QUALITY OF SOME BEERS BREWED IN NIGERIA

A THESIS PRESENTED TO THE AHMADU BELLO UNIVERSITY, ZARIA, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN ANALYTICAL CHEMISTRY

BY

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DEDICATION

Dedicated to my father, late mother, my wife, my brothers and sisters.

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I am grateful to Dr. K. Singh and Dr. M.S. Elmaraiby for supervising this work.

I am thankful to the Head of Chemistry Department for providing the necessary facilities.

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I am deeply indebted to Mrs. J.I. Komak, my wife, for patiently suffering various hardships as a result of my absence from home during the period of this study.

DECLARATION

The work contained in this thesis was carried out by the author in the Department of Chemistry, Ahmadu Bello University, Zaria, and has not been submitted to this University or any other institution for award of a degree.

I. Z. Komak.

A B S T B A C T

The analysis of some Beers such as Star and Guider from Nigerian Breweries Limited, Kaduna; Bock, Jos International Breweries Limited, Jos; Double Crown, North Brewery Limited, Kano; Stout, Nigerian Guiness Stout Limited, Lagos, and Noble, Kwara Breweries Limited, Ijagbo, has been carried out to determine the quality of various Beer samples. The exercise includes•

(l) estimation of alcohol by volume and by weight or mass, apparent extract, real extract and specific gravity;

(ii) determination of the nutritive value of beer, determined by estimating the total nitrogen (protein, reducing sugars and dextrine;

(iii) determination of the chemical compositions of the beers by estimating the ash;

(iv) determination of carbon dioxide content, colour, total acidity, volatile acidity and fixed acidity in the beers.

These constitute the organoleptic property of beers

(v) study of stability of the beers. This involves the estimation of apparent attenuation, real attenuation and determination of pH and Haze or turbidity of the bottled beer.

(vi) Last study is concerned with some factors which affect the quality of bottled beer. This requires the determination of

the concentration of copper, iron, and chloride ions present in the beer samples.

The quality of various samples of beer based on the analytical study suggests that deterioration in shelf-life of beers solely depends on the quantity of iron, copper or chloride ions present. Iron and copper have catalysing effects on the quality of beer since their presence introduces oxidation-reduction processes to occur, while the presence of chloride ion in high concentration may lead to deterioration in the beer taste.

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CHAPTER I

INTRODUCTION

Beer

Beer[1-6] is an alcoholic drink made from barley malt and hops. The malt extract solution flavoured by hops is fermented by yeast to alcohol and carbon dioxide gas.

The result is a sparkling, golden alcoholic beverage, with alcoholic content between 3-8 per cent, with a characteristic refreshing bitter taste.

Beer is often used as a generic[7] term for malt beverages in general, comprising both bottom-fermented lager beer and top-fermented ale, stout, porter, etc.

In bottom fermentation[7], for lager beer yeast is added to the cooled wort. After 15 to 24 hours in a settling tub, a white froth appears around the edges, indicating incipient fermentation.

In top fermentation for ale, which is distinguished by the use of special top-fermenting yeast and higher temperatures, refrigeration requirements are less. The higher temperatures favour the formation of aromatic and fast substances giving ales and top-fermented beverages in general their specific taste. The yeast-making occurs at and above the liquid surface.

HISTORY

Brewing of beer is both an art and a science.[8] Its beginning, according to recently established findings, dates back to the Sumerians.

1-1 IN AFRICA

Brewing in Egypt[1,3,9,11] began at a later date than in Babylon, but probably developed independently. There is documentary evidence of the drinking of beer by the Egyptians towards the middle of the 3rd millennium. Other sources[11] claim that the manufacturing of alcoholic liquour from grain was practised at least 5,000 years ago by the Egyptians.

Papyri of the period around 1300 B.C. referred to the regulation of beer shops to prevent persons over indulging in beer.

Beer was said to have been a gift of the god Osiris or his wife Isis about 2000 B.C. in Egypt[9], although it seems that several diffefent types of beer were brewed before then.

There is also archaeological evidence which indicates to its use as a mortuary offering in the 5th dynasty about 2800 B.C.[7], also in Egypty.

1-2 IN THE MIDDLE EAST

Brewing, however, seems to have originated in Babylon[9] where barley grew wild. There is archaeological evidence that beer made

from malted grain was being brewed in Mesopotamia by 6000 B.C.

Translation by the late Paul Haupt, Professor of Assyriology at John Hopkins University of Cuneiform writing on a day tablet found in ancient Nineveh and describing Noah's preparation for the flood indicates that beer was among the provision on the Ark.[9]

Brewing was largely a domestic function much as baking and cooking.[6] In the middle ages, brewing, like many other arts, was concentrated in the hands of Churchmen. Almost every monastery had its brewhouse.

Since the raw materials[9] are grain and yeast, brewing was usually carried out by bakers. The barley was soaked until it germinated and then was roughly ground. Yeast was added to this malted material and molded into cakes then used for production of beer.

The origin of the use of hop is unknown. Some authorities think herbs and other flavourings were widely used in these ancient brews.[6]

The Hebrews learned brewing during the Babylonian captivity.[6]

The Greeks[9] learned brewing from the Egyptians and also grew hops, although they did not appear to have used them particularly in brewing.

The Romans learned brewing from the Greeks.[9]

1-3 IN EUROPE

The Northern European[3,9,10] races probably discovered the technique of brewing long before the Christian era.

Beer has always been drunk most extensively in lands where soil and climate are inhospitable to the vine.

According to Diodorus Siculus,[3] in the second half of the first century B.C., Britons drank fermented liquour made from barley, honey or apples on festive occasion. At the time of the Roman invasion, Britons in the south of England made a kind of ale from barley and wheat and the inhabitants of Wales and Ireland also brewed ale, although it was a luxury only for the upper classes. From that time onward, beer has broadly speaking been a staple drink for adults in all classes in Britain.

Duty[9] on beer was first introduced in England in 1660 and the rate was 4s.9d. per barrel for small intermediate or table beer. At later dates the duty was assessed on the volume of malt used to make the beer, with separate duties on hop and sugar. The system of beer duty used more recently was brought into operation in Gladstone's[10] budget of 1880; by this system the duty is assessed on the original gravity of the beer before fermentation.

Tacitus and Pliny[3] both recorded that liquor made from corn and water was brewed in Germany, Spain, Russia and China.

1-4 IN AMERICA

A number of alcoholic beverages were made by the Indians long before the advent of Europeans.[6]

The history of American[9,11] brewing really begins in 1584 when the British brewed beer from corn (maize) during their first attempt to colonize Virginia.

During the era of exploration of the American continent beer was deemed an essential in the provisioning of ships.

The dwindling supply of beer on the Mayflower was a factor in the Pilgrims decision to seek harbour ahead of schedule. A journal of the voyage published in 1622, records that the Pilgrims landed at Plymouth Rock because "We could not take time for further search .... Our victual being much spent ... especially our beer."

The first commercial brewing in America was in New Amsterdam about 1633, in the vicinity of what is now the New York[6] City financial center.

The first formal licence for brewing was issued by the Massachusetts Bay Colony in 1637 to Captain Robert Sedgwick, a Commander of militia, at Charlestown, Mass.

The Massachusetts legislature, in 1789, passed an act exempting brewers from taxation for a five-year period, stating that "the wholesome qualities of malt liquors greatly recommend them to general use, as an important means of preserving the health of the citizen of this Commonwealth".[11]

In the 1840's, however, the introduction of lager-type yeast from Germany gave a new stimulus to the American[6] industry. American consumers took to the new beer which had better keeping qualities and seemed more palatable.

The advent of mechanical refrigeration which permitted longer aging under controlled temperature and airconditiong, which sterilized air in the fermentation room were important industry aids.

In the year 1863, an excise tax was applied on beer; American production then was 1.8 million barrels.[6]

In 1933, production taxes were collected on nearly 20.5 million barrels of beer, by 1934 this figure had increased to more than 40 million.

Jean Talor[9] established a brewery in Quebec city on the shore of the St. Charles river. This was the first brewery in Canada. Other breweries were established later.

The history of beer in different parts of the world has been cited for the following reasons:

(a) To establish the fact that the making of fermented beverages was discovered by primitive man, and has been practised as an art for thousands of years.

(b) That the basic principles of brewing have never changed, but rather that newer, better and more efficient machinery is now available for the production of beer.

(c) That beer has always been brewed and liked for its superior flavour, and positive keeping qualities.

(d) That better known beer brands today had their beig beginning in the past.

(e) That beer at various times has been subjected to different legislatures.

(f) That beer has provided the much needed source of revenue for several governments in different parts of the world including Nigeria.

In conclusion, nowadays careful scientific control of the raw materials, (the malt, water, hops and yeast), attention to detail at every stage, the skill and experience of the production staff, all help to produce a consistent high quality product.

1-5 RAW MATERIALS[12]

The three principal ingredients of beer are malt, hops and water, with yeast as an all-important operating agent. Very briefly, barley is converted into malt which is ground up with water to form a mash. The enzymes in the malt convert the starch into the fermentable sugar maltose. This is the wort, which is then boiled in a copper vessel or kettle with the addition of hops. After straining and cooling the hopped wort runs into a fermenting vessel into which yeast is added. In the course of a few days conversion of the sug

in the wort to alcohol and carbon dioxide has taken place and the resulting beer is run off to tanks from which in due course it is "racked" into cask or filled into bottles. Such are the basic principles of beer production with, in practice, many variations in detail.

The various raw materials used for production of beer are detailed hereunder.

1-5.1 Barley[7,13,14]

Barley has been used for making beer since historic times and now more than 10% of the world crop is used for this purpose. The two-rowed varieties of barley are used chiefly for malting, a process in which the grains are germinated in water and then killed by drying or gentle roasting.

A number of qualities are looked for in good malting barley; e.g., appearance and size of the corn. Such factors as ripeness, dormancy, water sensitivity, extract and loss during malting are also of real consequence.

The first stage of analysis of barley is careful sampling from bulk. Sample is not opened until it has reached room temperature, otherwise moisture might be absorbed from the air. The principal data required are (a) moisture (b) nitrogen (c) thousand corn weight (d) germination (e) percentage of foreign matter and half-

corns and (f) prediction of extract.

The normal moisture content of barley varies between 12-20% Barley with a lower moisture content than 16% keeps well, but if the moisture content be over this amount, the barley rapidly deteriorates, loses its germinating power and tends to go mouldy, and this process is accelerated the higher the moisture content over this value. The moisture content of a barley sample is therefore an estimation of the utmost importance. Moreover, the moisture content is also of economic importance, and it is indispensable to know this value, so that the analytical data can be calculated on a dry weight basis and comparable results can be obtained with other samples.

The analysis of the protein content of the barley corn is of the greatest importance and interest. Barley with a total protein content of 9-11% based on dry weight are to be preferred for brewing. Higher protein contents than this may lead to a lower extract figure and react unfavourably on the stability of the beer, while with lower amounts there is always the risk of obtaining a beer lacking palate fullness.

For thousand-corn weight, 1,000 grains are counted and weighed on a Tre'buchet balance. To obtain any exact value, the grains are weighed three times in all. The moisture content of the 1,000 grains is then subtracted from this figure, so as to obtain the dry weight of the sample. The dry weight of 1,000 corns varies between

30 - 50g. Equally, a 50-g sample can also be weighed three times and the grains counted.

Germination tests on barley are fundamental for the whole success of the brewing process. To obtain a good, sound malt and good beer, it is essential that the grains should be capable of even and regular germination. Barley grains do not germinate immediately after harvesting; the grains will germinate after they have passed through a dormant phase.

In addition to the determination of the percentage of foreign matter and half corns, the following routine checks are usually done: (a) colouring power (b) formation of haze in beer (c) iron content (d) extract (e) percentage of fermentable reducing sugars and (f) presence of foreign colouring materials.

Extract includes all the materials in the barley grain which can be broken down at mashing and pass into the wort. Estimation of barley extract in brewing gives an idea of the chemical constituents present and also gives an indication of the amount of extract obtainable from the subsequent malt made from the barley.

The malting process consists of steeping the barley corns in water, allowing them to sprout and then kilning. Malting is carried out under controlled conditions and for the purpose of developing their latent enzymatic powers, mellowing their original hard and raw condition, and, in general, making them suitable for

certain uses in the brewing. Originally, the sprouting stages took place on floors, in pieces a few inches deep and much malt is still prepared in this way, but drums and saladin "boxes" are replacing floor malting. Furthermore, there have been recent development in three directions; the use of re-steeping technique, the employment of gibberellic acid, and the development of continuous malting. These newer procedures can affect quality, and considerable economic claims are made for them. With gibberellic acid, whether applied in the steep or later, the claim is for increased extract and the saving of much time on the floor.

Malt as the sole source of enzymes for mashing and because of its decisive influence on the characteristic taste of beer barley malt is the one indispensable brewing material often referred to as the "soul" of the beer.[11] Chemically considered, it is composed of carbohydrates mainly starch 60 - 70% protein 10 - 14%, mineral salts about 3%, cellulose 10 - 13%, moisture 4 - 5%, small amount of fat.

1-5.2 Water[7,15,16]

Water constitutes about 90 per cent of the composition of average beer material. The mineral salts of brewing water exert far-reaching effects on the enzymatic actions during mashing and fermentation, on the extraction of the hops in the kettle, and on the soundness of the fresh yeast crops. These effects are either directly attributable to such ions as Ca^{++}, Mg^{++}, HCO_3^-, OH^-, SO_4^{--}

and Cl^-, present in the water, or they arise indirectly from fluctuations in the delicate hydrogen-ion balance (pH) or active acidity at any of the stages of the brewing process.

Brewing water must be clean, fresh, palatable, free from iron and preferably of a moderate degree of permanent or sulphate hardness.

The well water at Burton-on-Trent in England, which contains a high proportion of gypsum or calcium sulphate is used for brewing pale beer (ale). Such water requires no treatment beyond a boil and is also good for strong and mild beers. At Pilsen, where the beer brewed is pale lager, a very soft water with low salt concentration is used and is considered particularly suitable where a delicate hop flavour is involved. At Munich and Dublin, dark beers and stout are brewed from soft waters or even waters of slight temporary or carbonate hardness. These waters have low chlorides and sulphates and the Ca and Mg ions balance the CO_{-3} ions. To brew pale beers with such waters it would be necessary to remove the carbonates and replace by gypsum. Most natural waters require treatment with hardening salts containing sulphates and acidifying agents in order to insure a condition favourable to enzyme actions and hop extraction.

The brewer does not carry out a water analysis every time he brews, so that in that sense he does not have a quality control on his brewing liquor as water is referred to by the brewers. However,

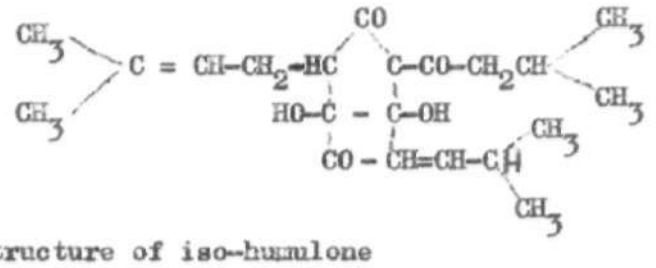
it is recognized that in most cases the mineral content of the water is fairly constant. The brewer, matches it to the type of beer he is going to brew. The influence of composition of brewing liquor from mash tun onwards is very marked. In addition to a tasteless, odourless, colourless, free from iron and manganese, free from suspended matter, brewing liquor must also be free of contamination by micro-organisms. A regular examination to test these points is usually necessary.

1-5.3 Hops[14-15,18]

Hops are used primarily for flavouring but they have important preservative qualities. There are three important groups of substances carried by the ripe hop:

(1) essential oils giving volatile aromatic flavours, (2) resins, giving bitterness and having preservative properties, and (3) tannin-like bodies which affect the classification and brilliance of the final beer.

The isomer of humulone, iso-humulone, which is formed when the wort is boiled, is responsible for the bitter flavour of beer. The structure of this substance is as shown:



Structure of iso-humulone

Iso-humulone has a more bitter flavour than humulone and is also more soluble in wort and beer.

Hops have been used in eastern Europe as a yeast preservative in baking and brewing for at least 2000 years. By the 13th century A.D., they were used in brewing throughout central Europe and in England around the 16th century. Their value has long been appreciated, if not understood. The alpha fraction of the soft resins in the cones. has a bacteriostatic action for prolonging the storage life of beer. The soft resins, and possibly other compounds, give beer its characteristic bitter taste. The hop oils in addition to the above confer a delicate flavour and aroma while the hop tannins assistin precipating protein hazes and classifying the beer.

Hops are normally used at the rate of about 1 lb hops per 36-gallon barrel. Hops belong to the genus HUMULUS within which four species have been recognized; namely, LUPULUS, AMERICONUS, JAPONICUS and NEOMEKICANUS. The first two species include all commercial hop varieties. For all practical purposes, HUMULUS LUPULUS is employed.

The hop plant, Humulus Lupulus, a long-lived dioecious perennial is propagated commercially from rhizome sections or root cuttings. The hop is one of the few crop plant species in which male and female flowers are borne on different plants. Exceptions to this general statement may be found, in which hop plants bear both staminate and pistillate flowers, in which case only one sex is usually fertile.

Hop plants are also occasionally found which are barren and fail to produce either flower type. These variants from the regular dioecious hop plants are not uncommon and appear rather frequently in plants grown from seed. The growing of hop plants is a type of breeding experiment.

The female flowers give rise to the hops of commerce, which are commonly referred to as hops, cones, or strobiles. Commercial hops are the dried and pressed cones from the female plant and these may develop without fertilization; the male are not indispensable in the culture of this crop. However, fertilization by the male plants do serve to stimulate the production of larger, heavier, and earlier maturing cones by the female plants. The fertilized female flowers contain seeds which contribute to the weight of the cones but have not reported value in the brewing process. As such, in some parts of the world, especially continental Europe, the presence of a male plant in yards is forbidden by law. In the United States and England about one male to 100 female plants, when properly spaced is considered sufficient for the production of seeded hops. Seedless hops usually command a higher premium on most markets.

The brewing value of hops depends largely upon the amount and quality of the lupulin which is produced in the female flower. This crop is of vital concern to brewers since beer and other malt beverages depend principally on hops for their characteristic flavours.

