A STUDY OF SHELF-LIFE OF SOME SOFT DRINKS AND A COMPARATIVE ANALYSIS OF GRAPE AND PALM WINES.

BY

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THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE MASTER OF SCIENCE.

DEPARTMENT OF CHEMISTRY,
FACULTY OF SCIENCE,
AHMADU BELLO UNIVERSITY,
ZARIA.

1982.

D E C L A R A T I O N

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any other University or Institution. Information derived from the published or unpublished work of others has been acknowledged in the text.

STUDENT SIGNATURE

DATE:.....

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TO:-

MRS. SARIAN OLAYINKA ONITIRI (MOTHER).

MISS. MOJISOLA OLUKEMI MARAIYESA (FIANCEE)

MR. ADESUMBO ADENIJI ONITIRI (BROTHER)

AND Post - humously to my late FATHER CHIEF SAMUEL AKANBI ONITIRI.

A C K N O W L E D G E M E N T S

I wish to express my profound gratitude to Dr. K. Singh, Dr. O. Olatunji both of the Chemistry Department of the Ahmadu Bello University and Dr. Emarakby for supervising this work, reading the draft and making suggestions.

My gratitude also goes to Dr. T. Bozimo, the acting Head of Department of Chemistry for making available all the reagents and equipments used for this study.

I am thankful to Mr. Smith for rendering help whenever called upon and the entire Technical and Store staff of the Chemistry Department for their cooperation.

I sincerely appreciate the help by the management and staff of the Nigerian Breweries Limited, Kaduna, Drinco Industries Limited, Kaduna in providing soft drinks. I am also grateful to the Director of the Federal Institute for Industrial Research Oshodi for providing palm-wines and also to the management and staff of the OZDE Distilleries Gbagada Estate, Lagos for supplying grape wines.

Above all, I am grateful to God.

ADENIYI SAMUEL ONITI

ABSTRACT

Shelf-life studies of Brahma Orange, Brahma Guarana, Brahma Lemon, Brahma Tonic and Green Sands Shandy drinks show that the pH, carbonation and specific gravity values increased constantly with time while the ascorbic acid and nutritive values decreased. The acidity value remained unchanged throughout the period of investigation (4 months) although it varied from one drink to the other. By the end of the third month virtually no ascorbic acid was present in any of the drinks; shelf-life of soft drinks analysed varied with temperature.

Comparative studies of palm and grape wines (table wines) showed that the former had higher initial sugar content, lower extract - to - alcohol ratio and lower pH values than the latter. Grape wines had an even distribution of all preservatives while an exceptionally high benzoic acid content was recorded for palm-wines. The determination of the amount of ethanol in palm-wine was affected by the presence of volatile acids.

Considering the two palm-wines, 'Uncle T' had more water content (dilution) than FIRRO.

CHAPTER 1

1 - 1 I N T R O D U C T I O N.

1 - 1.1 CARBONATED BEVERAGES.

Carbonated beverages [1-4] commonly known as soft drinks consist mainly of water impregnated with carbon dioxide gas and may contain added colour, flavour, sweetner and acid. They can be classified as sugar - sweetened, flavoured beverages or synthetically sweetened, flavoured beverages or carbonated waters of the club soda, seltzer and sparkling water variety. Sugar sweetened carbonated beverages contain 83 - 93% water, 3 - 17% sugar and sucrose, 0 - 200 grains of acid reported as citric acid per gal and 0.1 - 5 gas volume of carbon dioxide. The pH may vary from 2.5 - 7.0. Flavour ranges from birch beer, cola, ginger ale, and root beer to fruit flavours such as lemon, orange, raspberry and grape. The quality of the flavouring extract present is highly variable depending upon the particular flavour. Some citrus type beverages contain up to 5 - 6% reconstituted juice and essential oils. Colourants may be present in highly variable concentrations. The food preservatives, sodium benzoate may

or may not be present, when used, The concentration is usually 0.05 to 0.1%. The synthetically sweetened beverages in general have sugar replaced with saccharin which has roughly 300 times sweetening power of sucrose and/or one of the cyclamates with about 30 times sweetening power of sucrose. They may also be occasionally distinguished from their sugar sweetened counterparts by modification of flavour and acid level as well as by the addition of gums and stabilizers. Carbonated waters to which inorganic and/or organic salts are sometimes added constitute the class commonly known as sparkling water or club soda when salts are added, the carbonation rarely exceeds a few thousand parts per million (ppm). The salts are principally carbonates, bicarbonates and sulphates of calcium magnessiums and potassium.

1 - 1.2 WINES: Wines,[5-8] (which is defined by the wine Trade Association as "The alcoholic beverage obtained from the fermentation of the juice of freshly gathered grapes, the fermentation of which has been carried through in the district of its origin and

according to local traditions and practice") is basically the fermented juice of the grape derived from the fruit of vitis venifera and in some other cases from vitis labrusca. In most cases, fully ripe grapes are used. However overripe grapes are sometimes used which in the final event produces what is known as the saunterne. When not fully ripe grapes are used, champagne results.

There are two main classes of wine, "red" and "white",[5] the latter ranging in colour from the palest straw to brown. Red wines are made from coloured grapes; the stalks are removed as quickly as possible to avoid undue extraction of tanning. The marc (skins and seeds) is left in the fermenting must so that the colour may be extracted. White wine is made from either white or red grapes, the must being separated from the marc as quickly as possible. The juice and the first pressings are generally kept separate from the latter pressings which afford wine of darken colour (vins gris). If the must from red grapes is allowed to ferment on the marc for a short period, then separated, a pink-coloured wine results (vins rose).

1 - 2 PREPARATION PROCESSES FOR SOFT DRINKS

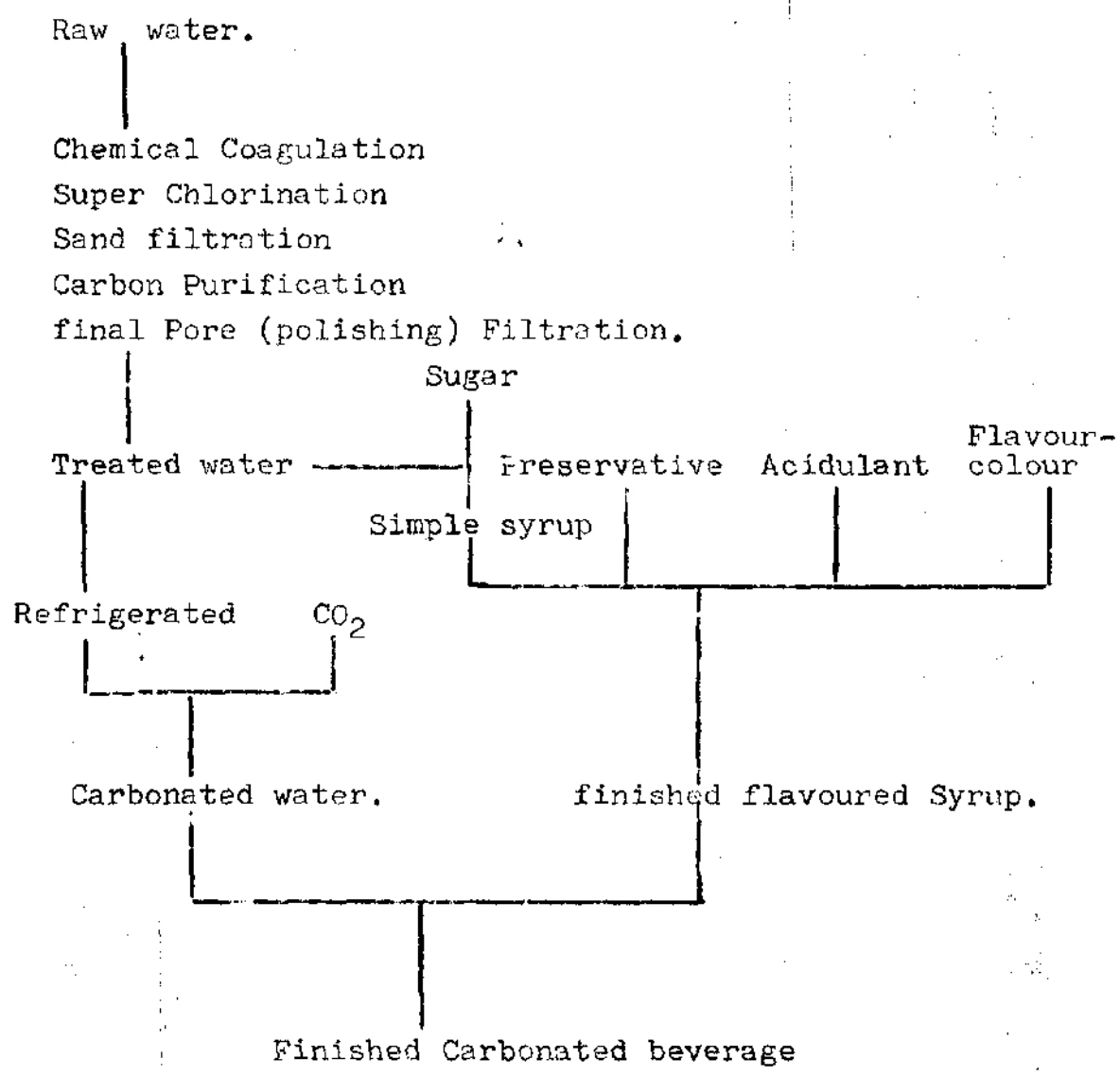
Raw materials for carbonated beverages are numerous depending on the manufacturers and the type required[2,4]. However, the basic raw materials for soft drinks include flavours, acidulants, sweetners, colouring matter, carbon dioxide and most important of all, water. The ratio of these materials vary from one type of drink to another and from manufacturer to manufacturer.

