause a poor flavour (Anon, 2005). Vinegar bacilli will change sugar to vinegar. Moulds give a stale flavour. To prevent these unwelcome intruders, cleanliness is the only answer. An effective agent is Sal soda (sodium carbonate). Baking soda is fairly effective if given time to work. Either of these agents will remove odours and flavours from the containers (Van Rooyen, *et al*., 1982; Riley, 1978). All these chemicals may reduce the wine quality if the right quantities are not added. To avoid this

situation, fruit juice for fermentation can be sterilised in stainless steel pans at a temperature of 85^{o}C in order to eliminate wild yeast after extraction. The juice is filtered and treated with either sodium or potassium metabisulphite to destroy or inhibit the growth of any undesirable types of micro-organisms - acetic acid bacteria; wild yeasts and moulds (Van Rooyen, *et al.,* 1982; Wimalsiri et al, 1971). Also, increasing temperatures above 60 ºC may kill wild yeast and other micro organisms (FAO, 2010; Robinson, 2006; Fleet, 1998).

During fermentation there are several factors that winemakers take into consideration. The most notable is that of the internal temperature of the must (Keller, 2010). The biochemical process of fermentation itself creates a lot of residual heat which can take the must out of the ideal temperature range for the wine (Keller, 2010). Thus fermentation is an exothermic process (it releases heat). But in wine-making, the temperature must not exceed 29.4 ºC for red wines or 15.3 ºC for (white wines), otherwise the growth of yeast cells will stop. Therefore a lower temperature is desirable because it increases the production of esters, other aromatic compounds and alcohol itself. This makes the wine easier to clear and less susceptible to bacterial infection (Anon, 2005; Amerine and Roesseler, 1983; Amerine and Ough, 1980; Wimalsiri, *et al*., 1971). In general, temperature control during alcoholic fermentation is necessary to facilitate yeast growth, extract flavours and colours from the skins, permit accumulation of desirable by-products, and prevent undue rise in temperature, that might kill the yeast cells. The low temperature and slow fermentation favours the retention of volatile compounds (Fleet, 1998).

Typically, white wine is fermented between 64-68 °F (18-20 °C) though a wine maker may choose to use a higher temperature to bring out some of the complexity of the wine (Battcock and Sue, 1998). Red wine is typically fermented at higher temperatures up to 85 °F (29 °C). In most cases, fermentation at higher temperatures may have adverse effect on the wine in stunning the yeast to inactivity and even "boiling off" some of the flavours of the wines. Some winemakers may ferment their red wines at cooler temperatures more typical of white wines in order to bring out more fruit flavours (Robinson, 2006; Fleet, 1998).

Yeasts are active in a very broad temperature range - from 0 to 50^{o}C, with an optimum temperature range of 20^{o}C to 30^{o}C (Mountney and Gould, 1988). The temperature of fermentation is usually from 25 to 30^{o}C this makes yeast an important micro organism for fermentation. White wines are fermented at 10 to 18º C for about seven to fourteen days. The low temperature and slow fermentation favours the retention of volatile compounds. Red wines are fermented at 20 to 30ºC for about seven days to fourteen days. This higher temperature is necessary to extract the pigment from the grape skins (Keller, 2010; Fleet, 1998). With reference to other organisms, different bacteria can tolerate different temperature which provides enormous scope for a range of fermentations. While most bacteria have a temperature optimum of between 20 to 30ºC, there are some (the thermophiles) which prefer higher temperatures (50 to 55ºC) and those with colder temperature optima (15 to 20ºC). Most lactic acid bacteria work best at temperatures of 18 to 22ºC. The Leuconostoc species which initiate fermentation have an optimum temperature of 18 to 22ºC. Temperatures above 22ºC, favour the lactobacillus species (FAO, 2010; Anon, 2005).

As soon as the desired degree of sugar disappearance and alcohol production has been attained, the microbiological phase of wine making is over (Stanier *et al*, 1972). The wine is then pasteurised at 50^{o} - 60^{o}C. Temperature should be controlled, so as not to heat it to about 70^{o}C, since its alcohol content `would vaporise at a temperature of 75^{o}-78^{o}C (Au Du, 2010).

2.3.2 Effect of pH on Fermentation and Wine Quality

According to Fleet (1998), pH directly affects wine stability. This may be as a result of the fact that at a pH close to neutral (7.0), most micro organisms like bacterial, moulds including some yeasts become more active for fermentation and subsequent spoilage of wine, whilst pH below 3.5 eliminate most of the microbes, and favours only few of the micro organisms for fermentation. Specifically, the optimum pH for most micro-organisms is near the neutral point (pH 7.0). Moulds and yeasts are usually low pH tolerant and are therefore associated with the spoilage of foods with low pH. Yeasts can grow in a pH range of 4 to 4.5 and moulds can grow from pH 2 to 8.5, but favour low pH (Mountney and Gould, 1988). A solution's pH is the measure of hydrogen ions (H^{+}) concentration of an acid solution such as pineapple and grape juice or wine or conversely the concentration of hydroxyl ions (OH^{-}) in alkaline solution such as lye. Because the numerical value of the hydrogen ions (H^{+}) concentration is often extremely small fraction (1×10^{-7}) the pH unit is used to express this concentration *(Encyclopedia Britannica*. 2000). A pH unit has been expressed as the negative logarithm of the hydrogen ion (H^{+}) concentration and it is determined by a pH meter (Lacroux *et al*., 2008; *Encyclopedia Britannica*. 2000; Gallander, *et. al*., 1987).

From the pH scale, the lower the pH value, the higher the concentration of H^+ ions, the higher the degree of acidity, thus there is an inverse relationship between decreasing pH value and increasing H^+ ions concentration. For example a wine at a pH of 3.0 is 10 times more acidic than a wine at a pH of 4.0, thus there is a ten fold change in acidity (*Encyclopedia Britannica*, 2000; Amerine and Roessler, 1983; Amerine and Ough, 1980).

The traditional process of fermentation involves extracting fruits juice and adjusting the pH to 4.0 using sodium bicarbonate and adding yeast nutrient (ammonium phosphate) at 0.14g per litre (Steinkraus, 1996). For example, during fermentation of fruit juice, reductions of soluble solids are possible from pH between 7.4 to 3.5 and 4.0 in worm fermentation (Steinkraus, 1992). A pH level of 4.0 may be conducive for the development of unwanted microbes like L. *oneos*, and this can be prevented by controlling the pH by reducing the wine pH to below 3.2 (Fleet, 1998). According to Rotter (2008), most fining and clearing agents such as Earths: bentonite, kaolin, Proteins: gelatine, isinglass, casein, pasteurised milk, albumen, yeast, Polysaccharides: alginate (agar), gum arabic (acacia), Carbons, Synthetic polymers: PVPP, silica gel, Tannins, Others: metal chelators, blue fining, enzymes are more effective in clearing the wine when pH is below 3.5.

pH plays an important role in aging, clarifying or fining. As the strength of the relative charge of suspended particles decreases in the wine, the pH of the wine increases. At high pH, organic protein fining agents may possess a positive charge insufficient to bind to the negatively charged particulates, thus potentially increasing the turbidity of the wine. This phenomenon is called "overfining" (Rotter, 2008).

2.3.3 Effects of Sugar Content on Fermentation

Sugar is the main substrate for fermentation of fruits juice into alcohol (Keller, 2010); although, other food nutrients such as protein and fats can be broken down by some micro organism in some cases where sugar is limited, but as long as sugar is present yeast cells will continue the process of fermentation until other factors that affect the growth of yeast become unfavourable (Dickinson, 1999). According to Garrison (1993), sugars are the most common substrate of fermentation to produce ethanol, lactic acid, hydrogen and carbon dioxide.

Although sugar is an important substrate of fermentation, higher sugar concentration inhibits the growth of micro-organisms (FAO, 2010). For example, during fermentation of the juices of the plant (*Agave Americana*), the soluble solids should be at the optimum, and should be reduced from between 25-30% to 6%; the sucrose content falls from 15% to 1% (Steinkraus, 1992). However, yeasts are fairly tolerant of high concentrations of sugar and grow well in solutions containing 40% sugar. At concentrations higher than this, only a certain group of yeasts – the osmophilic type – can survive. There are only a few yeasts that can tolerate sugar concentrations of 65-70% and these grow very slowly in these conditions (Board, 1983). A winemaker who wishes to make a wine with high levels of residual sugar (like a dessert wine) may stop fermentation early either by dropping the temperature of the must to stun the yeast or by adding a high level of alcohol (like brandy) to the must to kill off the yeast and create a fortified wine (Robinson, 2006).

2.3.4 Effect of Micro Organisms on Fermentation

For many traditional fermented products, the micro-organisms responsible for the fermentation are unknown to scientists. However there have been several researches to identify the micro-organisms involved in fruits fermentation. For example, the micro organism responsible for banana beer production is *Saccharomyces cerevisiae,* which is the same organism involved in the production of grape and other fruit wine. However many other micro-organisms that are associated with the fermentation have been identified? These organisms vary according to the region of production (Davis and Noble, 1995).

Yeast is a unicellular fungus which reproduces asexually by budding or division, especially the genus *Saccharomyces* which is important in food fermentations has the ability to reproduce much faster (Walker, 1988). Yeasts and yeast-like fungi are widely distributed in nature. They are present in orchards and vineyards, in the air, the soil and the intestinal tract of animals. Like bacteria and moulds, they can have beneficial and non-beneficial effects in foods. Most Yeast strains are larger than most bacteria. The most well known examples of yeast fermentation are in the production of alcoholic drinks and the leavening of bread. For their participation in these two processes, yeasts are of major importance in the food industry. Some Yeast strains are chromogenic and produce a variety of pigments, including green, yellow and black. Others are capable of synthesizing essential B group vitamins (Kawo and Abdulmumin, 2009; Adams and Moss, 1995; Walker, 1988).

Although there is a large diversity of yeasts and yeast-like fungi, (about 500 species), only a few are commonly associated with the production of fermented foods. They are all either

ascomycetous yeasts or members of the genus *Candida*. Varieties of the *Saccharomyces cervisiae* genus are the most common yeasts in fermented foods and beverages based on fruit and vegetables. All strains of this genus ferment glucose and many ferment other plant derived carbohydrates such as sucrose, maltose and raffinose. In the tropics, *Saccharomyces pombe* is the dominant yeast in the production of traditional fermented beverages, especially those derived from maize and millet (Adams and Moss, 1995).

Brewer's yeast, *Saccharomyces cerevisiae var ellipsoideus,* and *Saccharomyces uvarum* are very common in the brewery and the wine industry. These yeasts are the micro organisms that are responsible for fermentation in beer and wine (Keller, 2010). Yeast metabolises the sugars extracted from grains and fruits, which produces alcohol and carbon dioxide, and thereby turns wort into beer and fruits into wine respectively. In addition to fermenting the beer and wine, yeasts influence the character and flavour (Ostergaard *et al.,* 2000). The dominant types of yeast used in fermenting alcoholic beverages are the *Saccharomyces* species. For example, to make beer the ale yeast (*Saccharomyces cerevisiae*) and lager yeast (*Saccharomyces uvarum*) are used (Dittmer and Desmond, 2005), whilst in wine (*Saccharomyces cerevisiae* var ellipsoideus and (*Saccharomyces cerevisiae*) may be used (Keller, 2010). Other micro organisms used in fermentation wine and beer may include: *Brettanomyces species* for lambics (Hornsey, 1999), *Torulaspora delbrueckii* for Bavarian Weiss bier (Horwitz, 1999). Before the role of yeast in fermentation was understood, fermentation involved wild or airborne yeasts. A few styles such as lambics rely on this method today, but most modern fermentation adds pure yeast cultures (Hui and Khachatourians 1994).

The most common genera of wild yeasts found in winemaking include *Candida*, *Klöckera/Hanseniaspora*, *Metschnikowiaceae*, *Pichia* and *Zygosaccharomyces* (Wikipedia, 2010). Wild yeasts can produce high-quality, unique-flavoured wines; however, they are often unpredictable and may introduce less desirable traits to the wine, and can even contribute to spoilage (Keller, 2010). Traditional wine makers, particularly in Europe, advocate use of ambient yeast as a characteristic of the region's *terroir*; nevertheless, many winemakers prefer to control fermentation with predictable cultured yeast. The cultured yeasts most commonly used in winemaking belong to the *Saccharomyces cerevisiae* (also known as "sugar yeast") species (Wikipedia, 2010). Within this species are several hundred different strains of yeast that can be used during fermentation to affect the heat or vigour of the process and enhance or suppress certain flavour characteristics of the wine. The uses of different strains of yeasts are a major contributor to the diversity of wine, even among the same grape variety (Robinson, 2006). According to Panjai, *et al*., (2009), mixture of yeast thus dual culture (*T. delbrueckii* species and *S. cerevisiae*) can be used to produce a complex fruit wine from pineapple.

Yeast in general has a natural protein removal effect during fining or clearing. It is also sometimes used, in the dried (and dead) form, to remove copper sulphate, ethyl acetate, browning, oxidation and excess oak that may be associated with cloudy wine (Rotter, 2008). Doses commonly recommended are 240-1000 mg/l. It is important to rack the wine soon after yeast fining in order to avoid reductive aromas (Rotter, 2008).

According to Madigan and Martinko (2005), homolactic fermentation can occur in some kinds of bacteria (such as lactobacilli) and some fungi. It is this type of bacteria that

converts lactose into lactic acid in yoghurt, giving it its sour taste These lactic acid bacteria can be classed as homofermentative, where the end product is mostly lactate, or heterofermentative, where some lactate is further metabolized and results in carbon dioxide, acetate or other metabolic products (Axelsson, 1998; Dickinson, 1999).

Bacteria may not always be bad in fermentation; this is because to clarify the wine, the fermented juice maybe transferred into a settling vat, or if made on a smaller scale, into a demijohn (FAO, 2008). In these, suspended yeast cells, cream of tartar and particles of skin and pulp settle to the bottom of the container. As the yeast cells break down within the precipitate, they stimulate the growth of *Lactobacillus* bacteria that convert the wine's malic acid into lactic acid. This process is especially important in wines made from highly acidic grapes because lactic acid is a weaker acid than malic acid. (Bacteria decarboxylate malic acid, thus removing the acidic carboxyl group), therefore it mellows the wine's taste (Anon, 2005).

2.3.5 Effect of Acid on Fermentation and Wine Quality

Acid is said to directly affect wine quality, but wine owes its acid composition to citric acid, tartaric acid, and some traces of other acids like lactic acid which replaces malic acid during malolactic fermentation (Fleet, 1998). These acids in fruits juice or wine can be determined by titration (Gallander, *et al*., 1987; Lacroux *et al*., 2008; Amerine, and Ough, 1980; Iland, *et al*., 2000). Fruit acids are weak acids, compared to strong mineral acids such as sulphuric and hydrochloric. In solution, strong acids tends to yield their hydrogen ion (H^+) component nearly completely; weak acids dissociate only about one percent of their hydrogen ion. Thus such acid solutions like fruit wine have more hydrogen ions (H^+) than

hydroxyl ions (OH^-). As hydrogen ion concentration increases, the solution becomes more unfavourable for most micro organisms associated with spoilage of wine and acidic foods. However some moulds and yeasts which are needed in the fermentation of fruit juice into wine are usually acid tolerant therefore they are very important in the production of dry wine (wine with a very low or no sugar), (Mountney and Gould, 1988).