However, to attain the required hops flavour is very difficult, since the plant is extremely sensitive to sun, wind, heat, rain, insects and diseases, and the quality of the cone depends on type, colour, soundness, aroma, and contents of resins and essential oils. This is of paramount importance since no satisfactory flavour substitute than hops has ever been found.

The soft resins, alpha acids mentioned above in additinn also prevent the growth of certain types of bacteria in the wort, and thus have a preservative effect. The soft resins, alhpa acid and beta fraction, commonly referred to as alpha and beta resins, are derived principally from the humulon and lupulon portions of lupulin. Alpha resin consists of only alpha bitter acid or humulon, whereas the beta fraction is composed of beta bitter acid or lupulon, together with oxidation products of both humulon and lupulon.

Alpha acid and beta fraction are often used in computing a formula for "preservative value" or "brewing value" (P.V.), which might variously be expressed as 10 alpha, $10(\alpha + \frac{\beta}{3})$, $(\alpha + \frac{\beta}{9})$, etc. This is a measure of the inhibitory effect of the soft resins of hops as the growth of certain lactic acid forming bacteria in beer or wort.

In addition to its value in flavouring characteristics various other uses of the hop plant have been reported. For example, the Greek people are supposed to have used hops only as a salad plant.

Hops have also been used in prescriptions to cure many ills of man, and have been consumed green or in liquid concoctions. Some people use the dried cones in pillows since they believe that the aroma of the strobiles induces restful sleep. Fibre from hop stems has been used in the manufacture of twine, and a textile fabric resembling linen. An extract of hops has been used as a hair rinse, particularly for brunettes. Brewers' spent hops have been used as fodder and as a basis for manure in most countries producing beer.

The principl hop-producing countries in the world are the United States, England, Germany, Czechoslovakia, Yugoslavia, France Belgium and Sweden. The hop is a long-day plant and existing varieties are unsuitable for the tropics. Many varieties of hops have been grown in these countries. In U.S., three varieties, fuggles, late clusters, and early clusters, are planted on most of the hop acreage.

1-5.4 Yeast[16,19,20]

Yeast is a microbiological plant, which can change sugars into alcohol and/or carbon dioxide. In bread making, where there is plenty of air available (aerobic) yeasts produce only carbon dioxide. In beer production, in the absence of air (anaerobic) both alcohol and carbon dioxide in almost equal amounts are produced. Carefully selected strains of yeasts are used which produce the desired flavours. The brewing yeast which are specially selected

strains of SACCHAROMYCES CEREVISIAE and SACCHAROMYCES CARLBERGENSIS, ferment the principal sugars in wort (maltose and glucose) to give beer. The alcohol content of beers ranges from 3-8%. This shows that two main types of brewing yeast are recognized. Some strains tend to rise to the top of the wort during fermentation and so are termed top-fermentation yeasts. These are widely used in Britain and in some parts of the United States for brewing ales. But the bulk of the world's beer is produced by botton-fermentation yeasts.

The brewer looks for two main qualities in selecting a yeast. The first of these is the ability to bring about a vigorous fermentation in malt wort. Presumably, these yeasts are particularly well endowed with enzymes as well as with efficient mechanism for transporting sugars into the yeast. A brewer also requires that the yeast be capable of flocculating or depositing itself from the beer at the end of the fermentation. The flocculating ability of yeast depends on the chemical and physical properties of the outer layers of the cell wall and there is some evidence that flocculating yeast differs from non-flocculating strains in having a greater proportion of the polysaccharide mannan in their wall.

Few of the stages which finally lead to the production of alcohol and carbon dioxide by yeast are stated hereunder.

Malt supplies an enzyme, that is an organic catalyst, called diastase. Under this condition the starch is hydrolysed to a sugar maltose. With emperical formula for starch as $C_6H_{10}O_5$

$$2C_6H_{10}O_5 + H_2O \longrightarrow C_{12}H_{22}O_{11} \text{ (maltose)}$$

Yeast is then added at room temperature. One of its enzymes, maltase catalyses the hydrolysis of maltose to glucose

$$C_{12}H_{22}O_{11} + H_2O \longrightarrow 2C_6H_{12}O_6$$

Another enzyme of yeast, zymase catalyses the decomposition of glucose to alcohol and carbon dioxide

$$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + CO_2$$

It is believed today that to reach the end point shown in the equation the action passes through at least 12 different stages in which 12 different enzyme systems are involved. This indicates the great complexity of such natural changes and it is typical of the many which occur in the course of brewing beer.

Yeast and bacteria are unicellular and of very small dimensions. The yeast are irregularly oval and perhaps 0.004 to 0.010 mm in diameter. The yeast multiple by budding.

As already stated above two main strains of yeast are employed for brewing. Saccharomyces cerevisiae are top fermentation yeasts which operate at normal temperature ($60^0 - 70^0$F) and produce a large crop of yeast at the top of the wort; these are used throughout the U.K. and in a few places elsewhere, while saccharomyces carlsbergensis are bottom fermentation yeasts which function at lower tempera-

tures; e.g., 45°F and are consequently much slower in operation and largely 90 to the bottom of the wort. These are used exclusively in other parts of the world.

In top fermentation, yeast from a previous fermentation or from a pure culture plant is added to the wort or "pitched" at a temperature of about 60°F. After an initial lag period, multiplication of the yeast proceeds very rapidly, a yeast crop of 3 - 5 times the weight of the original pitching being obtained. As a result the temperature of the fermentation rises and is controlled to maximum of about 70°F by circulating chilled water through coils of pipes (attemperators). Fermentation takes 3 - 4 days and may be completed in one vessel from which the surplus yeast is skimmed off, or the wort may be "dropped" into "cleasing vessels" from which the yeast is similarly skimmed. At the end of fermentation, the beer is further cooled before "racking" into casks or storage for conditioning. Mild ales are usually sold a few days after racking while pale ales and bottled beers are stored for a week or more.

In botton fermentation, the pitching takes place at about 40°F and the major part of the fermentation in open vessels takes 10 - 14 days to complete; the beer is then chilled to between 34° and 36°F and dropped into closed tanks in cold cellars for lagering, a process which may take up to 6 months. After this the beer, highly charged with carbon dioxide gas, is filtered under pressure into

casks or bottles. It is stored and served cold.

In conclusion, the prerequisite conditions for a good fermentation process are:

(a) A micoorganism that forms a desired end product. This organism must be readily propagated and be capable of maintaining biological uniformity, thereby giving predictable yields.

(b) Economic raw materials for the substrate, e.g., starch or one of several sugars.

(c) Acceptable yields.

(d) Rapid fermentation.

(e) A product that is readily recovered and purified.

(f) That the particular strains used, apart from fermentation efficiency, can have an important effect on the flavour of the finished beer.

1-6 PROCESSES

However, good the quality of raw materials used in brewing process may be, much expense and effort will be wasted if the processing is not of a high hygienic standard. Quality control of processing starts at the reception of raw materials into the factory or brewery and is succeeded by further control up to the moment of consumption.

1-6.1 Milling[9,22]

Milling is the process whereby malt is crushed by rollers. The kernels are so crushed that the mealy endosperm is disintegrated as completely as is compatible with the preservation of the husk which is needed for subsequent filtration or lautering of the wort, and also with the avoidance of too much fine grits and flour. Too much breaking up of the husk material allows the release of excessive amounts of polyphenolic compounds. These exert adverse effects on both beer flavour and beer stability. The crushed grain is mixed with water in the mash tun (tank). The amylolytic enzymes which have lain dormant in the malt in its dried state now hydrate and begin to break down the starch material present in the malted barley grain. The enzymes will also break down any carbohydrate adjunct that is added.

Simple malt mills have only one pair of case - hardened steel rolls, 9 to 14 inches in diameter and 14 to 30 inches in length, plain or grooved, and motor-driven at equal or differential speeds of 150 to 560 rpm. Modern multi-roller mills have up to six rolls and are provided with screens which permit bypassing of some mill streams and selective regrinding of others. Automatic scales and scale-hoppers weigh the malt as well as the adjuncts.

1-6.2 Mashing[22]

Mashing is an extraction and enzymatic process in which amylases α and β liquefy starch and convert it into non-fermentable dextrins and the quantitatively more important fermentable sugars; i.e., glucose, maltose and trisaccharide materials. Proteases solubilise and modify the malt proteins; and phosphatases liberate phosphates which "buffer" the mash to a pH of 5.2 to 5.5. Control is exercised by observing or omitting those temperatures at which the different enzymes display their optimum activity or are inactivated, and by varying the corresponding time period.

In some countries, especially America, brewers prefer to brew a beer that is lighter in palate than the full flavoured, rather heavy beers produced when 100% malt is used. They do this by using unmalted cereal grains or parts of cereal grains that contribute essentially nothing to the brewing system but carbohydrate. The most commonly employed adjunct grains are corn and rice. Such cereal grain is ground and sent to a cereal cooker where it is gelatinized and mixed with the malt mash in a variety of ways. All these are designed to allow the saccharifying enzymes present in the malt to break down the grains starch producing fermentable sugars and intermediate fragments.

1-6.3 Lautering[9,22]

Lautering is the process where the insoluble malt substances are separated from the wort by filtering. The mash (the grain residue - liquid mixture) produced by the mashing operation is passed through a straining device called a lauter tun (tank) where the insoluble spent brewers grains are removed. These residual grains are high in protein and have values as a feed supplement.

1-6.4 Wort Boiling[9,22]

Wort boiling is the process where the wort is boiled. It is carried out in a vessel called a wort kettle, hence kettle boil. Here the clean liquid called wort is pumped into the brew kettle where either hops or extracts prepared from hops are added to give the characteristic hop aroma and better taste to beer. Atmospheric boiling is carried out in the kettle for slightly more than an hour generally. During this time, the characteristic beer colour develops in the kettle and a significant portion of the soluble barley protein becomes heat-coagulated. Some brewers add corn syrup at this juncture rather than use a cereal grain adjunct as in the mashing operation. The boiling process makes the wort sterile, changes hop resins into more bitter substances, and produces a precipitate called hot break.

In the whirlpool the hot break settles as a cone in the centre of the vessel. The hopped or bitter wort is pumped to the next stage

leaving this semi-solid cone behind.

Detailed study of the kettle process reveals great complexity in the oxidation, extraction, adsorption, emulsification and colloidal phenomena involved.

1-6.5 Wort Cooling and Trub Removal[9]

The freshly boiled wort is sent through a hop strainer to remove the spent hops and is then allowed to stand until the coagulated protein, commonly called hot trub, settles out. Flat or basket-shaped screens retain the hops and the coagulated sludge. Sparging with water increases the recovery of extract. The wort is then pumped through a coding system which aerates the wort and rapidly cools it down to about 10°C. This cooling causes more protein and protein-polyphenolic conjugated substances to fall out of solution.

Coolers are of several types, double pipe coolers and plate-type heat-exchangers, through which the wort flows counter-current; Bandelot coolers composed of horizontal tubes over which the wort flows in two thin sheets; or combinations of Bandelot and "surface coolers" shallow pans where the brew is allowed to cool to about 32°C simply by evaporation. Strict sanitation and airconditioning eliminate danger of infection at the critical transition temperature. Cold water brine or direct expansion ammonia are the usual refrigeration media. Finally, the material called cold trub must be removed by settling, by filtration and by centrifugation.

1-6.6 Yeast Pitching and Primary Fermentation[22]

Yeast is added to the cooled, aerated wort in a fermenting vessel where the yeast begins to grow and to ferment the sugars present to alcohol and carbon dioxide. Active growth of yeast (budding) takes place for about 48 hours after which budding practically ceases, but fermentation of sugar to alcohol continues actively. The steps involved in the biochemical conversion of glucose and maltose to alcohol are already given under yeast as a raw material.

Glucose is fermented first, it selectively permeates the yeast cell membrane and is metabolised. When it becomes sufficiently low in concentration the yeast cell membrane will begin to allow the permeating of maltose. As the maltose nears depletion the trisaccharides material present is taken up.

Production brewer's yeast is recovered from the primary brewing fermenter and is pumped to a yeast handling area where it is washed, reconditioned and prepared for re-use. In modern breweries, a pure culture propagation system is employed to provide a constant supply of fresh brewer's yeast that is used to replace older brewer's yeast in the brewing system. The older yeast is dried and sold as a fed supplement or it is mashed free of hop bittering substance, dried and sold into the human food market.

1-6.7 Lagering[22]

Lagering is the process where new beer is stored (german: to store - lager) and acquires a pleasant mature flavour. Undesirable off flavours are removed by yeast remaining in the stored beer. The lagering process is often called a secondary fermentation because the yeast is actively removing undesirable flavours and odours. After the primary fermentation which generally lasts 4-7 days, the yeast will have settled,down to the botton of the fermenter. The young beer, called Ruh beer, is pumped from over this yeast into an ageing tank. The yeast then recovered and is sent back to the yeast handling area mentioned above. Brewers vary in the lengths of time that they age their beers. Some age for only a few days while others will age for a month or more. The ageing step, called lagering, is generally done in a cold ($0^{\circ}-10^{\circ}C$) environment and is employed to improve flavour and stability of the beer.

1-6.8 Finishing and Packaging[23]

When lagering is completed the beer is given a brief chill-proofing treatment, filtered and carbonated (if the natural carbonation has not been retained). During filtration the beer becomes crystal clear with no visible trace of cloudiness or haze. Beer is usually passed through two filters, a rough filter and a polishing filter. The result is a bright sparkling beer. The beer is then ready for packing.

Packing means bottling, labelling and putting into cartons. Bottles are washed clean, rinsed and examined, then pre-evacuated (air removed), filled with pure carbon dioxide gas, filled with bright beer then crowned. The beer is then pasteurised to kill any yeast or other flavour spoilage.micro-organisms, by passing bottles through hot water sprays. The bottles of beer are passed by slighters who examine the bottles for any defects (such as short fills). The bottles are labelled then put into kegs or cartons and sent to the warehouse.

1-6.9 Marketing and Distribution[23]

Marketing and distribution of beer ensures that fresh beer supplies reach the customer. Beer does not improve in bottle and should be consumed before it is about three months old. Beer which is more than three months old will gradually deteriorate, especially to the taste of an experienced beer consumer. To maintain the flavour of beer as it is fresh from the brewery, it should be stored as cool as possible.

1-6.10 Quality Control

Quality control of the beer production process is achieved by attention to details at every stage, from purchasing and testing raw materials, through all the processes named above. The brewing, engineering, packaging and laboratory staff work as a team to maintain

rigid adherence to laid down production procedures. They assume the highest possible quality of the bottle of beer that reaches the customer.

Finally, when the beer is poured it should be cold, crystal clear, golden in colour, with sparkling bubbles rising upwards to produce a rich creamy head. It is now ready to drink for enjoyment and satisfaction.

1-6.11 Beer Flavour

Beer gets its characteristic flavour from three sources[37], malt, hops and yeast. Grain character and malty-to-caramel flavours come from the malt. Some fruity character, pleasing aromatic and bitterness come from the hops. The pleasing sourness, fruity aromatics and general etheral character of beer come from yeast fermentation by-products as do alcohol and carbon dioxide. Of the more than 200 chemical compounds identified in beer to date more than half are the result of yeast metabolism. Many of these are undesirable compounds, and proper brewing procedures must be followed to minimize their concentration. Some of the undesirable flavouring substances are diacetyl, hydrogen,sulphide. Some of the desirable metallic by-products found in beer are ethyl acetate, ethyl butyrate 2,3-pentane dione, 2,3-heptane dione, lactic acid, acetic acid and succinic acid.

MOTIVATION OF THE PROJECT

Beer is an important commodity consumed in Nigeria. Due to its pleasure making effects, as well as its digestive, nutritive values, many Nigerians, most of whom are literate, prefer beer as a drink to other available soft and alcoholic drinks.

Beer being a popular drink as well as an important industry it was deemed appropriate to carry a detailed analytical study of different brands brewed in Nigeria. The aim is to find its quality and shortcomings in its manufacture.

As there has been no analytical study on Nigerian beers,, I conducted this research on the following brands:

Star and Gulder Lager Beers from Nigerian Breweries Limited, Kaduna; Rock Lager Beer, Jos International Breweries Limited, Jos; Double Crown Lager Beer, North Brewery Limited, Kano; Stout, Nigerian Guinea Stout Limited, Lagos; and Noble Lager Beer, Kwara Breweries Limited, Ijagbo.

Different batches of the above six brands of beer were bought from shops in Zaria every 45 days. The samples were analysed for different parameters; important among them is the following:

(a) Alcohol

Alcohol content which determines the intoxicating power of beer has also been determined.

(b) Colour

A very pale beer is required by drinkers so this determination was necessary.

(c) Haze

Since beer is required to be brilliant to the eye, severe haze in beer is not required. Hence, its determination was necessary. And also for beer stability.

(d) Extracts

The extracts in beer express the sugars and carbohydrates present in beer. Specifically, it is required that sugars are completely converted into alcohol and carbon dioxide during fermentation. High values of extracts are not highly appreciated.

(e) Proteins

Drinkers require that beer should have some food value especially proteins. However, high concentration of proteins in beer is not required, since it introduces biological instability due to presence of yeast.

(f) Carbon dioxide

Correct concentration of carbon dioxide in beer is necessary both on account of head formation and palate. An adequate concentration of carbon dioxide serves as preservative for beer.

(g) Acidity

Beer requires some acidity in order to digest basic compounds in the stomach. So determination of acidity is also necessary.

(h) Attenuation Limit

The extent to which beer has been fermented is an essential control measure. Therefore requires to be determined.

(i) Metals

The presence of metals in beer determines its stability. High concentration of copper and iron accelerates oxidation which adversely affects the flavour and shelf-life of the packaged beer. The brewing industry requires little to none availability of metals in beer.

(j) Chlorides

High concentration in beer gives it a bad taste and smell. Hence it needs to be avoided.

(k) Bitterness

The characteristic flavour of beer comes from the addition of hops. Therefore the determination of α-acids produced from hops and substances responsible for the flavour of beer is necessary.

The results were compared and related to some International Standards and previous analytical results.

CHAPTER 2

ANALYSIS OF BEERS

Star, Gulder, Rock, Double Crown, Noble beers and Stout samples have been analysed for the following parameters.