Briefly, soft drinks are prepared by mixing syrups with water and carbon dioxide in the proper ratios. Simple syrup is prepared by dissolving a known quantity of sugar in water, filtered and coloured and preservatives added. This is then mixed well and flavour and acidulants added. In some cases, "concentrates" are prepared. This is done by the addition of flavour, colour and preservatives together. Soft drinks in Nigeria are generally supplied in this form whereby sugar, water and carbon dioxide are the remaining ingredients to be added.

P.T.O. for the flow diagram.

Fig. 1

Flow Diagram for Carbonated Beverage Preparation



1 - 3 PREPARATION PROCESSES FOR WINES

The most important raw material[6] for wine is the must which consists of crushed grape juice. It is important for the wine - maker to have a thorough knowledge of the must because the composition and quality of the finished wine depends largely on it. Other ingredients for wines include acidulants, sulphur dioxide and sometimes colouring material.

The first step in the preparation of wine is the crushing of grapes to release the juice referred to as must. In primitive practice, the grapes were crushed by treading on them with bare feet, a method which has the merit of avoiding the bruising of seeds and stalks. Nowadays, mechanical extractors are used.

The second and the most significant step in the preparation of wine is the fermentation process. The yeasts saccharomyce apiculatus and S ellipsoideus found in the juice do the fermentation. S ellipsoideus is the principal agent as S apiculatus ceases to be active when the liquor contains 4% of alcohol. The process of fermentation causes a rise in temperature of the liquor as such thermostated vats are usually employed.

The must of white wines is very thick and has to be clarified before fermentation. This is most commonly effected by allowing it to settle, after making a suitable addition of sulphurous acid to delay the onset of fermentation. This process is known as 'Mutage' in France.

When the fermentation ceases and the yeast and other insoluble materials settle at the bottom, the wine is drawn off and left to mature.

During the process of maturing which involves the storage of wines in cellars or caves, tannin and other compounds are deposited with consequent reduction of rough and acid flavours. The time required for maturing wines[10] varies considerably, most good wines are stored in casks for 1 - 5 years and then for some years in bottle. Some very choice wines require 10 or even more years before coming to perfection.

After maturation, the wine is then clarified[11], The clarification practices commonly used are filtration, fining, refrigeration and pasteurization. Filtration involves the passing of the wine through a layer of inert material with fine pore sizes. While fining involves the additions of a material that mechanically or chemically clarifies the wine. In Refrigeration, wines are chilled to about $25^{o}F$ or $20^{o}F$ in case of

dessert wines and the wine is filtered cold to prevent resolution of tartar. Pasteurization is used to stop progress of disease and to aid in clarification. The wine is heated to about 150°F or higher for a short period, allowed to cool and then filtered.

1 - 4 SPOILAGE OF SOFT DRINKS.

Spoilage[2] refers to any undesirable change in apperance, colour, taste or odour that takes place in the beverage . Spoilage may be due to chemical changes in the beverage or growth of microorganisims. Certain physical effects are brought about as a result of chemical reactions caused by heat or sunlight. Direct sunlight is detrimental to the flavour of almost all beverages, particularly those containing citrus fruit juices. Light causes a flavour change which in citrus - flavoured beverages may be described as oily or terebinthinate; in most other beverages there is merely a general loss of flavour or character. Some food colours, particularly Tartrazine, fade quickly in the sunlight. Heat, generally encountered

is not high enough to produce specific effects such as a cooked taste. However heat speeds up chemical reactions and will therefore hasten loss of freshness.

The chemical changes taking place in beverages are oxidation, enzyme action, reaction of free chlorine, saponification of esters, hydrolysis and other chemical reactions of flavouring ingredients, and breakdown of the complex molecules in caramel by reactions of acids and minerals. Oxidation may be due to air, which is present to some extent in all carbonated beverages. If the carbonating equipment is faulty, larger amounts of air may be introduced and the finished beverage will not have its proper degree of carbonation.

Free chlorine is frequently passed into the beverage from the water supply or from equipment not thoroughly rinsed. Free chlorine causes many radical and very undesirable flavour changes and will bleach the colour of the beverage. Alkaline waters used in the preparation of a beverage produce a highly insipid beverage with a decided off-odor. This off-odor is evidently due to some reaction with the flavouring ingredients. The odor of beverages generally changes

on standing. This is no doubt due in part to the reaction of the acid with the flavouring oils. Caramel has been known to become insoluble after standing for a few days. This is attributed to the breakdown of the complex molecules under the action of the acids and, in some cases, mineral salts.

The growth of microorganisms in the finished beverage may cause scums, clouds, sediments etc. The micro-organisms of importance to carbonated beverages are protozoa, algae, molds, bacteria and yeast. Protozoa are one-cell animals. They are always water borne, coming into the beverage from the water supply. These organisms may produce many off tastes in beverages and are otherwise undesirable. Algae are one-cell, chlorophyll-bearing plants. Light is required for their continued growth. Frequently sufficient members of these plants are introduced through the water supply to cause off-taste and sediments.

Molds are saprophytic fungi that require oxygen for their growth, and therefore a properly carbonated beverage does not normally have enough air to support them. Molds grow quickly on the surface of strong acid

solutions, on stored syrups, and in tanks not throughly cleaned. From these sources sufficient numbers may be introduced into beverages to produce a musty or moldy odor.

The typical acidified carbonated beverage does not support the growty of bacteria as the acid and carbon dioxide present exert a pronounced germicidal effect. Consequently, carbonated beverages have been found in sanitary survey to have a low "colour index". The colour index is the number of Escherichia coli in 100ml of beverage. Souring or fermentation in carbonated beverages is due to the growth of yeast. Yeast or Saccharomyces are microorganisms that feed on sugar. Yeast growth in considered responsible for 90% of spoilage in carbonated beverages. Low-acid or Low-carbonated beverages, or beverages with no carbonation are more succeptible to yeast growth, as acids seem to be the main inhibitor. Beverages made from water of high alkalinity sour easily owing to the neutralization of the acid. The typical carbonated beverage has an acidity of approximately 72% grains/gal of beverage and in such acid solutions, yeast growth is inhibited.

During the manufacturing process beverage syrups are continually succeptible to contamination. Metallic contamination has the most drastic action on the palatibility of the beverage. Acidified syrups, when exposed to any metals except stainless steel or monel, attack the metal. Handling equipment must therefore be of materials known to resist the action of the acidified syrups or carbonated water. Equipment that is lined with metals like silver must frequently be examined for possible points of metallic contamination.

The most insidious contamination because it is not drastic in its action, is contamination with absorbed foreign odours. These off-odors in the atmosphere may be caused by failure to keep the plant clean. A spoiled, soured smell is frequently associated with unclean plants. Plants are sometimes located in industrial centres where disagreable odors fill the air. Beverage plants must be kept free from offensive odors if their products are to be free of this sort of contamination.

Oil from the machinery used to manufacture the carbon dioxide gas sometimes finds its way into the

carbonated beverage. If dry ice converted are not kept drained of oil, the ~~oil~~ oil passes into the gas line and finally into the carbonator, filler head and eventually to the finished beverage.

To check spoilage in soft drink, certain chemicals are added. These chemicals are commonly referred to as PRESERVATIVES. Preservatives may be divided into three main categories - These are:

(1) Antimicrobials,[13] which includes benzoic acid, p - Hydorxybenzoic acid esters, sulphur dioxide, sorbic acid and Diethyl pyrocarbonate. The group acts as bacteriostatic and germicidal agents.

(2) Antioxidants, which includes ascorbic acid and certain of its derivatives, gum guaiac, lecithin and tocopherols. This group retards oxidation process in the drink. Another group of antioxidants are the synergists. These are Chemicals that have some antioxidant action themselves but give an enhance: effect when added together with another antioxidant. Some synergists are citric acid, phosphoric and tartaric acids.

(3) Antibiotics, which includes oxytetracyclines and chlortetracyclines.

The most commonly employed of all these preservations is the benzoic acid. It is added in the salt form i.e. sodium benzoate.

The addition of these preservatives to drinks does not imply that the drinks will become immune to spoilage rather it increases the time it takes for the drink to get spoilt i.e its "shelf life".

1 - 5 SHELF - LIFE

The period between manufacture and retail purchase of a food product during which the product is of satisfactory quality is commonly referred to as the "shelf life"[4], 14-16.

Shelf life or storage stability[15] of a drink depends on a large number of factors such as those of environmental origin, namely temperature, relative humidity, light intensity and so on. Soft drinks fall under the class "Semi perishable foods". These are foods that have received some type of preservation treatment for greater tolerance to environmental conditions.

The shelf-life of semi perishable foods is very important to the consumers. Soft drinks should have a shelf life that depends on the rate of consumption of that particular drink in least consumption area. This however is a very difficult thing. Most manufacturers tend to monitor their products by coding[16] thereby determining the shelf life. Better still, the shelf-life of soft drinks can be determined in the laboratory.

1 - 6 MOTIVATION OF THE PROJECT

Carbonated beverages are impulse drinks and are consumed to a very great extent, the world over. Their process of production is simple (see page 5) and the actual process of consumption is a matter of minutes. But there exists a time lag between the production of a drink and its consumption. This time lag depends on three factors.

(i) The number of middle men involved.

(ii) Transportation system in the locality and

(iii) The rate of consumption.

