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TECHNOLOGY

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Suitability of cassava starch as adjunct substitute for barley in the brewing of beer (stout beer).

A thesis submitted in partial fulfillment of the requirement for the degree of Master of Science on March 2008.

By

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MARCH 2008

DECLARATION

I declare that this work was carried out by me in the Department of Biochemistry and Biotechnology of the Kwame Nkrumah University of Science and Technology, under the supervision of Prof. W. O. Ellis

PRINCE K. ASANTE…….. ……..

Prince Kwame Asante Prof. W. O. Ellis (Supervisor)

DEDICATION

This work is dedicated to Jehovah God and my children, Nana Kwadwo Adjeibi Asante, Kwame Asante Jnr. and Aderemi Kofi Asante.

ACKNOWLEDGEMENT

From 2003, I pursued the course namely MSc. Food Science and Technology. During the course of the study, I was confronted with many problems which I took pains to overcome as the course progressed.

Emanating from the course was a project work titled "CASSAVA STRCH AS ADJUNCT SUBSTITUTE FOR BARLEY IN THE BREWING OF BEER (STOUT BEER)". I have the pleasure to state that whilst undertaking this project I had to spare a lot of time, energy and interest to work relentlessly at the project which I believe has received the approval of my supervisor and lecturers. I think I will be failing in my duty if I fail to make mention of all who gave me services that greatly contributed to my success. In this direction I will like to feature the following people who demonstrated benevolent gestures, professional services and

financial aid to put me through the course and especially the project work.

I first wish to express my profound and sincere thanks and appreciation to my supervisor Prof. W. O. Ellis for his professional services and benevolent gestures that have enabled me successfully complete this work. My sincere gratitude to my Dad for the financial aid he has provided throughout my education, to my Mum for her words of wisdom and motherly love that surpasses all and to my wife and children for their unwavering support that has contributed to what I am today.

ABSTRACT

Cassava starch has featured in many foods recipes and industrial applications. However its use as an adjunct in brewing has not received much attention. This work therefore sought to establish the suitability of cassava starch as an adjunct substitute for barley in brewing. The gelatinization temperature of cassava starch samples were determined and results ranged between 62 and 65°C. Other key parameters evaluated were wort perfect primaries, gravities and pH, attenuation limits, colour, and bitterness. Head retention (HR) and carbondioxide (CO_2) content of finished product were also determined. Results showed that the present gravities (PG), pH, bitterness (BITT) and colour (COL) obtained for the cassava starch substituted wort samples ranged from, 90.64-94.36°S, 5.3 – 5.5, 54 – 63EBU and 27 – 33 respectively. In addition to the above parameters, two additional parameters, original gravities (OG) and alcohol by volume (abv) were measured for the cassava starch substituted green beer with the following results, (OG) 87.21 – 91.43°S, (pH) 4.2 – 4.4, (BITT) 45 – 56EBU, (abv) 8.6 – 94. Results for head retention and carbondioxide for finished products were, (HR) 76 – 83sec, (CO_2) 3.08 – 3.43g/l. Sensory evaluation showed that the 25% cassava starch substituted adjunct sample was the most preferred based on estery flavour, tingly mouth feel, colour and bitterness.

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CHAPTER ONE

INTRODUCTION

Worldwide, the brewing industry is registering growth in both volumes and profits year on year. Ghana is no exception to this global phenomenon. Over the last two years, the biggest brewing industries in Ghana, Guinness Ghana Breweries Limited (GGBL) and Accra Breweries Limited (ABL) have made enviable strides, both in volume turnover and profit margins. The potential for the industry to grow has become unquestionable. The challenge however is how the industry can reduce production cost by the use of cheaper sources of raw materials such as malts and hops. Many brewing industries have taken remedial steps to accommodate the ever-soaring prices of these raw materials by the introduction of cheaper sources of carbohydrate as adjuncts in their recipes.

The important role adjuncts play in brewing has been stressed by many authors (Jones, 2006). In 2003 an estimated 1800tonnes of adjuncts was used in brewing all brands of beer in Guinness Ghana Limited (GGBL annual brewhouse report-Kaase site Kumasi, 2003). This accounted for a whopping 20% of the total extract yield of GGBL.

Nobody envisages a dramatic shift in grist materials used in the current beer market. Some brewers have shifted from sizeable use of adjuncts to grists that are largely composed of premium malted as they are convinced that this offers genuine quality. However, there remains a clear justification for many brewers to use adjunct materials, since they offer unique product attributes such as flavour and colour. The quality attributes of some of the world's leading global beer brands are heavily based on the adjunct used in their formulation. (Goode and Arendt, 2005). The foregoing underpins the employment opportunities this may offer to inhabitants in areas where brewing

industries are vibrant and are endowed with cereals that are commonly used as brewing adjuncts.

Brewing adjuncts are materials other than malted barley that bring additional sources of carbohydrate and protein into wort. Adjuncts have largely been limited to cereals such as corn, rice, sorghum, wheat and barley with little contribution coming from cane sugar. Globally, the use of adjuncts from roots and tubers has however received relatively little attention. The Bavarian purity law (Reinheitsgebot) defines an adjunct as " anything that is not malt, yeast, hops or water". However the definition is much broader today. The United Kingdom food standards committee interprets adjuncts to be "Any carbohydrate source other than malted barley which contributes sugars to the wort" (Institute of brewing, 2003). The latter definition seem to embrace roots and tubers alike that can provide sources of carbohydrate, which can meet the requirements for brewing.

It has been reported (Bamforth, 2003) that when the total cost of beer production is taken into consideration (from raw material purchase and processing through to packaging, sales and taxation), then malts costs in general have been estimated to represent just approximately 3.5% of the total cost. Therefore, it becomes apparent that grain costs represent only a relatively minor contribution to the total cost of beer production. The foregoing raises the question, why replace malted barley with an unmodified substrate 'adjunct'? In less developed countries, malting facilities and malting conditions are quite often less than optimal. Therefore, because of its lower price, locally produced adjuncts material can be used to supplement malted barley grain (Grujic, 1999). Apart from the direct cost benefits of using cheaper raw materials,

indirect costs (much greater than the direct costs) can also influence raw material selection.

In Kenya, for example, beer is made from malted grain, (Cege *et al.*, 1999). Kenyan brewers are therefore encouraged to develop beer from exclusively non-malted grain (mainly raw barley). Likewise in Japan, much lower rate taxation is applied to products containing high adjuncts levels (Happoshu)(Brewers Association of Japan; Shimizu *et al*., 2002). Therefore, Japan's brewers have a great incentive to brew products from grists containing adjunct levels in excess of 50%. Likewise, in Nigeria a 1988 government economic decision to ban the importation of malted barley forced local brewers to develop alternative brewing procedures to utilize locally grown sorghum and maize crops (Hallgren, 1995; Little, 1994). Additionally, factors associated with product quality, tradition and consumer product expectations can be the decisive reason to use adjuncts, such as the impact that rice has on flavour, colour and colloidal stability of an American pale lager, or the role that wheat plays in the taste and appearance of a Belgian or German style wheat beer (Delvaux et al., 2001). Also the use of liquid adjunct materials in today's high gravity brewing culture can increase production output and significantly reduce production costs, whilst contributing to product character.