2-1 Determination of Original Gravity

The original gravity of a beer may be defined as the specific gravity of the wort from which that beer has fermented.

The relationship between the composition of beer and its original gravity were first investigated by Balling.[15]

It is noted that the specific gravity of wort falls during fermentation since the fermentable sugars, which have a higher specific gravity than water are converted into alcohol, which has a lower specific gravity than water and carbon dioxide, which escapes as a gas. If beer is evaporated to a third of its volume the whole of the carbon dioxide and alcohol are eliminated, and if the original weight of the sample is readjusted with distilled water and residual gravity determined, the value of the residual extract (n) can be found.

Balling[15] has stated an equation for the determination of the original extract (p) of the wort in gram per 100 g or Plato given as

$$p = \frac{(2.0665\,A + n) \times 100}{100 + 1.0665\,A}$$

where A represents the percentage alcohol by weight in the sample.

The original gravity can then be calculated.from the attenuation quotient, original extract, alcohol factors given as p - n, p - n or n - n.

Balling figures for the attenuation quotient and alcohol factor for different percentage of original extract are given in Table 1. This determination must be carried out with a specific gravity bottle.

Other methods of determining original gravity are those such as the official British method of determining original Gravity[15] laid down by the British Custom and Excise.

Authorities and official Belgian method of determining original Gravity[15]. Then

Then finally in all cases the original gravity is determined as from Table 2.

EXPERIMENTAL

Apparatus

Specific gravity bottle 50 ml capacity

Thermometer 0 - 100°C range

Constant temperature water bath

Chemical balance

Table 1

BALLING'S TABLE*

% Original Extract	Attenuation Quotient q	Value of $\frac{1}{q}$	Alcohol Factors for Multiplying p-n, p-n, n-n to obtain % Weight of Alcohol		
1	0.221	4.524	0.3983	0.4864	2.2010
2	0.222	4.504	0.4001	0.4889	2.2024
3	0.223	4.484	0.4018	0.4915	2.2041
4	0.224	4.464	0.4036	0.4941	2.2058
5	0.225	4.444	0.4054	0.4967	2.2076
6	0.226	4.424	0.4073	0.4993	2.2096
7	0.227	4.405	0.4091	0.5020	2.2116
8	0.228	4.386	0.4110	0.5047	2.2137
9	0.229	4.366	0.4129	0.5074	2.2160
10	0.230	4.347	0.4148	0.5102	2.2184
11	0.231	4.329	0.4167	0.5130	2.2209
12	0.232	4.310	0.4187	0.5158	2.2234
13	0.233	4.292	0.4206	0.5187	2.2262
14	0.234	4,273	0.4226	0.5215	2.2319
15	0.235	4.255	0.4246	0.5245	2.2350
16	0.236	4.237	0.4267	0.5274	2.2381
17	0.237	4.219	0.4288	0.5304	2.2414
18	0.238	4.401	0.4309	0.5334	2.2448

Source of Table:- Reference 15.

Table 2

Table for Determining the Original Gravity of Worts of Beer

Spirit Indication	Degrees of Gravity	Spirit Indication	Degrees of Gravity	Spirit Indication	Degrees of Gravity	Spirit Indication	Degrees of Gravity
0.0	0.00	2.9	12.46	5.8	25.49	8.7	39.90
0.1	0.42	3.0	12.90	5.9	25.94	8.8	39.37
0.2	0.85	3.1	13.34	6.0	26.40	8.9	39.83
0.3	1.27	3.2	13.78	6.1	26.86	9.0	40.30
0.4	1.70	3.3	14.22	6.2	27.32	9.1	40.77
0.5	2.12	3.4	14.66	6.3	27.78	9.2	41.24
0.6	2.55	3.5	15.10	6.4	28.24	9.3	41.71
0.7	2.97	3.6	15.54	6.5	28.70	9.4	42.18
0.8	3.40	3.7	15.98	6.6	29.16	9.5	42.65
0.9	3.82	3.8	16.42	6.7	29.62	9.6	43.12
1.0	4.25	3.9	16.86	6.8	30.08	9.7	43.59
1.1	4.67	4.0	17.30	6.9	30.54	9.8	44.06
1.2	5.10	4.1	17.75	7.0	31.00	9.9	44.53
1.3	5.52	4.2	18.21	7.1	31.46	10.0	45.00
1.4	5.95	4.3	18.66	7.2	31.93	10.1	45.48
1.5	6.37	4.4	19.12	7.3	32.39	10.2	45.97
1.6	6.80	4.5	19.57	7.4	32.86	10.3	46.45
1.7	7.22	4.6	20.03	7.5	33.32	10.4	46.94
1.8	7.65	4.7	20.48	7.6	33.79	10.5	47.42
1.9	8.07	4.8	20.94	7.7	34.25	10.6	47.91
2.0	8.50	4.9	21.39	7.8	34.72	10.7	48.39
2.1	8.94	5.0	21.85	7.9	35.18	10.8	48.88
2.2	9.38	5.1	22.30	8.0	35.65	10.9	49.85
2.3	9.82	5.2	22.76	8.1	36.11	11.0	50.35
2.4	10.26	5.3	23.21	8.2	36.11	11.1	50.85

Table 2 (cont'd.)

Table* for Determining the Original Gravity of Worts of Beer

Spirit Indication	Degrees of Gravity	Spirit Indication	Degrees of Gravity	Spirit Indication	Degrees of Gravity	Spirit Indication	Degrees of Gravity
2.5	10.70	5.4	23.67	8.3	37.04	11.2	51.35
2.6	11.14	5.5	24.12	8.4	37.51	11.3	51.85
2.7	11.58	5.6	24.58	8.5	37.97	11.4	52.35
2.8	12.05	5.7	25.03	8.6	38.44	11.5	52.85

* Source of Table:- Reference 15

Reagents

All the reagents used were of analytical grade.

Ethanol (95% M/V)

Potassium Dichromate

Sulphuric acid (98% M/V)

Procedure for Determination of Specific Gravity

The specific gravity bottle was first thoroughly cleaned by immersion in a chromic acid bath, then washed with distilled water and rinsed with a little 95% (V/V) ethanol and dried by rinsing with little ether.

The bottle was weighed empty and then filled with freshly boiled and cooled distilled water, with the aid of the thistle funnel. The specific gravity bottle was immersed in a thermostatic water bath maintained at 20°C and allowed to come to equilibrium after 30 minutes.

The level of distilled water in the specific gravity bottle was gently adjusted so that the meniscus lied immediately above the graduation mark on the neck. After the level of the meniscus was adjusted, the specific gravity bottle was returned to the water bath for a few minutes.

The inside of the neck above the meniscus was wiped dry with a small piece of filter paper and the exterior of the bottle was carefully dried with a clean cloth. The weight of the water was

determined. The specific gravity of the different beer samples was similarly determined.

Calculation:

$$\text{Specific Gravity} = \frac{\text{weight of beer sample}}{\text{weight of equal volume of water}}$$

2-2 EXTRACTS

Carbohydrates and sugars which are not fermentable and other residual substances constitute the extracts in beer. Sugars and carbohydrates normally constitute about 80% of the extract. The remaining extracts are due to yeast starch and formaldehyde.

The greater the extract the lesser the attenuation limit that is the extent of fermentation.

Extracts are usually determined from the values of the specific gravity.

Procedure

Apparent extract and real extract corresponding to specific gravity were determined from Table (Reference 15).

2-3 ALCOHOL CONTENT

The alcoholic content of beer gives it its characteristic intoxicating power. The alcoholic content of beer ranges between 2 - 8% by weight.

The sample whose alcoholic content is to be determined is quantitatively transferred to a 300 - 500 ml. distillation flask and distilled. The distillate is collected in a tared receiver. The tip of the condenser carefully washed with distilled water and the distillate adjusted to the same weight as the original sample of beer with more distilled water. The specific gravity of the distillate is determined with a specific gravity bottle at 20°C and the amount of alcohol present in 100 g of the sample is found from the alcohol table (Reference 15).

Procedure for Determination of Alcohol (by volume)

100 ml of the sample was measured into 500 ml distillation flask, the temperature then noted. 50 ml of distilled water was added. The flask was attached to vertical condenser by means of bent tube. The flask and its content were distilled to almost 100 ml and the distillate diluted to 100 ml with distilled water at the same temperature. When foaming occurred it was prevented by adding small amount of antifoam material. The specific gravity of the distillate was then determined. The corresponding alcohol by volume was determined from Table (Reference 15).

Procedure for Determination of Alcohol (by weight)

50 g of sample was accurately weighed using clean and dried specific gravity bottle, then stoppered. The weighed sample was

transferred quantitatively to 500 ml distillation flask containing 50 ml of distilled water and few glass beads. The specific gravity bottle was rinsed three times bringing content of distillation flask to about 125 ml. This was distilled and then alcohol percentage by weight was determined. The specific gravity of the distillate was determined, and the corresponding alcohol content weight by weight, and weight by volume, were found (Reference 15).

2-4 Determination of Reducing Sugars in Beer Samples

Sugars and carbohydrates normally constitute about 80% of the extract. The determination of residual fermentable sugars left in the beer is of the utmost importance from the point of view of biological stability, and the determination of the attenuation limit is an essential control measure.

Sugar: which contains a free aldehyde group in the molecule are reducing agents in alkaline solution. The majority of determination of reducing sugars are based upon their power of reducing copper salts. Oxidation of potassium ferrocyanide or reduction of iodine are also fairly frequently used, but the reduction of silver, mercury or bismuth salts or the oxidation of bromine are rarely employed.

The following points must be borne in mind in the estimation of reducing sugar and the accuracy of the method very largely depend upon strict adherence to them. Length and intensity of boiling

concentration of the sugar solution and even the shape of the vessel in which the determination is carried out, all play an important role in the estimation, but above all, it is the concentration and composition of the particular salt solution employed for the determination which is extremely important. The presence of non-reducing sugars can also affect the determination, thus the presence of sucrose, which is non-reducing, can increase the reduction of copper solutions. This is a sufficient indaction that no stoichiometric reaction is involved, but it does show that the reduction of the copper salt is not due to the presence of aldehydric or ketonic groupings in the sugar molecule as was formerly supposed.[15] It is due, however, in part at least, to the presence of dienolic groups, such as are present in reductones which have a higher reducing power than the sugars themselves, and which are formed during the heating of the solution.

Reduction, should always be carried out in an alkaline medium because heating sugar solutions in an alkaline medium leads to the formation of reductones and perhaps other analogous compounds. The estimation of sugars by reduction method is really an indirect analysis.

The method used is based upon the reduction of copper sulphate in alkaline solution. Copper hydroxide, which forms in alkaline solution, normally precipitates, but is kept in solution by means of Rochelle salt (double tartrate of sodium and potassium). The

mixture of copper sulphate sodium hydroxide and Rochelle salt is known as Fehling solution.

A host of methods are available for the determination of reducing sugar, using copper salts and the determination can be carried out either gravimetrically or volumetrically.[15]

With gravimetric method of determination of reducing sugars an excess of the copper reagent is added and the cuprous oxide (Cu_2O) formed with a known amount of the sugar is determined gravimetrically. The cuprous oxide is either weighed directly or converted into cupric oxide (CuO) by strong igniting it, or the cuprous oxide can be reduced to metallic copper in a current of warm hydrogen.

EXPERIMENTAL

Reagents

(1) Alkaline tartrate solution - 173 g of KNa tartrate hydrate (KNa tartrate . $4H_2O$) i.e. Rochelle salt was dissolved in distilled water. 50 g of sodium hydroxide pellets was added then diluted to 500 ml. The flask and content was allowed to stand two days then filtered through prepared asbestos.

Soxlet modification of Fehling solution was prepared by mixing equal volumes of (1) and (2) immediately before use.

(2) Copper sulphate solution - 34.639 g of $CuSO_4 . 5H_2O$ was dissolved in distilled water, then diluted to 500 ml and filtered through glass wool or paper.

(3) Invert sugar standard solution - 1%. To a solution of 9.5 g of pure sucrose, 5 ml hydrochloric acid was added and then diluted with water to 100 ml. Then stored several days at room temperature (about 3 days), then diluted to 1L. The solution was neutralised with 1M sodium hydroxide.

Procedure

Carbon dioxide was removed by transferring sample to a large flask and shaking gently at first and then vigorously, keeping temperature of beer at $20^{\circ}-25^{\circ}C$. Suspended material was removed by passing the carbon dioxide - free beer through dry filter paper.

25 ml prepared sample was diluted to 100 ml with distilled water. Reducing sugar was determined on 25 ml of this solution by Munson-Walker method.[15]

25 ml each of copper sulphate and alkaline tartrate solutions were transferred to 400 ml beaker of alkali resistant glass and 25 ml reducing sugar solution was added. The beaker was heated on asbestos gauze over Bunsen burner, regulated flame so that boiling began in 4 minutes and continued boiling exactly 2 minutes.

The solution was filtered hot at once through asbestos mat in porcelain gooch using suction. The precipitate of cuprous oxide was thoroughly washed with distilled water at 60°, and weighed directly as cuprous oxide.

Blank determination was conducted using 50 ml reagent and 50 ml distilled water.

The weight of invert sugar equivalent to the weight of cuprous oxide was obtained from table (Reference 15).

2-5 Determination of Dextrins in Beer Samples

Determination by hydrolysis with acids. This method consists in determining reducing sugars before and after hydrolysis of the dextrins with acid. The difference is multiplied by the factor 0.9 to give the proportion of dextrins since 162 g of dextrin gives 180 g of glucose.[15]

EXPERIMENTAL

The same reagents used for the determination of reducing sugars were used also here.

Procedure

To 25 ml prepared sample measured at 20°C in erlenmeyer flask, 15 ml of hydrochloric acid (specific gravity 1.125) was added and diluted to 200 ml. The flask was attached to reflux condenser and kept in boiling water bath for 2 hours, cooled, nearly neutralised with sodium hydroxide solution (1+1) and was diluted to 250 ml. It was then filtered and glucose was determined by a procedure as that given for reducing sugars.

The dextrin values were determined from table (Reference 15).

2-6 Determination of Proteins

The determination of the more complex nitrogenous fractions in beer is a matter of considerable practical importance, because some indication may be obtained of the resistance of the sample to colloidal haze formation. A high concentration of residual assimilable nitrogen in bottled beer is undesirable and may lead to lack of biological stability since any excessive residue left in the beer can be utilized as a source of nitrogen by infecting micro-organism.[15]

The calorific value of beer can be calculated from the extract and alcohol content by using the following factors.[15]

For protein (N x 6.25)	4.0 calories/g
For extract	3.8 calories/g
For alcohol	7.1 calories/g

EXPERIMENTAL

Adequate ventillation was provided in the laboratory and accumulation of exposed mercury (Hg) was not permitted.

Apparatus

For digestion - Kjeldah flasks of hard moderately thick well - annealed glass with total capacity of 500 ml was used. Digestion was conducted over heating device adjusted to bring 250 ml of distilled water at 25°C to rolling boil in about 5 minutes. 3 boiling

chips were added to prevent super-heating.

For distillation - 500 ml Kjeldah flask was used, fitted with rubber stopper, through which passed lower end of efficient scrubber bulb to prevent mechanical carry over of sodium hydroxide during distillation. Upper end of bulb tube was connected to condenser tube by rubber tubing. Outlet of condenser was trapped in such a way as to ensure complete absorption of ammonia (NH_3) which was distilled over into acid in a receiver.

Reagents

Sulphuric acid 98% (M/V)

Sodium hydroxide - 450 g. Solid sodium hydroxide pellets were dissolved in distilled water, cooled and diluted to one litre (Specific gravity of solution was 1.36).

Zinc granules - analytical reagent grade

Methyl red indicator - 1 g methyl red was dissolved in 200 ml alcohol.

Hydrochloric acid (Specific gravity 1.125)

Sodium hydroxide standard solution - 0.1M

Standard solutions were each standardized with Primary standards and checked one against the other.

Procedure

To 25 ml prepared sample, as above, measured at 20° in Kjeldah flask 2 ml sulphuric acid was added and concentrated to sirupy consistency. The flask was placed in inclined position and heatedgently until frothing ceased. In some cases where it was necessary small amount of paraffin oil was added to reduce frothing. The flask was then boiled briskly until solution was cleared and then heated for 60 minutes.

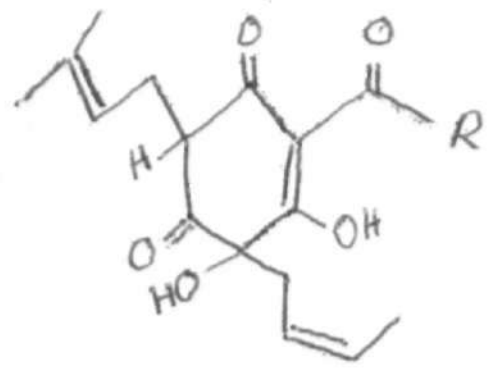
After cooling 200 ml of distilled water was added, then cooled below 25°C. 25 ml of the sulphide solution was added then mixed to precipitate mercury. Few zinc granules were added to prevent bumping. The flask was tilted and sodium hydroxide solution was added without agitation. Immediately, the flask was connected to distilling bulb on condenser and with tip of condenser immersed in standard acid with 5 drops of indicator added in the receiver. The flask was rotated to mix contents thoroughly, then heated until all ammonia was distilled. 150 ml of distillate was collected. The receiver was removed after the tip of the condenser was washed into it. Excess standard acid in distillate was titrated with standard sodium hydroxide solution. Blank determination was carried out on reagents.