The quality of beverages (with the exception of wines and certain distilled liquors) is known to deteriorate with time in the form of decrease in

nutritional value, colour change, development of off-flavour. The study of these changes with time (shelf-life) is therefore of prime importance. The study of the environmental factors contributing to these changes i.e. light and temperature, is also required. Important parameters in this study include: carbonation, acidity, ascorbic acid, sodium benzoate, colour, pH level, specific gravity, Refractive index, Protein content and Trace metals.

Naturally occuring wines known as 'emu' from palm trees and "Oguro" from Raffia palm are now bottled in Nigeria under different trade names like "Uncle T". These natural wines are gradually finding their way into the local market. Quantitative analysis of the major components of these wines and comparison of the data obtained with those of imported grape wines is therefore a necessity.

Factors that could determine the quality of these wines are:

(1) Alcohol to extract ratio.

(2) Total and volatile acids

(3) Ash content

(4) Free and total sulphur dioxide.

Samples for these analysis were obtained from Federal Institute for Industrial Research Oshodi and OZDE distilleries Lagos.

CHAPTER 2

ANALYSIS OF SOFT DRINKS

Drinks analysed include Brahma Orange, Brahma Guarana, Brahma Lemon, Brahma Tonic all obtained from the Drinco Industries Ltd, Kaduna, and Green sands shandy collected from the Nigerian Brewries Limited, Kaduna.

2 - 1 SAMPLING:

Bulk samples of the drinks were collected from the production plants on the day they were produced but at different times. The products were selected at random. Two sets of each type of drink were collected, one on the 4^{th} of September, 1981 and the other on the 15^{th} of the same month. Two sets were necessary so as to compare the differences if any in the drinks produced by the company. Each set of any particular drink chosen contained twelve bottles.

Two bottles of each type of drink were used for each determination and the results got were compared with the results obtained for the same parameter in the second set (i.e those collected on the 15^{th}).

For most of the parameters determined, degassed samples were used. This was obtained by measuring 250ml of the drink in a volumetric flask, pouring the measured quantity into a beaker and boiling to remove carbon dioxide. It was then cooled, filled back into the volumetric flask and made up to the 250 ml mark.

For the effects of refrigeration on the shelf-life of drinks, four bottles of each type of drink from each batch were kept in the refrigerator while the remaining bottles were left exposed to weather conditions. The temperatures in both cases were recorded and averaged.

2 - 2 EXPERIMENTAL

The conditions prevailing in bottled drinks at the time of production are such that microbiological organisms will not survive in them. As time goes on, decrease or increase in the values of some components of the drinks might encourage microbial spoilage and may also results in some new compounds in the drink. All these will lead to change in taste, colour and even smell of the drinks. Some of the drink components suspected to change during storage are therefore studied.

These include, acidity, pH, specific gravity, Ascorbic acid content, carbonation and trace metals.

All regents used are of analytical-reagent grade unless otherwise stated and the ZEAL thermometer of 30mm immersion calibrated from 0^{o} to $200^{o}C$ was used throughout.

DETERMINATION OF ACIDITY

Changes in the acidity values of soft drinks will indicate some type of reactions going on in the drinks that might lead to off-taste. Acidity values[6] of carbonated beverages are determined titrimetrically on degassed samples and the results reported in g/100ml.

APPARATUS

Automatic titrator: A Radiometer titrator type TTT 111, with a Pye Unicam pH meter model pHM 28, and an autoburette calibrated from 0.00 - 2.50ml.

REAGENT

Sodium hydroxide solution, 0.05M- Prepared by dissolving 0.5g of sodium hydroxide in 250ml distilled water.

PROCEDURE

10ml of the degassed sample (see page 19) was titrated with 0.05M sodium hydroxide solution to a pH of 8.3 as recommended[1]. The acidity was calculated as citric acid using the formula.

$$\text{citric acid g/100ml} = \frac{(V)(M)(6.404)}{v.}$$

V = Volume of NaOH used. (ml)

M = Molarity of NaOH used. (ml)

v = volume of sample used (ml).

Results on Tables 4 & 14

SPECIFIC GRAVITY DETERMINATION

Dissolution of some of the gases entrapped in the drinks, precipitation and evaporation of some of the components of the drinks can lead to changes in the specific gravity which will affects the shelf-life of the drinks.

The specific gravity was determined using the specific gravity bottle[17]. The weight of a certain volume of the drink was compared to the weight of

exactly the same volume of water at 20°C.

APPARATUS

50ml specific gravity bottles. Each bottle was washed with chromic acid solution, rinsed with distilled water and dried in an oven at 80°C, (for constant weight).

PROCEDURE

The specific gravity bottle was weighed (m_1), filled with distilled water and reweighed (m_2). The distilled water was poured away and degassed soft drink sample was filled into it and weighed (m_3).

The specific gravity of the drink

$$D = \frac{m_3 - m_1}{m_2 - m_1}$$ The results are given in Tables 1 and

REFRACTIVE INDEX DETERMINATION

The refractive index value when converted to °Brix[1] can be used in conjunction with the specific gravity values of the drinks to determine the sugar content. Changes therefore in the refractive index value implies changes in sugar content of the drink which will affect the shelf life of the drink.

APPARATUS

A refractometer type, Abbe 60, was used but without the Brix calibration for direct sugar content reading. The prevailing temperature was recorded with the ZEAL thermometer for correction.

PROCEDURE

The main prism of the refractometer was exposed by opening the hinged illuminating prism through 180°. Two drops of the soft drink sample were smeared on the main horizontal prism. The light was put on and the hair line adjusted to the centre. The refractive index value was read on the illuminated scale of the refractometer. Results are given in Table 2.

pH MEASUREMENT.

The pH of soft-drinks gives an indication of their possible resistance to microbial spoilage. It is therefore important in the determination of the shelf-life of these drinks. The pH can be determined using a pH meter.

APPARATUS.

Pye unican pH meter, model 290 MK 2 was used. The temperature knob of the pH meter was set to the temperature of the sample which was taken using the ZEAL

thermometer.

REAGENTS

Buffer solutions of pH 4.0 and pH 9.0 prepared by adding a satchet of the pH hydrion buffer of the specific pH to 500ml of distilled water.

The pH of the drinks were taken after the pH meter had been calibrated with the buffer solutions. The results are given in Tables 3 and 13.

ASCORBIC ACID DETERMINATION

Ascorbic acid is used in soft drink as an anti-oxidant and it also improves the shelf-life of drinks flavour[4]. The ascorbic acid content of soft drink was determined titrimetrically.

APPARATUS.

The automatic titrator was used (page 20).

REAGENTS.

Standard ascorbic acid - Prepared by dissolving 100mg of ascorbic acid in 100ml of 3% metaphosphoric acid (m/V).

0.01M iodine solution - Prepared by dissolving 1.27g of resublimed iodine in potassium iodide solution[18]

and making it up to 1 litre mark with distilled water.

PROCEDURE:

The iodine solution was standardised with 5ml aliquot of the standard ascorbic acid solution using starch indicator solution. 10ml degassed sample was added to 15ml of the 3% mataphosphoric acid. Then 5ml aliquot was titrated with standardised iodine solution to the starch end-point.

$$\text{Absorbic acid mg/ml} = \frac{\text{ml of iodine (M) (88.1)}}{\text{ml of sample in the aliquot.}}$$

The results are given in Table 5.

BENZOIC ACID DETERMINATION:

Sodium Benzoate is used as a preservative in carbonated beverages has an effect on the protection of the drinks from spoilage. The determination[19] is based on partition law using a solvent which is immiscible with the drink.

APPARATUS.

The automatic titrator was used (page 20).

REAGENTS

Solvents - Redistilled chloroform and 30% ethanol (V/V).

Sodium hydroxide, 0.05M.

Phenolphthalein indicator, 0.1 per cent. alcoholic solution.

PROCEDURE

10ml of degassed sample was extracted with 7.0 , 5.0 , 4.0 , and 3.0 ml portions of chloroform. The extractions were transferred into 250-ml beaker and evaporated just to dryness. The residue was dissolved in ethanol, two drops of phenolphthalein indicator were added and the solution was titrated with 0.05M NaOH until the colour changed to pink.

$$\text{Sodium Benzoate g/100ml} = \frac{(\text{ml of NaOH})(M)(0.144)(100)}{\text{volume of sample (ml)}}$$

The results are given in table 6.

PROTEIN DETERMINATION

Decrease in protein value of the drinks indicates a decrease in nutritional value making the drink less acceptable and might also cause a change in taste of the drink.

The Folin - lowry's method of protein assay[20] was followed, which involves the comparison of the intensity of the blue colour which developed in an unknown sample with that of a standard protein which in this case is Bovine Serum Albumin (B.S.A).

REAGENTS.

Solution 1. Standard B.S.A. 20% (m/V)

Solution 2. 2% soduim carbonate (m/V) in 0.1M NaOH

Solution prepared by dissolving 1.0g of sodium hydroxide in 250ml of distilled water.

Solution 3. Copper sulphate - 2% $CuSO_4$ (m/V) in 1% sodium potassium tartarate (m/V).

Solution 4. Folins ciocalteau reagent - commercial preparation was diluted with distilled water in the ratio 1:2.

PROCEDURE

50ml of solution 2 was mixed with 1ml of solution 3 to form the alkaline solution. 5ml of this was then mixed with 0.0, 0.1, 0.2, 0.3, 0.4, 0,5, 0.6 and 0.7ml of the B.S.A. and allowed to stand at room temperature for 10 mins. 0.5ml of the Folin-ciocalteau reagent was

then added to each and rapidly mixed. The microgram of protein in each tube was plotted against the absorbances obtained at 750nm which gave the calibration curve. Same procedure was followed again but this time, instead of the B.S.A. 0.1ml of the degassed soft drink sample was used. Results obtained are in Table 7.