In the past, the main drivers for the usage of brewing adjuncts have been cheaper cost of raw materials, together with opportunities of increasing product output capacity without the necessity of increasing brewhouse capacities (addition of syrups). In addition, usage of certain adjuncts has offered the brewer more control over product quality with regard to flavour, colour and colloidal stability. Likewise, governmental political decisions have encouraged the use of adjuncts; hence the manufacture of lager beer from

unmalted sorghum and maize in Nigeria, the manufacture of barley beer in Kenya and more recently the manufacture of happoshu in Japan. There are other drivers, which have the potential to increase adjunct usage. Very recent research efforts (Brauer et al., 2005; NicPhiarais et al., 2005; Wijngaard et al., 2005a,b, c; Zarnkow et al., 2005a,b) have concentrated on developing alternative beers and cereal-based beverages with the aim of fulfilling current consumers health needs and expectations. Two such beverages where both traditional and non-traditional adjunct materials will in the future play an important role in their recipe formulations are gluten-free beers and health promoting functional beers.

OBJECTIVE

This study seeks to evaluate the suitability of cassava starch as an adjunct substitute for barley in the brewing of beer.

Specific Objectives

- Assess wort production and fermentability
- Assess key quality indices such as foaming and head retention
- Assess flavour attributes of finished product such as diacetyl and estery aroma.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 ADJUNCTS

Several reasons have dictated the use of adjuncts in brewing. Prominent among these is the role it plays as a cheaper source of extract than malt (Grujic, 1999). The use of adjuncts in brewing has been determined by several factors among which availability, handling equipments, brewhouse operations are prominent (Coors, 1976). Adjuncts are sources of carbohydrates materials other than malted barley, which are employed in brewing to generally increase extracts and impart special characteristics to the final product. Coors, (1976) has pointed out that adjuncts can be considered an essential ingredient in American beers because of consumer preference for the lighter, more drinkable and more stable beer they make. A wide variety of cereal starches and sugars could be used as brewing adjuncts but due to economic factors, availability and utilization in the brewing process, their use is limited to a small number of types (Coors, 1976). At present the volume of beer produced with adjunct throughout the world has indeed increased much more than those produced with malt and this tendency is growing constantly, not only because of economic factors but also as a result of advantages related to the quality in the finished beer (Glienke, J. and Edwards, W. R., 1965).

The following advantages have been claimed for liquid adjuncts

- Shorter brewing cycle (higher turn around times)
- Increased production
- Reduced cost in the brewhouse

- Better sanitation control
- Cleaner fermentations and yeast
- Shortened fermentation cycles and more
- Tendency to give a smooth palatable high quality beer
- Production of stable product with good shelf life.

Among the plethora of reasons why brewers employ the use of adjuncts for brewing are:

- Adjust or mask flavour balance and head retention
- Diluting wort produced from malts that have high levels of nitrogenous materials to forestall the formation of haze in the finished product.
- Modify the body
- Modify the colour
- Save on mash tun capacity
- Save on cost per degree of starting gravity and alcohol.

The cost factor seems to have won much attention. For example it has been reported (Bamforth, 2003) that when the total cost of beer production is taken into consideration (from raw materials purchase and processing through to packaging, sales and taxation), then malt costs in general have been estimated to represent approximately 3.5% of the total cost. Therefore, it becomes apparent that grain costs represents only a relatively minor contribution to the total cost of beer production. The foregoing holistically suggests that breweries would not be adversely affected if they institute an all-malt brewing practice. However, cost only does present the fuller picture. Grujic, (1999) has stated that in less developed countries, malting facilities and malting conditions are quite often less than optimal. Therefore because of its lower price, locally produced

adjunct material can be used to supplement malted barley grain. Apart from the direct cost benefits of using cheaper raw materials, indirect cost can also influence raw material selection. In Kenya, for example, beer made from unmalted grain is taxed at 60% of the rate of beer made from malted grain (Cege *et al.,* 1999). A similar situation has been observed in Japan where much lower rate taxation is applied to products containing high adjunct levels (Happoshu, Brewers Association of Japan; Shimizu *et al*., 2002). Therefore Japan Brewers have a great incentive to brew products from grist containing adjuncts levels in excess of 50%. A 1988 government economic decision to ban the importation of malted barley in Nigeria forced local brewers to develop alternatives brewing procedures to utilize locally grown sorghum and maize crops (Hallgren, 1995; Little, 1194).

Adjuncts may be solids, which are usually added to the mash tun or liquids, which are added further downstream the brewing process in the wort boiler. Among the solid adjuncts are barley, maize, rice, wheat flour and sorghum. Corn syrups, caramels, sucrose and inverted sugars and malt syrups are some examples of liquid adjuncts. The point of introduction of solid adjuncts in the brew house greatly depends on their gelatinization temperatures. For those adjuncts whose gelatinization temperature is higher than that of malt, they are pre-gelatinized in special cookers known as adjunct cooking vessels before they are introduced into the mash (MacFadden and Clayton, 1989). Usually when solid adjuncts are used, the brewer employs decoction mashing. Decoction mashing is a kind of temperature programmed mashing where the adjunct or portions of the mash is withdrawn and boiled in a different vessel and later introduced into the mash to raise the temperature of the mash to the saccharifying temperature.

2.1.1 PROBLEMS ASSOCIATED WITH SOME ADJUNCTS

- Maize usually has some fats or undesirable lipids which may linger throughout the brewing process and finally into the packaged beer forming the basis of lipid auto oxidation leading to the formation of the undesirable trans-2-nonenal flavour.
- Barleys may provide solubilised ß-glucans in mashes which may give run-off and filtration difficulties.