Calculation:

$$\% \text{ Protein} = \frac{((\text{ml} \cdot 0.1\text{N acid} - \text{ml} \cdot 0.1\text{N base}) 1.4 \times 6.25 \times 100)}{(\text{Sp. gr.} \times \text{ml sample} \times 1000)}$$

2-7 Determination of Bitterness and Iso-alpha Acids in Beer Samples

The bittering potential of fresh hops is derived almost entirely from the α- acids shown below.[37] The β- acids are the softer α- acids.[37]



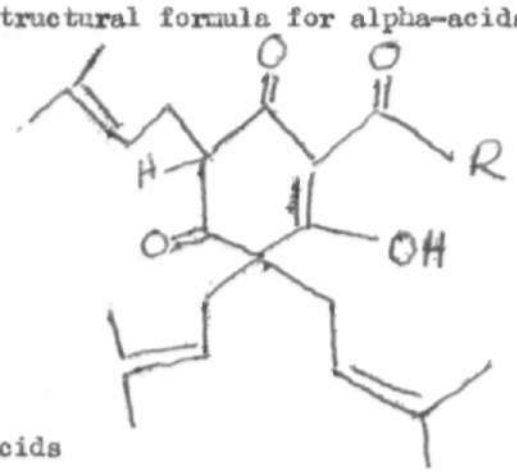
Alpha - Acids

HUMULONE where R = $CH_2CH(CH_3)_2$

COHUMULONE where R = $CH(CH_3)_2$

ADHUMULONE where R = $CH(CH_3)CH_2CH_3$

Structural formula for alpha-acids (humulone) of hops.



Beta-Acids

LUPULONE where R = $CH_2CH(CH_3)_2$

COLUPULONE where R = $CH(CH_3)_2$

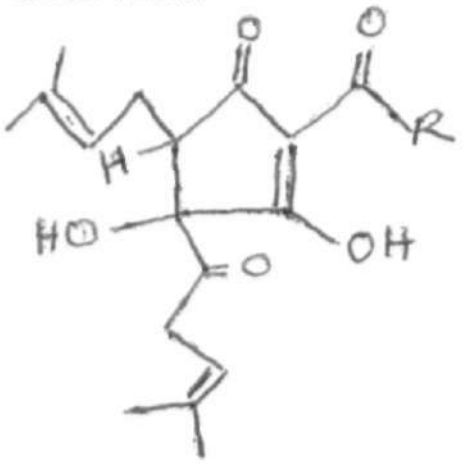
ADLUPULONE where R = $CH(CH_3)CH_2CH_3$

Structural formula for Beta-acids (lupulone) of hops.

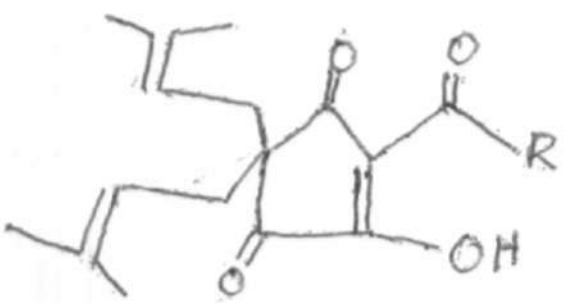
These are precipitable with lead acetate from a hexane extract of total resins. This property of the alpha-acids formed the basis of the first gravimetric method of determining the brewing value of hops.

The -acids comprise three principal and two minor analogues which can be saparated by counter-current distribution.

Humulone and its analogues are not themselves bitter, and they have limited solubility in wort. During boiling, however, they are transformed to the soluble bitter iso- alpha - acids with structural formulae shown below.



ISO - ALPHA - ACIDS
From Alpha - acids
Major Source of
bitterness.



HULUPONES
Minor Source of
bitterness.

When hops are stored, there is a decline in the content of α-acids which, as would be expected from their structure, readily undergo autoxidation.

Different varieties of hops have characteristic aromas and indeed it has been suggested[37] that the identity of an unknown sample of hops may be determined from a chromatogram of its oils. Few of the essential oils which are undoubtedly present in hops can be detected in beer and it seems possible that much of the hoppy aroma of beer emanates from degradation products of hop resins.

A spectrophotometric method was used for the determination of bitterness units (BU) and iso-α-acids. The beer was extracted with iso-octane and hydrochloric acid for bitterness while it was subjected to more treatment for iso-α-acids. At the end the absorbance was read at 275 nm for the first, and at 255 nm for the second. The method is very rapid to carry out in practice.

2-7.1 Determination of Bitterness

EXPERIMENTAL

Apparatus

Mechanical Shaker - The platform type with extending arm adjusted vertically so that tube held horizontally was used.

Spectrophotometer - SP 8000, Pye Unicam with 10 mm cuvettes

Centrifuge tubes - 50 ml glass stoppered with Teflon lining.

Reagents

2,2,4-Trimethylpentane (isooctane) - Analytical reagent grade.

Octyl alcohol - Redistilled. One drop was added to 20 ml iso octane to increase absorbance at 275 nm.

Procedure

10.0 ml chilled (50°F) carbonated beer was transferred to 50 ml centrifuge tube, using pipette which had minute amount of octyl alcohol in tip. 1 ml 3M hydrochloric acid and 20 ml isooctane were added. Centrifuge tube was tightly stoppered and shaken vigorously 15 minutes to separate phases. Immediately the clear upper portion was transferred to cuvette. The spectrophotometer was set to read 0 Absorbance at 275 nm for isooctane - octyl alcohol blank (involving 20 ml isooctane + 1 drop octyl alcohol).

Absorbance (A) was recorded in 10 mm cuvette at 275 nm.

Calculation:

Bitterness Units (BU) = A 275 x 50

BU reported to nearest 0.5 unit.

2-7.2 Determination of Iso- - Acids

EXPERIMENTAL

Apparatus

The same as for the determination of Bitterness Units.

Reagents

(a) 2,2,4-Trimethylpentane (isooctane) - Spectral grade

(b) Methanol - Reagent grade with Absorbance 0.04 at 260 nm in 10 mm cell compared with freshly redistilled water from all-glass still.

(c) Alkaline Methanol - Just before use, 1.0 ml 1.5 M Sodium hydroxide was mixed with 500 ml methanol

(e) Octyl alcohol - Reagent grade.

Procedure

15.0 ml chilled carbonated beer was transferred to 50 ml centrifuge tube, using pipette with minute amount of octyl alcohol in tip. 2.0 ml 6 M hydrochloric acid and 15.0 ml isooctane were added. Centrifuge tube then closed tightly and shaken vigorously on mechanical shaker more than thirty minutes until completely extracted. The tube then placed in centrifuge and set to run at highest permissible speed.

Centrifuged long enough to separate phases. Difficult emulsions were broken by adding one drop detergent to centrifuge tube.

10 ml clean upper layer was transferred to 50 ml glass-stoppered graduated cylinder containing 10.0 ml acid methanol. Then stoppered and inverted 100 times at rate causing content to pass from end to end and allow phases to separate.

5.0 ml of the upper layer was transferred to 25 ml volumetric flask and diluted to volume with alkaline methanol. Absorbance then read in 10 mm cell at 256 mm in a spectrophotometer set to read 0 Absorbance for reagent blank consisting of 5 ml isooctane diluted to 25 ml with alkaline methanol.

Calculation:

ppm iso-..-acids of beer = 96.15 A 255 + 0.4

Reported to nearest 0.5 ppm.

2-8 Determinttion of Carbon dioxide in Beer Samples

A correct concentration of carbon dioxide in beer is necessary both on account of head formation and palate.

The carbon dioxide content of beer should be approximately 4 g/1000 ml.[15] This figure varies with the season. In the hot season it should be between 3.5 - 4.0 g since there may be a tendency to fob on sale. While it is required that the value be slightly increased in cold season from 4.0 - 4.5 g.[15] While low gravity beers should contain the minimum quantities of carbon dioxide indicated above for hot and cold seasons, since there is a greater tendency with these beers to lose gas and they may gush.

A large number of methods are available for the determination of carbon dioxide in beer. The gas may be determined either gravimetrically, volumetrically, titrimetrically or manometrically, depending on whether weight, volume, titration or pressure is used.[15]

Each method has advantages for its own and whichever method is employed will depend on circumstances; e.g., if the beer is in tank, bottle or cask, whether or not a series of determinations have to be carried out, the degree of accuracy required, the saturation of the beer, etc.

The particular method employed for this project is the manometric[24] one where pressure is determined. This particular method has been chosen because of the following:

(a) Method of choice by most Nigeria's breweries.

(b) The nature of samples involved - liquor beer.

(c) Carbon dioxide content and head space volume are simultaneously determined.

(d) The beers involved are in bottles.

(e) A series of determinations were carried out and high degree of accuracy required.

The manometric methods for determining carbon dioxide are based on Henry's Law, which states that for a given temperature the partial pressure of a gas in equilibrium with a liquid is proportional to the quantity of gas dissolved in the liquid.[15]

In bottled beer, however, besides carbon dioxide, there is always a certain quantity of air present in the head space. The partial pressure of this air will affect that of the carbon dioxide and the partial pressure will also vary with the volume of free space

in the neck and these two factors must be taken into account to obtain accurate results.

In this circumstance, the air content of the bottle must be determined. This is an advantage because the determination of air in bottle plays an important part in the shelf-life of bottled beer.

Manometric methods of determining carbon dioxide have been extensively investigated in the U.S.A. and other parts of the world, and a large number of different types of apparatus have been devised for the rapid determination of the gas in bottle or tank. The manometric determination of carbon dioxide in bottled beer, however, requires some special means of piercing the stopper.

EXPERIMENTAL

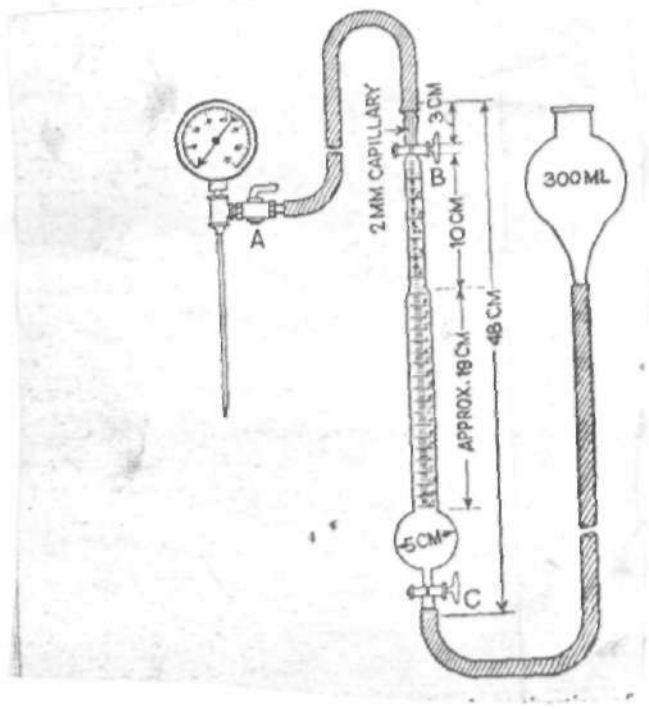
Apparatus

Piercing apparatus - for bottles -

This consisted of gas - tight packing box and fastening for adjustment over container and hollow spike connected to accurate pressure gauge and outlet valve. Gauges were checked frequently.

Absorption Burette - Consisted of graduated tube. Graduated in 0.05 ml division and ranged from 5 - 15 ml. The burette was connected to valve of piercing apparatus and to levelling bulb by transparent alkaline - resistant plastic.

Levelling bulb - approximately 300 ml with support was used.



Apparatus for the determination of Carbon dioxide in beer. (Fig. 1) 15

Procedure

Samples were brought to 25°C by immersion in water bath at 25°C. For the samples in the bottle scatches were made at beer level. The levelling bulb was filled and absorption burette was filled 15% sodium hydroxide solution. Air was displaced in tubing by connecting it to piercing apparatus completely with water. Care was taken so that no air was trapped in the system that could be carried into burette during determination.

With valve of piercing device closed the bottle crown was pierced by depressing hollow steel spike. The bottle was shaken

until the pressure had reached constant maximum value. The shaking was stopped and pressure reading recorded. The valve on piercing apparatus was opened cautiously and then allowed gas-foam mixture flow into absorption burette until pressure gauge read zero. The valve was closed and shaken until carbon dioxide had absorbed and gas volume in burette had reached maximum value. The levelling bottle was adjusted to equalize hydrostatic pressure and volume of head space air read.

In determination of "total air" the gas from the bottle was allowed to continue evolution by shaking it. Carbon dioxide evolved was absorbed by swirling and shaking burette.

Continued shaking and carbon dioxide absorption were carried out until there was no further increase in volume of unabsorbed gas in burette. Final volume of unabsorbed gas was considered the "air content" or "total air" of container was determined.

The piercing device was disconnected from package and then a thermometer was inserted to be sure that temperature was 25°C. The headspace volume was determined as follows:

The bottle was filled to with water and poured from it into 100 ml graduated cylinder until liquid level in bottle corresponded to scatched mark placed on it. Volume in ml of liquid poured off was considered as headspace volume.

Calculation:

% CO_2 by weight was calculated as follows:

$$\% CO_2 \text{ by wt.} = \frac{P - \text{ml "head-space air"} \times 14.7/\text{ml headspace}}{\times\ 0.00965}$$

where P = absolute pressure in psi = gauge pressure + 14.7

Volume CO_2 = % CO_2 by wt. x Sp. gr. of beer/0.1976

= % CO_2 by wt. x 5.0607 x Sp. gr. beer

% by wt. reported to second decimal and volume of CO_2 to first decimal.

2-9 Determination of Beer Colour

The appearance of beer is described as bright, slightly opalescent, opalescent, turbid, while it is dark brown, dark for stout.

Formerly the practice was to measure the colour of beer by comparison with iodine solution.[15] The result is expressed by the number of millilitres of N/10 iodine which had to be added to 100 ml of distilled water to obtain the same colour as that of the sample. However, it was discovered that for pale beers, especially the colours of the iodine solution and of the sample could not match satisfactorily and the determinations became difficult.

In order to overcome this difficulty Brand[15] employed solutions of various dyes, known as the Brand Scale, with which it was possible to obtain a very much more exact colour match. The following mixture

of dyes were used for this purpose:

Victoria yellow	16.0 g
Patent blue	1.0 g
Fast brown	2.5 g
Bordeaux	4.0 g

1.15 g of this mixture were dissolved in a litre of 20% ethanol which gave a colour closely equivalent to that of decinormal iodine.

One particular difficulty in the measurement of colour arose when opalescent or turbid beers were examined. The tendency was to give a deeper colour than was really the case. So it was suggested[15] that Barium sulphate be used as a compensation.

De Clerck[15] preferred to classify turbid beers by mixing with 0.1 - 0.2% Kieselguln and then filtering. Several tests with this method have shown that there was no loss of colour.

Colour was always to be measured with the same light intensity and preference was given to a day light bulb.

To avoid the direction of light problem, measurement of colour was made by an objective method such as that due to Oswald[15] or photometrically using e.g. the Pulfrich photometer or colorimetrically using photo-electric cells. However, results obtained from these methods were not reliable since beers contain turbid matter which upset photometric readings by absorbing light and secondly the colour

of different beers can be of fundamentally different composition.

Stone and Gray[27] recently used a spectrophotometer for measuring colour in beers. They employed a wavelength of 440 nm in order to obtain maximum absorption. An allowance was made for the turbid matter present by making a second reading with light of wavelength 660 nm which gave minimum absorption. They considered that with this wavelength the absorption of light was entirely due to the haze present and subtracted the result from that obtained with wavelength 440 nm.

Other methods are those due to the European Brewery Convention (E.B.C.), a method which originated from Bishop[28] and a method due to American Society of Brewing Chemist.

The spectrophotometric method has been the method of choice for the determination of colour of beers in this project.

EXPERIMENTAL

Spectrophotometer - SP 8000 Pye Unicam with 10 mm cell.

Preparation of Sample

Sample was partially degassed by opening bottle at room temperature, pouring contents into 1 litre erlenmeyer and swirling gently. Formation of turbidity was avoided while partial degassing and readings were conducted as rapidly as possible.

Procedure

The prepared sample was placed in 10 mm cell and Absorbance (A) was determined at 430 nm and 700 nm.

Calculation

A was calculated from the thickness of the cell in which the sample was read to $\frac{1}{2}^{n}$ ($A\frac{1}{2}$).

Colour was calculated as follows:

Beer colour intensity = 10 x ($A\frac{1}{2}$ at 430 nm)

In case where ($A\frac{1}{2}$ at 700 nm) was greater than 0.039 x $A\frac{1}{2}$ at 430 the sample was further clarified by centrifuge and A redetermined.

Colour intensity then reported in values to nearest 0.1 unit.

2-10 Determination of Total Acidity in Beer Samples

The beer is degassed by boiling in a beaker with constant stirring. The moment the beer begins to boil, the flame is removed and the beaker quickly cooled to avoid loss of volatile acids. The beer must be completely degassed, otherwise the carbon dioxide will be included as non-volatile acid.

Measurement of acidity[15] or alkalinity by titration simply consists in bringing a known volume of a solution to a known pH by the addition of standard acid or alkali. When a pure acid or alkali is titrated the end-point of the titration is taken as that pH at which the acid or alkali is completely neutralised.[24]

In other cases, such as beer, which contains a mixture of buffer salts of which the titration curves overlap it is impossible to determine single substances by simple titration. By the separation of one of the components present in the liquid it is possible to separate and carry out a quantitative titration.[15]

Acidimetric titration are usually made with indicators which show a change of colour at the desired pH. In the titration of hydrochloric acid with standard, a rose pink colour is given by the indicator, phenolphthalein but in mixed buffered solutions such as beer, it is essential to titrate to a known pH.[15]

EXPERIMENTAL

Reagents

Phenolphthalein indicator

Sodium hydroxide - standard solution of 0.1M.

Procedure

Indicator Titration Method

250 ml of distilled water was brought to boil and continued boiling for two minutes. From fast flowing pipette, 25 ml beer, previously decarbonated by shaking and filtering was introduced. After emptying pipette, heating was continued for 60 seconds, regulating the heat so that the solution resumed boiling during final 30 seconds. Then removed from the heat, stirred 5 seconds and cooled rapidly to room temperature.

0.5 ml of 0.5% phenolphthalein indicator was added and the beer solution was titrated with 0.1 M sodium hydroxide solution against white background. Frequent colour comparisons were made with sample of equal volume and dilution to which approximate anticipated amount of alkali but no indicator were added. Titration was carried out to first appearance of faint pink. Burette was read, 0.2 ml more alkali was added. A permanent definite pinkish-red colour was obtained indicative of over-titration. The first burette reading was taken as end-point. All details of method were strictly observed.

Calculation

Results were reported as % lactic acid to nearest 0.01%

1 ml 0.1 M alkali = 0.0090 g lactic acid.