DETERMINATION OF CARBON DIOXIDE CONTENT

Canges in the carbon dioxide content implies change in taste of the drink which automatically affects its shelf-life. Carbonation in drinks is most commonly determined using the pressure of the gas at the temperature of measurement - which is usually very low between 6 - 10^{o}C.

APPARATUS

Zahn Carbonation tester with psi pressure gauge unit was used. For shandy, the tester of the Nigerian Brewries, Kaduna was used and for the Brahma products, tester from the Drinco Industry was used.

PROCEDURE

The valve of the pressure gauge of the Zahn tester was closed and the sample placed securely in the frame of the tester. The crown was pierced and the valve was closed when the gauge read zero. The

bottle was then vigorously shaken until a constant pressure was achieved. The bottle was vent again and the temperature read. The air content was also obtained from this test. Results given in Table 8 and 10.

DETERMINATION OF THE COLOUR

Changes in the colour of a drink might be due to either changes in the composition of the colouring material or changes in certain other components of the drink. Colour analysis of the carbonated beverage is best accomplished by the CIE tristimulus system using the selected ordinate method[21]. For routine purposes, the colour determination may be simplified to an observation of the absorbance of an appropriate dilution at the dominant wavelength.

APPARATUS

A Unicam, SP1750. ultraviolet spectrophotometer, a wavelength selector type, SP1803 of the Pye Unicam model and cells of 1 cm width were used.

PROCEDURE

For this determination, the sample was degassed by pouring 100ml of the drink forth and back until gas

bubbles emerging from the drink were reduced to the barest minimum. The cells were then filled with the sample and scanned from the ultraviolet region to the visible region. The dominant wavelength was recorded for each sample and was used for subsequent determinations. Results are given in tables 9 and 16.

DETERMINATION OF ASH CONTENT

The ash content is a measure of the inorganic components of the drinks. This may affect the shelf-life of the drinks depending on the concentrations and types of metals present. The ash content is determined by evaporation and incineration of the drink at high temperatures.

APPARATUS

A muffle furance with Gallenkamp hotbox oven size one were used.

Platinum dishes brought to constant weight (m_1) by heating in the furnace at 600°C were also used.

PROCEDURE

10ml degassed sample was pipetted into the dish and evaporated to dryness in the oven at 100°C. It was then incinerated in the furnace at 525°C for 3 hours.

The dish was then removed from the furace, cooled and weighed (m_2).

$$\text{Ash content} = \frac{(m_2 - m_1)}{v} \quad 1000 \quad g/L$$

v = volume of sample used, (ml).

Results obtained are in table 11

DETERMINATION OF AVERAGE LIQUID CONTENT AND HEAD SPACE

Determination of the average liquid content of the drinks is as a check of the stipulated quantity, helps in some determination involving amount per bottle, and possible changes that may occur in volume during storage.

The head space is an indication of entrapped air in the drinks and this is deliberated to help microbial growth.

PROCEDURE

The head space and the average liquid content were determined at the factories where the drinks were produced. Heights of three bottles of same type of soft-drink were marked. The bottles were emptied and refilled with distilled water to the marks. These were

were later poured into a measuring cylinder and averaged. This gave the average liquid content. The three bottles were again filled to the brim with distilled water, emptied into a measuring cylinder and averaged. The average liquid content was substracted from this value to give the head space value. Results are given in Table 11.

TRACE METALS DETERMINATION

Traces of metals especially copper, in beverages act to accelerate oxidative changes which adversely affects the flavour and shelf-life of the packaged drink[22,23]. The presence of these metals can be tracedto both the raw materials used for the production of these drinks and the metallic fittings of the production plant. In the present, study, we are concerned with the determination of copper, Lead and Zinc.

The analysis of these trace metals involves the use of the Atomic Absorption spectrophotometer which is able to detect as low as 0.1 part per million. The sample is sprayed into a flame which converts it to atomic vapour by absorbing light at characteristic wavelengths. If the element is the same as the cathode

of the generating lamp, then a resonance line is attenuated. It is the intensity of this line that is measured either as percentage absorption or absorbance and the concentration of the element is determined by comparing the reading with those obtained for a series of standards[18].

APPARATUS

Pye Unicam SP1900 Atomic Absorption Spectrophotometer.

REAGENTS

All reagents used were of analytical - reagent grade. Stock solutions: Iron - Prepared by dissolving 9.8g of Ferrous Ammonium sulphate, $(NH_4)_2SO_4$. $FeSO_4$, $6H_2O$. in 1 litre distilled water.

Copper - Prepared by dissolving 1.5g of Cupric sulphate, $CuSO_4$, $5H_2O$ in 1 litre of distilled water.

Lead - Prepared by dissolving 1.8g of lead nitrate, $Pb(NO_3)_2$ in 1 litre distilled water.

Zinc - Prepared by dissolving 1.5g of Zinc oxide, ZnO in 1 litre.

All these solutions were then standardised with EDTA[24] and adjusted with distilled water to a known volume so as to contain 1 mg/ml of the metals and labelled "STOCK SOLUTIONS".

Calibration Curves - A series of standards ranging from 0 to 10 ppm were prepared by appropriate dilutions of the stock solutions to prepare the calibration curves.

CHAPTER 3

3 - 1 ANALYSIS OF WINES

Wines analysed include white table wines (white Lady), red table wines (dark Lady) both collected from the OZDE dilleries in Gbagada estate Oshodi, Lagos, "Uncle' T" palm wine that was bought and "emu" bottled by the Federal Institute of Industrial research Oshodi (FIIRO). Two samples each were collected from three batches in case of the grape wines while four samples each were collected from three batches in case of the palm wines except "uncle' T" that was bought.

Using standard analytical methods[6, 25 - 31], the following parameters were determined and results obtained were compiled in Table 17 on page 68.

pH MEASUREMENT

The determination of the pH of wines is very important because of its effect on microorganism, oxidation-reduction potential and susceptibility to iron phosphate cloudiness.

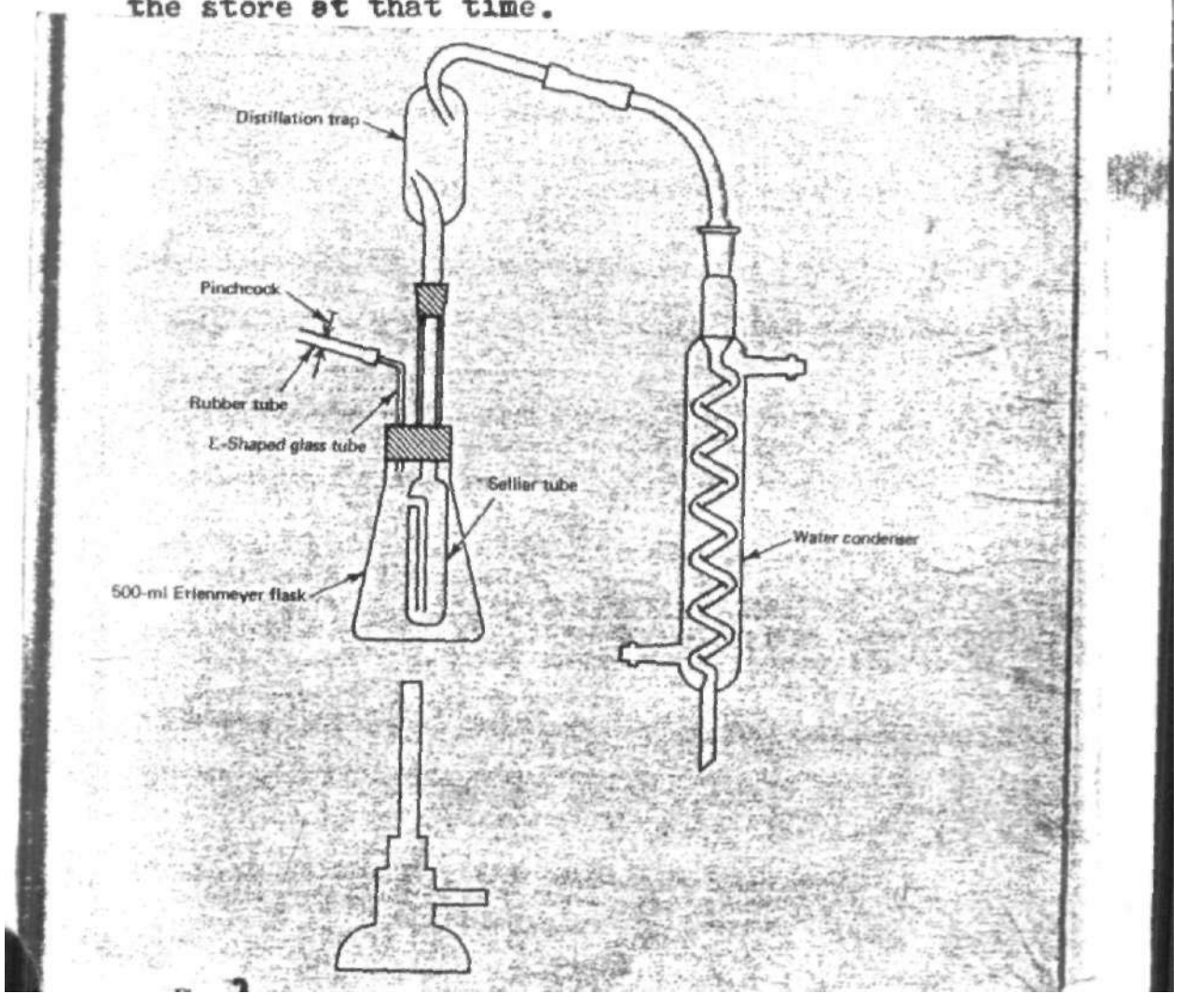
EXPERIMENTAL - As on page 23 in the pH measurement of soft drinks.