The economies of using adjuncts are not simple, since many require special plants to be installed for cleaning, handling, storing and milling. Plants needed to cook grits, or handle flours, flakes, micronised, raw grains or syrups are specialized, and it is not feasible to change easily from using one type of adjunct to another. Consequently users must ensure that a continuing supply of good quality, competitively priced material is available before committing themselves to installing plant to allow it to be used. (Dennis, E. 1998)

2.2 GELATINIZATION TEMPERATURE OF SOME CEREAL STARCHES

Starch in the uncooked stage is insoluble in water. It forms a temporary suspension of large particles which are undissolved in the surrounding medium and will settle to the bottom of the container of liquid unless agitated (Vaclarik, and Christian, 2003). The uncooked starch molecule is a highly ordered crystalline structure that refracts light in two directions exhibiting a maltese cross formation or birefringence on the granule when it is viewed under polarized light with a microscope (Vaclarik, and Christians, 2003). When starch is heated in water, imbibition or intake of water into the granules occurs. This occurs first in less dense areas and subsequently in the more crystalline regions of the starch molecule. As heating continues, the starch granules take up more

water irreversibly and swell, losing their ordered crystalline structure and becoming opaque and more fragile. Some short chain amylose comes out of the granules. This process is called gelatinization and is responsible for the thickening of food system (Vaclarik, and Christian, 2003). Cereals such as maize and rice are commonly used as adjuncts in brewing (Bentley, 2006). The gelatinization temperature of the starch in these adjuncts are higher than that used for Saccharification in mashing. Therefore it is necessary to cook the adjunct prior to addition to the mash to ensure complete gelatinization and liquefaction (Bentley, 2006). Adjunct cooking is traditionally carried out using the addition of some malt into the cereal cooker along with the adjunct. The α-amylase of the malt has sufficient activity at the higher temperatures of the cooker to liquefy the starch. However a more efficient method is the use of a heat stable α-amylase (Bentley, 2006). Below are some cereal starches and their gelatinization temperatures.

STARCHES	**GELATINIZATION TEMP (ºC).**
Maize	70 – 75
Sorghum	70 – 75
Rice	68 – 75
Wheat	52 – 54
Barley	61 – 62
Potato	56 – 69

(http://brewery.org/brewery/library/GelTemps_RL0796.html)

2.3 BARLEY

The barley plant has been described in many publications (Cook, 1962; Brigss, 1978). It is a monocotyledenous cereal grain of the family Graminae. Sound ripe barleys are usually pale golden brown with darker or even reddish veins. There are however some known varieties in which grains are of various shades of black, blue, green or red.

2.3.1 CULTIVATION AND USE OF BARLEY

Barley is cultivated from the sub artic Scandinavian to near the equator; in the mountains of Ethiopia and in the South American; from below sea level near the Dead sea to great altitudes in the Andes and Himalayas; from normal temperature regions like western Europe to dry land areas in parts of Northern America to irrigated areas in deserts such as the Sahara (Hunter, 1962; Briggs, 1978; Rasmusson, 1985) Barleys will grow on a wide variety of soils. The English-type, low nitrogen malting barleys are best grown on light soils with pH values about 6.5. Chalky sub-soils are suitable.

Usually fertilizers are used to supply major quantities of Nitrogen, Potassium and Phosphorus. Usually the application of nitrogenous fertilizers has the most dramatic effects on grain yield and quality. Barley is a usual material for making beer. It lacks friability for easy milling; it provides a highly viscous extract deficient in amino acids and lacks the colour and flavour required for making beer (Lewis and Young, 1995). Barley for brewing is considered sound if it has well defined analytical, agronomic and physiological properties. It has to be dry, about 12% moisture content free of diseases, infestation and discoloration. It should be reasonably free of debris including dust, weeds and broken corns. The grain should preferably be plump since these types

contain relatively less husk and hence more starch to increase brewers' extract (Lewis and Young, 1995).

2.4 CASSAVA

Cassava, Manihot esculenta is second in importance only to sweet potato as a tropical root crop with world production estimated to be around 100metric tones in 1973. Like many other new world crops it was taken to West Africa by the Portuguese in the 16th century, but its spread throughout all the world's tropics has been a relatively recent event and it is only during this century that Cassava has achieved its present great importance as a food crop. Annual production in Africa is about 42metric tons, 37metrics tons in South America and 25metrics tons in Asia. Brazil, which may have been the centre of origin, remains the world's chief producer with more than 30metric tons each year. Under the best conditions cassava gives very large yields of tubers up to 50 tons per hectare. Ironically it is often grown with little attention on poor or exhausted soils and average yields are 8 tons per hectare.

It is easy to propagate from the stem cuttings and the tubers keep so well in the ground that cassava is an important famine relief crop Cassava is the most important tropical root crop. Its starchy roots are a major source of dietary energy for more than 500 million people. It is known to be the highest producer of carbohydrates among staple crops. According to United Nations Food and Agriculture Organization (FAO), cassava ranks fourth of the food crops in developing countries after rice, maize and wheat. The edible leaves are relatively rich in protein. Cassava can be stored in the ground for several seasons, and thereby serve as a reserve food when other crops fail. Cassava is also increasingly used for animal feed and in different industrial processes and products.

All cassava plants and tubers are to a certain degree poisonous because they contain various amounts of cyanogenic glycosides linamarin, which breaks down to yield prussic acid. The cultivars of cassava are classified in two groups, the sweet types in which the prussic acid tends to be confined to the outer rind and the bitter types in which it is more generally distributed throughout the tissues of the tuber.

2.4.1 Application of Cassava in food industries

Cassava is also used to produce starch for industrial use and other products used in processed food. Starch is a multibillion-dollar business worldwide and it is finding application in several industries. Cassava starch can perform most of the functions where maize, rice and wheat starch are currently used. Starch is utilized in sizing and dyeing in the textiles industries to increase brightness and weight of the cloth. In the pharmaceutical industries, starch serves as a filler material and bonding agent for making tablets.

Cassava starch also have several other numerous uses such as an additive in cement to improve the setting time, and it is used to improve the viscosity of drilling muds in oil wells. It is also used to seal the walls of boreholes and prevent fluid loss. Starch is also the main raw material in glue and adhesive industries. In paper production, cassava starch is currently used as glue to achieve brightness and strength. Starch is also an important raw material for powder in the cosmetics industries. In detergent soap manufacture, starch is used to get better recovery and to improve the shelf life of detergents. While in the rubber and foam industries, starch is employed for getting better foaming and colour. Stabilize Cassava starch can be converted to maltotriose,

maltose, and glucose as well as to other modified sugars and organic acids (Tan *et al*. 1984). Starch from cassava can be used to make fructose syrups (Vuilleumier, 1993) and formulate gelatin capsules (Nduele *et al*. 1993). The use of cassava as a source of ethanol for fuel is already being exploited and very promising. Recently, Roble *et al*. 2003 demonstrated the production L-Lactic acid from raw cassava starch in a bioreactor using *Aspergillus awamori* (fungus) and *Lactococcus lactis* spp. *lactis* (bacteria). Furthermore, cassava dregs could be employed for phytase production after the addition of a nitrogen source and mineral salts (Hong *et al*. 2001), while activated carbons prepared from waste cassava peel (Rajeshwarisivaraj *et al*. 2001) are efficient as adsorbents for dyes and metal ions.

2.4.2 Application of cassava in fermented products

2.4.2.1 *Cassava Alcohol*

Cassava is one of the richest fermentable substances for the production of alcohol. The fresh roots contain about 30 percent starch and 5 percent sugars, and the dried roots contain about 80 percent fermentable substances, which are equivalent to rice as a source of alcohol. Ethyl alcohol is produced from many carbohydrate materials. In Malaysia and some other countries, many factories are equipped to use cassava roots, starch or molasses (by-product of the sugar industry), the type of product depending on the costs of the raw materials. When cassava is used, the roots are washed, crushed into a thin pulp and then screened. Saccharification is carried out by adding sulfuric acid to the pulp in pressure cookers until total sugars reach 15-17 % of the contents.