2-11 Determination of Volatile Acids

Volatile acids, principally acetic acid, are derived from alcohol and therefore from the sugar originally present in the wort.

Acetic acid is formed from hexose by the following reactions

$$C_6H_{12}O_6 \longrightarrow 2(2H_5OH + 2CO_2$$

$$2C_2H_5OH + 4O \longrightarrow 2CH_3COOH + 2H_2O$$

180 g 120 g

180 g of hexose gives rise to 120 g of acetic acid.

In order to determine volatile acids the beer is distilled to

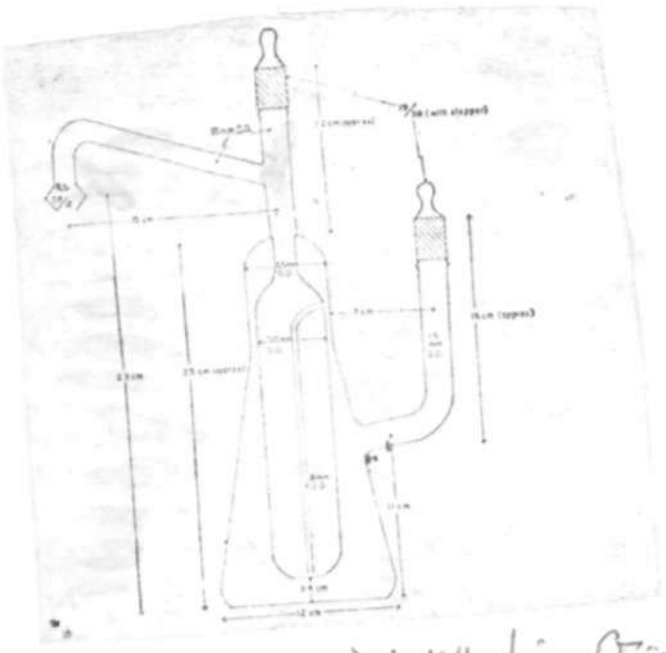
about a third of its original volume and the distillate collected. The acidity of the distillate is determined by titration with standard alkali using phenolphthalein as indicator. The litre in millilitres multiplied by the factor 0.006 and divided by the specific gravity of the beer gives the volatile acid content in gram acetic acid per 100 g of beer. It should be noted that with beers rich in volatile acids up to 500 ml of distillate may have to be collected so as to be certain that all volatile acids are removed from the sample.

EXPERIMENTAL

Apparatus

Cash electric still, consisting of outer chamber, inner chamber, trapped 2-way stopcock, electric coil heater and glass "T" inlet-outlet for water. All parts were of pyrex. Residue in inner chamber was flushed out automatically by vacuum action when current was shut off. Addition of water through funnel above stopcock gave automatic spray bath to inner chamber and waste drained through outlet in glass "T". Two way stopcock permitted introduction of sample, served as escape vent for carbon dioxide and allowed introduction of washed water.

Steam distillation apparatus.



Apparatus for Steam Distillation (Fig. 2) [24]

Procedure

To 100 ml prepared sample about 600 ml boiled water was added to outer chamber of still. 25 ml freshly prepared sample was pipetted into the inner chamber and stoppered. The water was boiled for three minutes with sidearms opened. Then closed and was distilled into 300 ml erlenmeyer flasks. 0.5 ml phenolphthalein indicator then added to the distillate and titrated rapidly with 0.1 M sodium hydroxide solution until pink persisted 15 seconds.

Calculation

Results were expressed as of acetic acid (HOAc) per 100 ml.

1 ml 0.1 M alkali = 0.0060 g HOAc

2-12 Determination of Fixed Acidity

From the results obtained above for total acidity and volatile acidity fixed acidity was determined by subtraction.

2-13 Determination of Apparent Attenuation

The determination of attenuation limits give an indication of the extent of the percentage of extract left in the beer. If the beer has been poorly attenuated, or primed with sugar at racking they naturally form a greater percentage of the extract.

The determination of residual fermentable sugars left in beers is of the utmost importance from the point of view of biological stability and the determination of the attenuation limit is therefore an essential control measure.

EXPERIMENTAL

Procedure

100 ml of sample was accurately weighed to 0.1 g and was evaporated on water bath maintained at temperature of about 80°C to about $\frac{1}{3}$ the original volume. This was cooled and made to original weight with distilled water and the specific gravity was determined with a specific gravity bottle at 20°C.

From this specific gravity value the corresponding extract was determined.

Calculation

$$\text{Apparent extract found} \times \frac{\text{Sp. gr. of dealcoholised beer}}{\text{Sp. gr. of beer}} = \frac{\text{g extract}}{100 \text{ g beer}}$$

Extract of original wort 0 was calculated as follows:

$$0 = \frac{(A \times 2.0665) + E \times 100}{100 + (A \times 1.066))}$$

where 0 = extract of original wort

A = % alcohol by weight of /100 of beer

E = % real extract

Apparent attenuation is then calculated as follows:

$$A = (\text{Original extract} - \text{apparent extract}) \times \frac{100}{\text{original extract}}$$

Real attenuation then calculated as follows:

$$R = (\text{Original extract} - \text{real extract}) \times \frac{100}{\text{original extract}}$$

2-14 Measurement of pH of Beer Samples

pH of beer samples may be determined either electrometrically or colorimetrically.[15] For this project the former method has been employed. Electrometric methods serve as the standard of reference for the determination of pH values. Colorimetric methods are simpler to carry out and are useful in routine work where no higher degree of accuracy than 0.1 - 0.2 pH is required. Colorimetric methods of determinations are however not sufficiently exact for brewing operation.[15]

Reagents and Apparatus

Acid potassium phthalein buffer solution - 0.0496 M; 10.12 g dried acid potassium-phthalein standard sample was dissolved in water and diluted to 1 litre. pH 7.

Apparatus Pye Unicam pH meter.

Procedure

The pH of the undiluted sample was determined using glass calomel electrode system. The pH meter was checked before and after used against standard acid potassium phthalein buffer.

2-15 Determination of Haze in Beer Samples

By means of the nephelometric[29] method, the complete range of turbidity developed by bottle beer during shelf-life from brilliant, through slight to severe haze can be quantitatively followed.

The determination is preferably made in small 125 ml white bottles fitted with screw caps.[15] With such an arrangement there is no interference from bubbles of gas and the development of haze in bottle may be followed from its earliest inception, which is a great advantage.

The Zeiss nephelometer is the only instrument which is particularly well adapted for following the development of haze in bottle.[15]

The results should be reported as follows:

Sample	Reading on Zeiss nephetometer
Brilliant	Less than 0.001
Bright	Between 0.001 and 0.005
Slight haze	Greater than 0.005
Haze	Greater than 0.020

The Zeiss nephetometer has the great advantage that haze can be measured in bottle without removing the beer and the development of haze can be followed in the same bottle from the start of storage.

The chill haze content of beer plays an important part in the subsequent shelf-life of the beer from the point of view on non-biological stability. The determination of chill haze in beer is therefore a matter of some importance.

The white bottle is immersed for some time in broken ice and the increase in the intensity of the haze is determined. The second value minus the first will give the chill haze content of the sample.

The haze is first examined microscopically to see if it is of biological origin, i.e. due to the presence of yeast, bacteria, etc., or non-biological in character and due to the presence of colloidal and amorphous matter. If the haze is non-biological in origin it may be due to a wide variety of different causes, such as chill haze, oxidation haze, the presence of certain metallic ions in too high concentration starch, formaldehyde or for some accidental reason.

EXPERIMENTAL

Reagents and Apparatus

Turbidity free distilled water was used throughout.

Hydrazine sulphate solution 1% - 1.000 g hydrazine sulphate was dissolved in water and diluted to 100 ml.

Stock formazine suspension - 2.500 g hexamethylene tetramine (formin) was dissolved in 25 ml distilled water in 125 ml erlenmeyer flask. 25 ml hydrazine sulphate solution was pipetted in and the flask stoppered. Formazine began to precipitate in 6 - 8 hours, and the precipitation was completed within 24 hours. This stock solution was prepared every 3 months.

1000 turbidity standard - 14.5 ml well mixed stock suspension was diluted to 1000 ml. with distilled water in a volumetric flask. This standard solution was prepared weekly and constituted the 1000 formazine turbidity unit (FTU). On empirical formazine turbidity scale this represented reflectance of insoluble reaction products of 0.0725 g hydrazine sulphate with 0.7250 g hexamethylene tetramine diluted to 1000 ml.

Apparatus - Nephelometer, Evans Electroselenium.

Calibration

Nephelometer: Calibration curve was prepared at 580 nm for instrument employed by use of series of working standards, i.e. the 1000 turbidity standard. The readout device of nephelometer

ranged from 0 - 100 scale of arbitrary units. The needle was set to indicate 0 units when the cuvet was filled with turbidity - free distilled water and 100 units when it was filled with selected formazine turbidity standard.

Procedure

Containers of beers to be tested were placed in upright position in 0^{o}C bath and held for 24 hours. The nephelometer cuvette was prechilled in a small ice-water bath containing wetting agent (external contact only).

Beer containers which were removed carefully from bath and without disturbing the sediment, were used to rinse and fill the cuvette with the test sample. The cuvette was placed in an ice-water bath containing wetting agent and degassed beer by stirring with thermometer. When beer temperature was 0^{o} the cuvette was placed in sample chamber of nephelometer and reading was taken. (Beer was at 0^{o} when taking reading).

Calculation

FTU total haze was calculated from the calibration curve and the formula: FTU = R x S/100, where R = nephelometer reading on galvanometer scale.

S = FTU of formazine turbidity standard used for calibration of nephelometer.

CHAPTER 3

ANALYSIS OF BEERS FOR ASH, METALS AND CHLORIDE CONTENTS

The determination of the total ash content of beer is a measure of the total salt content of beer, whereas the determination of metal constituents may be important where haze formation is suspected. Analysis of chloride content in beer is of equal importance. The presence of high concentrations of the chloride ion does not suit some types of beer.

3-1

3-1 Determination of Ash

In the determination of the ash content of beer generally, care must be taken to carry out the combustion at as low temperatures as compatible with the complete combustion of the carbon, because the ash contains salts of the alkali metals, which readily fuse with particles of carbon, and prevent complete combustion.

The estimation of the ash content of beer is carried out as follows:

50 ml of beer are evaporated to dryness in a tared evaporating basin and then ashed. The preliminary stage of the combustion is carried out with an ordinary bunsen burner until all fumes and smoke have ceased. The combustion is completed in a muffle furnace at 550°C, until a pure white ash is obtained. If higher temperatures

than this are used, there is danger of partial volatilization of the ash. If the colour of the ash should remain obstinately grey, it is necessary to continue the combustion to constant weight.

The ash content of beer normally varies between 0.20 - 0.30%.[15] The value will depend to a certain extent upon the salt content of the mashing liquor, particularly if the liquor has been treated with calcium sulphate or other salts. Two-thirds of the ash consists of potassium (one-third) and phosphate (one-third) expressed as P_2O_5.

If a complete quantitative analysis of the ash is required, it is redissolved in dilute hydrochloric acid, and the various ions estimated.

EXPERIMENTAL

Preparation of Sample

Carbon dioxide was removed by transferring sample to large flask and shaking gently at first and then vigorously, keeping temperature of beer at 20° - 25°C. If necessary, suspended material is removed by passing the carbon dioxide - free beer through dry filter paper.

Procedure

50 ml prepared sample was evaporated to dryness. The sample then of appropriate weight was heated in 100 ml platinum dish at 100°C until water was expelled. Few drops of pure olive oil were added then heated slowly over bunsen flame until swelling stopped.

The dish was then placed in a muffle furnace at about 550°C and left until white ash was obtained. The ash was moistened with water, dried on steam bath and then on hot plate and re-ashed in muffle at 550°C to constant weight.

3-2 Determination of Traces of Copper in Beer - Non-ashing Technique[24,30,31]

Traces of copper in beers and ales act to accelerate oxidation changes which adversely affect flavour and shelf-life of the packaged beverage.

The brewing industry requires a simple, rapid and precise method for the determination of those traces of copper. A colorimetric procedure has been developed which requires no ashing of the sample and yields results in about one hour.

Sample is digested with nitric and sulphuric acids. Copper is then isolated and determined colorimetrically at pH 8.5 as diethyldithiocarbamate in presence of chelating agent EDTA. Bismuth and Terricin may also give coloured carbamates at pH 8.5 with 1N sodium hydroxide. However, these elements are normally not present in beers. Copper complex is stable. Good recovery of added copper is obtained. Routine application of this method will provide another quality control tool, enabling the brewing industry to produce more uniform and stable products.

The presence of traces of copper and iron of the order of a fraction of a part per million (ppm) to several parts per million

has a strong catalytic effect on the oxidation reactions occurring in beer. These reactions adversely affect the shelf-life and taste of the packaged product.

The industry is cognisant of this and requires simple, sensitive and rapid test methods for these elements.

Before arriving at the non-ashing procedure for copper determination, several methods have been used. These procedures include the destruction of the organic matter,[31] either by wet or dry ashing before the copper could be determined. This destruction of organic matter made the method cumbersome and time consuming and was unsuited for routine work in the laboratory of the industry.[32]

In this project a simple, direct non-ashing method has been used for the determination of the traces of copper which may be found in beers.

Martin and Githens[33] published a paper, discussing the use of zinc dibenzyldithiocarbamate for the colorimetric determination of small amounts of copper in dyes and rubber chemicals. This reagent complexes with the copper to give a yellowish or brownish organic solvent - soluble colour in strongly acid solution. The commercial availability of the reagent adds to the convenience of the method. The use of this reagent was adapted to beer as a direct non-ashing technique and the method was suitably modified for this purpose.

In the strongly acid solution employed none of the common

metallic constituents which may be present in beer interfere with the copper colour development. The test was conducted entirely in a corked 50 ml centrifuge tubes and results were obtained within one hour.

The sample was acidified to 1 N sulphuric, then heated in a boiling water bath for 0.5 hour, cooled and then shaken out with a measured portion of a carbon tetrachloride solution of the reagent. The tube was centrifuged and the coloured carbon tetrachloride layer transferred to a cell and read in a photometer. The copper content is calculated from the photometer reading. The photometer used was a Klett-Summerson photoelectric colorimeter.

In the present project a similar method was adopted except that diethyldithiocarbamate reagent was used in the presence of EDTA as a chelating agent.

EXPERIMENTAL

Preparation of Sample[24]

Carbon dioxide was removed by transferring sample to a large flask and shaking gently at first and then vigorously keeping temperature of beer at 20 - 25°C. If necessary, suspended material is removed by passing the carbon dioxide - free beer through dry filter paper.

To 25 ml prepared sample measured at 20° - 25°C in Kjeldah flask of hard moderately thick well-annealed glass with total capacity of

500 ml, 2 - 3 ml of sulphuric acid and 5 ml of nitric acid were added. Digestion is then conducted over a heating device adjusted to bring 250 ml water at 25°C to rolling boil in about 5 minutes. 3 - 4 boiling chips were added. Similarly, reagent blank was also determined.

Reagents

(a) Sodium diethyldithiocarbamate, carbamate solution: - 1 g of the salt is dissolved in distilled water, then diluted to 100 ml and filtered. (Stored in refrigerator and prepared weekly).

(b) Citrate - EDTA solution:- 20 g of dibasic ammonium citrate and 5 g sodium EDTA were dissolved in water and diluted to 100 ml. Traces of copper are removed by adding 0.1 ml carbamate solution and extracting with 10 ml carbon tetrachloride. Extraction is repeated until carbon tetrachloride was colourless.

(c) Copper standard solution (1) stock solution:- 1 mg/ml
0.2000 g of copper wire or foil was placed into 125 ml erlenmeyer flask. 15 ml of nitric acid (1 + 4) was added. The flask was covered with watch glass and copper was allowed to dissolve, warming to complete solution, then boiled to expell fumes, cooled and diluted to 200 ml.

(2) Intermediate solution - 100 μg/ml
20 ml stock solution was diluted to 200 ml.

(3) Working solution - 2 ug. / ml. Prepared daily by diluting 5 ml intermediate standard to 250 ml with 2.0N sulphuric acid.

(d) Ammonium hydroxide - 6N purified as (b) above.

Preparation of Standards and Calibration Curves

0,1, 2.5, 5, 10, 15, 20 and 25 ml of standard copper solution 2 ug/ml were transferred to separators and 2.0N sulphuric acid was added to make the total volume to 25 ml.