Wines produced from grapes grown in colder climates tend to have a higher concentration of malic acid and a lower pH (3.0 to 3.5) and the taste benefits from this slight decrease in acidity. Wines produced from grapes in warmer climates tend to be less acidic (pH > 3.5) and a further reduction in acidity may have adverse effects on the quality of the wine. Decreasing the acidity also increases the pH to values which can allow spoilage organisms like L. *oenos* to multiply to embark on malo-lactic fermentation (Fleet, 1998).

During fermentation of palm sap, within 24 hours pH can be reduced from 7.4-6.8 to 5.5 and the alcohol content ranges from 1.5 to 2.1 percent. Within 72 hours the alcohol level increase from 4.5 to 5.2 percent and the pH is 4.0. Organic acids present are lactic acid, acetic acid and tartaric acid (Odunfa, 1985).

During fermentation, the pH of the wine reaches a value of 3.5 to 3.8, suggesting that an acidic fermentation takes place at the same time as the alcoholic fermentation. Final alcohol content is about 7 to 8% within a fortnight (Steinkraus, 1996). Fruit juices often have all that yeast needs all by themselves. Notably grape juice is a favourite, as it has the acids, tannins and sugars needed. Apple juice stands on its own quite well too. Other juices may need acids (not just for the yeast, but for flavour!), and many commonly need tannins to be

added. Yeasts are very hardy micro organisms that will get by with most fruits sugar and juices in fermentation. They can even work on plain white sugar so far as the right acid and nutrient blend is available, although this is difficult to do by most micro organisms (Garrison, 1993). Acids present in wine enhance the taste, aroma and preservative properties of the wine (Van Rooyen and Tromp, 1982; Wimalsiri *et al*., 1971).

2.4 RELATIOSHIP BETWEEN SUGAR CONTENT AND ALCOHOL PRODUCTION

Sugar is essential for making wine, as without it yeast may not be able to produce alcohol. Natural sugars in some fruits are often insufficient to produce anything stronger above 8% by volume of alcohol. This means that to produce an alcoholic fruit wine of stronger alcoholic strength above 8% by volume of alcohol a white granulated cane or beet sugar must be added (Dull, 1971). Corn sugar can be used in direct proportions to granulated sugar. Fructose (fruit sugar) is sweeter than other kinds and should be used only when a sweet wine is desired or in sweetening a wine after fermentation (Keller, 2010).

Duration and pH variances also affect the sugar composition of the resulting must during fermentation (Kunze, 2004). As fermentation time increases, more sugars are digested, more antioxidants will be produced and the pH will probably settle around pH 3.5 making a drier acidic drink. There will also be a greater yeast activity producing more scum and sediment from dead bodies of yeasts and lactobacilli (Sulz, 2011). Fermentation stops naturally when all the fermentable sugars have been converted to alcohol or when the alcoholic strength reaches the limit of tolerance of the strain of yeast involved. Fermentation can be stopped artificially by adding alcohol, by sterile filtration or centrifugation (Ranken *et al*., 1997). Any wine that is absolutely bone dry (little or no

sugar) will stabilize itself within a few days to weeks, as no food remains to keep the yeast alive. For bone dry wines (specific gravity of 0.990 or lower), allow them to sit for 30 days before bottling (Van Rooyen and Tromp., 1982; Anon, 2005: Davis and Noble, 1995).

A wine given a hydrometer reading of a specific gravity of 0.990 and a sugar °Brix of below 3.5 is a dry wine; therefore, addition of sugar would be required if the wine is to be sweetened. On the other hand, a wine with a specific gravity of 1.020 and a sugar °Brix of 5.08 is a sweet wine (Wine World FDW, 2002; Ed Kasper, 2007).

2.5 CONTAINERS USED IN FERMENTATION

Traditionally, fermentation was carried out in large wooden barrels or concrete tanks. These tanks were very difficult to clean, and were easily contaminated by unwanted micro organisms which interfere in subsequent fermentation given varying tastes and flavours. This indicates that the quality and stability of wine depends very largely on preventing further microbial activity, both during the "ageing" in wooden casks and after bottling (Stanier *et al.*, 1972). Due to this and other reasons, modern wineries now use stainless steel tanks as these are more hygienic and provide better temperature control (Ranken Kill and Baker, 1997). Typical example of wine produced from the fermentation in stainless steel tanks, are white wines like Riesling, and other sparkling wines (Robinson, 2006)

Most breweries today use cylindro-conical vessels, (CCVs) made of stainless steel. CCCs have a conical bottom, cylindrical top cone's aperture typically fitted around 60° angle to allow yeast to flow towards the cones apex, but not so steep as take up too much vertical space. CCVs can handle both fermenting and conditioning in the same tank. At the end of

fermentation, the yeast and other solids which have fallen to the cones apex can be simply flushed out a port at the apex (Kunze, 2004; Davies, 1994).

Simple cylindrical stainless steel tanks with bevelled ends are normally arranged vertically, as opposed to conditioning or wooden tanks, which are usually laid out horizontally. Only a very few breweries and wineries still use wooden vats and concrete tanks for fermentation are difficult to keep clean of infections and must be repitched more or less yearly (Keller, 2010; Kunze, 2004).

Modern fermentation tanks made of stainless steel may be fitted with cooling rings to regulate temperature or with a bung device (German: *Spundapparat*) to allow CO_2 produced by the yeast during fermentation to naturally carbonate the wine or the beer. This bung device can be set to a given pressure to match the type of beer being produced. The more pressure the bung holds back, the more carbonated the beer becomes (Keller, 2010; Kunze, 2004).

Fermentation may take place in open or closed vessels. There may be a secondary fermentation which can take place in the brewery, in the cask or in the bottle. The primary fermentor most often used by the home wine maker is a glass gallon jug (Van Rooyen and Tromp, 1982; Riley, 1978).

After fermentation and clearing, wine must be aged. Aging can be done by storing the wine in wooden or the wine bottle. Aging is done in a wooden vat for least six months in most red wine to add the flavour of the wood to the wine (Wimalsiri *et al.,* 1971).

After the demijohn stage, the wine is repeatedly racked to leave behind less and less precipitate. During the repeated pouring, the wine is also given a chance to rid itself of the excess carbon dioxide from fermentation. As the CO_2 escapes, oxygen enters the wine with each transfer, helping eventually to age the wine (Amerine and Roessler, 1983**;** The ANON, 2005).

Precipitation of combined particulates is faster at lower temperatures. Protein fining is therefore more effective at lower temperatures (except for bentonite). However, some fining agents are less temperature sensitive than others (e.g. isinglass is less temperature sensitive than gelatine. According to Rotter (2008), wines should be low in dissolved CO_2 when fined, since dissolved CO_2 will tend to keep particulates in solution and inhibit settling

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 LOCATION OF EXPERIMENT

The research was carried in the chemistry laboratory of Teshie Presbyterian Senior High School in the Greater Accra Region.

3.2 YEAST STRAINS AND MEDIA

Brewer's yeast *(Saccharomyces cerevisiae var. ellipsoideus)* was obtained from the Microbiology and Biochemistry section of Accra Brewery Limited (ABL), Accra Ghana. Baker's Yeast (*Saccharomyces cerevisiae*) was also obtained. Yeasts were grown at 25 ^{o}C on YEPD (Yeast Extract-Peptones, Dextrose) medium; glucose, 20 g/l; yeast extract, 10 g/l; peptone, 20 g/l; and agar, 15 g/l. Total and living cell numbers of yeasts were estimated microscopically by using a counting chamber slide. Cells (450 µ l) were added to 50µl of methylene blue solution [0.4% methylene blue, 10% ethanol and 0.4 M potassium phosphate (KH_2PO_4)] and mixed. Blue cells were counted as dead cells, while cells without obvious colour were counted as live or viable cells.

3.3 PRODUCTION OF PINEAPPLE JUICE

Pineapples were obtained from Gomoa West in the Central Region of Ghana. After peeling they were freshly pressed using a juice squeezer (Figure 3.1).

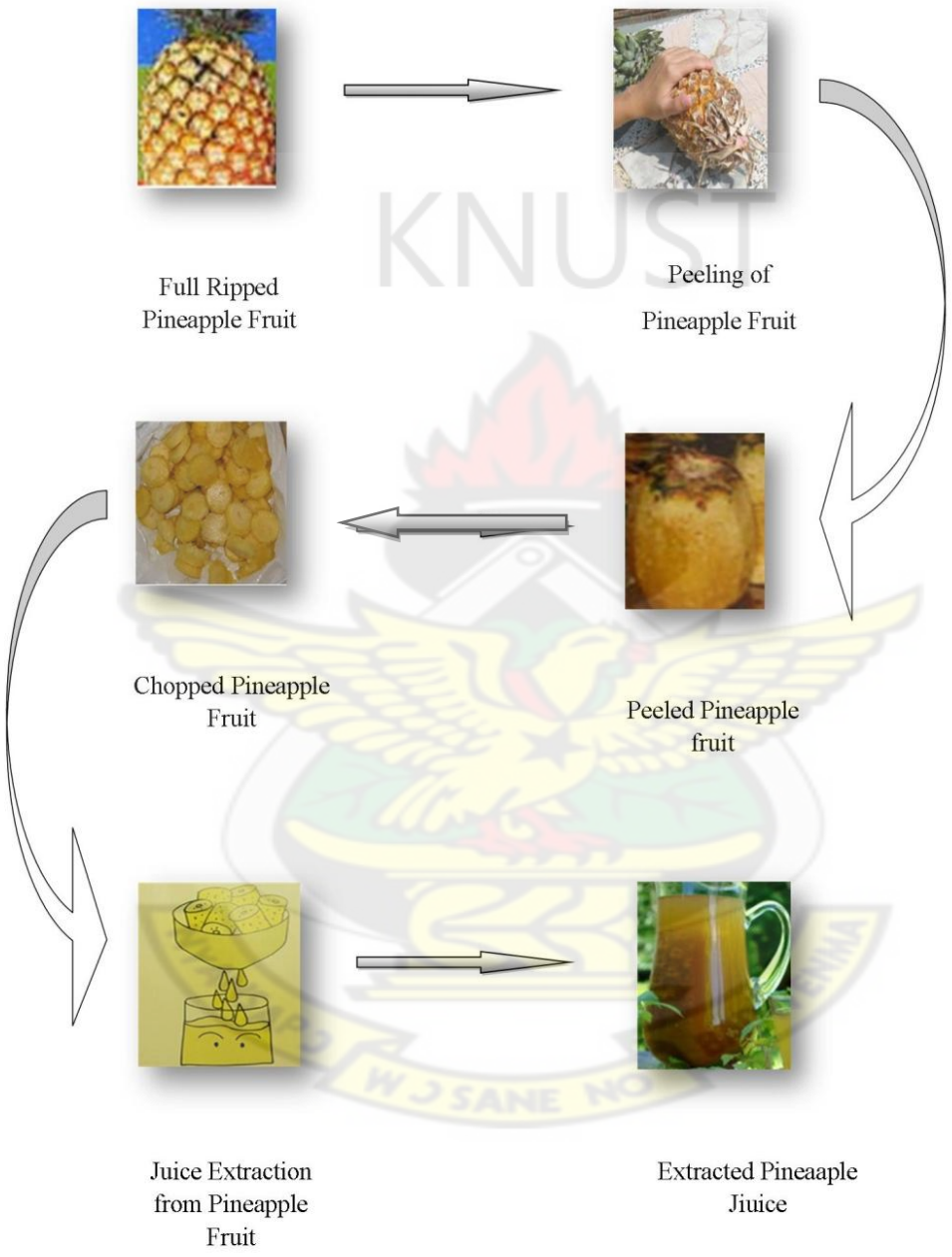

Figure 3.1: Flow Chart for Pineapple Juice Extraction

3.4 FERMENTATION OF PINEAPPLE JUICE

Seven rings were drawn on one-metre high bench at 50cm interval. The bench was placed one-metre away from the wall. 800ml of the pasteurised pineapple juice was drawn into each of the 27 round bottomed flasks. The yeasts were pre-cultured for 24 hours at room temperature (28±2^{o}C) before being used. The respective quantities of the yeasts were measured and were used to pitch the various units. For *Saccharomyces cerevisiae*, 20ml (1.0×10^{6} *cfu/g*) and 10ml (6.0×10^{5} *cfu/g*) of the yeasts types were used to pitch the pasteurised pineapple juice. A control treatment (without yeast) was also set up. For *Saccharomyces cerevisiae var. ellipsoideus*, 20ml (1.0×10^{6} *cfu/g*) and 10ml (6.0×10^{5} *cfu/g*) of the yeast was used to pitch the pasteurised pineapple juice. Again a control treatment (without yeast) was setup. For the dual cultures (*S. cerevisiae* + *S. cerevisiae var. ellipsoideus*), 20ml (1.0×10^{6} *cfu/g*) and 10ml (6.0×10^{5} *cfu/g*) of the yeast was used in pitching the pasteurised pineapple juice. Also a control (without yeast) was set up alongside the others. The units were then corked for fermentation except for the control which was aerated for twenty-four hours. The set up was replicated three times. The samples were incubated for 14 days at room temperature (28±2^{o}C).

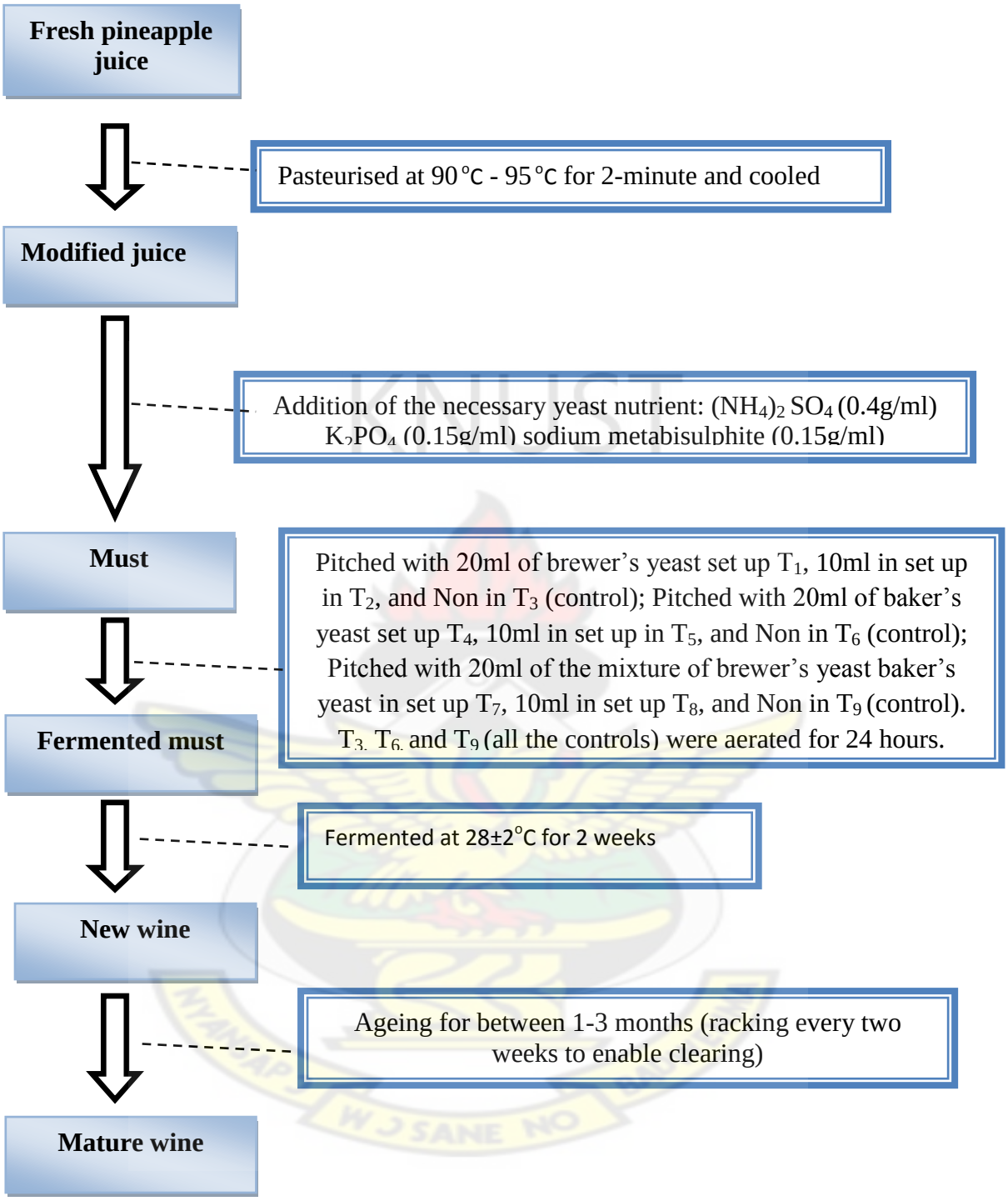

Figure 3.2: A Flow diagram for the production of pineapple wine by controlled fermentation

3.5 EXPERIMENTAL DESIGN

A 3 x 3 factorial in Complete Randomised Design (CRD) was used.