The pH value is adjusted by using sodium carbonate, and then yeast fermentation is allowed for three to four days at a suitable temperature for the production of alcohol, carbon dioxide and small amounts of other substances from sugar. Alcohol is then separated by heat distillation. The yield of conversion is about 70-110 liters of absolute alcohol per ton of cassava roots depending on the variety and method of manufacture. The crude alcohol of cassava is described as average in quality. It has a disagreeable odor, but can be improved if the first and last fractions in the distillation process are discarded. It is usually utilized for industrial purposes, as in cosmetics, solvents and pharmaceutical products. If the production is required for human consumption, special care should be taken in handling the roots to rid them of hydro cyanic acid.

2.4.2.2 Dried Yeast Production

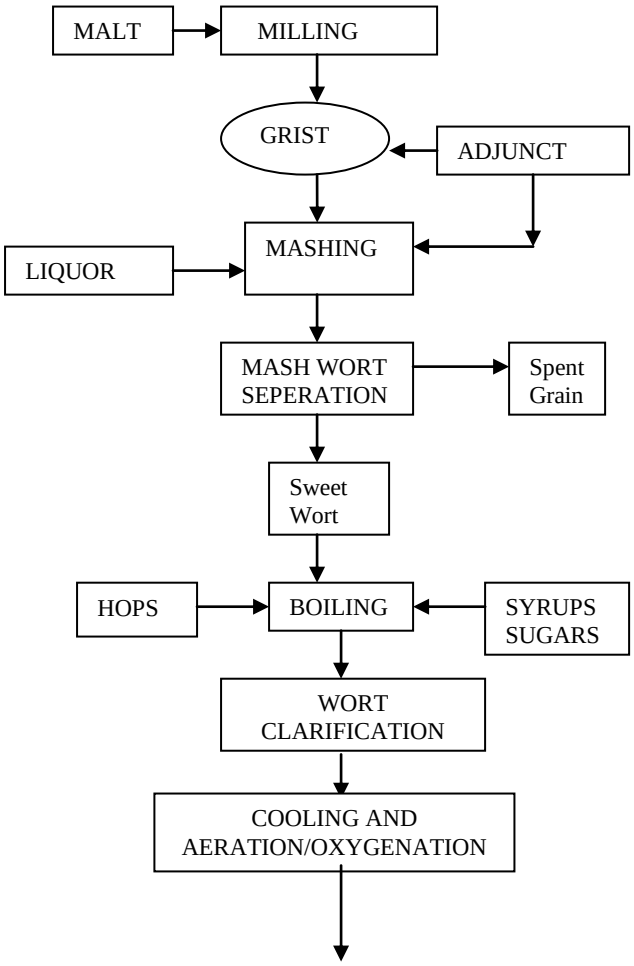
Microbial protein is attracting growing interest owing to the enormous protein requirements of the world. Among the microorganisms, which are considered possible food sources, yeast has perhaps stirred the greatest interest. Candida and saccharomyces yeasts have had a well-established place for many years as feed, and the technology of production, the composition and the nutritive value of yeast are well known (www.foodmarketexchange.com/datacenter/product/feedstuff/tapioca/detail/dc_pi_ft).

Most of the production of yeast is based on such low-cost raw materials as waste liquids, wood hydrolyzates and molasses. Starch-rich plant materials from wastes or surplus production are also utilized as substrata for yeast production. Cassava starch and cassava roots are being used in Malaysia and some other countries for the production of yeasts for animal feed, human diet and for bakery yeast The starch is hydrolyzed into simple sugars (predominantly glucose) by means of mineral acid or by enzymes. Certain yeasts are then propagated which assimilate the simple sugars and produce microbial cellular substances. The dry, inactive yeast contains about 7% moisture and

the raw protein content can vary between 40 and 50% depending on the raw material. The yield of yeast production also depends on the raw material. In some applications of cassava starch conversion into substances obtained from yeasts, a 38-42% yield of yeast product containing 50% raw protein has been obtained.

OVERVIEW OF THE BREWING PROCESS

FLOW DIAGRAM OF THE BREWING PROCESS



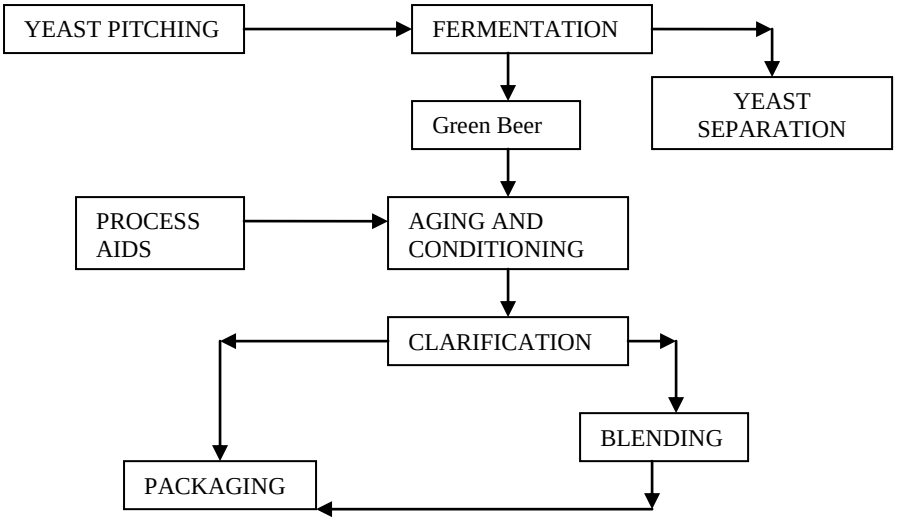


Fig. 1

2.5.1 MILLING

The brewing process starts with size reduction of the basic raw material (malt and any grainy adjunct) with the principal objective of enhancing optimum enzymatic action during mashing and stand-on or hold of the mashing process. Two kinds of milling exist, wet and dry milling. A choice of any of them dictates the kind of filtration equipment available to the brewer.

Milling is a size reduction unit operation in which the grain sizes are reduced by a mechanical action of some sort. Milling of any substrate must achieve two objectives; particle size reduction and particle size control, (Lewis and Young, 1995). Various types of laboratory mills have been described. Recently, Buckee *et al* (1976) reported a comparative study of several laboratory mills. Finding that a disc mill (DLFU, Büihler – Miag) had several advantages over the Miag and Casella cone mills for hot water extract. For the institute of Brewing analyses, Hudson, (1976) recommended that disc mill of Büihler – Miag should be used from the beginning of 1977. Milling must be

regulated to obtain the derived proportion of husk fragments, coarse grits, fine grits and flour (Briggs, 1998). To produce very fine grits, hammer mills may be employed. Sometimes the surface of the malt is dumped with steam or fine sprays of water to soften the husk and reduce its tendency to shatter before the malt reaches the rollers. In an extreme case, termed wet milling the malt is steeped before it is rolled.