DETERMINATION OF VOLATILE ACIDITY

The determination of volatile acidity present is necessary so as to meet the legal limits, to follow the development of volatile acidity during storage and as a measure of spoilage.

APPARATUS

The instrument used is shown in Fig. 2 below. The sellier tube was constructed according to specification by the glass-blowers of the chemistry department of the Ahmadu Bello University as there was none available in the store at that time.



REAGENTS

Sodium hydroxide, 0.1M.

Phenolphthalein indicator, 0.1 per cent. alcoholic solution.

PROCEDURE

250-ml of water was poured into the 500-ml Erlenmeyer flask while 10ml of the wine was pipetted into the sellier tube. Flame was applied and the water brought to boil until steam started to issue from the rubber outlet. The pinchcock was then closed thereby forcing steam into the sellier tube and commencing the distillation. The distillate was collected into a 250ml flask.

After about a 100ml of distillate had been collected, the flame was removed and the pinchcock opened. The distillate was then heated over a gentle heat plate until it boiled for about 30 secs. Three drops of 1% phenolphthalein indicator solution was added and titrated while still hot with 0.1M sodium hydroxide solution to a faint pink end point.

The volatile acidity can be expressed as acetic acid thus:

$$\text{Acetic Acid (g/100ml)} = A = \frac{V_1 \times M_1 \times 60 \times 100}{1000 \times v}$$

V_1 = volume of sodium hydroxide solution used.

M_1 = Molarity of sodium hydroxide solution.

v = volume of sample, ml

Results are given in table 22

DETERMINATION OF THE TOTAL (TITRATABLE) ACIDITY

The acids present in musts and wines (tartaric, malic, lactic, acetic) are relatively weak organic acids. Thus when musts or wines are titrated with a strong base, the true end point will be greater than pH 7.0, usually between 7.8 and 8.3. The accepted procedure of the American society of Enologists and the A.O.A.C methods [32] indicate the end-point pH as 8.2.

Visual indicator (using phenolphthalein) is also applicable as long as the wine colour does not interfere with the pink colour end point.

APPARATUS

The automatic titrator described on page 20 was used.

REAGENTS

1% phenolphthalein indicator solution.

0.1M sodium hydroxide solution.

PROCEDURE

200ml boiling water was added to 5ml of the wine sample and titrated to the pH 8.2 end point.

The result, expressed as tartaric acid is:-

$$\text{Tartaric acid g/100ml} = \frac{V \times M \times 75 \times 100}{1000 \times v}$$

V = volume of NaOH used, ml.

M = Molarity of NaOH used

v = volume of sample, ml

DETERMINATION OF ETHANOL CONTENT

There are a number of formular to predict the amounts of ethanol present in wine. The ^{o}Brix is usually the base for these calculations. Actually the amount of ethanol formed in wines depend on a number of factors:-

(a) amount of fermentable sugar (b) yeast species (c) fermentation temperature (d) nutrient level of grapes and (e) general fermenting conditions.

For the estimation of the ethanol content of wines in this project, the method used is the macro-distillation of the wines followed by the determination of the specific gravity and/or refractive index of the distillate.

APPARATUS

The macrodistillation set up below was used.

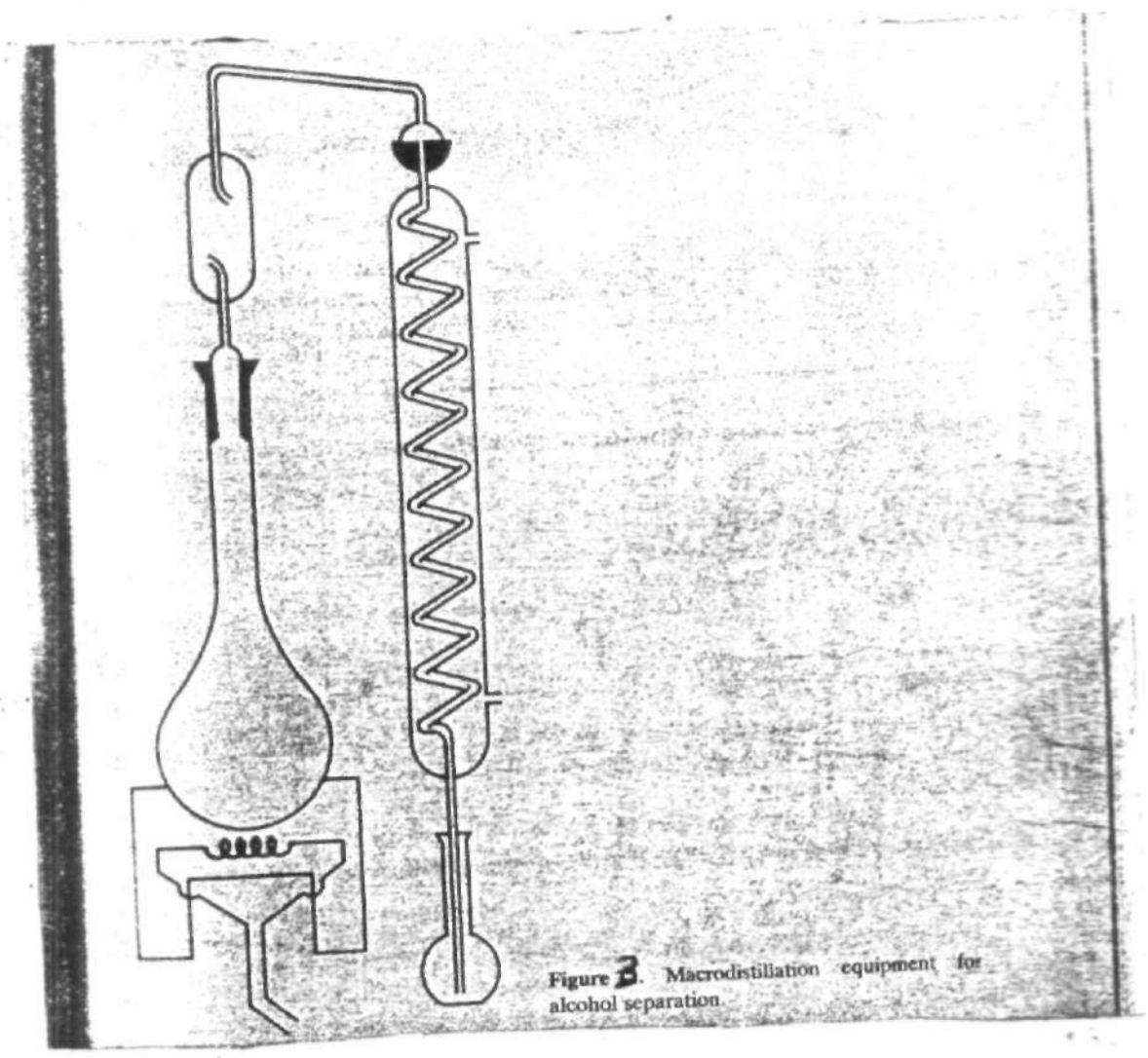


Figure 3. Macrodistillation equipment for alcohol separation.

PROCEDURE

100-ml volumetric flask was filled to the mark with the wine sample at 20°C and then poured into the distillation flask. The volumetric flask was rinsed with 20ml and 30ml of distilled water consecutively and poured into the wine. Antibumping granules

were added and the wine distilled until only about 5% of the original volume remained. Some distilled water was added to make the 100ml mark. The liquor was the neutralised with 0.4M NaOH solution and redistilled. This was to eliminate the volatile acids present.

SPECIFIC GRAVITY DETERMINATION

The Pycnometer method is considered to be of international standard,[17,33] and approved by the A.O.A.C.[34]. The weight of a certain volume of an alchol distillate is compared to the weight of exactly the same volume of distilled water. The ratio of the weights of the two solutions gives the specific gravity of the distillate. The value of the alcohol content can be got as volume or weight percent.

APPARATUS

50ml specific gravity bottle and ZEAL thermometer calibrated to 200°C.

PROCUDURE

The specific gravity bottles were cleaned by soaking them in chromic acid over night - and later washing with soap solution and dried in an oven at 80°C. It was cooled in dessicator and weighed (a) Filled with distilled water dried and weighed again (b) The

weight of the distilled water alone is b - a.

The specific gravity bottle was then filled with the wine distillate and weighed too (c) The weight of the distillate is c - a.

Thus the specific gravity of the distillate at room temperature 26°C is obtained by the formula.

$$D = \frac{c - a}{b - a}$$

But for comparative analysis and as recommended by A.O.A.C., the specific gravity should be obtained at 20°C.

i.e.

$$D_{20^\circ/20^\circ C} = \frac{1}{M_w}\left\{P_a - M_o - d_i\left(\frac{P_a}{db} - \frac{M_o}{dg}\right)\right\} + \frac{d_i}{d_w}$$

$D_{20^\circ/20^\circ C}$ = Specific gravity at 20°C.

M_w = weight of water content of the specific gravity bottle at the measuring temperature

P_a = Weight of the S.G bottle filled with sample.