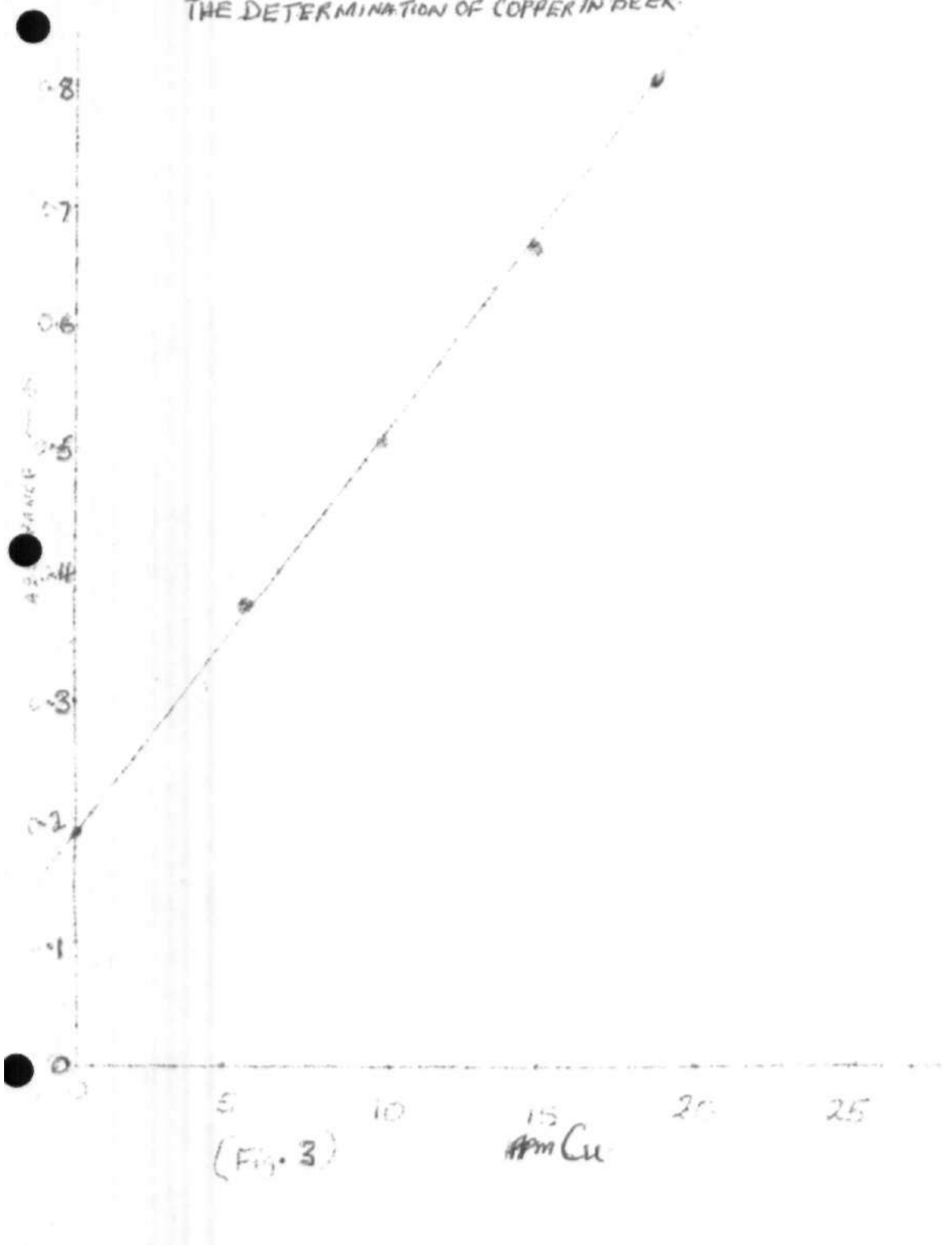
10 ml citrate - EDTA reagent was added and then 2 drops of thymol blue indicator, after that 6N ammonium hydroxide dropwise until solution turned green or blue-green. It was cooled and 1 ml carbamate solution added and then 5 ml carbontetrachloride. Then shaken vigorously two minutes. The layers were let to separate, and carbon tetrachloride was drained through cotton pledget into glass stoppered tube or flask. The Absorbance (A) was determined on a SP8000 Pye Unicam spectrophotometer at 400 nm. (A) was then plotted against µg copper on ordinary graph paper (Fig.)

3-2.4 Isolatin and Determination of Copper

Procedure

25 ml prepared beer sample solution was pipetted into 250 ml short stem separator and 10 ml citrate - EDTA reagent, 2 drops of thymol blue indicator were added then 6N ammonium hydroxide dropwise until solution turned green or blue-green. It was then cooled

CALIBRATION CURVE OF ABSORBANCE AGAINST ppm Cu FOR THE DETERMINATION OF COPPER IN BEER.



(Fig. 3)

and 1 ml carbanate solution and 5 ml carbon tetrachloride were added. It was then shaken vigorously for two minutes and layers were let to separate, then carbon tetrachloride was drained through cotton pledget into glass stoppered flask. The Absorbance (A) was similarly determined as above at 400 nm, and the ppm Cu were calculated from the standard graph (Fig. 3).

3-3 Determination of Iron in Beers[24,34]

The presence of traces of iron and copper of the order of a fraction of a part per million (ppm) to several parts per million has a strong catalytic effect on the oxidation reactions occurring in beer. These reactions adversely affect the shelf-life and taste of the packaged product as earlier pointed out under the determination of copper.

Also the determination of iron is of interest in most laboratories as it occurs either as a major constitutent or at the trace level in so many industrial and natural products. Iron is frequently found in natural waters used in brewing processes and also for most purposes, it is considered that its concentration in a water supply should not exceed 0.5 ppm. The presence of even this amount of iron can be a considerable nuisance in some industrial applications such as laundries, brewing and textile processing plants. Standard methods for the control of the iron content of water supplies have

been specified.

Iron may be determined in pale beers without ashing, using either α : α'-dipyridyl or O-phenathroline.

The temperature of beer is adjusted to 20° - 25°C and it is then degassed by shaking in a large erlenmeyer flask. The beer should not be filtered unless the sample is turbid. If filtration is necessary, the filter paper should be tested to see that it is free from iron, by spotting the paper with a drop of the colour reagent containing 2.5 mg of ascorbic acid in about 5 ml of the solution. If the sample of beer is suspected of having a high iron content the head of fob formed after degassing should be allowed to subside before sampling.

The method depends on measurement of the colour developed in the reaction between 1, 10-phenanthroline and iron; i.e., orange red orthophenanthroline complex with iron at 508 nm.

$$Fe^{2+} + 3Ph\ H^{+} = FE(Ph)_3^{2+} + 3H^{+}$$

$$K_{eq} = 2.5 \times 10^{6} \text{ at } 25^{\circ}C \quad ^{24}$$

In using this reagent an excess of reducing agent is added to the solution to keep the iron in the reduced state. Hydroquinone or hydroxylamine is convenient for this purpose.

EXPERIMENTAL

Apparatus

SP8000 Pye Unicam spectrophotometer 10 mm cell set at about 505 nm.

Reagents

(a) Colour reagent, O-phenanthroline - 0.3%. 1.5 g of O-phenanthroline was dissolved in 500 ml water at 70°C.

(b) Iron standard solutions: 0.1 mg/ml ferrous ammonium sulphate, 3.512 g $Fe(NH_4)_2SO_4.6H_2O$ was dissolved in water, 5 ml of concentrated hydrochloric acid added then transferred quantitatively to 500 ml volumetric flask, then diluted to volume with distilled water and mixed. 50 ml of this solution was transferred to 500 ml volumetric flask, diluted to volume with water and mixed.

(c) Ascorbic acid - USP, ground to fine powder.

(d) Hydroquinone 1% in acetate buffer pH 4.5. Kept in refrigerator.

Preparation of Standard Curve

Series of beer standards containing 0.0, 0.25, 0.50, 1.00, 2.00,

and 3.00 ppm iron were prepared as follows:
0.0, 0.25, 0.50, 1.00, 2.00 and 3.00 ml of iron standard solution were pipetted to series of 100 ml volumetric flasks. Enough water then added from pipette to total 3.00 ml and diluted with decarbonated beer as prepared below. Depending upon size of cell to be used colour was developed in 25 ml aliquot of each of the beer standards as in determination below.

Preparation of Sample

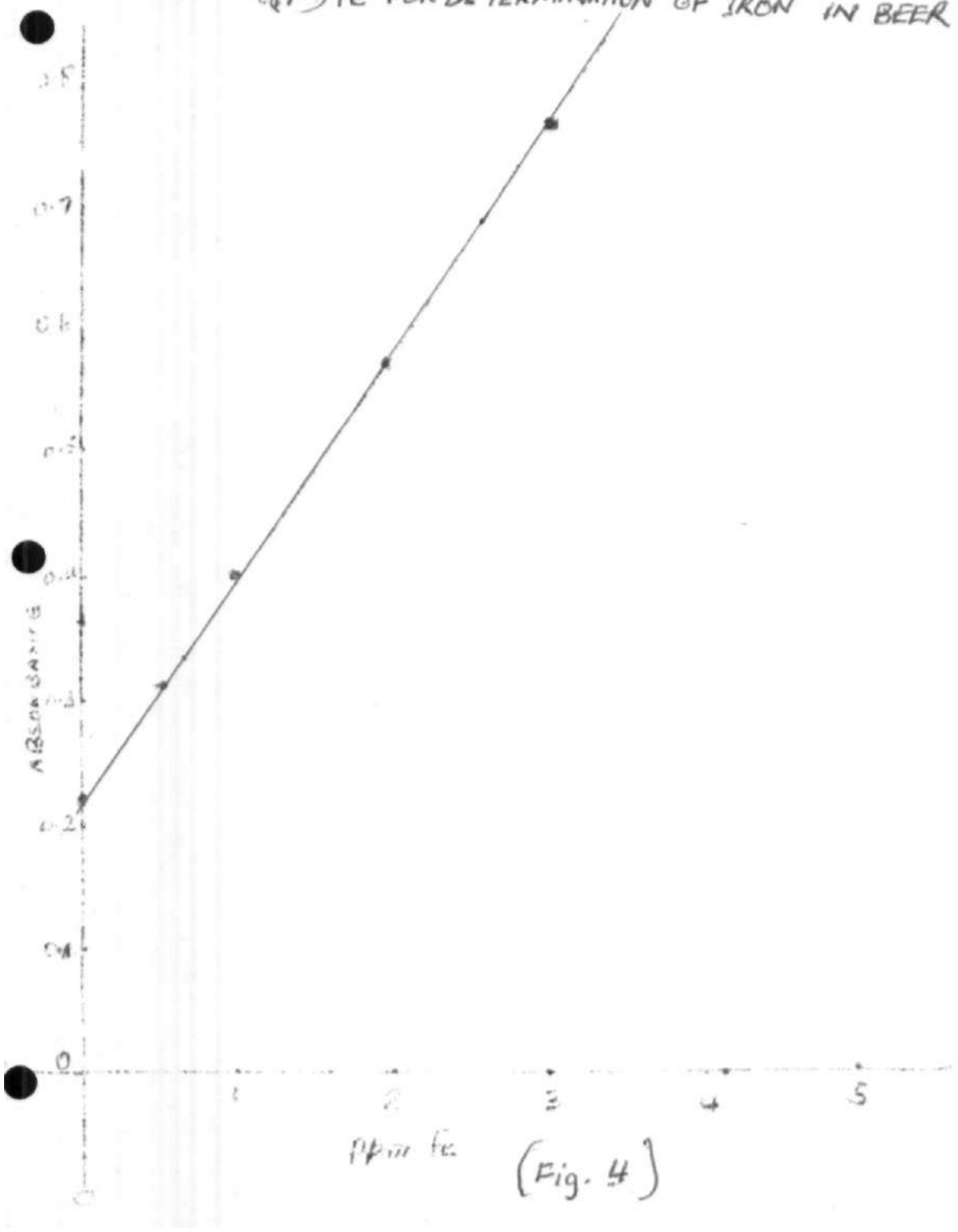
Temperature of beer was adjusted to 20° - 25°C. Then decarbonated by transferring sample to large erlenmeyer flask and shaking first gently and then vigorously until all gas was released. The prepared sample was not filtered since filter paper was proved to contain iron.

Since beer was suspected to contain not relatively high iron decarbonation took place as stated above, and then permitted foam to subside before further sampling.

Procedure

Two aliquots of degassed beer (25 ml as used in preparation of standard curve) were pipetted into 50 ml erlenmeyer flasks. 25 mg ascorbic acid was added to each aliquot and 2 ml colour reagent above added to one while 2 ml distilled water was added to the other. The two then allowed to stand thirty minutes at room temperature.

CALIBRATION CURVE OF ABSORBANCE AGAINST PART PER MILLION (ppm) Fe FOR DETERMINATION OF IRON IN BEER



(Fig. 4)

Both solutions were cooled and read in photometer against water as reference. The same size of cell and wavelength were used both in preparation of standard curve and in real determinations.

3-4 Determination of Chloride in Beer Samples

The volumetric determination of chloride is based upon titration with silver nitrate using potassium chromate as indicator. The end point is indicated by a red precipitate of silver chromate.

If the sample is very rich in organic matter (more than 100 mg $KMnO_4$/1000 ml required), the organic matter must be destroyed by boiling with excess of potassium permanganate.

High concentration of iron also interfereswith the determination and the iron must be removed by shaking 100 ml of the sample with 1 g of zinc oxide and filtering.

Hydrogen sulphide must be removed by the addition of potassium permanganate until a rose tint is obtained. The colour from the permanganate is then discharged by the addition of a drop of hydrogen peroxide.

The titration in the determination is carried out to the point when a reddish colour is produced when the solution is well shaken.

Weaker standard silver nitrate solution is used since a more accurate result is obtained.

The concentration of chloride ions in water is very variable,

and the presence of high concentrations does not suit all types of beer.

The method of determination of chlorides adopted here depended on precipitation of chlorides with standard silver nitrate solution and back titration with standard ammonium thiocyanate solution.

EXPERIMENTAL

Reagents

Silver nitrate standard solution - 1 ml = 0.00355 g Cl.

A solution stronger than 0.1 M was prepared then standardized and adjusted to exactly 0.1 M.

Ammonium thiocyanate standard solution - 0.1 M solution was prepared slightly stronger than 0.1 M then standardized and adjusted to exactly 0.1 M.

Nitric acid was freed from lower oxides of nitrogen by diluting pure acid with $\frac{1}{4}$ volume of distilled water, and boiling until perfectly colourless.

Preparation of Solution

Complete retention of chloride was verified first in each kind of sample by trial, since lost of beer sample occurred when insufficient sodium carbonate was present during ignition or when excessive temperature was used.

50 ml sample was placed in platinum dish then 20 ml 5% sodium carbonate solution added. The platinum dish content then evaporated.to dryness and ignited as thoroughly as possible at temperature less than 500°C. Then extracted with hot water, filtered and washed. Residue returned to platinum dish and ignited to ash; dissolved in nitric acid (1 + 4), filtered, washed thoroughly and added this solution to water extract.

Procedure (Volumetric Method)

To prepared solution above known volume of standard silver nitrate solution was added in slight excess. Stirred well filtered and silver chloride precipitate was washed thoroughly. To combined filtrate and washings 5 ml ferric indicator and few ml nitric acid were added then excess silver titrated with thiocyanate standard solution to permanent light brown. From ml silver nitrate used, the quantity of chloride was calculated.

C H A P T E R 4

RESULTS AND DISCUSSION

Table 3 summarises the analytical results obtained from the analyses of different brands of beer.

Based on the obtained analytical data, Nigerian beers are compared, where possible with values given by the American Society of Brewing Chemists (Am. Soc. Brew. Chem.), National Bureau of Standards (NBS). Official Methods of Analysis of the Association of official Analytical Chemists (AOAC); European Brewing Convention (EBC).

Finally, conclusions and suggestions for further work are made.

ALCOHOL

From the data obtained for alcohol by volume, Gulder Lager Beer from Nigerian Breweries Limited, Kaduna appears to have the highest value between 5.98 - 6.00% followed by Double Crown Lager Beer from North Brewery Limited, Kano. The value obtained ranges from 5.61 - 5.62%.

Similarly, the same trend is followed for alcohol by weight. Gulder Lager beer has a value 4.50 - 4.52%. While that of Double Crown Lager is 4.38 - 4.40%.

Specifically, the percentage alcohol by weight or by volume decides the intoxicating power of any alcoholic beverage. Hence it should be expected that from these data Gulder consumers are

more liable to intoxication than those who consume other brands. However, the quality of beer does not depend solely upon its alcoholic content; flavour,[35] colour, etc. also have their parts to play which are discussed later.

Other Lager beers and Stout follow closely in values for both alcohol by volume and alcohol by weight.

The value for Star Lager Beer from Nigerian Breweries Limited, Kaduna, ranges from 5.38 - 5.40% for alcohol by volume and 4.25 - 4.27% for alcohol by weight.

As for Rock Lager Beer from Jos International Breweries Limited, Jos, values obtained ranges from 5.10 - 5.15% for alcohol by volume and 4.00 - 4.05% for alcohol by weight. Noble Beer from Kwara Breweries Limited, Ijagbo value obtained ranges from 5.39 - 4.28% for alcohol by weight, while for Guinea Stout, from Nigerian Guinea Stout Limited, Lagos, values ranges from 5-54 - 5.56% for alcohol by volume and 4.31 - 4.33% for alcohol by weight.