2.5.2 MASHING

This operation involves the addition of water at a relatively high temperature to the grist amidst gentle stirring. Various factors dictate the choice of grist-liquor ratio. High or sales gravity brewing or beer type (stout or lager) is paramount among these factors. This stage however transcends the mere addition of water to the grist. It requires the skill of the brewer to manipulate the interplay of temperature and pH to enhance the optimal activity of the malt enzymes. Usually pH must be measured with only pH meter with a pH electrode. The often-used pH indicator paper is not precise enough and the resulting colour change depends not only on the pH value, but also on the colour of the mashes (Pieper and Senni, 2001). If well executed, the mash does not only yield high extracts but also its filterability is improved. It should be noteworthy that the filtration process in brewing is a time consuming one and poor mashes may further extend this time and therefore the total cycle time for a batch. Many interrelated chemical and physical changes begin and proceed simultaneously (Hough, 1971).

Brewers' mash by one of three classical methods with minor variations as prescribed by individual breweries. They are namely; infusion; decoction and double mashing (Lewis and Young 1995). The principal objective of mashing therefore is to produce extract by dissolving the starch and other substrates and converting them by the action of enzymes

present to a desirable spectrum of wort components. A choice of a mashing programme may largely be dependent on the extent of modification of the malts, a particular desirable flavour and brew house equipment.

2.5.2.1 INFUSION MASHING

This is mashing at a single temperature usually called the conversion temperature (Lewis and Young, 1995). The temperature of the mash in infusion is critical since changing it may involve serious dilution with water (Hough, 1985). It has been established that the best mash temperature for this system of mashing is in the range of 62 - 67^{0}C and usually is 65^{0}C (Hough, 1985). To achieve this, the water used should be some 4 – 5^{0}C higher. Since infusion mashing employs a single temperature stand particularly for starch hydrolysis, protein and glucans may not be hydrolysed. Lewis and Young (1995) stated that this method requires well-modified malt and unsuitable for conversion of adjuncts that require gelatinization. This method is attractive in small breweries because the vessels are simple and cheap, and easily scaled to their low output. A study of infusion mashing is instructive, because the factors in mashing that influence wort properties are most easily researched, explained and understood in infusion mashing models; much of the information about mashing was derived from such systems (Lewis and Young, 1995).

2.5.2.2 DECOCTION MASHING

This method was introduced in traditional German brewing to cope with poorly modified malts (Hough, 1985). The principle of decoction mashing commonly used in Germany is to mash in the grist at a rather low temperature, say 40^{0}C and then withdraw a quarter of the mash to a Kettle and boil it. The boiling mash is later mixed with the

remainder to say 54^0C. This process can be repeated until a final temperature stand of 65^0C. It is clear that decoction mashing is a type of temperature programmed mashing and therefore particularly suited to dealing with under-modified malts (Lewis and Young, 1995)

2.5.3 FILTRATION

This is the recovery stage in brewing. The filtration of the mash at 75^0C is slow both for fine and coarse grist though quicker for the fine grist. The rate of filtration is related to the degree of modification of the malt; a well modified finely ground malt giving a mash filtering in one hour, while a mash from poorly modified malt takes six hours or more (Pollock, 1979). A new method of filtration of mash under reduced pressure has been described by Moll *et al,* (1977). Wort separation must be achieved as promptly as possible without leaving a large portion of the extract in the spent grains (Briggs, 1998). Usually sparging, the process of washing the last extractives from the mash is performed by spraying water that is hotter than the mash with the following results

1. Reduced wort viscosity and hence a faster run-off
2. A more rapid dissolution of insoluble materials
3. Almost complete inactivation of the enzymes (Hough, 1985)

Various equipments have been described for the purpose of mash filtering. They include the Isothermal Mash Tun, Lauter Tun and the Modern Mash Filter. (O'Rourke, 2003)

2.5.3.1 Isothermal Mash Tun.

This is a combined conversion and wort separation vessel. Since it has no agitation or heating, it operates at a single temperature in the range of **65°C**. Mash tuns have the smallest filter surface area with the deepest bed depth, which applying Darcy's equation

will explain why it has the slowest filtration and poorest extract recovery. The poorer run performance is partially compensated by using coarse grist but this could lead to poorer extract recovery. It does produce the brightest wort, (O'Rourke, 2003). Extract performance is a result of the combined effects of the malt grist and the bed depth. The poorer potential performance of the mash tun is partially offset by using a low volume of water in mashing. This allows for a larger volume of sparge water to optimize the leaching effects.

The flow rate of wort from a mash tun is usually controlled manually. The run-off taps are set and adjusted to prevent pulling the bed down on to the plates. Unlike the other wort separation systems the mash in a mash tun floats on the wort, at least during the strong wort recovery. During the initial run-off the flow rate is low to allow for the high viscosity of the wort and to prevent the floating bed of mash being drawn down on to the false bottom of the vessel. The flow rate can be increased during sparging as the wort viscosity falls. Excluding the time taken for the mash conversions the mash tun is the slowest wort separating system. Mash tuns are well suited to their traditional use in producing wort from well-modified malt. They are the cheapest system in terms of capital outlay and are the simplest to operate with little or no automation, (O'Rourke, 2003). Mash tuns can only use a single temperature for mash conversion and as a result poor quality malts or malts requiring a protein or glucanase stand cannot be handled. Mash tuns are also less well suited to modern large batch production where high brew house utilization and extract efficiency are expected.

2.5.3.2 The Lauter Tun

Before transferring mash from the mash conversion vessel a layer of brewing water or "underlet" is added to cover the plates in the lauter tun. The transferred mash is allowed

to settle on the lauter plates. The bed in the lauter tun is shallower and the vessel has a larger diameter than the mash tun. This gives it a better filter performance and allows the use of finer grist which helps extract performance. The lauter may be run in one way or the other according to the beer type, installation and tun design. Most lauter tuns are fully automated and as well as controlling the wort run-off rate, they also measure and control the differential pressure above and below the lauter plates.

It is essential to measure the following properties to control the lauter run off.

- Wort flow rate
- Flow rate and volume of underlet and sparge.
- Differential pressure, which is the difference in pressure above and below the lauter tun false bottom. This directly measures resistance to flow through the filter bed.
- Wort clarity
- Wort gravity
- Temperature of sparge
- Dissolved oxygen. (O'Rourke, 2003)

The above measurements may be used to control lauter tun run-off.