3.6 TREATMENTS USED

Three Yeast strains namely Baker's Yeast (*Saccharomyces cerevisiae*), Brewer's yeast (*Saccharomyces cerevisiae var. ellipsoideus*) and a dual culture (Baker yeast + Brewer yeast) were used. Three concentration of yeast used included 20ml (1.0×10^6 *cfu/g*), 10ml (6.0×10^5 *cfu/g*) and a control treatment (no yeast). The following were the treatments combinations used during the fermentation period.

- T1 - Baker's Yeast @ 20ml (1.0×10^6 *cfu/g*)
- T2 - Baker's Yeast @ 10ml (5.0×10^5 *cfu/g*)
- T3 - Control for Baker's Yeast
- T4 - Brewer's yeast @ 20ml (1.0×10^6 *cfu/g*)
- T5 - Brewer's yeast @ 10ml (5.0×10^5 *cfu/g*)
- T6 - Control for Brewer's yeast
- T7 - Dual Culture @ 20ml (1.0×10^6 *cfu/g*)
- T8 - Dual Culture @ 10ml (5.0×10^5 *cfu/g*)
- T9 - Control for Dual Culture

3.7 PARAMETERS STUDIED

3.7.1 Ambient and Must Temperatures (^{o} C)

Daily ambient and must temperatures were taken during the experimental period. Average daily ambient and must temperatures were recorded.

3.7.2 Sugar Content (^{o}Brix)

Fructose, glucose, sucrose and total sugar concentrations were obtained using ^{o}Brix Refractometers (Model: HI 96801 Sucrose, HI 96802 Fructose, HI 96803 Glucose and HI 96804) and hydrometer and confirmed by HPLC. Readings were taken for 14 days during fermentation.

3.7.3 Alcoholic Content

The alcoholic percentage levels (%/ vol.) in all the fermented must were determined using alcohol meter (HI-96813) and an alcohol hydrometer Readings were taken for 14 days during fermentation and mean alcohol levels (%/ vol.) were calculated.

3.7.4 pH of Must

The pH value of the must was determined using a pH meter. Readings were taken for 14 days during fermentation.

3.7.5 Acid Levels

Titratable acid (TA) levels were determined by a titration method (Iland, 2000; Ed Kasper, 2007). TA was recorded for 14 days during fermentation. The determination was as follows:

200 ml of boiled and cooled distilled water was placed into a 500 ml. Erlenmeyer flask and 1 ml of phenolphthalein indicator was added. The distilled water was titrated with 0.1 N, Sodium hydroxide (NaOH) to a definite pink end point. 5 ml of must/wine sample was added to the flask. The sample was titrated with 0.1 NaOH to the same distinct end point. The volume of NaOH used in the titration was noted. Using the formulae

$$\text{TA as citric acid (g/100 ml)} = \frac{(V)\ (N)\ (75)\ (100)}{(1000)\ (v)}$$

Where

V = ml of NaOH solution used for titration, N = Normality of sodium hydroxide solution, v=sample volume (ml)

3.7.6 Sensory Analysis

The sensory properties in the young pineapple wine were evaluated. White wines were served simultaneously with the pineapple wine obtained from the three set-ups to ten (10) panellists for their assessment. The scale used was Outstanding (Best), Standard (Better), Commercial acceptability (Good), Below commercial acceptability (Bad), and Completely spoiled wine (Worst) using the score card in Table 3.1. According to Davis (1995) 20-points scale, to achieve a score of 17 to 20 a wine must have an outstanding characteristics and no marked defect, 13 to 16 is a standard wine, which has neither an outstanding characteristics nor defects; 9 to 12 have commercial acceptability but with a noticeable defect; 5 to 8 wine is below commercial acceptability; 1 to 4 is a completely spoiled wine

Table 3.1: Score card for sensory analysis

SCORE CARD										Commercial Wine
Name…… Phone No……..…… Age…….										
Name / Sample Number	*S. cerevisiae*			*S. cerevisiae var ellipsoideus*			*S. cerevisiae* + *S. cerevisiae var ellipsoideus*			
	A	B	C	A	B	C	A	B	C	
Appearance 2										
Colour 2										
Aroma /Bouquet 4										
Ace scent 2										
Total Acid 2										
Total Sugar 1										
Body 1										
Flavour 2										
Astringency 2										
General Quality 2										
Total 20										

Source: Adopted and modified from Davis (1995)

3.8 ANALYSIS OF DATA

Data on all the parameters studied were collected, and their respective means calculated. The mean values were subjected to statistical analysis using analysis of variance (ANOVA). The statistical package used was Statistix version 9 software. Differences in treatment means were determined using Tukey HSD test at P=0.05 (5%)

CHAPTER FOUR

4.0 RESULTS

4.1 NUTRITIONAL COMPOSITION OF PINEAPPLE JUICE AND WINE

Table 4.1: Nutritional composition of pineapple juice and wines

Content	Fresh Juice	Wine produced from all the yeast types		
		20ml (1.0×10^{6} cfu/g)	10ml ($6.0\text{x}10^{5}$ cfu/g)	Control (no yeast)
Total sugar (^{o}Brix)	17.3	5.0	5.4	9.20
Reducing sugar	+	+	+	+
Non-reducing sugar	+	+	+	+
Titratable acid (Citric acid)	0.64	0.82	0.80	0.77
Lactic acid	+	+	+	+
Malic acid	+	+	+	+
Vitamin A	+	+	+	+
Vitamin B	+	+	+	+
Vitamin C (mg/ml)	8.20	1.9	1.97	1.99
Protein	+	+	+	+
Ph	4.82	3.80	3.82	3.92
Alcohol (%)	-	7.60	7.45	5.35
Methanol	-	-	-	-

(+) present (-) absent

Table 4.1 shows the nutritional composition of the fresh pineapple juice and the wine produced. The fresh pineapple juice had a high sugar level (17.3^{o}Brix) than the wines produced. Wine produced from the 20ml (1.0×10^{6}cfu/g) yeast concentration recorded the lowest sugar level of 5.0^{o}Brix. Titratable acid (citric acid) of the wine produced from 20ml (1.0×10^{6}cfu/g) yeast concentration had a high content of 0.82 and had a low content of 0.64 in the fresh juice. pH of the fresh pineapple juice was high (4.82) than that of the wine produced from the 20ml (1.0×10^{6}cfu/g) yeast concentration (3.80) and 10ml (6.0×10^{5}cfu/g) yeast concentration (3.82). The fresh juice had high vitamin C content (8.20mg/ml) than the wine produced from 20ml (1.0×10^{6}cfu/g) yeast concentration (1.98) and 10ml (6.0×10^{5}cfu/g) yeast concentration (1.97). Both the fresh juice and wine had reducing and

non-reducing sugars, protein, vitamin A and B, malic and lactic acids present. Alcohol content of wine produced from 20ml (1.0×10^6cfu/g) yeast concentration was higher (7.6%) than that of wine produced from 10ml (6.0×10^5cfu/g) yeast concentration. However, methanol was absent in both the fresh juice and wine.

4.2 MICROORGANISMS PRESENT IN PINEAPPLE JUICE AND WINE

Table 4.2: Microorganisms present in raw pineapple juice and wine

Micro Organism	Fresh Pineapple Juice	Sterilized juice	Wine produced from all the yeast types		
			20ml (1.0×10^6 cfu/g)	10ml (5.0×10^5cfu/g)	Control (no yeast)
Bacterial	*Lactobacillus sp., Gluconobacta sp., Streptococcus sp., Leuconostoc sp.*	-	-	-	*Gluconobacta oxydans, Streptococcus sp.*
Yeast	*Sacharomyces sp. Candidda sp.*	-			*S. cerevisiae, S. cerevisiae var ellipsoideus*
Mould	*Aspergillus sp.*	-	-	-	(Traces)

+ Microorganism present - Microorganism absent

Results of the micro-biological analysis of fresh and sterilized juice presented in Table 4.2 showed that most of the organisms in the raw pineapple juice were eradicated. Yeast *(Sacharomyces sp. Candidda sp.*) mould (*Aspergillus sp.*) and bacteria (*Lactobacillus Sp, Gluconobacta sp. Streptococcus sp, Leuconostoc sp.*), were absent in the juice after the sterilization process. There were however bacteria (*Gluconobacta oxydans, Streptococcus sp),* yeast *(S. cerevisiae, S. cerevisiae var ellipsoideus*) and some traces of mould

(*Aspergillus sp.*) in the wine from the control. The baker and brewer yeast used were recovered from their respective wines.

4.3 AMBIENT AND MUST TEMPERATURE

The temperature pattern for both the incubation room and the must showed a similar trend. The temperature range for the fermentation room was between 25.6^{o}C to 28.2^{o}C. The lowest temperature reading was recorded 7days after incubation and the maximum temperature was recorded 2days after incubation. For the yeast treatment, the temperature ranged between 20.1^{o}C to 28^{o}C. The minimum must temperature was recorded at the set-up stage while the maximum must temperature was recorded 10days after incubation in the dual cultures and 7days in the baker yeast and the brewer yeast (Figure 4.1).

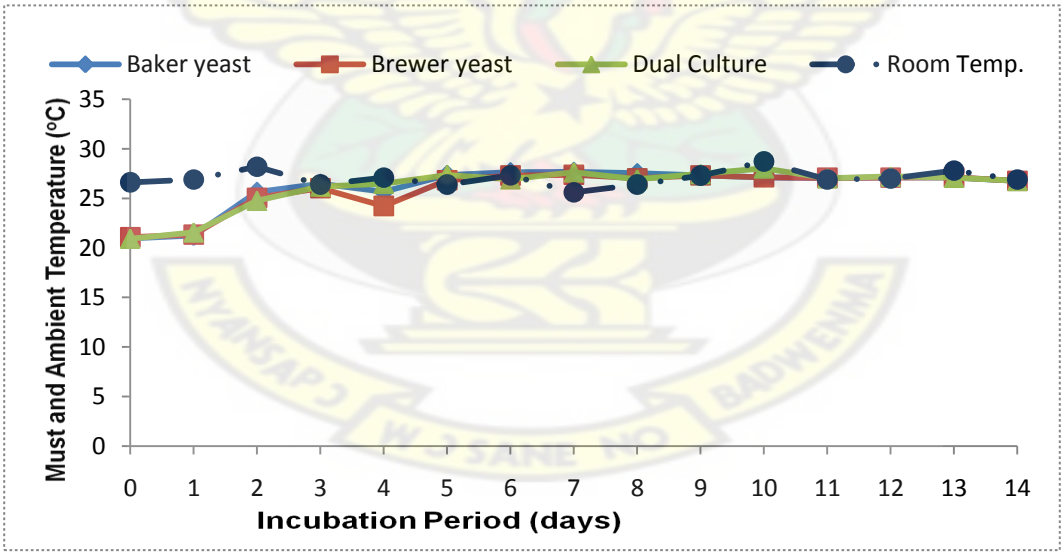

Figure 4.1: Effect of yeast on must temperature

For the yeast concentration setup, both the incubation room and the must temperatures showed a similar trend. The temperature readings from the yeast concentration must range

between 21.0°C to 29.2°C for the 20ml (1.0×10^6cfu/g), 21.0°C to 28.3°C for the 10ml (6.0×10^5cfu/g) and 21.0°C to 27.3°C for the control. The lowest temperature reading was recorded at the start of the experiment and the maximum must temperature was recorded 9days after incubation in the 20ml (1.0×10^6cfu/g), 6days in the 20ml (6.0×10^5cfu/g) and 9days after incubation for the control (Figure 4.2).

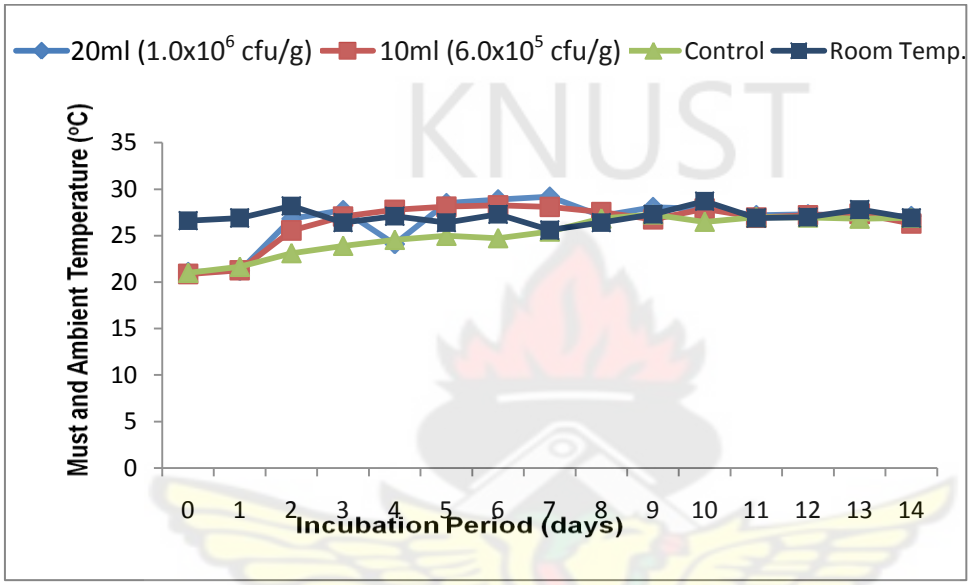

Figure 4.2: Effect of yeast concentration on must temperature

4.4 GLUCOSE CONTENT OF PINEAPPLE WINE

Figure 4.3 shows the effect of yeast on glucose content of pineapple must. There was a gradual decline in glucose content of the pineapple must fermented by the yeast from day 1 to day 14 of the incubation period. The glucose content of the must decreased from 5.4°Brix to 0.8°Brix in the baker's yeast, brewer's yeast and dual yeast must. However, there was no significant differences observed between the Yeast strains ($P>0.05$).