It is noted that the values for alcohol content lie within the needed range of % weight by weight and % weight by volume as required by the E.B.C., with value which ranges from 4 - 4.5% of alcohol by weight.[14]

Table 3

Results of the Analysis of the Physical Properties of Beers

Brand	Colour	Clarity	Odour	Taste	Foam
STAR	Pale yellow	Clear	Aromatic	Bitter	Persistent, non-creamy
GULDER	Pale yellow	Clear	Aromatic	Bitter	Firm, white, persistent and creamy
ROCK	Pale yellow	Clear	Aromatic	Bitter	Firm, white, persistent and creamy
DOUBLE CROWN	Pale yellow	Clear	Faint smell	Bitter	Firm, white, persistent and creamy
NOBLE	Pale yellow	Clear	Faint smell	Bitter	Persistent, non-creamy
STOUT	Dark brown	Turbid	Aromatic	Malty and very bitter	Brownish feeble foam

Table 3

Results of Analyses of Beer Samples

BATCH NO.	ALCOHOL BY VOLUME %	ALCOHOL BY WEIGHT %	SPECIFIC GRAVITY	APPARENT EXTRACT %	REAL EXTRAC%	EXTRACT OF ORIGINAL WORT %
1	5.39 ± 0.00	4.25 ± 0.00	1.0013	0.334	2.85 ± 01	8.78 ± 0.00
2	5.40 ± 0.01	4.27 ± 0.02	1.0013	0.334	2.87 ± 00	8.78 ± 0.00
1	5.99 ± 0.00	4.50 ± 0.00	1.0037	0.949	2.53 ± 01	11.29 ± 0.00
2	5.99 ± 0.00	4.50 ± 0.00	1.0038	0.949	2.54 ± 00	11.29 ± 0.00
1	5.10 ± 0.01	4.00 ± 0.04	1.0070	1.795	3.19 ± 00	10.99 ± 0.00
2	5.15 ± 0.04	4.05 ± 0.01	1.0070	1.795	3.20 ± 01	10.98 ± 0.01
3	5.10 ± 0.01	4.05 ± 0.01	1.0071	1.820	3.18 ± 01	10.99 ± 0.00
4	5.10 ± 0.01	4.05 ± 0.01	1.0070	1.795	3.19 ± 00	10.99 ± 0.00
1	5.61 ± 0.01	4.40 ± 0.01	1.0008	0.206	2.84 ± 00	11.40 ± 0.01
2	5.62 ± 0.00	4.38 ± 0.01	1.0009	0.231	2.84 ± 00	11.39 ± 0.00
3	5.62 ± 0.00	4.38 ± 0.01	1.0009	0.231	2.85 ± 01	11.39 ± 0.00
4	5.62 ± 0.00	4.40 ± 0.01	1.0009	0.231	2.84 ± 00	11.39 ± 0.00
1	5.39 ± 0.01	4.27 ± 0.01	1.0024	0.616	3.12 ± 00	11.42 ± 0.00
2	5.40 ± 0.00	4.28 ± 0.00	1.0024	0.616	3.13 ± 01	11.42 ± 0.00
3	5.40 ± 0.00	4.28 ± 0.00	1.0024	0.616	3.12 ± 00	11.43 ± 0.01
4	5.39 ± 0.01	4.28 ± 0.00	1.0024	0.616	3.12 ± 00	11.42 ± 0.00
1	5.54 ± 0.01	4.31 ± 0.01	1.0222	5.629	6.35 ± 00	14.59 ± 0.01
2	5.54 ± 0.01	4.32 ± 0.00	1.0221	5.605	6.35 ± 00	14.58 ± 0.00
3	5.56 ± 0.01	4.32 ± 0.00	1.0221	5.629	6.36 ± 01	14.57 ± 0.01
4	5.55 ± 0.00	4.33 ± 0.01	1.0222	5.629	6.35 ± 00	14.59 ± 0.01

Table 3

Results of Analyses of Beer Samples

BRAND	BATCH NO.	TOTAL ACIDITY % LACTIC ACID	VOLATILE ACIDITY ACETIC ACID (g)/100ml	FIXED ACIDITY (g)	REDUCING SUGARS %	DEXTRIN %	PROTEIN %
STAR	1	0.17 ± 0.01	0.0165	0.0249	0.056 ± 0.002	0.03 ± 0.00	0.40 ±
	2	0.18 ± 0.00	0.0160	0.025	0.056 ± 0.002	0.03 ± 0.00	0.41 ±
GULDER	1	0.18 ± 0.00	0.017	0.027	0.065 ± 0.001	0.03 ± 0.00	0.42 ±
	2	0.18 ± 0.00	0.017	0.027	0.066 ± 0.000	0.03 ± 0.00	0.45 ±
ROCK	1	0.19 ± 0.01	0.016	0.030	0.191 ± 0.001	0.09 ± 0.00	0.45 ±
	2	0.20 ± 0.00	0.017	0.030	0.191 ± 0.001	0.08 ± 0.01	0.46 ±
	3	0.20 ± 0.00	0.016	0.031	0.190 ± 0.000	0.09 ± 0.00	0.40 ±
	4	0.19 ± 0.01	0.017	0.030	0.190 ± 0.000	0.09 ± 0.00	0.41 ±
DOUBLE CROWN	1	0.18 ± 0.00	0.017	0.030	0.058 ± 0.000	0.04 ± 0.00	0.41 ±
	2	0.18 ± 0.00	0.017	0.030	0.058 ± 0.000	0.05 ± 0.01	0.40 ±
	3	0.17 ± 0.01	0.016	0.030	0.058 ± 0.000	0.04 ± 0.00	0.41 ±
	4	0.18 ± 0.00	0.017	0.029	0.057 ± 0.001	0.04 ± 0.00	0.41 ±
NOBLE	1	0.18 ± 0.00	0.009	0.037	0.138 ± 0.000	0.065 ± 0.000	0.43 ±
	2	0.18 ± 0.00	0.010	0.037	0.139 ± 0.001	0.066 ± 0.001	0.42 ±
	3	0.18 ± 0.00	0.010	0.036	0.138 ± 0.000	0.065 ± 0.000	0.41 ±
	4	0.18 ± 0.00	0.009	0.037	0.138 ± 0.000	0.065 ± 0.000	0.41 ±
STOUT	1	-	0.012	-	0.361 ± 0.000	0.17 ± 0.00	0.48 ±
	2	-	0.012	-	0.361 ± 0.000	0.18 ± 0.01	0.48 ±
	3	-	0.013	-	0.361 ± 0.000	0.17 ± 0.00	0.47 ±
	4	-	0.012	-	0.361 ± 0.000	0.17 ± 0.00	0.48 ±

- No Results Obtained

Table 3

Results of Analyses of Beer Samples

BRAND	BATCH NO.	BITTERNESS UNITS (BU)	ISO-ALPHA ACIDS (ppm)	COLOUR INTENSITY	HAZE (FTU)	CARBON DIOXIDE %	pH VALUE
STAR	1	24.0 ± 0.4	47.03 ± 0.12	5.60 ± 0.15	0.76	0.56 0.00	4.35 ± 0.0
	2	25.0 ± 0.4	47.05 ± 0.10	5.60 ± 0.15	0.78	0.56 ± 0.00	4.35 ± 0.0
GULDER	1	27.0 ± 0.01	52.2 ± 0.01	4.7 ± 0.00	0.83	0.50 ± 0.00	4.20 ± 0.0
	2	27.0 ± 0.01	52.0 ± 0.01	4.7 ± 0.00	0.83	0.50 ± 0.00	4.20 ± 0.0
ROCK	1	25.5 ± 0.0	50.4 ± 0.00	6.22 ± 0.02	0.50	0.505 ± 0.000	4.10 ± 0.0
	2	25.5 ± 0.0	50.3 ± 0.00	6.20 ± 0.00	0.50	0.510 ± 0.005	4.00 ± 0.0
	3	25.5 ± 0.0	50.4 ± 0.00	6.20 ± 0.00	0.50	0.500 ± 0.005	4.10 ± 0.0
	4	25.5 ± 0.0	50.4 ± 0.00	6.20 ± 0.00	0.51	0.505 ± 0.000	4.00 ± 0.0
DOUBLE CROWN	1	23.75 ± 0.01	46.60 ± 0.05	8.89 ± 0.02	1.00	0.455 ± 0.000	4.20 ± 0.0
	2	23.75 ± 0.01	46.50 ± 0.05	8.85 ± 0.02	1.00	0.454 ± 0.001	4.20 ± 0.0
	3	23.70 ± 0.04	46.50 ± 0.05	8.87 ± 0.00	1.00	0.455 ± 0.000	4.20 ± 0.0
	4	23.74 ± 0.00	46.60 ± 0.05	8.87 ± 0.00	0.99	0.455 ± 0.000	4.20 ± 0.0
NOBLE	1	25.0 ± 0.01	49.40 ± 0.00	5.71 ± 0.00	0.80	0.45 ± 0.00	4.10 ± 0.
	2	25.0 ± 0.01	49.50 ± 0.10	5.70 ± 0.01	0.79	0.45 ± 0.00	4.10 ±
	3	25.0 ± 0.01	49.40 ± 0.00	5.70 ± 0.01	0.79	0.45 ± 0.00	4.10 ±
	4	25.2 ± 0.01	49.40 ± 0.00	5.71 ± 0.00	0.80	0.45 ± 0.00	4.10 ±
STOUT	1	-	-	-	0.41	0.45 ± 0.01	3.85 ± 0
	2	-	-	-	0.42	0.45 ± 0.01	3.80 ± 0
	3	-	-	-	0.41	0.46 ± 0.00	3.85 ± 0
	4	-	-	-	0.41	0.46 ± 0.00	3.85 ± 0

- No Results Obtained.

Table 3

Results of Analyses of Beer Samples

BRAND	BATCH NO.	ASH CONTENT	COPPER (ppm)	IRON (ppm)	CHLORIDE %	APPARENT ATTENUATION %	REAL ATTENUATION %
STAR	1	0.21 ± 0.02	0.50 ± 0.00	0.19 ± 0.00	0.16 ± 0.00	96.19 ± 0.00	67.44 ± 0.0
	2	0.23 ± 0.00	0.51 ± 0.01	0.18 ± 0.00	0.15 ± 0.01	96.19 ± 0.00	67.44 ± 0.0
GULDER	1	0.25 ± 0.02	1.25 ± 0.02	0.45 ± 0.00	0.15 ± 0.00	91.59 ± 0.00	77.59 ± 0.01
	2	0.21 ± 0.02	1.25 ± 0.02	0.45 ± 0.00	0.15 ± 0.00	91.60 ± 0.01	77.60 ± 0.00
ROCK	1	0.21 ± 0.01	0.05 ± 0.00	0.04 ± 0.00	0.15 ± 0.00	83.66 ± 0.01	70.97 ± 0.00
	2	0.23 ± 0.01	0.06 ± 0.01	0.04 ± 0.00	0.15 ± 0.01	83.65 ± 0.00	70.96 ± 0.01
	3	0.21 ± 0.01	0.05 ± 0.00	0.05 ± 0.01	0.16 ± 0.00	83.65 ± 0.00	70.97 ± 0.00
	4	0.23 ± 001	0.05 ± 0.00	0.04 ± 0.00	0.16 ± 0.00	83.65 ± 0.00	70.97 ± 0.00
DOUBLE CROWN	1	0.28 ± 0.01	2.50 ± 0.00	0.05 ± 0.00	0.16 ± 0.00	98.19 ± 0.01	75.09 ± 0.0
	2	0.27 ± 0.02	2.50 ± 0.00	0.05 ± 0.00	0.16 ± 0.00	98.20 ± 0.00	75.08 ± 0.0
	3	0.29 ± 0.00	2.50 ± 0.00	0.05 ± 0.00	0.17 ± 0.01	98.20 ± 0.00	75.08 ± 0.0
	4	0.30 ± 0.01	2.50 ± 0.00	0.05 ± 0.00	0.16 ± 0.00	98.20 ± 0.00	75.09 ± 0.0
NOBLE	1	0.25 ± 0.00	1.25 ± 0.00	0.125 ± 0.01	0.15 ± 0.01	94.60 ± 0.01	72.68 ± 0.0
	2	0.26 ± 0.01	1.25 ± 0.00	0.126 ± 0.00	0.16 ± 0.00	94.62 ± 0.01	72.68 ± 0.0
	3	0.25 ± 0.00	1.26 ± 0.01	0.126 ± 0.00	0.16 ± 0.00	94.60 ± 0.01	72.65 ± 0.0
	4	0.24 ± 0.01	1.25 ± 0.00	0.125 ± 0.01	0.16 ± 0.00	94.60 ± 0.01	72.68 ± 0
STOUT	1	0.84 ± 0.00	1.50 ± 0.01	-	0.16 ± 0.00	81.42 ± 0.00	56.48 ± 0
	2	0.83 ± 0.01	1.50 ± 0.01	-	0.16 ± 0.00	81.43 ± 0.01	56.48 ± 0
	3	0.84 ± 0.00	1.51 ± 0.00	-	0.17 ± 0.01	81.42 ± 0.00	56.47 ± 0
	4	0.84 ± 0.00	1.51 ± 0.00	-	0.16 ± 0.00	81.42 ± 0.00	56.47 ± 0

- No Results Obtained.

4-2 EXTRACT

Apparent extract, Real extract and extract of original wort are concenred here.

Apparent extract for Stout has the highest value which ranges from 5.605 - 5.629% followed by Rock Lager Beer with value which ranges from 1.795 - 1.820%.

Other lager beers tend to have lower values of apparent extract, the lowest being for Double Crown; the value ranges from 0.206 - 0.231%.

These apparent extract values were obtained from tables (Reference 15). The apparent extract expresses sugars and carbohydrates present in beer. Hence the high value obtained for Stout shows it contains much more carbohydrates than other lager beers.

The values for real extract and extract of original wort in most cases follow the same trend as for those of apparent extract. Guinea Stout ranking first in each case.

Values for real extract and extract of original wort obtained for Stout ranges from 6.35 - 6.36% and 14.57 - 14.59%, respectively.

For Rock Lager Beer values obtained ranges from 3.18 - 3.20% and 10.98 - 10.99%, respectively; for Gulder values range from 2.53 - 2.54% and 11.28 - 11.29%; Noble Lager Beer values range from 3.12 - 3.13% and 11.42 - 11.43%. For Double Crown values range from 2.84 - 2.85% and 11.39 - 11.40%, and then values for Star Lager Beer range from 2.86 - 2.87% and 8.78%.

Similarly real extract and extract of original gravity can be said to be due to sugars and carbohydrates, since these substances normally constitute about 80% of the extract.[15]

4-3 SPECIFIC GRAVITY

For specific gravity Stout again has the highest value which ranges from 1.0221 - 1.0222. This is followed by Rock Lager Beer again as for extract with value which ranges from 1.0070 - 1.0071. Gulder has a value which ranges from 1.0037 - 1.0038; Noble Lager Beer value obtained is 1.0024. For Star the value ranges from 1.0013 - 1.0017 and for Double Crown value ranges from 1.0008 - 1.0009.

The specific gravity of beer compares with the weight of equal volumes of beer and water.

This could also give some indication of the extract present in beers. Evidently, the results for extract and specific gravity follow the same trend.

The values for specific gravity are in good agreement with that of the American Society of Brewing Chemists standard with value which ranges from 1.0015 - 1.0018.

4-4 REDUCING SUGARS

Stout has the highest value of reducing sugar 0.361%. This is followed by Rock Lager Beer with a value which ranges from 0.190 -

0.191%. Noble Lager Beer has a value which ranges from 0.138 - 0.139%; Gulder 0.065 - 0.066%; Double Crown 0.057 - 0.058%, and Star 0.056 - 0.059%.

Sugars which contain a free aldehydic group in the molecule are reducing agents for copper sulphate in alkaline solution. However, those which lack these properties are non-reducing sugars hence the differences in values above. The estimation of reducing sugars are based upon their power of reducing copper salts.

The extract specific gravity trend is still markedly observed from the values obtained for reducing sugar. Proving without doubt the earlier observation that sugars present in beer constitutes about 80% of the extract.

4-5 DEXTRINS

From the data obtained Stout has value which ranges from 0.17 - 0.18% dextrins. Rock 0.08 - 0.09%; Noble 0.065 - 0.066%; Double Crown 0.04 - 0.05%; Gulder 0.03 - 0.04% and Star 0.03 - 0.04%.

Like reducing sugars, dextrins determine how much extract will be present in beer. The higher the dextrins the higher the extract content of beer. This is clearly illustrated in the case of Stout which contains higher extract, it also contains higher dextrins. Another observation is that when the percentage of reducing sugars is high, the percentage of dextrins will be similarly high. Compared with the European Brewing Convention standard the values

obtained were low. The range for EBC is 0.3%.[14]

4-6 PROTEINS

Stout seems to take the lead again in protein content. Stout has a value which ranges from 0.47 - 0.48%; followed by the other brands, Rock Lager Beer again with value which ranges from 0.41 - 0.43%; Noble 0.41 - 0.43%; Star 0.40 - 0.45% and Double Crown 0.40 - 0.41%.

The determination of these more complex nitrogenous fractions in beer is a matter of considerable practical importance, because some indication may be obtained about the resistance of the sample to colloidal haze formation. However, a higher concentration of residual assimilable nitrogen in bottled beer is undesirable and may lead to lack of biological stability, since yeast present in beer will readily feed on this proteins.

From the point of view of food value in beer it is readily seen that Stout is the best source of protein from beers and stouts.

The values obtained are within the range of the American Society of Brewing Chemists standard which is 0.40%.[15]

4-7 CARBON DIOXIDE

The value for carbon dioxide ranges from 0.56 - 0.57% for Star; followed by Gulder with a value of 0.50%; Rock appears to have almost the same value as Gulder, its value ranges from 0.500 - 0.510%; Double

Crown 0.454 - 0.455; Noble 0.45%; Stout 0.45 - 0.46%.

It should be noted that a correct concentration of carbon dioxide in beers is necessary, both on account of head formation and palate.

The carbon dioxide content as specified by the European Brewery Convention (EBC)[15] should be approximately 4 g/1000 ml which gives a percentage within the range of the above data.

In hot season the value of carbon dioxide content may be slightly lowered while it will be slightly higher in the cold season. Low gravity beers should contain the minimum quantities of carbon dioxide, since there is a greater tendency with these beers to lose gas and may gush.

4-8 COLOUR

The colour intensity for Double Crown has a value which ranges from 8.85 - 8.89. This is followed by Rock Lager Beer from 6.20 - 6.22; Noble 5.70 - 5.71; Star 5.60 - 5.90; Gulder 4.7 - 4.8. No value was obtained in the case of Stout for the same concentration used, since Stout is highly coloured.

The colour intensity describes the appearance of a particular beer brand whether it is bright, slightly opalescent, opalescent or turbid. The higher the value of the colour intensity the less bright the beer or the more turbid the beer compared to others.

What attracts a beer consumer is its clarity or paleness. So colour is one of the criteria to be judged.

The colour intensity of beers should be 5.75 according to the American Society of Brewing Chemists.[47] The results obtained are within that range.

4-9 TOTAL ACIDITY

The total acidity as lactic acid for the different brands of beer is almost the same. For Rock, it is 0.19 - 0.20%; Gulder 0.18%; Noble 0.18%; Star 0.17 - 0.18%; Double Crown 0.17 - 0.18%, while no value was obtained for Stout.

The fact that beer when taken in the right quantity can accelerate digestion of basic foods in the stomach stresses the need for beers to contain some acids. These values agree with the American Society of Brewing Chemists value of 0.16%.[47]

4-10 VOLATILE ACIDITY

The volatile acidity expressed as gram acetic acid (HOAc) for Gulder the value ranges from 0.017 - 0.018 g. Rock 0.016 - 0.017 g; Double Crown 0.016 - 0.017 g; Star 0.0160 - 0.0165 g. Stout 0.012 - 0.013 g; Noble 0.009 - 0.010 g.

Compared to the European Brewing Convention (EBC)[14] standard which ranges from 0.017 - 0.020 g the values obtained are within the required range.

4-11 FIXED ACIDITY

The fixed acidity in gram for Noble, the value ranges from 0.036 - 0.037 g; Rock 0.030 - 0.032 g; Double Crown 0.029 - 0.030 g; Gulder 0.026 - 0.028 g; Star 0.0249 - 0.0250 g. While no value was obtained for Stout, since the process involved an indicator titration method which cannot be carried in this case because of the high colour density of this brand.

Real Degree of Fermentation - Apparent Degree of Fermentation

4-12 REAL DEGREE OF FERMENTATION

In respect of the tested brands real degree of fermentation can be arranged in the following decreasing order.