M_o = weight of empty S.G bottle.

dg = Density of glass - for pyrex that was used it is 2.23 g/l

db = Density of the weights = 8.4 g/ml

d_w = Density of water at 20°C = 0.99823.

d_i = Density of air under weighing condition.

With the value of the specific gravity of the distillate, the ethanol content of the wine is got by contacting tables[6,34].

RAFRACTIVE INDEX

The ethanol content of the distillate can also be checked by measuring the refractive index. Ethanol causes the most significant contribution to changes in refractive index; The other components that interfere in the distillate are primarily the secondary alcohols.

Tables are available for dilution corrections[34] and for relating the refractive index to percent alcohol at several temperature[17].

APPARATUS

Refractometer: Abbe 60 Refractometer see page 23.

Thermometer : ZEAL 30mm immersion thermometer. calitrated from 0° to 200°C.

PROCEDURE

The main prisum was exposed by opening the hinged illuminating prism through 180°. Two drops of the distillate were put on the main horizontal prism. The light was put on and the hair line adjusted to the centre. And the refractive index read from the illuminated callibrated scale.

EXTRACT DETERMINATION

The extract content of grape wines indicate the initial sugar content of the must. Thus the higher the initial sugar content of the must, the higher the non-alcoholic residue of the resulting wine.

The extract content can be determined using the formula

$$E = R - 0.9T - 0.8A - 0.05ET.$$

where:

E = (sugar free) Extract

R = Indicated extract - reducing sugar.

T = Titratable acidity

A = Ash.

ET = Ethanol content.

Also for official use, a modified formula is applied.

$$E = R + 0.125 + 0.75V - 0.9T - 0.8A - 0.05ET - 0.2TA$$

L = Lactic acid.

TA = Tartaric acid

V = volatile acid.

All the other notations are as given above. Both the formulas given above are important in Europe where wine sophistication is a more serious problem.

In the United States, the extract content is determined by the Taberie formula[17] which is what was used in this thesis.

$$dr = d_w - d_a + 1.0000$$

dr = specific gravity of the wine residue

d_a = specific gravity of the wine distillate

d_w = specific gravity of the wine.

PROCEDURE

See SPECIFIC GRAVITY DETERMINATION ON PAGES 41-42

The value of the dr got was converted to the amount of extract expressed as grams per 100ml with the help of a table (see ref. 6, 33)

FREE SULPHUR DIOXIDE (SO_2)

Sulphur dioxide (SO_2) is one of the chemical additives added to wine as antiseptic agents.

SO_2 is introduced into wines by using potassium metabisulphite, cylinders of compressed SO_2 or solutions of SO_2 in water.

The determination of SO_2 is based on the oxidation - reduction reaction.

$$SO_2 + I_3^{-} + H_2O \longrightarrow SO_3 + 3I^{-} + 2H^{+}$$

APPARATUS

An Autoberette A.B.U 11 was used with a ZEAL thermometer 30mm immension calibrated to 200°C.

REAGENTS

0.02M iodine solution prepared in potassium iodide solution (see page 24)

Conc. sulphuric acid of specific gravity, 1.84.

PROCEDURE

25ml of the wine samples were pipetted into three 250-ml Erlenmeyer flasks and 5ml of 1:3 sulphuric acid was added and rapidly titrated with 0.02M iodine solution freshly prepared by adding calculated amount of resublimed iodine solid into concentrated solution of potassium iodide and made to the mark. The H_2SO_4 was added to reduce the oxidation of polyphenols by iodine.

For red wines, yellow light was shone so as to enhance the end-point visibility.

The free SO_2 was calculated from the formula.

$$\text{Free } SO_2(mg/l) = \frac{(V)(M)(64)(1000)}{v}$$

V = volume of iodine solution used
M = molarity of the iodine solution.
v = volume of the wine sample.

Other methods include that modified by Burroughs and Spar

TOTAL SULPHUR DIOXIDE

Since the free sulphur dioxide is the unbound oxide to acetaldehyde, to determine total sulphur dioxide[32,35] content, it is necessary to hydrolyse the acetaldehyde - α - hydroxysulphonate and other bisulphite complexes. The use of strong alkalis like sodium hydroxide and acidifying with sulphuric acid then titrating with iodine are preferred. The temperature of the liquor is kept below 20°C.

APPARATUS

The autoburette type ABU 11 and ZEAL themometer of 30mm immersion were used.

REAGENTS

Iodine solution, 0.02M

Sodium hydroxide solution, 1.0M.

Concentrated sulphuric acid of specific gravity, 1.84

PROCEDURE

25ml of the wine sample was pipetted with 25ml of 1M sodium hydroxide solution into a 250-ml conical flask. This was thoroughly mixed and allowed to stand for 15 mins. Thus permitting hydrolysis of the acetaldehyde - sulphurous acid. 5ml of starch indicator solution was added with 10ml of 1:3 sulphuric acid. The

liquor was titrated immediately and rapidly with 0.02M iodine solution to a bluish end point. The total sulphur dioxide content was calculated using the formula:

$$\text{Total } SO_2 \text{ (mg/l)} = \frac{(V)(M)(64)(1000)}{v}$$

V = volume of iodine solution used, ml.

M = molarity of iodine solution

v = volume of the wine samples ml.

BENZOIC ACID DETERMINATION

All methods of benzoic acid determination depend on its solubility product in the wine. An immiscible solvent like ether will extract the acid which can later be evaporated and the residue dissolved in 30% alcohol.

APPARATUS

The automatic titration set-up was used.

REAGENT

Solvents - 30% ethanol (V/V) diethyl ether distilled at 34.5°C.

Petroleum ether 100° to 120°C.

Hydorchloric acid, concentrated 1.18g wt. per ml.

Sodium hydroxide, 0.05M. AQUEOUS.

PROCEDURE

10ml of the sample was extracted with 2.0 5.0, 4.0 and 3.0 ml of ethyl ether but in the case of palm wines where emulsion was obtained, some petroleum ether was added. The extracts were evaporated almost to dryness, dissolved in 30% ethanol and titrated with 0.05M sodium hydroxide solution.

The amounts of sodium benzoate in the wines were calculated using the formula below.

$$\text{Sodium benzoate g/100ml} = \frac{(V)(M)(0.144)(100)}{v}$$

V = volume of NaOH solution used, ml

M = Molarity of NaOH solution used,

v = volume of sample used, ml.

ASH CONTENT DETERMINATION

After evaporation and incineration of wines, the ash, which is the inorganic matter of the wine remain. Ashing can be determined at temperatures between 500 - 550^{o}C and not more because of possible loss of chlorides.

APPARATUS

A muffle furnace was used in conjuction with a Gallenkemp Hot box oven size one. Platium dishes were washed, dried and kept in Davisil heat resisting desiccator.

PROCEDURE

The platinum dish was first heated to constant weight (m_1) at 600°C in the muffle furnace. 10ml of wine was pipetted into the dish and evaporated first on water bath and later in the oven at 100°C. The dried sample was then taken into the muffle furnace for 10 mins, removed and allowed to cool in a desiccator. 5ml of distilled water was then added dropwise and later dried in the oven again. It was replaced back into the furnace but this time, for 20 hrs after which the ash was greyish white. It was removed from the furnace, cooled and weighed (m_2).

$$\text{Ash content g/l} = \frac{m_2 - m_1}{V} \times 1000$$

V = volume of sample used, ml.

CHAPTER 4

RESULTS AND DISCUSSION

SOFT DRINKS

Brahma products were collected from Drinco Company Nigeria Limited while Green Sand Shandy from Nigerian Brewries Limited both in Kaduna. Some of the samples were exposed to weather while others were kept in the refrigerator at about $4^{o}C$.

The analysis was conducted from the 4th to the 9th and from 15th to 20th of each month, starting from September to December, in a predetermined sequence followed throughout the duration of the analysis.

The following abbreviations will be encountered -

G.S.S. - Green Sands Shandy.

B.L - Brahma Lemon.

B.T. - Brahma Tonic

B.O. - Brahma Orange

B.G. - Brahma Guarana

Tables 1 - 16 show the results obtained both when exposed to weather condition at an average of $26.4^{o}C$ and when kept in the refrigerator at an average temperature of $4^{o}C$.

The Specific Gravity (S.G,)

A regular increase was observed in the specific gravity values of the drinks (Table 1). Since the containers were sealed, the Law of conservation of mass holds. However, certain reactions and/or growth of yeasts and moulds can affect the specific gravity value in some way. If for instance, the yeasts increased in number through reproduction, an increase will therefore be noticed in the specific gravity. If alcohol was produced as a result of some of the reactions going on in the drinks, and that to determine the specific gravity the drinks had to be degassed by boiling thus eliminating the alcohol. This will increase the specific gravity value.

B.O. had the greatest increase amongst the drinks examined followed by B.L. If it is assumed that the increase in S.G. was due to increase in the number of bacteria then B.O. had the highest amount of bacteria over the four months.

S.G. is a measure of the sugar content of soft drinks. Changes in the S.G. value indicates changes in the sugar content and as such the taste of the drink.

The production of alcohol and some other products might affect both the smell and the eye-appeal of the drinks.

At 4°C however (Table 15) B.L. recorded a change of 0.0057 units over the four months while it was as much as 0.0164 units when exposed to weather condition over the same period. This indicates a slower rate of reaction in the cold than at higher temperatures.

The Preservatives.

The preservatives used in soft drinks are benzoic acid and ascorbic acid.