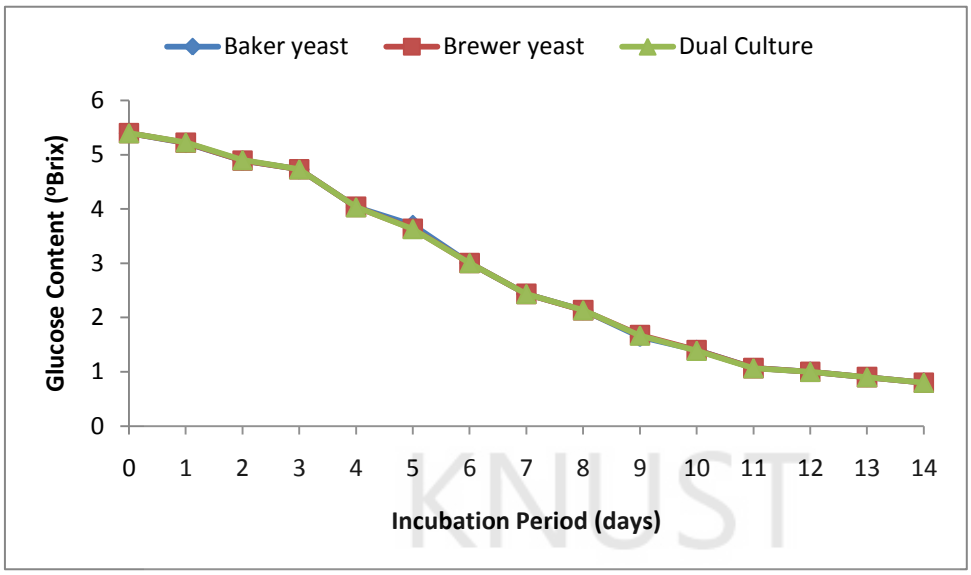

Figure 4.3: Effect of Yeast on Glucose Content of Pineapple Must

The different yeast concentrations showed a gradual decline in glucose content of the pineapple must from day 1 to day 14 of the incubation period (Figure 4.4). The glucose content of the must containing 20ml (1.0×10^6cfu/g) of yeast decreased from 5.4°Brix to 0.3°Brix. In the must containing 10ml (6.0×10^5cfu/g) of yeast also decreased from 5.4°Brix to 0.4°Brix. The control (must without yeast) also showed a gradual decrease in glucose content from 5.4 °Brix to 1.7°Brix. Significant differences were observed between the yeast concentrations and the control ($P<0.05$).

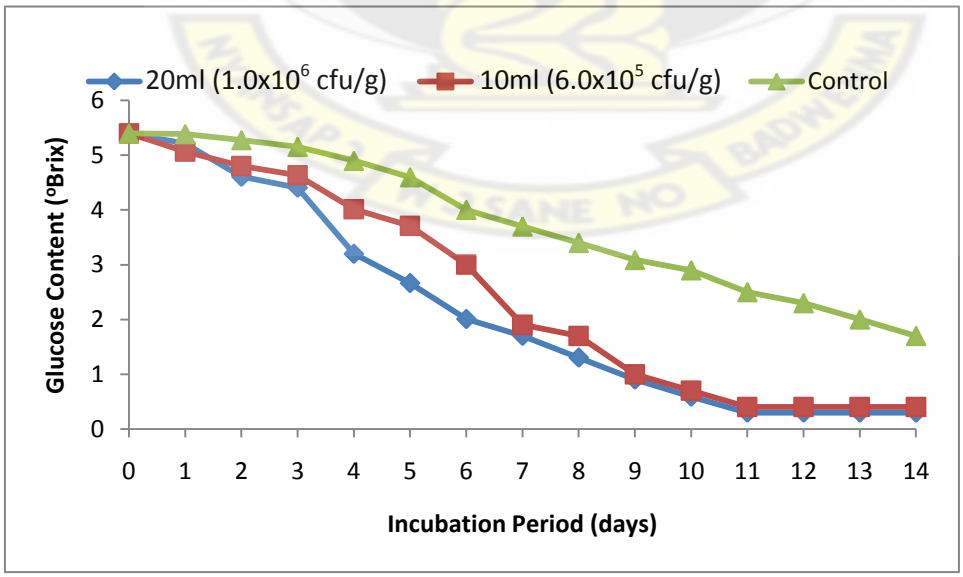

Figure 4.4: Effect of yeast concentration on glucose content of pineapple must

Table 4.3: Effect of yeast and concentration on glucose content of must

	Yeast concentration (cfu/g)			
	20ml (1.0×10^6 cfu/g)	10ml (6.0×10^5 cfu/g)	Control	Mean
Baker yeast	0.30	0.40	1.70	0.80
Brewer yeast	0.30	0.40	1.70	0.80
Dual Culture	0.30	0.40	1.70	0.80
Mean	0.30	0.40	1.70	

Tukey $HSD_{(0.05)}$ Yeast = 0.007

Tukey $HSD_{(0.05)}$ Concentration = 0.007

Tukey $HSD_{(0.05)}$ Yeast x Concentration = 0.016

Table 4.3 above show the effect of the yeast and its concentration on glucose content of wine produced. Glucose level of 0.8°Brix was recorded in the Yeast strains. The glucose content of the Yeast strains were the same ($P>0.05$). For the yeast concentrations, the control recorded higher glucose level of 1.7°Brix than 20ml (1.0×10^6 cfu/g) which recorded 0.3°Brix and 10ml (6.0×10^5 cfu/g) which also recorded 0.4°Brix. The three yeast concentrations were different from each other ($P<0.05$). The different yeast used at different concentration in the fermentation recorded different glucose levels but statistically, there were no difference observed between the treatments ($P>0.05$).

4.5 SUCROSE CONTENT OF PINEAPPLE WINE

The effect of yeast on sucrose content of the pineapple must is presented in Figure 4.5. There was a steady decline in sucrose content of the must from day 1 to day 14 of the incubation period. The sucrose content of the must decreased from 3.8°Brix to 0.8°Brix in the must containing the baker's yeast, brewer's yeast and dual yeast must. No significant differences were observed between the Yeast strains ($P>0.05$).

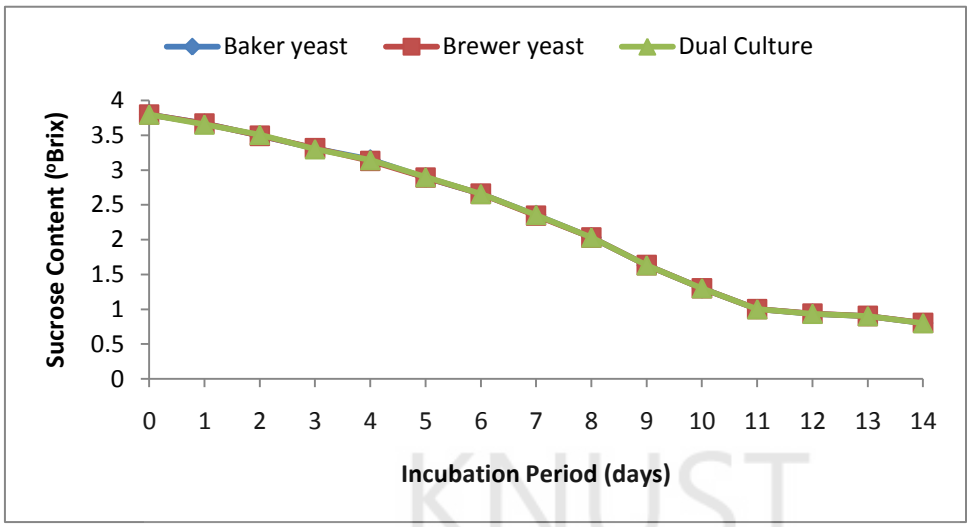

Figure 4.5: Effect of yeast on sucrose content of pineapple must

There was a mild decline in sucrose content of the pineapple must in the yeast concentrations from day 1 to day 10 and a steady decline up to day 14. A gentle decline was observed in the control over the incubation period (Figure 4.6). The glucose content of the must containing 20ml (1.0×10^6cfu/g) and 10ml (6.0×10^5cfu/g) of yeast decreased from 3.8°Brix to 0.4°Brix respectively. The control (must without yeast) also showed a gradual decrease in sucrose content from 3.8°Brix to 1.6°Brix. Significant differences were observed between the yeast concentrations and the control ($P<0.05$).

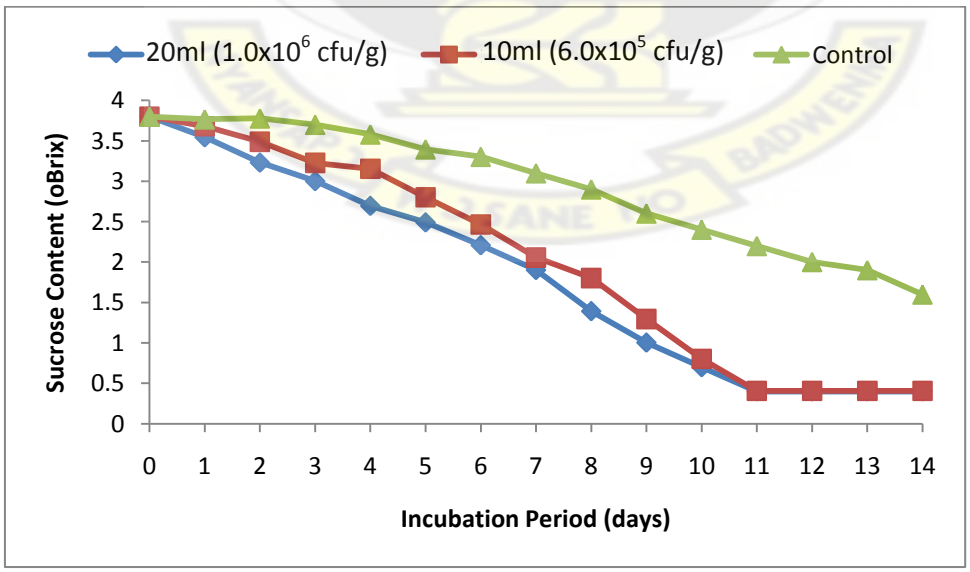

Figure 4.6: Effect of yeast concentration on sucrose content of pineapple must

Table 4.4: Effect of yeast types and their concentration on sucrose content of must

	Yeast types and their concentration			
	20ml (1.0×10^{6} cfu/g)	10ml (6.0×10^{5}cfu/g)	Control	Mean
Baker yeast	0.40	0.41	1.60	0.80
Brewer yeast	0.40	0.41	1.60	0.80
Dual Culture	0.40	0.40	1.60	0.80
Mean	0.40	0.41	1.60	

Tukey HSD $_{(0.05)}$ Yeast = 0.007

Tukey HSD $_{(0.05)}$ Concentration = 0.007

Tukey HSD $_{(0.05)}$ Yeast x Concentration = 0.016

The effect of the yeast and its concentration on sucrose content of wine produced is presented in Table 4.4. Sucrose level in the Yeast strains recorded was 0.8^{o}Brix. Thus the sucrose content were the same (P>0.05). For the different yeast concentrations, the control recorded higher sucrose level of 1.60^{o}Brix than 20ml (1.0×10^{6} cfu/g) and 10ml (6.0×10^{5} cfu/g) which recorded 0.4^{o}Brix respectively. The three yeast concentrations were different from each other (P<0.05). The different yeast used at different concentration in the production wine were different from each other and statistically different (P>0.05).

4.6 FRUCTOSE CONTENT OF PINEAPPLE WINE

The effect of yeast on fructose content of pineapple must is shown in Figure 4.7. There was a gradual decline in fructose content of the pineapple must from day 1 to day 14 of the incubation period. The fructose content of the must decreased from 4.7^{o}Brix to 1.1^{o}Brix in the baker's yeast, brewer's yeast and dual yeast must. However, there was no significant differences observed between the Yeast strains (P>0.05).

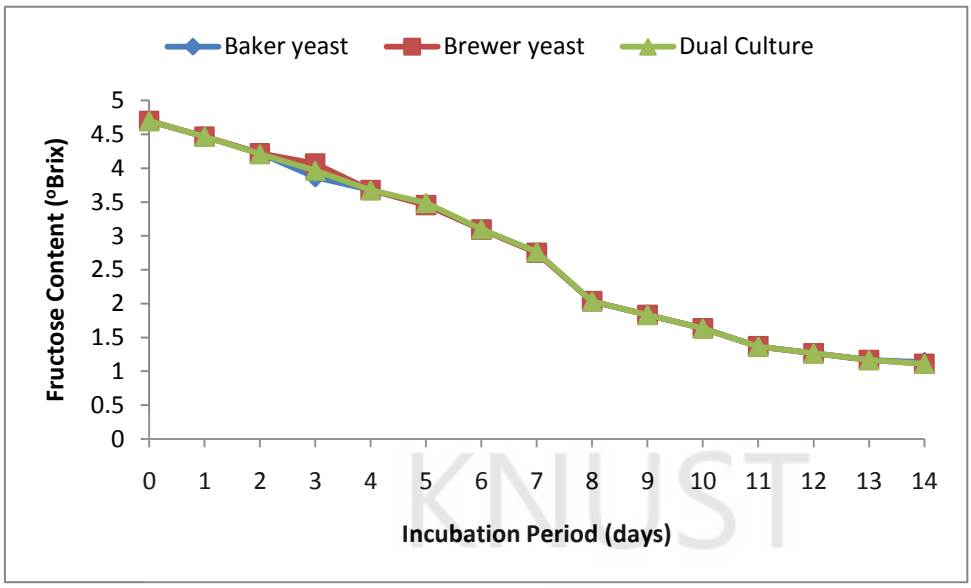

Figure 4.7: Effect of yeast concentration on fructose content of pineapple must

There was a steady decline in fructose content in the must with different yeast concentrations from day 1 to day 14 of the incubation period (Figure 4.8). The fructose content of the must containing 20ml (1.0×10^6cfu/g) and 10ml (6.0×10^5cfu/g) of yeast decreased from 4.7°Brix to 0.6°Brix respectively. However, the fructose content in the control must decrease from 4.7 °Brix to 2.2°Brix. Significant differences were observed between the yeast concentrations and the control ($P<0.05$).

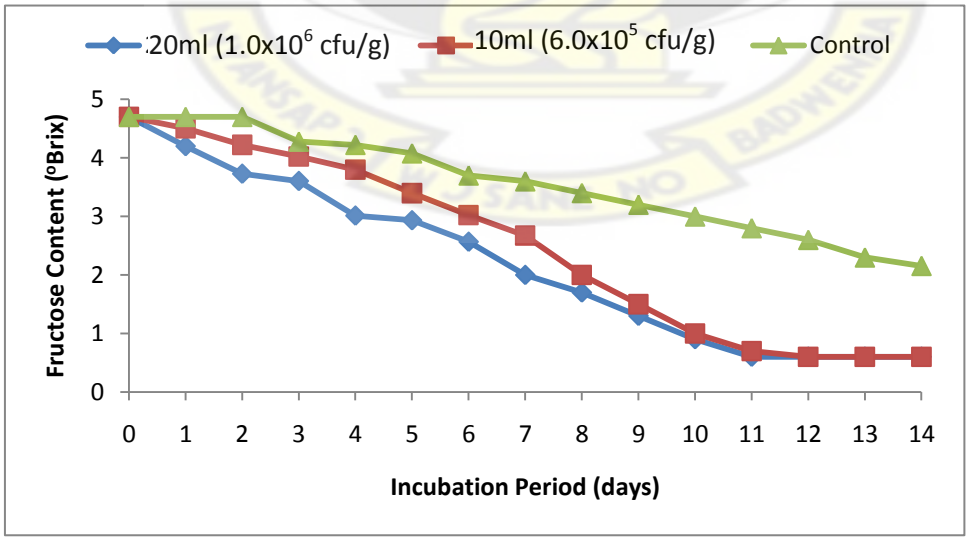

Figure 4.8: Effect of yeast concentration on fructose content of pineapple must

Table 4.5: Effect of yeast and concentration on fructose content of must

	Yeast concentration (cfu/g)			
	20ml (1.0×10^6 cfu/g)	10ml (6.0×10^5 cfu/g)	Control	Mean
Baker yeast	0.60	0.60	2.20	1.13
Brewer yeast	0.60	0.60	2.13	1.11
Dual Culture	0.60	0.60	2.13	1.11
Mean	0.60	0.60	2.15	

Tukey $HSD_{(0.05)}$ Yeast = 0.069

Tukey $HSD_{(0.05)}$ Concentration = 0.069

Tukey $HSD_{(0.05)}$ Yeast x Concentration = 0.164

Table 4.5 above show the effect of the yeast and its concentration on fructose content of wine produced. The fructose level of 1.1^{o}Brix was recorded by the Yeast strains but were not significantly different from each other ($P>0.05$). The control for the yeast concentrations used recorded a higher fructose level of 2.2^{o}Brix than 20ml (1.0×10^6 cfu/g) and 10ml (6.0×10^5 cfu/g) which recorded 0.6^{o}Brix each respectively. The fructose content of the three yeast concentrations were different from each other ($P<0.05$). The different yeast used at different concentration in the fermentation were not statistically significant ($P>0.05$).