Gulder 77.59 - 77.60%; Double Crown 75.08 - 79.09%; Noble 72.65 - 72.68%; Rock 70.76 - 70.97%; Star 67.44%; Stout 56.47 - 56.48%.

These values are in agreement with the American Society of Brewing Chemists standard of around 60.6%.[43]

4-13 APPARENT DEGREE OF FERMENTATION

For the apparent degree of fermentation, Double Crown takes the lead with a value which ranges from 98.19 - 98.20%; Noble 94.60 - 94.62%; Gulder 91.59 - 91.60%; Rock 83.65 - 83.66%; Star 96.20% and Stout 81.42 - 81.43%.

The determination of Real degree of Fermentation (Real Attenuation) and Apparent degree of Fermentation (Apparent Attenuation) gives an indication of the extent of the percentage of extract left in the beer.

If the beer has been poorly attenuated, or primed with sugar at racking, they naturally form a greater percentage of the extract.

As expected, Stout having the lowest degree of attenuation (fermentation) has the highest content in respect of real extract reducing sugars and dextrins.

The estimation of residual fermentable sugars left in beers is of the utmost importance from the point of view of biological stability and the determination of the attenuation limit is, therefore, an essential control measure.

Also the values for apparent degree of fermentation are in good agreement with the American Society of Brewing Chemists standard which is about 69.9%.

4-14 HYDROGEN ION CONCENTRATION

The value for Star beer ranges from 4.30 - 4.35; Guider 4.20 - 4.25; Double Crown 4.20; Rock 4.00 - 4.10; Noble 4.10 and Stout 3.80 - 3.85.

The measurement of pH is of the greatest importance in brewing and the pH of various solutions must be determined in a large number of samples.

The measurement of pH gives an indication of the stability of beer biologically and non-biologically. It also gives an indication of the extent of attenuation of beer.

The American Society of Brewing Chemists standard is from 4 - 4.60. These values are within that range.[15]

4-15 HAZE

Haze in (FTU) units for Double Crown ranges from 0.99 - 1.00; Gulder 0.83 - 0.84; Star 0.76 - 0.78; Noble 0.79 - 0.80; Rock 0.50 - 0.51 and Stout 0.41 - 0.42.

The determination of haze gives the complete range of turbidity developed by bottle beer during shelf-life from brilliant, through slight to severe haze.

The higher the haze value the less bright or the more severe the turbidity of the beer.

Investigation into the nature of beer hazes is first carried out microscopically to see if it is of biological origin, i.e., due to the presence of yeast, bacteria, etc. or non-biological in character and due to the presence of colloidal and amorphous matter. If the haze is non-biological in origin, it may be due to a wide variety of different causes, such as chill haze, oxidation haze, the presence of certain metallic ions in too high concentration, starch, formaldehyde or some accidental reason.

From the available data it is assumed that Double Crown and Gulder Beers with high haze values are likely to be the result of high concentration of copper and iron and may be also other substances which cause haze.

The approximate value required for fresh beer by the European Brewing Unit (EBU)[14] is 0 - 1 (FTU). This value agrees with those obtained.

Ash - Copper - Iron - Chloride

4-16 ASH

The ash content for Stout, 0.83 - 0.84%, is considerably higher than any of the values obtained for the other lager beers. Double Crown follows with a value which ranges from 0.27 - 0.30%; Noble 0.24 - 0.25%; Gulder 0.21 - 0.25%; Star 0.21 - 0.25%. Rook 0.21 - 0.23%.

The determination of the total ash content of beer is of no great practical importance but certain constituents of the ash may be important where haze formation is suspected of being due to metallic contamination as stated under discussion of haze in beers.

The ash content of beer normally as accepted by the European Brewery Convention (EBC) should vary between 0.20 - 0.30%. The values obtained during the project are in agreement with this range except in the case of Stout.

4-17 COPPER

From the data obtained the concentration of copper in Double Crown is 2.50 ppm.; Stout 1.50 - 1.51 ppm.; Gulder 1.25 - 1.28 ppm.; Noble 1.25 - 1.26 ppm; Star 0.50 - 0.51 ppm. and Rock 0.05 - 0.06 ppm.

The presence of copper in beers act to accelerate oxidation changes which adversely affect flavour and shelf-life of the packaged beverage.

The presence of traces of copper of the order of a fraction of a part per million (ppm) has a strong catalytic effect on the oxidation reactions occurring in beer. These reactions adversely affect the shelf-life and taste of the packaged product.

The brewing industry requires little to none availability of copper in beers.

In the past brewing processes were carried out in copper vessels. However, due to the above effects, copper vessels are no more in use in most brewing industries.

Probably cases where concentration of copper is of the order of 3.0 ppm., copper vessels are used.

Double Crown, Stout, Noble and Gulder beers having more than 1 ppm are expected to be less stable than Star and Rock beers.

Irwin, Romola and Carlyle[30] stated that the approximate content of copper in beer lies between 0.05 - 3 ppm. Values obtained are within that range.

4-18 IRON

The concentration of iron in Gulder ranges from 0.45 - 0.46 ppm; Star 0.18 - 0.19, Noble 0.13 Double Crown 0.05 ppm and Rock 0.04 - 0.05 ppm.

The effects which have been stated in case of copper in beers also apply to iron in beers.

Gulder lager beer, having the highest iron content, is expected to be less stable.

4-19 CHLORIDES

All brands of beer tested have almost the same content of chlorides; Star has a value 0.15 - 0.17%; Stout 0.16 - 0.17%; Rock 0.15 - 0.16%; Noble 0.15 - 0.16%; Gulder 0.15%, and Double Crown 0.16 - 0.17%

Chloride content is one of the predominating factors governing the quality of beer. It affects mainly its gustatory properties. High concentration of chloride gives beer a bad taste and smell. That is why it should be avoided.

Chloride content as reported by the American Society of Brewing Chemists[37] standard should lie between 0.15 - 0.18%. The values obtained are in agreement.

Bitterness Units (BU) - Iso-Alpha Acids

4-20 BITTERNESS UNITS (BU)

The Bitterness Unit (BU) value for Gulder ranges from 27.0 - 27.2; Rock 25.5; Noble 25.0 - 25.2; Star 24.0 - 24.8; Double Crown 23.70 - 23.75, while no value was obtained for Stout since the process was carried out spectrophotometrically.

Bitterness Units for beers should be between 18 - 25.[14] Results obtained are within that range.

4-21 ISO-ALPHA ACIDS

Also from the data obtained the iso-alpha acids in parts per million (ppm) for Gulder, it ranges from 52.0 - 52.2 ppm.; Rock 50.3 - 50.4 ppm.; Noble 49.40 - 49.50 ppm.; Star 47.02 - 47.50 ppm.; Double Crown 46.50 - 46.60 ppm. No result was obtained for Stout also due to the same reason given for Bitterness Units.

As expected the results of iso-alpha acids content correlate well with the bitterness units for each brand of beer; e.g., Gulder has the highest figure for both parameters while Double Crown has the lowest values.

At one time beer was evaluated by its flavour alone which comes from the bitter substances or iso-alpha acids produced from hops.

Higher bitterness units and higher iso-alpha acids mean an increase in bitterness or flavour in beers. However, the addition of even pure isomerized preparations to beer is accompanied by some degree of haze formation, particularly when high local concentration of iso-α- acids are allowed to develop because of a failure to ensure rapid intimate mixing with the beer. This calls for caution to the quantity of hops that should be added after fermentation.

The economics of post-fermentation[37] bittering are naturally related to the cost of the product and to the success a brewer has in hopping by conventional means, and this varies greatly from brewery to brewery. Not least among the advantages of using isomerized extracts, however, is the ability to control bitterness to any desired level, a matter of increasing importance when a company has breweries in different areas which are striving to produce an identical brand of beer.

According to the European Brewing Convention[43] the range for iso-alpha acids in ppm should be between 50 - 60 ppm. Results obtained correspond to this range.

CONCLUSION

1. Comparing the analytical values obtained for the different brands of Nigerian beer to International Specifications, one can conclude that the locally produced beers are not lacking in quality.

2. From the analytical results, one can say without doubt that the different batches of the same brand retain their quality i.e., the local beer producers can be relied upon in brewing products of reasonably constant values. It was noted that different batches of the same brand showed very little deviation from mean values for the different parameters analysed.

3. Finally, from the results of the different brands, the following conclusions can be made:

   (i) A consumer interested in becoming intoxicated after taking few bottles of beer should go on Gulder Lager Beer, since its alcoholic content is relatively high compared to other brands of beer.

   (ii) A consumer who is interested in some food value in beer, for example, proteins, carbohydrates and sugars, should drink Stout.

(iii) A consumer who is interested in beer with keeping quality should drink Rock Lager Beer and Star Lager Beer, since their copper, iron and chloride contents are very low, so haze will not develop easily.

(iv) Star, Gulder and Rock are more biologically stable than other brands of beer, because their carbon dioxide content is higher.

(v) A drinker interested in very pale beer should go on Gulder, Star and Noble beers. This is readily seen from the values of their colour intensity. They are relatively low compared to other brands of beer.

(vi) A consumer with high sense for flavour (bitterness) should drink either Stout or Gulder.

(vii) Drinking beer for its acidic content, one should choose Stout.

Based on flavour, paleness, carbon dioxide content, keeping quality, intoxicating ability, Gulder Lager Beer can be considered the best of the six brands of beer studied.

RECOMMENDATIONS FOR FURTHER WORK

Some aspects of analytical studies on beers deserve further work:

(a) Some elements such as Fluorine, Bromine, Tin, Aluminium, Lead, Nickel and Arsenic have not been determined. Recent investigations indicate that some of these elements, though only present in trace quantities in beers, may have important effects.

(b) Determinations such as the estimation of sucrose, trisaccharides, pentisan glycerol, higher alcohols, esters succinic acids, diacetyl, dissolved oxygen, reducing power in the cold (I.T.T. value) have also not been determined. A further analysis of the foregoings will give a better understanding of the chemical composition of beers.

APPENDIX

The results obtained on the analyses of some beers brewed in Nigeria have already been presented in Chapter 4.

In this appendix results obtained by other workers for lager beer and ale are shown.

Also shown is the classification of beers.

APPENDIX I

CLASSIFICATION OF BEERS

Lager and Ale

The principal difference between Lager and Ale is in the fermentation process. Lager beer is fermented at a relatively cool temperature, with a yeast that falls to the bottom at the end of fermentation. Ale is fermented at a warmer temperature, with a yeast that rises to the top.

Lager can range from the pale highly-hopped Pilsener to the dark aromatic less bitter Munich types.

Stout

The most obvious characteristic of Stout, is the characteristic dark colour which is conferred on it by inclusion in the grist of roasted barley (6% or more) or caramel. Within the genus these are sweet stouts, and dry highly hopped stouts with a characteristic grainy flavour and particularly foam head-retention properties - Nigeria's stout is of the nature of the latter type.

Appendix II

Typical Analyses of Brewery Products, The Amer. Soc. Brew. Chem.[47]

	Lager Beer	Ale
Appearance on arrival	Clear	Clear
Colour	5.75° Lovibond	6.0° Lovibond
Odour	Pure	Mildly aromatic
Taste	Pure, somewhat full-bodied, good hop flavour	Good hop flavour
Foam keeping capacity	Good	Good
Specific gravity	1.01598	1.01399
Saccharometer indication	4.07° Plato	3.57° Plato
Alcohol by volume	4.89%	5.40%
Alcohol by weight	3.80%	4.20%
Extract	5.79%	5.49%
Sugar	1.63%	1.54%
Dextrin	2.52%	2.23%
Unconverted Starch	None	None
Acidity as lactic	0.16%	0.16%
Protein	0.40%	0.42%
Heavy metals	None	None
pH value	4.60	4.30
Carbon dioxide, 2 bottles	0.48%; 0.48%	0.45%; 0.45%
Original gravity	13.1° Plato	13.6° Plato
Real degree of fermentation	55.8%	59.6%
Apparent degree of fermentation	68.9%	73.8%
Chilling test, clarity (absolute turbidity) on 2 bottles chilled 24 hours in ice pact	(a) -0.0029 (b) -0.0030	(a) -0.0028 (b) -0.0028

REFERENCES

1. The New Caxton Encyclopedia, Volume Two, The Caxton Publishing Company, London, New York, Toronto, Sydney, Tokyo, Johannesburg 1972. p.626.
2. Encyclopedia Americana, International Edition, Volume 1, Americana Corporation, New York, 1966. p.356.
3. Chambers's Encyclopaedia, New Revised Edition, Volume II, International Learning Systems Corporation Limited, London, 1973. p.197.
4. Encyclopaedia Britannica, Volume 3, Encyclopaedia Britannica, Inc., William Benton Publisher, Chicago, London, Toronto, Geneva, Sydney, Tokyo, Manila, 1969. p.379.
5. Chemical Synonyms and Trade Names, A Dictonary and Commercial Handbook, by William Gardner, Eight Edition, The Technical Press Ltd., Oxford, England, 1978.
6. Collier's Encyclopedia, Volume 3, Crowell-Collier Educational Corporation, 1968. p.772.
7. Encyclopedia Americana, Volume 4, International Edition, Americana Corporation, New York, 1966. p.475.
8. Bennett, H., The Chemical Formulary, Volume V, Chemical Publishing Co., Inc., New York, 1941.
9. Encyclopedia Britannica, Volume 4, Encyclopedia Britannica, Inc. William Benton Publisher, Chicago, London, Toronto, Geneva, Sydney, Tokyo, Manila, 1969. p.163.
10. Chambers's Encyclopaedia, New Revised Edition, Volume II, International Learning Systems Corporation Limited, London, 1973. p.530.

11. Sienko, M.J., Plane, R.A.; Chemistry International Student Edition, McGraw-Hill Book Company, New York, London, Kogakusha Company, Ltd., Tokyo, 1971.

12. Shreve, R.N., "Chemical Process Industries (CPI)" 3rd Edition, McGraw-Hill Book Company, New York, 1967. p.603.

13. The New Caxton Encyclopedia, Volume 2, The Caxton Publishing Company, London, New York, Toronto, Sydney, Tokyo, Johannesburg, 1969, p.566.

14. Pearson, D.; Chemical Analysis of Food, Seventh Edition, Churchill Livingstone, London, 1976.

15. De Clerck, J., "A Textbook of Brewing", 2 Volumes, (Trans. by K. Barton-Wright) Chapman and Hall, London, 1957, 1958.

16. Everyman's Encyclopaedia, Fifth Edition, Volume 2, J.M. Dent and Sons, Ltd., London, 1967. p.449.

17. Chambers's Encyclopaedia, New Revised Edition, Volume VII, International Learning Systems Corporation Limited, London, 1973. p.212.

18. Encyclopedia Americana, Volume 14, International Edition, Americana Corporation, New York, 1966. p.377.

19. Roger, J.W., Edwin, M,L., "The Encyclopedia of Biochemistry", Reinhold Publishing Corporation, New York, 1967.

20. Holderness, A., Lambert, J., "A New Certificate Chemistry, Third Edition, Heinemann Educational Books Ltd., London, 1964.

21. Shreve, R.N., "Chemical Process Industries (CPI), McGraw-Hill Book Company, New York, 1967. p.591.

22. Peterson, M.S., Johnson, A.H., "Encyclopedia of Food Science", The AVI Publishing Company, Inc., Westport, Connecticut, 1978.

23. Reports on the Progress of Applied Chemistry, Volume XXXIV, The Society of Chemical Industry, London, 1949.

24. Official Methods of Analysis of the Association of Official Analytical Chemists, Published by Association of Official Analytical Chemists, Washington, 1970.

25. Gray, J., Wallerstein Laboratory Communication, Volume 1, 1938. p.21.

26. Enders; Woch, Fur, Brauerei, 53, 1936. p.89.

27. Stone, I., Gray, J., Wallerstein Laboratory Communication, Volume 10, 1946, p.209.

28. Bishop, L.R., J. Inst. Brew., 56, 373, 1950.

29. Vogel, A.I., "A Textbook of Quantitative Inorganic Analysis Including Elementary Instrumental Analysis", Third Edition, The English Language Book Society, Longman, 1945.

30. Irwin, S., Romola, R, and Carlyle, G., Analytical Chemistry, 25, 893, 1953.

31. Stone, I., Industrial Engineering Chemical Analysis. Ed. 14, 479, 1942.

32. American Society of Brewing Chemists Proceeding, Report of Sub-Committee on Copper in Beer, 1952.

33. Martin, R.I., and Githens, R.E. Sr., Analytical Chemistry, 24, 991 (1952).

34. American Society of Brewing Chemists, 1950; p.166.

35. Martin, E.B.C., The Brewers Digest, 1972.

36. Souliotis, A.G., Grimanis, A.P., Tsanos, N.A., Analyst, 90, 449, 1965. 1965.

37. Rose, A.H., "Alcoholic Beverages", Volume 1, Academic Press, London, New York, San Francisco, 1977.

38. Encyclopedia Americana, Volume 2, International Edition, Americana Corporation, New York, 1966; p.346.

39. Encyclopaedia Britannica, Volume 1, Encyclopaedia Britannica, Inc., William Benton Publisher, Chicago, London, Toronto, Geneva, Sydney Tokyo, Manila, 1969; p.539.

40. Roger, J.W., Edwin, M.L. Jr., "The Encyclopedia of Biochemistry" Reinhold Publishing Corporation, New York, 1967.

41. The New Caxton Encyclopedia, Volume One, The Caxton Publishing Company, London, New York, Toronto, Sydney, Tokyo, Johannesburg, 1973; p.117.

42. Everyman's Encyclopedia, Fifth Edition, Volume I, J.M. Dent and Sons, Ltd., London, 1967; p.186.

43. Trolle, B., Nyborg, P. and Buchmann-Olsen, B., Journal of the Institute of Brewing 57, 347, 1951.

44. Klopper, W.J., Wallerstein Laboratories Communications, 18, 123, 1955.

45. Bishop, L.R., "Proceeding of the American Society of Brewing Chemists", 1970; p.145.

46. Noris, F.W., Journal of the Institute of Brewing, 74, 192, 1946.

47. Riegel, E.R., "Riegel's Handbook of Industrial Chemistry" 7th Edition, Van Nostrand Reinhold Company, New York, 1974; p.188.