Ascorbic acid acts as an antioxidant to prevent rancidity which is generally due to oxidation through the formation of peroxides at the double bonds of fat molecules with subsequent breakdown of these peroxides to form aldehydes, ketones and acids of lower molecular weights.[37] Ascorbic acid when added to drinks is preferentially oxidised and lost.[4] Thus a decrease in asorbic acid content was expected and at the begining of the third month, there was virtually no ascorbic acid left in the drinks suggesting commencementofspoilage. Talking about

shelf life therefore, B.L. should have the shortest followed by G.S.S. and B.G.

Benzoic acid on the other hand acts as a germicidal or bacteriocidal agent[37]. It is expected to react with the cells of the microorganisms thus killing them. A decrease was therefore expected in the benzoic acid content (Table 6). The decrease also points to the fact that there was constant increase in bacterial growth in the drinks unless of course if the benzoic acid was involved in another reaction occuring in the drinks.

The pH

Microorganisms are known to develop most rapidly at the pH optimum and ceases to develop at their pH minimum. If the environmental pH is lower than the minimum for a given type of microorganism, it will be unable to develop and will eventually die[4].

Results show a regular increase in pH value (Table 3) with time thus making the drink more succeptible to bacterial growth or in other words the pH approaches the optimum for the microorganisms. The increase in the pH values can be attributed to some supposed reactions occuring within the drinks

involving some of the acids present most probably the preservatives.

By the begining of the third month, B.L. had a pH of 2.9 second to B.G with a pH of 3.0. There is therefore the highest possibility of bacterial growth in these drinks.

At $4^{o}C$, the pH of B.L was 2.75 at the begining of the third month. (Table 13).

Acidity

The acidity value is a measure of the weak acids (acetic, lactic, formic, Tartaric) present in the drinks and according to Nigerian Standard Organisation[38] should not exceed 0.5%

From the analysis, the acidity value seems to remain constant over the four months period (Table 4). This implies that either no acid was produced or involved in any of the reactions going on in the drinks or that the rate of consumption of the acids equals the rate of their production.

The acidity value is highest in B.O. followed by B.L. and does not seem to have any vissible effect on the shelf life of the drinks.

RECEIVED

Carbonation

Increase in carbon dioxide content of the drinks (Table 8) can be as a result of (1) chemical reactions going on in the drinks and (2) respiration by yeasts believed present in the drinks.

Since carbon dioxide has a noticeable effect on the taste of the drinks, an increase therefore will affect the taste of the drinks differing them from their specific tastes.

Protein

The protein values of drinks decreased with time implying decrease in nutritional value of the drinks. B.T. seems to have no protein at all whereas at the end of the third month, B.L. also recorded no protein. Highest protein value of 480 Mg/ml was recorded in G.S.S

Air content

Except in P.O, air content increased significantly. This points to the fact that some of the reactions occuring in the drinks involve production of gases - which increased the air content of the drinks. This also enhances the bacterial growth by supplying air moreover if oxygen is involved.

Ash and Trace Metals

Ash is the inorganic components of the drinks. G.S.S. was found to contain the highest concentration of inorganic components of 3.8 mg/10mls followed by B.G. while B.L had the lowest (Table 11). Ash may therefore affect the shelf-life of soft drinks i.e. the lower the ash, the lower the shelf life.

Table 12 showed B.O. having the highest concentration of Iron followed closely by G.S.S. iron in the Ferrous state is easily oxidised to the Ferric State with the gain of an electron, and since the resulting ferric ion have a brown colour and a rusty taste, this can greatly affect the shelf-life of the drink. The other trace metal examined may have no effect on shelf-life.

Colour

The absorbance measured in the colour determination is believed to involve more than one absorbing species because an increase in the absorbance was observed but brighter colouring of the samples was not observed. If a system contains more than one absorbing components, it is assumed that the species act independently of

each other and their absorbance is additive[18]. The increase in the absorbance may not be due to increase in the principal absorbing species but rather an increase in the minor components. These minor components may increase as a result of reactions going on in the drinks. These will eventually affect the eye appeal of the drinks.

At $4^{o}C$, the rate of increase in the absorbance was not as high as when exposed to weather condition. This also shows the dependence of chemical reactions on temperature.

Table 1

SPECIFIC GRAVITY VALUES

	A Sept.	Oct.	Nov.	B Dec.	Diff. (B-A)
G.S.S.	1.0339	1.0392	1.0435	1.0488	0.0449
B.L.	1.0358	1.0412	1.0468	1.0522	0.0164
B.T.	1.0308	1.0349	1.0388	1.0421	0.0113
B.O.	1.0436	1.0775	1.0599	1.0639	0.0203
B.G.	1.0437	1.0483	1.0542	1.0586	0.0149

Table 2

REFRACTIVE INDEX VALUES

	Sept.	Oct.	Nov.	Dec.
G.S.S.	1.348	1.652	1.675	1.682
B.L.	1.346	1.666	1.669	1.671
B.T.	1.344	1.659	1.666	1.671
B.O.	1.348	1.678	1.685	1.693
B.G.	1.350	1.664	1.676	1.684

Table 3

pH. Values

	Sept.	Oct.	Nov.	Dec.
G.S.S.	2.35	2.5	2.7	2.9
B.L.	2.50	2.7	2.9	3.0
B.T.	2.30	2.5	2.7	2.8
B.O.	2.40	2.6	2.8	2.9
B.G.	2.65	2.8	3.0	3.1

Table 4

ACIDITY VALUES

g/100ml

	Sept.	Oct.	Nov.	Dec.
G.S.S.	0.221	0.242	0.240	0.237
B.L.	0.163	0.184	0.1825	0.182
B.T.	0.237	0.245	0.2431	0.243
B.O.	0.229	0.261	0.275	0.262
B.G.	0.160	0.179	0.1721	0.175

Table 5

ASCORBIC ACID VALUES mg/ml

	Sept	Oct.	Nov.	Dec.
G.S.S.	0.0488	0.0075	0.0022	
B.L.	0.0488	0.0044	0.0012	
B.T.	0.0675	0.0076	0.0030	
B.O.	0.0525	0.0134	0.0084	
B.G.	0.0638	0.0075	0.0025	

Table 6

SODIUM BENZOATE VALUES g/100ml

	Sept.	Oct	Nov.	Dec.
G.S.S.	0.1011	0.0698	0.0521	0.0489
B.L.	0.0652	0.0373	0.0373	0.0361
B.T.	0.0714	0.0368	0.0328	0.0304
B.O.	0.050	0.0480	0.0411	0.0389
B.G.	0.1236	0.0746	0.0604	0.0599

Table 7

PROTEIN VALUES

µg/ml

	Sept.	Oct.	Nov.	Dec.
G.S.S.	430	520	200	180
B.L.	240	120	00	00
B.T.	0	180	00	00
B.O.	260	240	140	90
P.G.	200	400	100	60

Table 8

CARBONATION VALUES

	Sept.	Oct.	Nov.	Dec
G.S.S.	0.52	0.53	0.53	0.55
B.L.	3.3	3.3	0.46	0.48
B.T.	3.2	3.2	0.45	0.45
B.O.	3.2	3.2	0.46	0.46
B.G.	2.9	3.0	0.45	0.46

Table 9

COLOUR nm.

	Sept.	Oct.	Nov.	Dec.
G.S.S.	1.074	1.138	1.182	1.196
B.L.	0.0210	0.034	0.048	0.052
B.T.	0.027	0.068	0.073	0.072
B.O.	1.338	1.562	1.602	1.651
B.G.	1.016	1.156	1.161	1.175

Table 10

AIR CONTENT

	Oct.	Nov.
G.S.S.	5.2	7.5
B.L.	6.0	6.5
B.T.	3.4	4.2
B.O.	2.3	2.3
B.G.	2.8	3.6

Table 13

LEFT AT .°C (IN THE REFRIGERATOR)

pH VALUES

	Sept.	Oct.	Nov.	Dec.
B.L	2.50	2.6	2.7	2.75
B.T.	2.3	2.4	2.5	2.6
B.O.	2.4	2.5	2.6	2.7

Table 14

ACIDITY VALUES g/100ml

	Sept.	Oct.	Nov.	Dec.
B.T.	0.237	0.2492	0.258	0.251
B.L.	0.163	0.183	0.184	0.198
B.O.	0.229	0.259	0.258	0.261

Table 11

	Ash mg/10ml	Average liquid content, ml	Head Space ml
G.S.S.	3.8	273.33	26.67
B.L.	2.2	296.67	16.56
B.T.	2.4	296.67	16.56
B.O.	2.4	296.67	16.56
B.G.	2.8	296.67	16.56

Table 12

TRACE METALS ppm

	Cu	Fe	Pb	Zn	Co	Cr.
G.S.S.	0.02	0.02	ND	ND	ND	ND
B.L.	0.03	0.06	ND	1.1	ND	ND
B.T.	ND	ND	0.1	0.6	ND	ND
B.O.	ND	0.27	0.1	0.4	ND	ND
B.G.	ND	0.06	ND	0.5	ND	ND

ND = Not Detected.

Table 15

SPECIFIC GRAVITY VALUES

	Sept.	Oct.	Nov.	Dec.
B.T.	1.0308	1.0325	1.0334	1.0351
B.L.	1.0358	1.0362	1.0388	1.0415
B.O.	1.0436	1.0461	1.0677	1.0781

Table 16

C O L O U R

	Sept.	Oct.	Nov.	Dec.
B.T.	0.027	0.052	0.061	0.073
B.L.	0.0210	0.023	0.029	0.037
B.O.	1.338	1.372	1.482	1.610

WINES

FIIRO - represents the palm wine obtained from the Federal Institute of Industrial Research Oshodi.