4.7 TOTAL SUGAR CONTENT OF PINEAPPLE WINE

There was a gradual decline in total sugar content of the pineapple must from day 1 to day 14 of the incubation period (Figure 4.9). The total sugar content of the must decreased from 17.3^{o}Brix to 6.6^{o}Brix in the baker's yeast and dual yeast must and to 6.5 in the brewer's yeast. However, there was no significant differences observed between the Yeast strains ($P>0.05$).

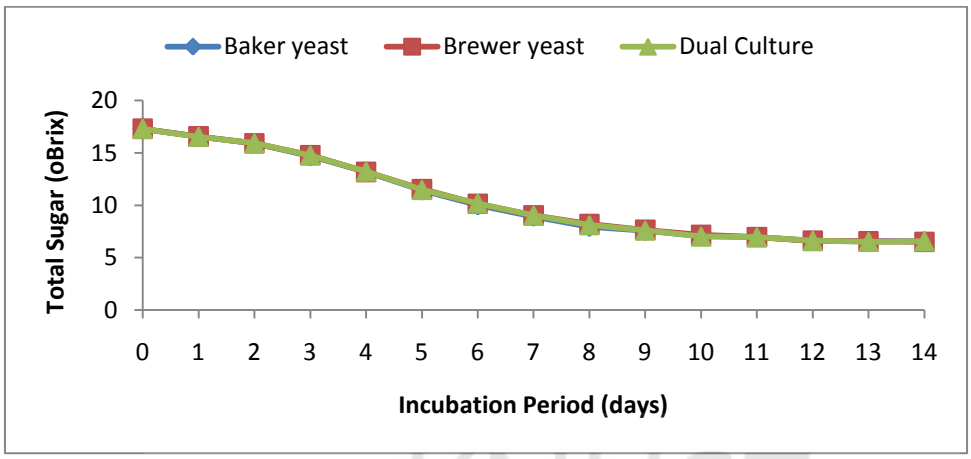

Figure 4.9: Effect of yeast on total sugar content of pineapple must

The different yeast concentrations showed a gradual decline in total sugar content of the pineapple must from day 1 to day 14 of the incubation period (Figure 4.10). The total sugar content of the must containing 20ml (1.0×10^6cfu/g) of yeast decreased from 17.3^{o}Brix to 5.0^{o}Brix, in the must containing 10ml (6.0×10^5cfu/g) of yeast there was also a decrease from 17.3^{o}Brix to5.3^{o}Brix. The control (must without yeast) also showed a gradual decrease in glucose content from 17.3^{o}Brix to 9.3^{o}Brix. Significant differences were observed between the yeast concentrations and the control ($P<0.05$).

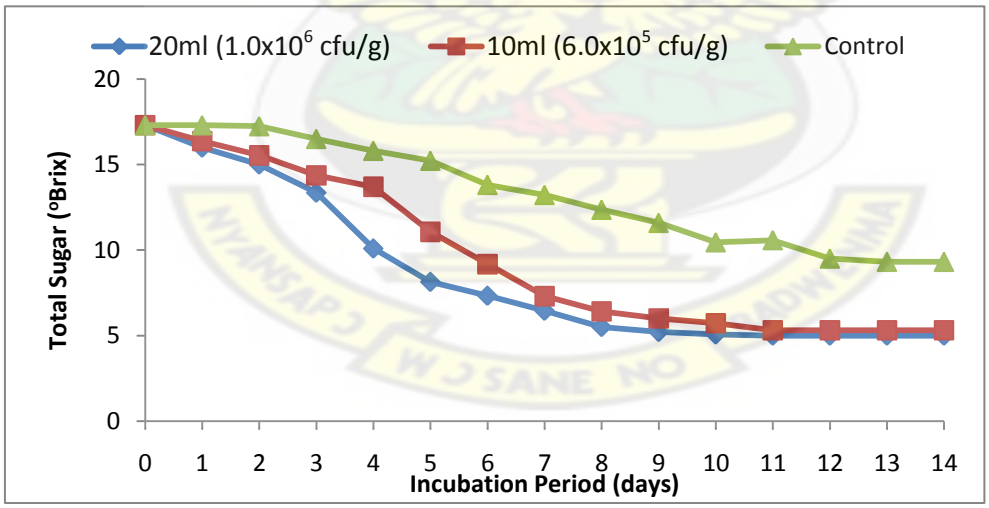

Figure 4.10: Effect of yeast concentration on total sugar content of pineapple must

Table 4.6: Effect of yeast types and their concentration on total sugar content of must

	Yeast concentration (cfu/g)			
	20ml (1.0×10^{6} cfu/g)	10ml (5.0×10^{5} cfu/g)	Control	Mean
Baker yeast	5.01	5.29	9.37	6.56
Brewer yeast	5.00	5.33	9.20	6.51
Dual Culture	5.00	5.33	9.37	6.57
Mean	5.00	5.32	9.31	

Tukey $HSD_{(0.05)}$ Yeast = 0.322

Tukey $HSD_{(0.05)}$ Concentration = 0.322

Tukey $HSD_{(0.05)}$ Yeast x Concentration = 0.770

The total sugar content of the yeast used was 6.5^{o}Brix respectively. The total sugar content of the wine were the same for the three Yeast strains ($P>0.05$). For the different yeast concentrations used, the control recorded higher sugar level (9.31^{o}Brix) than 20ml (1.0×10^{6} cfu/g) and 10ml (6.0×10^{5} cfu/g) which recorded 0.5^{o}Brix respectively. The three yeast concentrations were different from each other ($P<0.05$). The yeast at different concentration were not different from each other ($P>0.05$) as shown in Table 4.6.

4.8 ALCOHOL CONTENT OF PINEAPPLE WINE

The effect of yeast on alcohol content of pineapple must is shown in Figure 4.11. There was a gradual increase in alcohol content of the pineapple must from day 1 to day 10 and a decline in day 11 with a linear increase from day 11 to day 14 of the incubation period. The alcohol content of the must increased from 0% to 7.0% 10days after incubation and a decrease to 6.8 14days after incubation in the baker's yeast, brewer's yeast and dual yeast must. There was no significant differences observed between the Yeast strains ($P>0.05$).

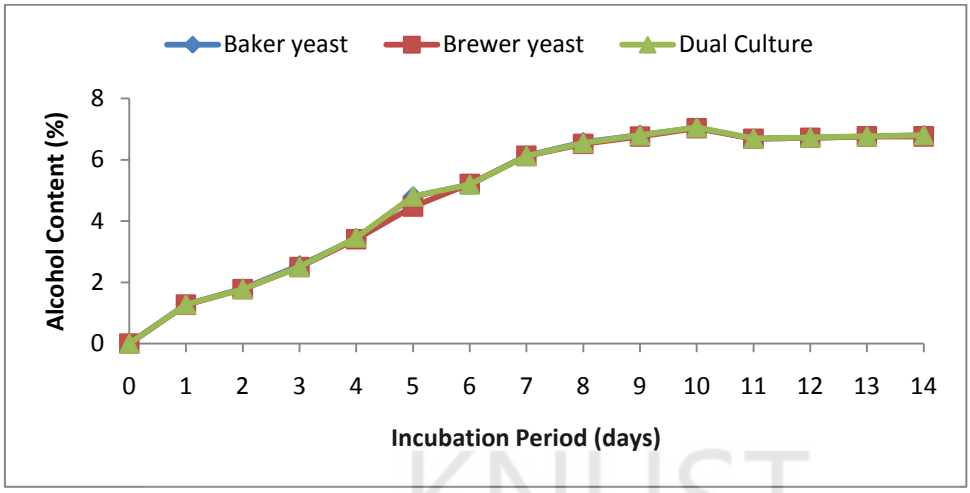

Figure 4.11: Effect of yeast on alcohol content of pineapple must

The different yeast concentrations showed a sharp rise in the alcohol content of the pineapple must from day 1 to day 10 and a drop in day 11 with a linear increase from day 11 to day 14 of the incubation period (Figure 4.12). The control however showed a gradual increase in alcohol content during the incubation period. The alcohol content of the must containing 20ml (1.0×10^{6}cfu/g) of yeast increased from 0% to 8.5% 10days after incubation and then decreased to 7.6% 14days after incubation. The must containing 10ml (6.0×10^{5}cfu/g) had an alcohol content of 0% to 8.2% 10days after incubation and then declined to 7.45 14days after incubation. The control (must without yeast) also showed a gradual increase in alcohol content from 0% to 5.3% 14days after incubation. Significant differences were observed between the yeast concentrations and the control ($P<0.05$).

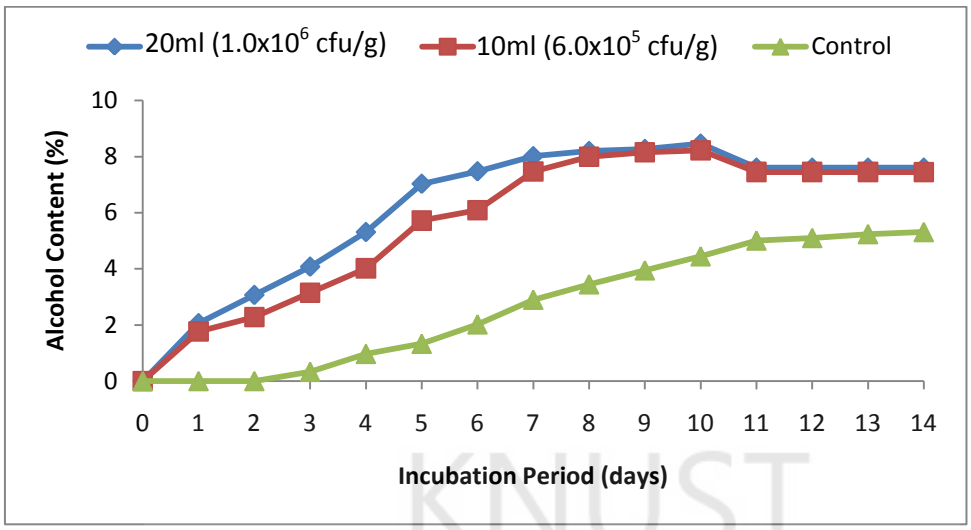

Figure 4.12: Effect of yeast concentration on alcohol content of pineapple must

Table 4.7: Effect of yeast types and their concentration on alcohol content of must

	Yeast concentration (cfu/g)			
	20ml (1.0×10^{6} cfu/g)	10ml (5.0×10^{5} cfu/g)	Control	Mean
Baker yeast	7.60	7.45	5.35	6.80
Brewer yeast	7.60	7.45	5.24	6.76
Dual Culture	7.61	7.45	5.35	6.80
Mean	7.60	7.45	5.31	

Tukey $HSD_{(0.05)}$	Yeast	= 0.002
Tukey $HSD_{(0.05)}$	Concentration	= 0.002
Tukey $HSD_{(0.05)}$	Yeast x Concentration	= 0.006

The effect of the yeast and its concentration on alcohol content of the wine is presented in Table 4.7. Alcohol level of 6.80% and 6.80% for both baker yeast and dual culture were statistically significant from the alcohol content of 6.76% for the brewer yeast ($P<0.05$). The yeast concentrations also recorded alcohol content of 7.6% at 20ml (1.0×10^{6} cfu/g), 7.5% at 10ml (6.0×10^{5} cfu/g) with the control recording the lowest of 5.3%. Statistical

differences existed between the concentration (P<0.05). The yeast at different concentration also recorded significant alcohol contents (P<0.05).

4.9 pH OF PINEAPPLE WINE

The effect of yeast on pH of pineapple must is shown in Figure 4.13 below. There was a drop in pH a day after incubation then a gradual decrease from day 2 to day 14 of the incubation period. The pH of the must decrease from 4.82 to 3.85 14days after incubation in the baker's yeast, brewer's yeast and dual yeast must. There were significant differences observed between the Yeast strains (P>0.05).

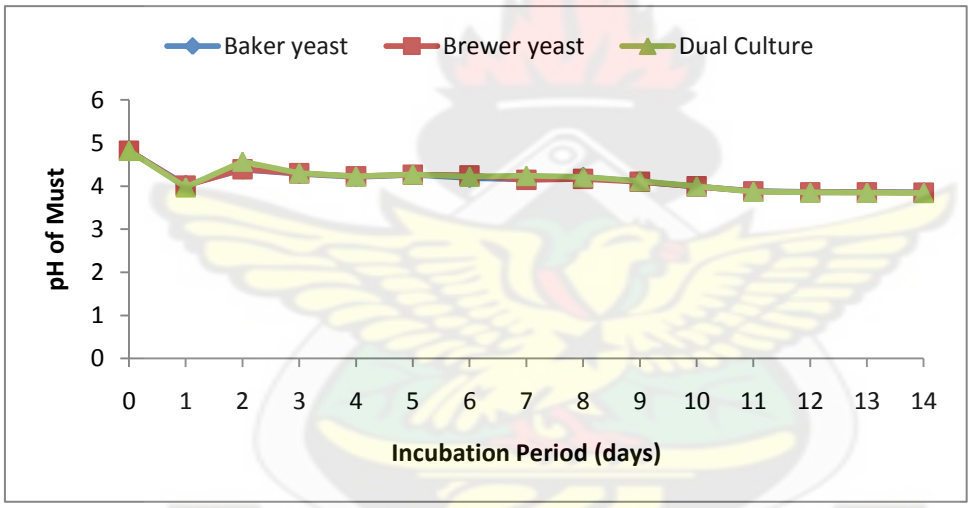

Figure 4.13: Effect of yeast on pH of pineapple must

The different yeast concentrations showed a drop in pH a day after incubation then a gradual decrease in pH 14 days after incubation period (Figure 4.14). The control however showed a gradual decrease in pH during the incubation period. The pH of the must containing 20ml (1.0×10^{6}cfu/g) and 10ml (6.0×10^{5}cfu/g) of yeast, both decreased from 4.8 to 3.8 14days after incubation. The control also showed a gradual decrease in pH from 4.8 to 3.9 14days after incubation. Significant differences were observed between the yeast concentrations and the control (P<0.05).