- Rate of run-off or flow rate
- Sparge rate
- Sparge temperature
- Re-circulation (O'Rourke, 2003)

2.5.3.3 Modern Mash Filter

The Meura 2001 typifies the modern generation of mash filter. This filter has a large surface area because of the number of filter plates; it uses a very thin filter bed a few millimeters thick, and operates at up to 1.5 bar pressure (O'Rourke, 2003). This provides a significant pressure to aid filtration. Through its design, the filter is able to optimize the filtration conditions defined in the Darcy's equation. It is therefore able to handle very fine grist. Mash filter grist is produced using a hammer mill; the very fine grist ensures an excellent extract recovery. The mash filter is charged with converted mash from the mash mixer, the mash filter is fitted with fine polypropylene filter sheets suitable for fine grist, without particles bleeding in a tight filter bed which means that no recirculation is required before first wort are drawn off which can run straight to the kettle. The large number of plates and shallow bed depth gives a high filter flow rate and the fine grist coupled with a thin filter bed results in high extract efficiency without the reduction in wort quality.

The sequence below shows the series of events during a mash filter run.

Filling: - Mash is pumped at low pressure from the mash conversion vessels. **Filtration: -** The solids in the mash form a cake on the surface of the filter clothe. Clear wort is run off to the kettle.

Pre-Compression: - After all the mash has been transferred from the mash mixing Vessel, gentle air compression is applied to the membrane which forces the strong wort through the bed.

Sparging: - When most of the strong worts has been squeezed from the grain, the membrane pressure is slowly released and sparge water is pumped through the mash inlet.

Final Compression: When all the sparge has been supplied the membrane is compressed at high pressure and the grain bed squeezed.

Cake Discharge: Once all the extract has been squeezed from the grain, the pressure is released and the filter is opened up. The spent grains fall into a grain hopper for removal.

The new mash filter use very fine grist, which allows a high extract recovery usually in excess of 100% laboratory extract. In addition because it requires a lower sparge volume than the other systems it can readily handle high gravity worts from an all malt brew (O'Rourke, 1999)

2.5.4 WORT BOILING

Lewis and Young (1995) have described this stage as the wort stabilization stage since the wort is subjected to high temperature, which consequently brings it to the boil. This largely reduces the microbial load to a considerable minimum. Hough, (1985) has outlined the following as the principal effects of work boiling.

- Sterilization of wort
- Halting Enzyme Action
- Concentration of wort
- Isomerisation of bitter substances
- Removal of volatiles
- Increase in colour
- Reducing wort pH
- Reducing wort Nitrogen levels
- Extraction and precipitation of tannins / polyphenols
- Producing Reducing compounds.

2.5.4.1 Sterilization Of Wort

Brewing raw materials such as malt, hops and occasionally brewing water itself are infected by microorganisms, which have to be killed during the brewing process to prevent wort and beer spoilage. After boiling the wort is largely free from microbial contamination. Some microorganisms, primarily Bacillus sp. and other thermophilic bacteria are able to form spores which can withstand and survive the heat treatment, including boiling. If present in the raw materials or the brewing water may persist into the finished beer (O'Rourke, 2002).

2.5.4.2 HALTING ENZYME ACTION

Enzymes rely on their three dimensional structure for their activity. Above certain temperatures (usually the range of 50 – 70°C) the tertiary structure of the enzyme becomes denatured, and they lose their activity. By the time the wort has reached boiling point there is usually no residual enzymes activity. The continued action of enzymes after the normal mashing program will affect the fermentability of the wort and hence in a programmed mash there is a final mash temperature rise to between 76°C and 79°C, which is sufficient to halt the malt enzyme activity (O'Rourke, 2002).

2.5.4.3 CONCENTRATION OF WORT

During wort boiling water is driven off as steam, thus concentrating the wort. The amount of water removed during the boil is directly proportional to the rate of evaporation (and hence the amount of energy supplied) once boiling has been achieved (O'Rourke, 2002).

2.5.4.4 ISOMERISATION OF BITTER SUBSTANCE

During boiling the insoluble acids extracted from hops are converted to a more soluble iso-alpha acids and this reaction is accelerated by temperature rise. Isomerization is a relatively rapid reaction with the production of over 90% of wort bitterness occurring within the first 30 minutes of boil. Maximum isomerisation usually occurs within 60 to 70 minutes of boiling and accounts for around 60% of the total alpha acids present. Iso-alpha acids continues to be lost during the fermentation and maturation processes and is lost in any foam produced so that the final conversion value of alpha acids into iso-alpha acids in the beer is around 40%(O'Rourke, 2002).

2.5.4.5 REMOVAL OF VOLATILES

During the evaporation stage of wort boiling unsuitable volatile compounds are driven off with the steam. The principal malt derived volatile lost during wort boiling is dimethylsulphide (DMS), which comes from lager malts and gives lagers a taste described as sweet corn. It is produced by thermal decomposition of s-methyl-methionine in a first order reaction, with a half-life of around 35minutes (O'Rourke, 2002).

2.5.4.6 WORT COLOUR

The colour of wort increases during the boil. The reactions responsible for colour development fall into three broad categories.

- Maillard's reaction between carbonyls and amino compounds.
- Caramelisation of sugars, which is limited in steam heated coppers.
- Oxidation of polyphenols(O'Rourke, 2002).

2.5.4.7 REDUCING WORT pH

Control of pH throughout the brewing process from brewing water to final package, is fundamental for product consistency. Wort pH starts to decrease during mashing and continues to fall during wort boiling. The principal fall in pH is due to the reaction of $Ca2^{+}$ compounds with phosphates and polypeptides to form an insoluble compounds releasing H^{+} (hydrogen) ions (O'Rourke, 2002).

2.5.4.8 REDUCING WORT NITROGEN LEVELS

During the brewing process it is necessary to decrease the level of high molecular weight nitrogen which comes from the malt and if allowed to persist can affect the pH, colloidal stability (chill haze and permanent haze), fining and clarifying properties, fermentation and taste of the beer. Wort boiling is an important stage in the reduction of nitrogen. The effect in reducing the amount of wort nitrogen (measured by the Kjeldahl method) for a standard boil at 100^{0}C is shown below (O'Rourke T. 2002).

Table. 1

Percentage Nitrogen Removal after different Boiling times for a Standard boil

Time of boil (hrs)	Percentage Nitrogen Removal
0	**0**
0.5	**5.4**
1	**6.2**
1.5	**7.7**
2	**9.9**
3	**10.4**

Ref: Hough, Briggs and Stephen " Malting and Brewing Science"

2.6 WORT CLARIFICATION

Prior to wort cooling after boil, the wort should be clarified. Clarification ensures the separation of hop debris and the coagulant protein (trub) from the wort. The constituents of trub, explains why it should be removed from the wort. Insufficient removal may cause poor yeast performance and unsatisfactory attenuation (O'Rourke, 2002). Fermentation may be slow rendering the beer susceptible to infection. Filtration runs may be very slow and or poor.