'Uncle 'T' - represents the palm wine bottled by Uncle T Company Lagos called 'emu'.

'White' - refers to 'white Lady' which is a white grape wine bottled by OZDE distilleries Lagos.

'Red' - refers to 'Dark Lady' which is a red grape wine bottle by OZDE distilleries Lagos.

White and red are table wines

The results obtained from the analysis of wines are compiled in table 17 overlieaf.

Table 17
TABLE OF RESULTS OBTAINED FOR THE ANALYSIS OF WINES

Parameter	'Uncle T"	FIIRO	White	Red
pH	2.95	3.00	3.05	3.35
Total (titratable) acidity g/100ml	0.26%	0.41%	0.61%	0.84%
Volatile acidity g/100ml	0.089	0.143	0.116	0.142
Specific Gravity of wine samples	1.0227	1.0233	0.9900	0.9912
Specific Gravity of wine Distillate	0.9957	0.9952	0.9771	0.9822
Ethanol content % m/V % V/V	 2.33 2.93	 2.61 3.28	 14.82 18.31	 11.04 13.72
Free Sulphur Dioxide mg/l	3.375	3.99	14.483	9.675
Total sulphur Dioxide mg/l	37.32	20.82	359.19	238.08
Extract content g/100ml	6.99	7.25	3.36	2.32
Ash content g/l	0.58	1.50	1.92	2.36
Benzoic Acid g/100ml	0.7640	0.6016	0.0648	0.0707

pH

The pH value is of very great importance in the resistance of wines to disease. The smaller the pH value[5], the greater its effect on microorganisms. Nonetheless, the pH of wines should not exceed 3.6.

The pH values obtained for the wines analysed lie between 2.9 and 3.4 which are within specified limit. The least succeptible to disease is 'uncle T' with the lowest pH value of 2.95 followed closely by FIIRO. Both being palmwines, it is safe to say that palmwines are less succeptible to disease than grape wines.

Total (titratable) acidity

The titratable acidity[24,39] of wines is used during processing and finishing operations to standardize the wines. For table wines, the acidity values should range between 0.6 - 0.9% (as grams of tartaric acid per 100ml of wine).

From the results obtained, both 'white' and 'red' have titratable acidities within the specified range. The titratable acidity of palm wines are relatively low compared to the grape wines analysed. Although the

titratable acidity value has no particular singular effect on the quality of wines, the Eastern United States government does not allow titratable acidity value of less than 0.5%.

Volatile Acidity

Wines of high volatile acidity[25] indicate that spoilage organisms, particularly 'Acetobacter', are present and that the wine may eventually turn to vinegar.

Table 18

Maximum Permitted Volatile Acidity in Wines

Wine Type	United States		France	German	Italy
	Federal	California			
Red table wines	0.140	0.120	0.110	0.160	0.25
White table wines	0.120	0.110	0.110	0.120	0.25
All other wines	0.120	0.110	0.110	0.250	0.25

High ethyl acetate and acetic acid content are know to be responsible for spoiled odour. It is also known that both are produced simultaneously and proportionately, the amount of acetic acid therefore is a good measure of the amount of ethyl acetate present, hence of the degree of spoilage in the wine.

The grape wines analysed fall into specification (red and white German table wines).

'Uncle T' has the lowest volatile acidity value of 0.089g/100ml which falls below the United States legal limit of 0.06 - 0.090g/100ml.

Ethanol Content

Ethanol[6] is important for the taste, adds little odour and "body" to the wines. Wines of less than 10% V/V ethanol tend to enhance bacterial growth easily. Thus for wines in this category, enough antiseptic is used.

Table 19

Legal Ranges and limits for Ethanol in Wines[1]

Table wines	Ethanol (% V/V)		
	California (minimum)	United States	
		Min.	Max.
White and rose	10.0	-	14.0
Red	10.5	-	14.0

The results obtained show that the ethanol content of white wine is above the maximum acceptable limit.which suggests fortification (see table 17).

On the other hand, the ethanol contents of both FIIRO and 'uncle T' (i.e. 3.28 and 2.93% V/V) fall below the value of 4% v/v indicated on their labels and also below spoilage limit of 10% V/V.

Table 20

Changes in Ethanol Content within 24 hours

	Fresh Samples		24 hours laterat 4°C	
	Ethanol Content % V/V	S.G	% V/V Ethanol content	Specific Gravity
'Uncle T'	2.65	0.99613	0.60	0.99911
FIIRO	3.00	0.9956	1.21	0.99822
white	18.31	0.97712	18.31	0.97709
red	13.72	0.98215	13.72	0.98212

The specific gravity which is an indicator of the ethanol content was found to change rapidly within 24 hours for palm wines and did not change noticeably in grape wines (Table 20). The change can be due to loss of ethanol or bacterial growth since bendable crown[40] are used to cover palm wines.

Free and Total Sulphur dioxide.

Wine bacteria are well inhibited by SO_2, As little as 100mg/l of total SO_2 in some red wines is sufficient to inhibit growth of malo-lactic bacteria.

As a rule, SO_2 is maintained in wines between 5 and 40mg/ℓ as free SO_2. The free SO_2 if present in excess reacts vigorously with the receptors in the nose causing sneezing, pain and choking sensations. Wine with high pH are known to have very little SO_2 in the gaseous form[6]. In levels usually present in wine when consumed (60 - 150mg/l with less than 15mg/ℓ of free SO_2), The adverse sensory effects noted are very slight.

Results obtained show palm wines to have less than 5mg/ℓ which is below legal limit. Also grape wines have high total SO_2 than expected in wines when consumed (see Table 17).

Benzoic acid

The use of benzoic acid[6] in wines has largely disappeared mainly because of its inferior antiseptic properties, Amounts less than 100mg/ℓ are acceptable in wines.

About 600 - 800mg/ℓ was observed in palmwines while grape wines show as little as 60 - 70mg/ℓ. Benzoic acid is noticed to be the major preservative in palmwine.

For the determination of benzoic acid, the volume of the ethyl ether/petroleum ether had to be increased to enhance the separation of the benzoic and ether layer.

Extract

The higher the initial sugar content of the must the higher the extract content of the resulting wine. Red wines have higher residual extract than do white wines.

Extract values[5,6] range from 0.7g to 3g/100ml with an average of 2.0g/100ml.

It was noticed from experiments that palmwines have as much as twice extract content than grape wine analysed (see table 18). This indicates that palm wines

have higher initial sugar concentration than do grape wines. FIIRO has the highest extract content of 7.25g/100ml as compared to 'uncle T' with 6.99g/100ml. This implies that FIIRO had a higher initial sugar content than 'uncle T'. This is not expected since the raw material for both was the same (emu). There are two deductions that can be arrived at with the above result. Either that sugar was added to the raw material for FIIRO or that the raw material for 'uncle T' was diluted.

In France, for comparative analysis, alcohol - to - extract ratio is used.

Alcohol-to-extract ratio

The alcohol-to-extract[5] ratio is not expected to exceed 4.5 in red or 6.5 in white. To determine this ratio, the alcohol is first expressed in grams per litre by multiplying the vol.% by 7.94. If the ratio should exceed stipulated values, the wines are said to be fortified with spirit.

Table 21

	Extract g/l	Ethanol		Ethanol-to-extract ratio
		V%	g/l	
'Uncle T'	69.9	2.93	23.27	0.33
FIIRO	72.5	3.28	26.01	0.36
White	33.6	18.31	145.38	4.33
Red	23.2	13.72	108.94	4.70

Grape wines have alcohol - to - extract ratio in the specified range. Palm wines have extremely low values as expected because of their relatively high extract content.

Ash.

The ash content of wine is usually from 0.1 - 0.4% or 1.0 to 4.0g/l if lower, dilution may be suspected and an abnormally high ash indicates plastering.

1.92g/l was observed for white wine analysed while 2.36g/l for red showing higher ash content in white than in red as expected. 'Uncle T' has the lowest ash content suggesting dilution.

Q - value can also be used to detect watering or plastering in wines and it is given by

Q - value = sugar free extract (g/l) - alcohol (V%) + ash g/l.

The Q-value is known to decrease as the raw material is watered. 'Uncle T' and FIRRO have same raw material (emu) therefore their Q-values can be compared:

For 'uncle T'

Extract = 69.9g/l.
Alcohol = 2.93V%
ash = 0.58g/l
∴ Q - value = 69.9 - 2.93 + 0.58
= 67.55

For FIIRO

Extract = 72.5g/l
alcohol = 3.28V%
Ash = 1.5g/l
∴ Q - value = 72.5 - 3.28 + 1.5
= 70.72

Lower Q - value for 'uncle T' indicates watering of the wine.

C O N C L U S I O N

From the data obtained from this investigation, the lower the temperature and pH of the soft drinks, the longer the shelf-life of the drinks. The amounts of trace iron present seems to affect the shelf-life of soft drinks by its change of oxidation states. Given the type of climatic condition prevailing in this part of the world, the shelf-life of soft drinks is probably three months with Brahma ~~Orange~~ Lemon having the shortest.

Palm wines compare favourably with grape wines although certain differences do occur for example in the type of preservative used. While SO_2 is predominantly used in grape wines, benzoic acid is preferred in palm-wines. Bendable crowns used in palm-wines bottling afford some losses in alcohol contents of the wines. Since palm wine trees grow to a large extent in this part of the world, foreign exchange can be conserved if palm-wine should replace grape imported wines. There could be some difficulty in