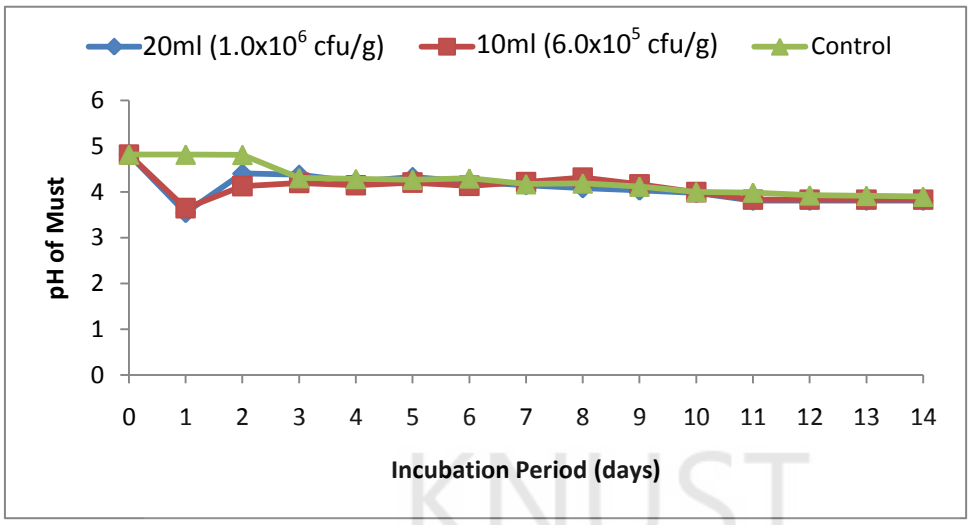

Figure 4.14: Effect of yeast concentration on pH of pineapple must

Table 4.8: Effect of yeast and concentration on pH of must

	Yeast concentration (cfu/g)			
	20ml (1.0×10^{6}cfu/g)	10ml (5.0×10^{5}cfu/g)	Control	Mean
Baker yeast	3.82	3.84	3.90	3.85
Brewer yeast	3.80	3.84	3.90	3.85
Dual Culture	3.81	3.82	3.90	3.84
Mean	3.81	3.83	3.90	

Tukey $HSD_{(0.05)}$	Yeast	= 0.000
Tukey $HSD_{(0.05)}$	Concentration	= 0.000
Tukey $HSD_{(0.05)}$	Yeast x Concentration	= 0.000

Table 4.8 depicts the effect of the yeast and its concentration on pH of the wine. pH of 3.85 and 3.65 for baker yeast and brewer yeast respectively were statistically significant from pH of 3.84 for the dual culture ($P<0.05$). The yeast concentrations also recorded pH value of 3.90 in the control, 3.81 in 20ml (1.0×10^{6} cfu/g) and 3.83 in 10ml (6.0×10^{5} cfu/g). Statistical differences existed between the concentration ($P<0.05$). The yeast at different concentration also recorded significant pH value ($P<0.05$).

4.10 TITRATABLE ACIDITY OF PINEAPPLE WINE

The effect of yeast on titratable acidity of pineapple must is shown in Figure 4.15 below. There was a gradual increase in total titratable acidity of the pineapple must from day 1 to day 14 of the incubation period. The total titratable acidity of the must increased from 0.41 to 0.8% 14days after incubation in the baker's yeast, brewer's yeast and dual yeast must. There were significant differences observed between the Yeast strains (P>0.05).

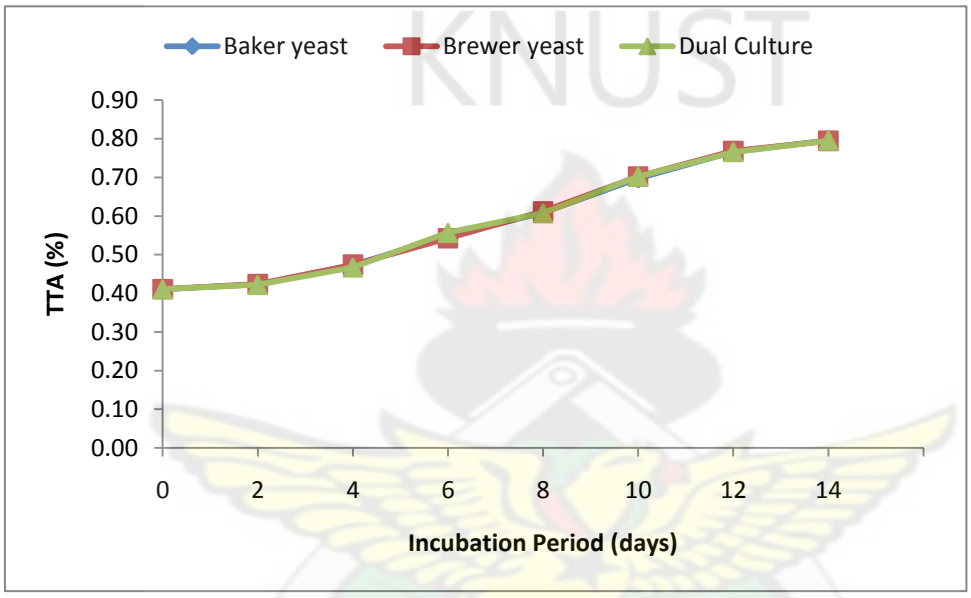

Figure 4.15: Effect of yeast on total titratable acidity of pineapple must

The different yeast concentrations showed a gradual rise in the total titratable acidity of the pineapple must from day 1 to day 14 of the incubation period (Figure 4.16). The total titratable acidity of the must increased from 0.4 to 0.8% 14days after incubation. Significant differences were observed between the yeast concentrations and the control (P<0.05).

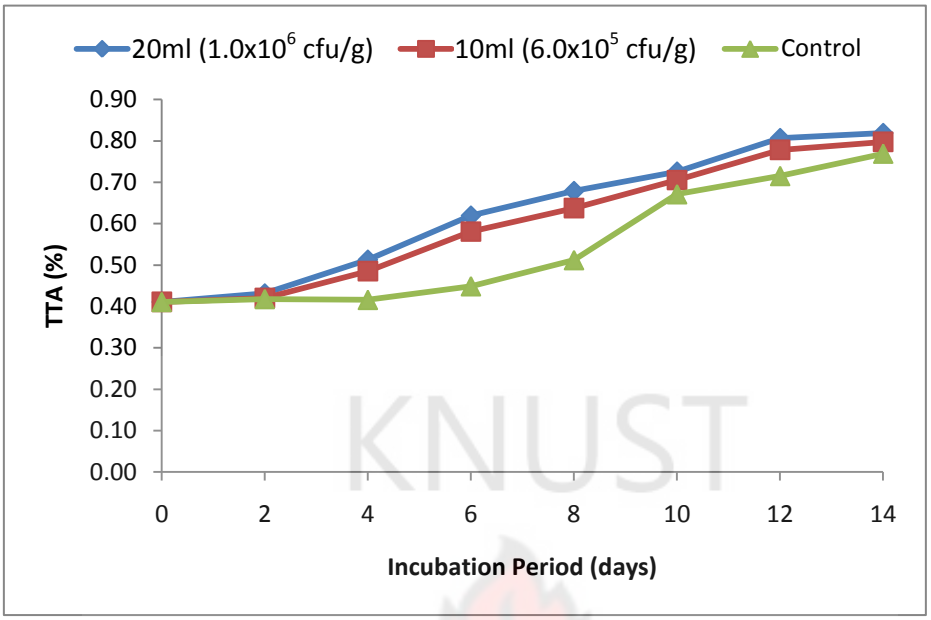

Figure 4.16: Effect of yeast concentration on total titratable acidity of pineapple must

Table 4.9: Effect of yeast and concentration on total titratable acidity of must

	Yeast concentration (cfu/g)			
	20ml (1.0×10^{6} cfu/g)	10ml (5.0×10^{5} cfu/g)	(Control)	Mean
Baker yeast	0.82	0.80	0.77	0.80
Brewer yeast	0.82	0.80	0.77	0.80
Dual Culture	0.82	0.80	0.77	0.80
Mean	0.82	0.80	0.77	

Tukey $HSD_{(0.05)}$	Yeast	= 0.002
Tukey $HSD_{(0.05)}$	Concentration	= 0.002
Tukey $HSD_{(0.05)}$	Yeast x Concentration	= 0.006

The effect of the yeast and its concentration on total titratable acidity of the wine is presented in Table 4.9. Total titratable acidity of 0.8 was recorded for baker yeast, brewer yeast and the dual culture respectively. Statistically no significant differences existed between the Yeast strains ($P<0.05$). The yeast concentrations also recorded total titratable

acidity of 0.82% at 20ml (1.0×10^6 cfu/g), 0.80% at 10ml (6.0×10^5 cfu/g) with the control recording the lowest of 0.77. Statistical differences existed between the concentrations ($P<0.05$). The yeast at different concentration were not significantly different ($P>0.05$).

4.11 CONSUMERS ACCEPTABILITY OF PINEAPPLE WINE

Table 4.10: Consumers acceptability of pineapple wine

Number or name	Baker yeast			Brewer yeast			Dual yeast			COMMERCIAL
	20ml	10ml	C	20ml	10ml	C	20ml	10ml	C	
Appearance	2	2	1	2	1	1	2	2	1	2
Colour	2	2	2	2	2	2	2	2	2	2
Aroma or Bouquet	2	2	2	2	3	2	2	2	2	2
Ace scent	2	1	1	1	1	1	1	1	1	1
Total Acid	1	1	1	1	1	1	1	2	1	1
Total Sugar	1	1	1	1	1	1	1	1	1	1
Body	1	1	1	1	1	1	1	1	1	1
Flavour	1	1	1	2	2	1	1	1	1	2
Astringency	1	2	1	1	1	1	1	1	1	1
General Quality	1	1	1	1	1	1	1	1	1	2
Total	15	14	13	15	15	13	15	14	13	14

Scale: 17-20- outstanding, 13-16- standard wine, 9-12- commercial wine, 5-8- below commercial wine, 1-4- spoiled wine

Sensory evaluation of the wine produced from the different yeast combinations and concentration are presented in Table 4.10. From the table, wine produced from the Yeast strains at different concentrations had a scored of 14 to 15 which implies that the wine was in the category of a standard wine. Wine from the control treatments had a score of 13 which implied that it also fell in the category of standard wine

CHAPTER FIVE

5.0 DISCUSSIONS

5.1 NUTRITIONAL COMPOSITION OF PINEAPPLE JUICE AND WINE

The nutritional composition of the pineapple juice showed that fresh juice was high in sugar than the wine produced at different yeast concentrations. Titratable acid (citric acid) content of the wine produced was relatively high than the fresh juice. The pH of the wine was more acidic than the fresh pineapple juice. The alcohol content of the wine produced was relative mild. The high citric acid content, low pH and mild alcohol content gives the wine its characteristic sour taste in the mouth. The wine also had appreciable amount of vitamin C compared to the high levels in the fresh juice. The fresh pineapple juice after fermentation retained most of its nutrients although the levels were lower in the wine produced. The observed trend was due to the fact that as the nutrients available in the fresh juice were utilised, alcohol an end product of fermentation was produced. Au Du (2010) in his study of cashew juice fermentation reported that as pH, total sugar, specific gravity decreased, titratable acidity and alcohol levels increased.

5.2 MICROORGANISMS PRESENT IN PINEAPPLE JUICE AND WINE

The main bacteria contaminants isolated in the fresh pineapple juice included *Lactobacillus sp., Gluconobacta sp., Streptococcus sp.* and *Leuconostoc sp.* Fungal or wild yeast contaminants also isolated included *Sacharomyces sp. Candidda sp.* and *Aspergillus sp.* After sterilization of the juice, the microorganisms isolated were all destroyed with the sterilization process. Au Du (2010), Robinson (2006) and Fleet (1998) reported that increasing the must temperature above 60^{o}C and the addition of Potassium Meta-bisulphate

may kill or destroy or inhibit the growth of wild yeast, acetic acid bacteria, wild yeast and moulds present in wine.

Aeration of the control treatment for 24 hours probably accounted for the traces of *Aspergillus sp., Gluconobacta sp.* and *Streptococcus sp.* Adams and Moss (1995) and Walker (1988) reported that bacteria, moulds, yeasts and yeast-like fungi were widely distributed in nature and may be present in orchards, vineyards, in the air or the soil. Also, Kawo and Abdulmumin (2009), in their work on microbiological quality of re-packaged sweets sold in Nigeria identified specific bacteria and fungi present in the air that may enter the re-packed sweets.

The wine produced from the different concentration of yeast had no contaminant (bacteria or mould) as was seen in the fresh juice and the control. *S. cerevisiae* and *S. cerevisiae var ellipsoideus* used in the fermentation process inhibited the growth of any wild microbe. Bacteria and mould that might have entered the fermenting must during the period of incubation and ageing were destroyed by the acidic conditions created by the inoculated yeast types during fermentation period, thus enhancing the growth and rapid multiplication of the inoculated yeast. Keller (2010) and Dittmer and Desmond (2005), reported that *Saccharomyces* species were the most common Yeast strains used in fermenting alcoholic beverages. They further reported that ale yeast (*S. cerevisiae*) and lager yeast (*S. uvarum*) were used in making beer whilst *S. cerevisiae var ellipsoideus* and *S. cerevisiae* were used in wine production. Panjai *et al*. (2009), working on dual culture used in wine production reported that *Torulaspora. delbrueckii* and *S. cerevisiae* were used in producing a complex fruit wine from pineapple.

5.3 AMBIENT AND MUST TEMPERATURE

Temperature played an important role during the fermentation process. There was a general rise and fall in the ambient temperature as well as the must temperatures. The temperature range was very conducive for the growth and other microbiological and enzymatic activities of the yeast. Robinson (2006), Fleet (1998) and Mountney and Gould (1988) reported that yeasts perform best within an optimum temperature range of 20^{o}C to 30^{o}C. They further stated that higher temperatures during fermentation may have adverse effect on the wine in stunning the yeast to inactivity and even "boiling off" some of the flavours of the wines. Keller (2010) in his work on fermentation stated that temperature range of 20^{o}C to 30^{o}C favour red wine fermentation whilst a temperature range of 10^{o}C to 20^{o}C was conducive for fermenting white wine. By definition a white wine is a wine produced by the extraction of only the juice for fermentation. Wimalsiri *et al*. (1971) in their research work on fruits wine production stated that in wine-making, the temperature must not exceed 29.4^{o}C for red wines or 15.3^{o}C for (white wines), otherwise the growth of yeast cells will stop.

5.4 EFFECT OF YEAST ON SUGAR CONTENT OF THE WINE

In general, there was a substantive reduction in the soluble solids available in pineapple juice. Glucose, sucrose, fructose and total sugar showed a gradual reduction with the different yeast and their concentrations during the incubation period. This conforms to the assertion by Van Rooyen and Tomp (1982) and Sulz (2011), working on fruit wine production, stated that as fermentation time increases, more sugars are digested. Also Steinkraus (1992), working on wine production from *Agave americana* stated that during fermentation of the juices of the plant (*Agave americana*), the sucrose content fell from

15% to 1%. According to FAO (2010), higher sugar concentration between 25-30% inhibits the growth of micro organisms and must be reduced to 5% for efficient fermentation.

Keller (2010), reported that reducing the fermentation time produced sweet wine high in fructose, a natural sugar often used in sweetening wine after fermentation. Robinson (2006), therefore concluded that winemaker who wishes to make wines high in residual sugar like dessert wine may have to stop fermentation early either by dropping the temperature of the must to stun the yeast or by adding a high level of alcohol like brandy to the must to kill off the yeast and create a fortified wine.

According to Keller (2010), a wine maker uses baker's yeast (*S. cerevisiae*) and brewer's yeast (*S. cerevisiae var ellipsoideus*) because they are highly efficient in reducing sugar over the fermentation period. Ostergaard *et al*., (2000) also reported that yeasts were the common micro organisms used in fermentation of beer and wine. Au Du (2010) reported that there was selective utilisation of sugar by wine yeasts in cashew fruit juice fermentation. However, Steinkraus (1996), reported that pH, temperature and yeast nutrients were major factors that affect the optimum growth and efficient sugar utilisation by the yeast.