Presently many breweries use a settling tank called the whirlpool to achieve fast settling. Wort from copper at the end of boil is cast a high velocities in the range of 3.5m/s through a tangential inlet of around 20^0. This creates a vortex, which accelerates the trub particles towards the centre of the vessel. They form large flocks, which then settle down the centre line of the vessel to form a trub cone.

The clarified wort is run off from above the trub cone through a series of run off points such that trub cone is not disturbed. The wort running out of the whirlpool into a target fermentor does that via wort cooler (O'Rourke, 2002).

2.6.1 COOLING AND AERATION

The objective of this unit operation is to bring the temperature of wort at 79°C to a fermentation temperature of 22ºC depending on the type of yeast and beer type. However other critical operations are carried concurrently during wort cooling. These are aeration or oxygenation of wort to specification and yeast pitching. Cooling ensures that the high temperature of wort from the whirlpool does not kill the yeast. Its also ensures that the lag phase of fermentation is achieved so as to control beer flavours. Too

high fermentation temperature will end up with beers with undesirable esters in tailing fermentations (O'Rourke, 2002).

It is important to control the following during this unit operation

a) Microbial contamination
b) Cooling
c) Aeration/oxygenation
d) Pitching rates (O'Rourke, 2002).

2.6.2 MICROBIAL CONTAMINATION

Brewhouse operations usually ensure the sterility of wort during boiling. However wort is an ideal growth media for yeast and a large range of bacteria. Any microbial contamination during wort cooling may not only affect beer flavour but also yeast performance and hence tailing fermentation or premature attenuation.

2.6.3 COOLING WORT TO FERMENTATION TEMPERATURE

Brewer's wort usually contains nutrients and other growth factors such as amino acids, vitamins and nutrients to support healthy yeast growth. However wort is deficient in certain phospholipids necessary to form the yeast's cell wall membrane. Yeast requires oxygen in order to synthesize the cell wall material.

Under aerated or oxygenated wort will usually lead to poor yeast growth and hence sluggish fermentations. Fermentations that are sluggish usually result in beer with poor quality in terms of flavours. The degree of aeration /oxygenation varies with yeast strain and the original gravity of the wort. The required amount of dissolved oxygen is usually in the range 7 to 18ppm. Wort is usually aerated in line on transfer to the wort

clarification vessel (whirlpool tank) through the wort cooler to the fermenting vessel prior to yeast addition. Most breweries oxygenate the wort on the cold side after the wort cooler. It is surprisingly difficult to get oxygen to dissolve into wort (O'Rourke, 2002).

2.5 FERMENTATION (WORT METABOLISM BY YEAST)

The cooled aerated wort made in the brew house is fermented by yeast to make an immature beer called green beer. Fermentation is an exercise in nurturing and cajoling yeast to do the work of a living cell in an environment specified by the brewer. This work from the standpoint of the yeast is to grow and multiply (Lewis and Young, 1995). It is the by-product of this labor, which both makes a major contribution to the chemical composition of beer (alcohol, carbon dioxide and flavour compounds) and generates new yeast for subsequent fermentation. For yeast to live and grow, wort must contain a sufficient supply of nutrients. Yeast needs fermentable carbohydrate, assimilabe nitrogen, molecular oxygen, vitamins, sources of phosphorous, sulphur, calcium, magnesium ions and trace elements such as copper and zinc ions, (Lewis and Young, 1995).

The dissolved oxygen content of wort is an important parameter. Research has shown that molecular oxygen must be supplied at the beginning of the fermentation to satisfy the yeasts' requirements. Once this is done no more is needed and indeed anaerobic conditions are established and necessary to prevent undesirable oxidation reactions which spoils finished beer (Lewis and Young, 1995). In modern brewing practices 8ppm dissolved oxygen is often used and in some cases more. The quantity of oxygen

in solution is inversely proportional to both specific gravity and temperature. Therefore for wort of different gravities at the same temperature, air saturation gives different dissolved oxygen concentrations and vice versa. Brewers therefore resort to the use of molecular oxygen as source and carefully monitor the level in wort using in-line oxygen electrodes. Too much results in too vigorous fermentation on (with consequent changes in beer flavour) and excessive yeast growth at the expense of alcohol production. Too little causes accelerated loss in yeast vitality and viability (Lewis and Young, 1995). Since fermentation is an exothermic reaction, it is vital to control temperatures to enhance quality product. However this effect on the rate of yeast respiration has other effects, such as high ester production and the pattern of monitor fermentation performance by measuring the following.

(a) Rate of sugar depletion.

(b) Rate of alcohol formation and CO_2

and several other indices to enable the process to be controlled. At the desired attenuation, cooling or chilling the wort to very low temperatures in the range of –2 – 2°C may slow the fermentation process down.

The principle that the rate of fermentation is a function of rate and extent of cellular growth is well established, (Masschelein, 1989). It has indeed been shown that each growth period is characterized by a maximal fermentation capacity for glucose, maltose and maltotriose per unit yeast, the extent of which is specific for each yeast strain and that this diminishes rapidly during the stationary phase to a maintenance level.

Inadequate growth on only reflects on the economies of inconsistent fermentation times but the limit also results in poor attenuations and altered beer flavour notes (Masschelein, 1989).

Cellular growth per se is of little interest to the brewer whose primary interest is in production of ethanol and quality beer with the desired sensory and analytical profile. Sufficient yeast growth is required for repitching purposes and to ensure that the yeast is in a reasonable condition for the next fermentation (Masschelein, 1989). The brewer is therefore faced with the challenge to achieve sufficient yeast growth, to gain an optimal rate and extent of attenuation and desired flavour development whilst balancing but not over-expending nutrients and energy for accumulation of reserved material (Masschelein, 1989).

The next stage in the processing of fermented wort is to remove the yeast cells. Yeast may naturally aggregate to form a mass on top of decanted or at the bottom of the fermentation vessel, which may be purged till clear green beer, is seen. Modern trends in brewing has lead to the formation of genetically engineered yeast which may be non flocculent and remain in the beer at attenuation. Since natural settling may take longer periods to achieve clear green beer, the settling is accelerated with the use of large centrifuge plants to remove yeast cells from the beer.

The clear green beer may be processed further before packaging. Among such processes may include

- Blending down the high gravity to sales gravity.
- Incorporation of $C0_2$ to the specified levels.
- Correcting the bitterness by the addition of pre isomerised hops.
- Allowing the green beer to undergo maturation.

2.7.1 POST FERMENTATION TREATMENTS

Beer run from the fermentor is not ready for drinking because it contains suspended particles and is therefore hazy, lack sufficient carbonation, the flavour is not fully

matured, it is physically and microbiologically unstable and flavour and colour may need to be adjusted (Lewis, and Young, 1995). It requires a variety of treatments before it is dispatched from the brewery.