5.5 ALCOHOL CONTENT OF PINEAPPLE WINE

The result of the analysis revealed that baker's yeast (*S. cerevisiae*), brewer's yeast (*S. carevisiae var ellipsoideus*) and the dual culture were very effective in the conversion of sugar to alcohol. In general, there was a gradual increase in alcohol content among the treatments from the start of fermentation to the time of termination of fermentation. The rise in alcohol content of the wine could be attributed to the ability of the yeast to utilize sugar in the presence of favourable must condition such as favourable must temperature,

sufficient quantity of sugar, right acids and pH, absence of wild yeast and microbes. The differences observed among the yeast type and yeast concentration could be due to the fact that the two yeasts were from the same genera though different species and their concentration were above the minimum requirement for the fermentation. Perhaps this enabled the yeast thrive best in similar conditions and performed equally. Panjai, *et al.* (2009) working on two different culture; *T. delbrueckii* species and *S. cerevisiae* observed difference in performance in the production of alcohol.

According to Keller (2010) internal temperature of the must is very critical during fermentation. Though fermentation is an exothermic process and must releases heat, the temperature must not exceed 29.4^{o}C for red wines or 15.3^{o}C for (white wines), otherwise the growth of yeast cells will stop. Odunfa (1985) working on palm wine indicated that during alcoholic fermentation, acid and pH level are critical for yeast growth and activities. Furthermore, the gradual increase in alcohol content may be due to the absence of wild microbes such as lactobacilli that causes homolactic fermentation and certain fungal species that utilize alcohol to produce acids (Dickinson, 1999 and Axelsson, 1998).

The linear level in alcohol production at the tail end of fermentation indicates an inhibition in fermentation attributable to high alcohol content or low pH that slowed activities of the yeasts although Mountney and Gould (1988) argued that yeast can grow in a wide pH range. Also, Fleet (1998) suggested that undue rise in temperature might kill the yeast cells. However, Dickinson (1999) concluded that as long as sugar is present in the must, yeast cells will continue the process of fermentation.

5.6 pH OF PINEAPPLE WINE

The pH of the wine produced from the pineapple juice was slightly acidic for the Yeast strains used and the different yeast concentration. According to Fleet (1998) pH directly affects wine stability. This may be due to the fact that at a pH close to neutral (7.0) most micro organisms like bacteria and moulds including some yeasts become more active for fermentation and subsequent spoilage of wine, whilst pH below 3.5 eliminates most of the microbes, and favours only few of the micro organisms for fermentation. Mountney and Gould (1988) reported that yeasts can grow in a pH range of 4 to 4.5 and moulds in a pH range of 2 to 8.5. According to Amerine and Ough (1980), a wine at a pH of 3.0 is more acidic than a wine at a pH of 4.0. Steinkraus (1996) working on the traditional process of fermentation reported that during fermentation, the pH of the fruit juice is adjusted to pH of 4.0 using sodium bicarbonate and yeast nutrient (ammonium phosphate) at 0.14g per litre to enhance the performance of the yeast.

5.7 TITRATABLE ACIDITY OF PINEAPPLE WINE

The titratable acidity of the wine produced increased with fermentation period. Similar patterns from the yeast strains and the different yeast concentrations also showed a gradual increase in titratable acidity over time. Acid is known to directly affect wine quality. According to Fleet (1998) wine acid composition is made up of citric acid, tartaric acid, and some traces of acids such as lactic acid which replaces malic acid during malo-lactic fermentation. Fruits acids are usually weak acids compared to strong mineral acids. Wines with higher concentration of malic acid have lower pH (3.0 to 3.5) and the taste benefits from this slight decrease in acidity. On the other hand, wines with less acidity (pH > 3.5) may have an adverse effect on the quality of the wine. Decreasing the acidity also increases

the pH which allows spoilage organisms to undergo malo-lactic fermentation (Fleet, 1998). Odunfa (1985) reported that fermentation of palm sap within 24 hours reduced pH from 7.4 to 5.5 and the alcohol content ranged from 1.5% to 2.1%. He further reported that within 72 hours, the alcohol levels increased from 4.5% to 5.2% with a pH of 4.0.

Also, Steinkraus (1996) concluded that during fermentation, the pH of the wine reaches a value of 3.5 to 3.8, suggesting that an acidic fermentation takes place at the same time as the alcoholic fermentation resulting in an alcohol content of about 7 to 8% within a fortnight. Garrison (1993) concluded that most fruit juices have all the acids needs by themselves. Grape juice contains tannins and sugars, other fruit juices may need acids for flavour. Acids presence in wine enhances the taste, aroma and preservative properties of the wine (Wimalsiri et al, 1971).

5.8 CONSUMERS ACCEPTABILITY OF PINEAPPLE WINE

Generally, the wine produced from the pineapple juice was highly accepted by the 10 panellists. In order to assess the general acceptability of the produced wine, it was compared to an already existing commercial wine. The views of 10 panellists were sought by using the Davis 20 point scale. The results basically revealed no difference, which means that wine produced from pineapple juice may be commercially accepted. This may also imply that the process of ageing in the bottle, fining and racking the wine every two weeks for the three months period enhanced the quality of the wine. This is in agreement with several reports that during ageing, and subsequent maturing in bottles, woods, and other containers, many reactions, including oxidation, occur with the formation of traces of esters and aldehydes, which together with the tannin and acids already present enhance the

taste, aroma and preservative properties of the wine (Rotter, 2008: Wimalsiri *et al.*, 1971 and Au Du, 2010).

This may also mean that naturally pineapple contains enough fining agents that enhanced the quality and clarity of the wine. Rotter (2008), Akhimien *et al*., (1987), Izuagbe, (1982), and Wimalsiri *et al.*, (1971) reported that most fining and clearing agents such as Earths: bentonite, kaolin, Proteins: gelatine, isinglass, casein, pasteurised milk, albumen, yeast, Polysaccharides: alginate (agar), gum arabic (acacia), Carbons, Synthetic polymers: PVPP, silica gel, Tannins, Others: metal chelators, blue fining, enzymes are more effective in clearing the wine when pH is below 3.5. Also the wine produced using the three yeasts were exactly the same as the commercial wine.

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 SUMMARY

An experiment was conducted to study the effect of baker's yeast (*Saccharomyces cerevisiae*) brewer's yeast (*Saccharomyces cerevisiae var. ellipsoideus*) and dual culture (*Saccharomyces cerevisiae var. ellipsoideus* and *Saccharomyces cerevisiae)* on the fermentation of pineapple juice into wine. The study revealed that the sugar levels in the wine produced decreased with time at different concentration levels. The nutritional composition of the fresh pineapple juice and the wine produced showed that the sugar content of the fresh pineapple juice was higher than that of the wine. Titratable acidity (citric acid) of the wine was higher than the fresh juice. The vitamin C content of the fresh juice was higher than the wine. Reducing and non-reducing sugars, protein, vitamin A and B, malic and lactic acids were all present in the fresh juice as well as the wine. Alcohol content of the wine was also higher than the fresh juice. Methanol was however absent in both the fresh juice and wine.

The microbial analysis of the fresh pineapple juice revealed that yeast species (*Sacharomyces sp* and *Candidda sp*.), mould organisms (*Aspergillus sp.*) and bacteria species (*Lactobacillus sp, Gluconobacta sp. Streptococcus sp*. and *Leuconostoc sp.*) were present but were eliminated when the juice was sterilized. The bacteria *Gluconobacta oxydans* and *Streptococcus sp*, and traces of mould (*Aspergillus sp.*) were found in the wine from the control. However, the baker and brewer yeast used were successfully recovered from the individual wine produced. The room and must temperature showed a similar pattern with the fermentation room temperature ranging between 25.6^{o}C to 28.2^{o}C and the must temperature ranged between 20.1^{o}C to 29^{o}C.

The glucose content of the pineapple must gradually decreased in the baker's yeast, brewer's yeast and dual yeast 14 days after the incubation period. The different yeast concentrations also showed similar trend 14 days after fermentation. The glucose content of the wine was high in the control treatments than in the yeasts at different concentration.

The sucrose content of the pineapple must also decreased during fermentation in the baker's yeast, brewer's yeast and dual yeast. The yeast concentrations also showed a similar trend in sucrose reduction during fermentation. The sucrose content of the wine was high in the controls treatments than in the yeasts at different concentration.

Fructose content of pineapple must reduced in the baker's yeast, brewer's yeast and dual yeast must during fermentation. The different yeast concentrations also recorded reduction of fructose during the incubation period. Again yeast at both concentrations had lower fructose than the control.

Total sugar content of the pineapple must decreased during the incubation period in the baker's yeast, brewer's yeast and dual yeast. In all the instances the treatments with higher yeast concentration performed better than those with lower or no yeast concentrations in utilising total sugar content during fermentation. The total sugar content of the wine was high in the controls and lower in the treatments with higher yeast concentrations.

The alcohol content of pineapple must increased during fermentation in the baker's yeast, brewer's yeast and dual yeast treatments. Differences in all the yeast strains and the control were statistically significant. The treatment with higher yeast concentration was efficient in utilising sugar to produce alcohol than those with lower or no yeast concentrations. The

different yeast concentrations showed a sharp rise in alcohol content of the pineapple must from day 1 to day 14. The alcohol content of the wine produced from the dual yeast was not statistically different from the baker and the brewer yeast, but produced a distinct wine flavour and taste characteristics far above the individual yeast concentration and the control treatments.

The pH of pineapple must showed gradual decrease 14 days after fermentation in the yeast treatments used. The different yeast concentrations exhibited a similar pattern in pH 14 days after fermentation. The pH of the wine was high in the controls treatments than in the yeasts at different concentration. The pH of the wine was lower in the wine from the yeasts at different concentration than the wine from the control treatments which had higher pH values.

Titratable acidity increased in the pineapple must during fermentation in the baker's yeast, brewer's yeast and dual yeast must. The different yeast concentrations also increased in titratable acidity of the pineapple must during fermentation. Titratable acidity content of the wine was high in the yeasts at different concentration than the control treatments.

6.2 CONCLUSION

The seasonality of pineapple fruits as well as poor handling and transportation of the fruits, absence of industrial utilization of the pineapples as raw material, rejection from the international market coupled with inadequate local market conditions contribute to the high post harvest losses of pineapple in Ghana. Measures put in place to reduce this wastage were very effective. This included the extraction of pineapple juice and conversion of the

juice to wine by fermentation. These measures would greatly enhance the keeping qualities of pineapple juice wine, thus reducing wastage.

The wine produced had low sugar levels, low pH and higher acidity suitable for wine stability. This means that all the yeast strains and their concentrations performed better during the incubation period.

The alcohol content, sugar level, pH and acidity of wine produced from the various yeast types were not different from each other, but yeast types with higher concentrations produced more alcohol than those with lower concentrations.

The pineapple wine had sufficient levels of vitamins A, B and C making it nutritious, palatable and nourishing drink hence all the yeast strains had a positive effect on the musts during the fermentation.

The yeasts were very efficient in converting the natural sugar in the pineapple juice into alcohol. This also improved the flavour, colour, aroma and taste which made it highly acceptable to the panellist; and improved the keeping quality of the pineapple wine.

6.3 RECOMMENDATION

Based upon the findings the following recommendations are made to the various stake holders:

Pineapple should be fermented into wine as wine has higher demand with high economic value in the market in that pineapple wine is highly acceptable; more so, fermentation is low energy requiring and efficient in extending the keeping quality of pineapple juice.

Subsequent experiment should be carried out on using other beneficial and edible micro organisms and yeast with concentration lower than 6×10^5 cfu/g.

REFERENCES

Adams, M. R. and Moss, M. O., (1995). *Food Microbiology*. The Royal Society of Chemistry, Cambridge, UK [online]. Available from: http://www.fao.org/docrep [Accessed 10th 0ctober, 2010]

Akhimien A. N., Uriaiah and Y. S. Izuagbe (1987) Production of wine from Plantain Acta Biotech [online]. Available from: http://www.fao.org/docrep/x0560e/x0560e14.htm 8.295-298 [Accessed 10th 0ctober, 2010]

Amerine, M. A. and C. S. Ough. (1980). *Methods for Analysis of Musts and Wines*. John Wiley and Sons, New York. Pg 105

Amerine, M. A., and Roessler, E. B. (1983). *Wines: Their Sensory Evaluation*, W. H. Freeman and Co., San Francisco. Pg 432.

Appert, J. (1987). *Tropical Agriculture: The Storage of Grain and Seed.* C.T.A. Macmillan Publishers Ltd, London. Pg 145

Au Du, O. J (2010). Comparative Studies of Wine Produced by Spontaneous and Controlled Fermentation of Preserved Cashew (*Anacardium occidentale*) Juice. *Research Journal of Biological Sciences* 5 (7): Pg 460-464

Axelsson, L., (1998). Lactic Acid Bacteria: Classification and Physiology. In: *Lactic Acid Bacteria, Microbiology and functional Aspects*. Ed. S Salminen and A von Wright, Marcel Decker Inc, New York, USA [online]. Available from: http://www.fao.org/docrep/x0560e/x0560e14.htm [Accessed 10th 0ctober, 2010]

Bartolomew, D.P., and Kadzimann S.B. (1977). Ecophysiology of tropical crops. P.T. Alvin and T.T. Kozlowski [Ed]. New York. *Academic Press*. Pg 502.

Battcock, M. J. and Sue, A. (1998). Fermented Fruits and Vegetables: A Global Perspective. FAO Agricultural Services Bulletin No. 134.

Board, R. G., (1983). *A Modern Introduction to Food Microbiology*. Blackwell Scientific Publications, Oxford, UK [online]. Available from: http://www.fao.org/docrep/x0560e/ x0560e14.htm

Collins, J. L. 1960. *The pineapple*. Leonard Hill. London. Pg 294.

Collins, J.L. 1949. History, taxonomy and culture of the pineapple. *Economic Botany* 3(4): Pg 335

Coppens, G. (2001). Uses of Pineapple [online].available from: http://www.ciat.cgiar.org/ipgri/fruits_from_americas/frutalesGeo.[Accessed November, 2010].

Comisión Veracruzana de Comercialización Agropecuaria[COVECA] (2002). History, taxonomy and culture of the pineapple. *Economic Botany* 3(4): Pg 335,156,143

Davies, G., (1994), Domestic Banana Beer Production in Mpigi District Uganda, ETC paper, Netherlands [online]. Available from: http://www.fao.org/agap/ frg/afris/espanol.[Accessed 7TH August, 2010]

Davis, U.C and Noble, C. A. (1995). Davis 20-point Scale. Department of Viticulture and Analogy, University of California, USA [online]. Available from: http://www.fao.org/agap/frg/afris/espanol. [Accessed 16TH August, 2010]

Dickinson, J. R. (1999). Carbon metabolism. In the Metabolism and Molecular Physiology of Saccharomyces cerevisiae, ed. J. R. Dickinson and M. Schweizer, Philadelphia, PA: Taylor & Francis. Pg 591–595

Dirar, H., (1992), *Sudan's Fermented Food Heritage*, in Applications of Biotechnology to Traditional Fermented Foods. National Academy Press, USA [online]. Available from: http://www.fao.org/agap/frg/afris/espanol. [Accessed 2nd August, 2010].

Dirar, H., (1993). *The Indigenous Fermented Foods of the Sudan: a study in African Food and Nutrition*. CAB International, UK [online]. Available from: http://www.fao.org/agap/frg/afris/espanol. [Accessed 2nd August, 2010].

Dittmer, P. R. and Desmond J. (2005). *Principles of Food, Beverage, and Labor Cost Controls*, John Wiley and Sons. Pg 375-386

Dull, G. G. (1971). The pineapple: general In Hulme A. C. (ed.), *the biochemistry of fruits and their products*. Academic Press, New York. vol. 2: Pg 303-324.

Ed Kasper L.Ac, (2007), Make your own Fresh Organic Probiotics Safely at Home. Acupuncturist & Herbalist, 417 Laurent St. Santa Cruz, CA 95060 [online]. Available from: http:// www.HappyHerbalist.com. [Accessed July, 2011]

Elfic J. (2004). Pineapple. School of Education, University of Queensland, Brisbane, Australia [online]. Available from: http://www.fao.org/agap/frg/afris/espanol. [Accessed 2nd August, 2010].

Encyclopaedia Britannica DVD (2000) - Free software downloads [online]. Available from: http://www.download.cnet.com/Encyclopaedia-Britannica [Accessed 20TH April, 2010]

FAO (1998). Fermented Fruits and Vegetable: A Global Perspective. FAO Agricultural Services Bulletin No. 134 [online]. Available from: http://www.fao.org/agap/frg/afris/espanol. [Accessed 2nd August, 2010].

FAO (2002). Tropical foods commodity notes, Statistical Database [online]. Available from: http://www.fao.org/statisticedatabas. [Accessed 15th October, 2010].

FAO (2004). Postharvest Operations of Pineapple [online]. Avaialables from: http://www.fao.org/agap/frg/afris/espanol. [Accessed 2nd August, 2010].

FAO (2010). Fermented Fruits and Vegetables [online]. Available from: http://www.fao.org/docrop/x056oe14.htm. [Accessed 16th August, 2010].

Fleet, G. H., (1998). The microbiology of alcoholic beverages. In: "Microbiology *of Fermented Foods"*, Blackie Academic and Professional, London, UK [online]. Available from: http://www.fao.org/agap/frg/afris/espanol [Accessed October, 2010]

Gallander, J. F. Klingshirn, L. M., Liu, J. R., (1987). Higher alcohol formation in wines as related to the particle size of soluble solides. *American Journal of Enology and viticulture.* Pg 38, 207-210

Garrison, E. C. (1993). Making Simple Fermented Beverages [online]. Available from: http://www.homebrew.net/ferment/ [Accessed October, 2010]

Gastronomía (2004). Medicinal of some Tropical Fruit Crops [online]. Available from: htpp://www.peru.com /gastronomia/docs2/. [Accessed 12th September, 2010].

Hornsey, S. I (1999). Brewing. *Royal Society of Chemistry*. Pg 221-222.

Horwitz D. (1999), Torulaspora delbrueckii. *Royal Society of Chemistry*, web.mst.edu/~microbio/BIO221_2001/torulospora_delbrueckii.htm. [Accessed September, 2008]

Hui, H. Y., and Khachatourians G. G. (1994), *Food Biotechnology*, Wiley-IEEE. Pg 847-848.

IIand, P., Ewart, A., Markides, A., Sitters, J. and Bruer, N. (2000). Techniques for chemical analysis and quality monitoring during winemaking. Campbelltown, Patrick Iland Wine Promotions [online]. Available from: http://www.fao.org/agap/frg/afris/ espanol. [Accessed 10th August, 2010].

Izuagbe, Y. S (1982) Malo-lactic Fermentation os Leoconostoc Species Isolated from Oregon wine. PhD Thesis. Oregon State University USA [online]. Available from: http://www.fao.org/docrep/x0560e/x0560e14.htm [Accessed 11th August, 2010].

Journal of Bilogical Chemistry (2005),The Chemistry of Wine Making: What are the benefits of resveratrol, an antioxidant found in wine? [Online]. Available from: www.emsb.qc.ca/laurenhill/science/wine.html [Accessed 15TH April, 2010]

Kawo, A.H. and Abdulmumin, F.N. (2009) Microbiological Quality of Re-Packaged Sweets Sold in Metropolitan Kano, Nigeria Microbiology Unit, Department of Biological Sciences, Bayero University, P.M.B. 3011, Kano, *Nigeria Bayero Journal of Pure and Applied Sciences*, 2(1): Pg 154 – 159

Keller, J. B. Jr. (2010). Pineapple Wine: Directions for Pineapple Wine Baskets [online]. Available from: http://www.ehow.com/way_5810589_directions-pineapple-wine-baskets.html#ixzz14iXAYA5s. [Accessed 16th November, 2010].

Klein, D. W., Lansing, M. and Harley, J. (2005). *Microbiology*. New York: McGraw-Hill[online]. Available from: www.bdu.ac.in/syllabi/affcol/pg/mb8.pdf [Accessed 18th November, 2010]

Kunkee, R. E., and Vilas, M. R., (1994). Towards the Understanding of the Relationship between Yeast Strains and Flavour Production during Vinification: Flavour Effect in Vinification of a Nondistinct Variety of Grape by Several Strains of Wine Yeast. *Win-Wissentchaft* 49. Pg, 46-50

Kunze, W. (2004). *Technology Brewing and Malting*. VLB Berlin, Germany [online]. Available from: http://en wipidia.org/wiki/special. [Accessed 2nd August, 2010].

Lacroux F., Tregoat, O., Van Leeuwen, C. A., Tominaga, T., Lavigne-Cruege, V. and Dubordieu, D. (2008). Effect of foliar nitrogen and sulphur application on aromatic

expression of *Vitis vinifera* L. cv. Sauvignon blanc, *International Journal of Vine and Wine Science* 42 (3): Pg 125-132.

Madigan M. ,and Martinko J. (2005), *Brock Biology of Microorganisms* (11th ed.), the free encyclopedia [online]. Available from: en.wikipedia.org/wiki/*Lactococcus_lactis* [Accessed 15TH April, 2010]

Medina, J. De La Cruz and García H.S. (2005) *PINEAPPLE: Post-harvest Operations.* Instituto Tecnologico de Veracruz. Danilo Mejía, PhD Ed: - Agricultural and Food Engineering Technologies Service (AGST). Pg 1-37, [online]. Available from: http://www.itver.edu.mx [Accessed October, 2010]

Mountney, G. J. and Gould, W. A. (1988). *Practical Food Microbiology and Technology*. AVI Books, Van Nostrand Reinhold Company, New York, USA [online]. Available from: [online]. Available from: http://www.fao.org/agap/frg/afris/espanol. [Accessed 20TH August, 2010].

Mundogar (2004). Pineapple Post-harvest operations: accelerates cicatrisation [online]. Available from: http://www.fao.org/fileadmin/user.../Post_Harvest_Compendium_-Pineapple.pdf[Accessed 14TH August, 2010].

Odunfa, S.A., (1985), African Fermented Foods, in *"Microbiology of Fermented Foods*", Elsevier Applied Science Publishers, UK [online]. Available from: http://www.fao.org/agap/frg/afris/espanol. [Accessed 14TH August, 2010].

Ostergaard, S., Olsson, L., Nielsen, J., (2000), Metabolic Engineering of *Saccharomyces cerevisiae*, Microbiol. Mol. Biol [online]. Available from:. http://www.fao.org Rev 64: Pg 34-50

Panjai, L., Ongthip, K. and Chomsri, N. (2009). Complex fruit wine produced from dual culture fermentation of pineapple juice with *Torulaspora delbrueckii* and

Saccharomyces cerevisiae, *Asian Journal of Food and Agro-Industry* 2(2): Pg 135-139.

Pedersen, C.S. (1979). *Microbiology of Food Fermentation*, AVI Publishing Co, USA [online]. Available from: www.caritasuni.edu.ng/pro/natural/Mb11.do [Accessed 18th October, 2010]

Py. C., Lacoeuilhe, J. J. and Teisson, C (1987). *The pineapple, cultivation and uses*. Paris: G.P. Maisonneuve & Larose. Pg 568.

Ranken, M.D., Kill, R.C. and Baker, C.G.J., (1997), Food Industries Manual, Blackie Academic and Professional, UK [online]. Available from: http://www.fao.org/docrep/x0560e/x0560e14.htm [Accessed 24th 0ctober, 2010]

Riley J. M., (1978), *Making Wine from Rare Fruit*, CRFG Yearbook. Vol. 10, Pg. 57-62.

Robinson, J. (2006). *The Oxford Companion to Wine*. Third Edition, Oxford University Press. Pg 267-269, 779-787.

Rose, A. (1961), *Industrial Microbiology*, Butterworths, UK [online]. Available from: http://www.fao.org/docrep/x0560e/x0560e14.htm [Accessed 10th August, 2010].

Rotter, B. (2008). Fining [online]. Available from: www.brsquared.org/wine. [Accessed 11th July, 2011].

Sarah, J. L., Mesnidrey, L., Maguerite, E., and Biosseau, M. (1997) Laboratory screening of pineapple germplasm for resistance to the lesion nematode *Pratylenchus brachyurus. Acta Horticulture* 425, pg 179-186. [Online] Available from: http://www.fao.org/fileadmin/user_upload/inpho/docs/Post_Harvest_Compendium_-_Pineapple.pdf [Accessed 10th 0ctober, 2010]

Stanier, R.Y, Doudoroff, M. and Adelberg, E.A., (1972), *General Microbiology*, Macmillan, UK [online]. Availablefrom: http://www.fao.org/docrep/x0560e/x0560e14.htm [Accessed 15TH September, 2010]

Stanton, R.W. (1985). Food Fermentation in the Tropics, in "*Microbiology of Fermented Foods*", edited by Wood, B.J.B., Elsevier Applied Science Publishers, UK [online]. Available from: http://www.fao.org/docrep/x0560e/x0560e14.htm [Accessed 11th July, 2011].

Steinkraus, K.H. (1992). Lactic Acid Fermentations, in "*Applications of Biotechnology to Traditional Fermented Foods*". Report of an Ad Hoc Panel of the Board on Science and Technology for International Development, National Academy Press, Washington D.C. USA [online]. Available from: www.fao.org/docrep/x0560e/x0560e14.htm [Accessed 11th July, 2011]

Steinkraus, K. H. (1996). *Handbook of Indigenous Fermented Foods*. Marcel Decker Inc, New York. Pg 389–398

Sulz, C. H. (2011). A Treatise on Beverages or The Complete Practical Bottler [online]. Available from: www. Chestofbook.com/food/beverages/A-treatise-on-beverages/index.html. [Accessed 8th July, 2011].

Van Rooyen, P. C. and Tromp, A. (1982). The Effect of Fermentation Time (as Induced by Fermentation and Must Conditions) on the Chemical Profile and Quality of Chinin blanc Wine. South African Journal of Enology and Viticulture 3(2). Pg 75

Walker, P. M. B. (1988) *Chambers Science and Technology Dictionary*, Cambridge University Press, UK [online]. Available from: www.worldcat.org/.../chambers-science-and-technology-dictionary [Accessed 10th July, 2011]

Wikipedia (2010) *Lactococcus lactis* -, the free encyclopedia [online]. Available from: en.wikipedia.org/wiki/*Lactococcus_lactis* [Accessed 15TH April, 2010]

Wine World FDW (2002) home brews wine kits under the name of Wine World [online]. Available from: www.wineworldfdw.com [Accessed 20TH April, 2010]

Wimalsiri, P., Sinnatamby, A., Samaranayake, S and Samarasinghr, C.R., (1971*), Cashew Apple Wine,* Industry Prospect Report 44, Industrial Development Board, Sri Lanka

Yokotsuka, T. (1985). Fermented Protein Foods in the Orient, with Emphasis on Shoyu and Miso in Japan, in "*Microbiology of Fermented Foods*", edited by Wood, B.J.B., Elsevier Applied Science Publishers, UK [online]. Available from: library.wur.nl/wda/dissertations/dis3672.pdf Accessed 10th August, 2010].

APPENDICE

Appendix 1: Analysis of Variance Table for Glucose Content

Source	DF	SS	MS	F	P
rep	2	7.407E-06	3.704E-06		
yeast	2	5.185E-05	2.593E-05	0.90	0.4250
conc	2	10.9640	5.48201	190986	0.0000
yeast*conc	4	3.704E-05	9.259E-06	0.32	0.8587
Error	16	4.593E-04	2.870E-05		
Total	26	10.9646			
Grand Mean 0.8007		CV 0.67			

Appendix 2: Analysis of Variance Table for Sucrose Content

Source	DF	SS	MS	F	P
rep	2	0.00007	0.00003		
yeast	2	0.00002	0.00001	0.38	0.6892
conc	2	8.59227	4.29613	147296	0.0000
yeast*conc	4	0.00004	0.00001	0.38	0.8190
Error	16	0.00047	0.00003		
Total	26	8.59287			
Grand Mean 0.8022		CV 0.67			

Appendix 3: Analysis of Variance Table for Fructose Content

Source	DF	SS	MS	F	P
rep	2	0.0027	0.00134		
yeast	2	0.0031	0.00156	0.49	0.6208
conc	2	14.4978	7.24890	2283.45	0.0000
yeast*conc	4	0.0058	0.00145	0.46	0.7667
Error	16	0.0508	0.00317		
Total	26	14.5602			
Grand Mean 1.1193		CV 5.03			

Appendix 4: Analysis of Variance Table for Total Sugar Content

Source	DF	SS	MS	F	P
rep	2	0.226	0.1131		
yeast	2	0.017	0.0083	0.12	0.8886
conc	2	103.777	51.8884	739.56	0.0000
yeast*conc	4	0.042	0.0104	0.15	0.9610
Error	16	1.123	0.0702		
Total	26	105.184			
Grand Mean 6.5444		CV 4.05			

Appendix 5: Analysis of Variance Table for Alcohol Content

Source	DF	SS	MS	F	P
rep	2	7.407E-06	3.704E-06		
yeast	2	0.00865	0.00433	1168.00	0.0000
conc	2	29.5284	14.7642	3986332	0.0000
yeast*conc	4	0.01504	0.00376	1015.00	0.0000
Error	16	5.926E-05	3.704E-06		
Total	26	29.5521			
Grand Mean 6.7885		CV 0.03			

Appendix 6: Analysis of Variance Table for pH

Source	DF	SS	MS	F	P
rep	2	4.930E-30	2.465E-30		
yeast	2	4.667E-04	2.333E-04	9.5E+27	0.0000
conc	2	0.03927	0.01963	8.0E+29	0.0000
yeast*conc	4	9.333E-04	2.333E-04	9.5E+27	0.0000
Error	16	3.944E-31	2.465E-32		
Total	26	0.04067			

Grand Mean 3.8478 (WARNING: The model error mean square is too small to continue.The model may fit the data exactly.)

Appendix 7: Analysis of Variance Table for Total Titratable Acidity (TTA)

Source	DF	SS	MS	F	P
rep	2	0.00001	3.704E-06		
yeast	2	0.00001	3.704E-06	1.00	0.3897
conc	2	0.01094	0.00547	1477.00	0.0000
yeast*conc	4	0.00001	3.704E-06	1.00	0.4362
Error	16	0.00006	3.704E-06		
Total	26	0.01103			

Grand Mean 0.7963 CV 0.24

Appendix 8: Analysis of Variance Table for Must Temperature

Source	DF	SS	MS	F	P
rep	2	0.00519	0.00259		
yeast	2	0.00074	0.00037	0.28	0.7625
conc	2	3.14741	1.57370	1172.14	0.0000
yeast*conc	4	0.00148	0.00037	0.28	0.8893
Error	16	0.02148	0.00134		
Total	26	3.17630			

Grand Mean 26.770 CV 0.14