Six processes are involved

- Carbonation
- Flavour and aroma modification
- Colour standardizing
- Stabilization against non-biological haze and flavour changes
- Clarification
- Biological stabilization

2.7.2 PRODUCT QUALITY INDICES

2.7.2.1 EXTRACT

The quantitatively most important biochemical reaction taking place during mashing is the enzymic(amylolytic) conversion of starch to fermentable sugars and dextrins (Palmer, 1990)

Extract is a measure of the amount of sugar recovered from the malt after mashing. The extract value is based on laboratory mash. The only two basic laboratory procedures used for measuring extracts are [O'Rourke, 2002]

- The institute of brewing method, which involves mashing 10% malt, with, distilled water and letting the mash stand for 60 minutes at 65^0C. The extract is measured as the specific gravity of the filtered solution at 20^0C. The results are expressed as litre degree per kilogram.
- The European Brewery conversion method employs two mash stand temperatures of 45^0C and 70^0C. The extract is expressed as percentage sugar

(sucrose) over total weight of malt. Table. 2 shows the extract values of standard ale and lager malts as reported by the Institute of Brewing and the European Brewing Convention.

Table. 2

Extract value for typical malt made from standard 2-row barley

Malt extract "dry"	IOB 1^0/kg	EBC Plato
Standard ale malt	305 – 315	81 – 82
Standard lager malt	300 – 310	80 – 81

[Ref – The Brewer International, 2002]

2.7.2.2 COLOUR

The colour of wort originally emanates from the kilning stage of the malting process (O'Rourke, 2002). The kilning stage does not only generate colour compounds but also flavours. Chemical reactions take place between the malt components to produce colour compounds. The principal reaction is between amino acids and reducing sugars called the "Maillard reaction". The reaction produces both colour and flavour active compounds. The higher the kilning temperature, the greater the amount of colour compounds produced. Wort boiling also contributes to beer colour, the major contributing reactions are the Maillard, Caramelization of sugars and oxidation polyphenols(O'Rourke, 2002).

2.7.2.3 pH

pH is the measure of acidity which is the concentration of hydrogen ions H^+ in solution. The pH changes in the brewing process are principally governed by the mineral

composition of the brewing water and mineral treatment added to the brewing water. It is interesting to note that pH decrease as the brewing process progresses. A typical pH trend for the brewing operation for a standard lager is shown as follows (O'Rourke T. 2002). Table. 3 shows the pH trend for brewing operations for a standard lager brew. pH figures show a downward trend from mashing to fermentation.

Table. 3

pH Trend for Brewing Operation for a Standard Lager

Brewing water	pH	7.0 (Neutral)
Mash	pH	5.6 $\pm$ 0.2
Boiled wort	pH	5.4 $\pm$ 0.2
At end of fermentation	pH	4.0 $\pm$ 0.2

[Ref: The Brewer International, 2002]

The principal decrease in pH during mashing comes from the precipitation of phosphates and amino acids or polypeptides derived from the malts. The presence of calcium in brewing water is a major contributory factor to pH decrease and the higher the concentration of calcium, the lower the pH of the mash. This is as show in the table.4 below (O'Rourke, 2002).

Table. 4

The effect of mineral composition of mash water on wort pH

Water Composition	**Pre Boil**	**After Boil**
50ppm Ca^{2+}	5.5	5. 4
50ppm Ca^{2+} and 100 ppm $CO3^{2-}$	5.8	5.6
350ppm Ca^{2+}	5.1	5.0
350ppm Ca^{2+} and 100 ppm $CO3^{2-}$	5.4	5.3

(Ref: Taylor D MBAA T.Q $N6_4$ 1990)

The phosphates present in the malt are precipitated as calcium phosphates leaving $3H^+$ in solution thus decreasing the pH.

$3Ca^{2+} + 2H_3PO_4 \longrightarrow 6H^+ + Ca_3(PO_4)_2 \downarrow$

A similar reaction will occur with amino acid and polypeptides present in the wort.

Amino group – COOH $\longrightarrow 2H^+ + Ca(\text{Amino group} - COO)_2 \downarrow$

2.7.2.3.1 Effect of pH on the brewing process

pH has a major effect on the rate of reaction, solubility and electrostatic charge of many molecules. This will have an important influence on beer quality and production throughout the brewing process.

- Physical e.g. Colloidal stability of the beer
- Chemical e.g. Isomerisation of alpha acid
- Enzymatic e.g. Malt and yeast enzyme activity (O'Rourke, 2002).

2.7.2.3.2 PHYSICOCHEMICAL CHANGES

Most of the reactions in brewing are organic reactions in aqueous systems and these are affected by pH. Biological macromolecules act as acids and bases by donating and accepting protons. However due to the size of these molecules, they often contain several different groups that accept or donate protons, thus having both acidic and basic groups rather than behaving as purely acids or bases (O'Rourke, 2002).

These acidic and basic groups act as weak acids and bases. Changes in the pH around the macromolecule will determine which groups are protonated and which are not, which in turn determines properties of the molecule. A typical example is amino acids, which are small molecules containing both amino and carboxyl groups. Since each amino group can be protonated and each carboxyl group de-protonate, the structure of and amino acid depends on the pH of the solution it is in (O'Rourke, 2002).

It therefore follows that all the protein and polypeptides molecules in beer which are charged will affect their physical properties such as their ability to coagulate and settle out as hot and cold break. There is also the tendency to form hydrogen bonds leading to the formation of chill haze and foam (O'Rourke, 2002).

2.7.2.3.3 CHEMICAL CHANGES - Isomerization of alpha acid

When hops or hop products are boiled in wort, the single most important reaction of hop component is the isomerization of $\propto$ - acids to iso - $\propto$ - acids. Generally alpha acids are insoluble in wort at normal temperature and pH. Iso – alpha acids account for the desirable bitter quality of hops. Success in the enterprise of kettle boiling is therefore best measured by the amount of iso - $\propto$ - acids in beer (Lewis and Young, 1995). Alpha acids more or less isomerize and may participate in many side reactions at the pH and

temperature of boiling wort. High wort pH promotes α - acid dissolution and isomerization but as the pH of boiling wort falls, isomerization slows.

2.7.2.3.4 ENZYMATIC REACTIONS (e.g.) Malt and yeast enzyme activity

Among the critical factors, which requires keen monitoring, and control during mashing and fermentation are temperature and pH. The activities of the two enzyme of brewing during mashing are greatly facilitated by pH changes. A pH of 5.7 is conducive for the optimum activity of β – amylase and at a pH 5.3, more favorable to α - amylase. Since these pHs cannot be achieved at one particular time during mashing, a compromise is achieved in practice, for the optimal activity of both enzymes. pH can be adjusted by the elimination of carbonates and bicarbonates ions in the brewing water and ensuring that there are adequate calcium ions present.

Table. 5

Optimum temperatures and pH values for infusion mashing

	Temperature (^{0}C)	pH
Greatest extract	65 – 68	5.2 – 5.4
Most fermentable wort	65	5.3 – 5.4
α - amylase activity	70	5.3 – 5.7
β - amylase activity	60 – 65	4.6 (mash)
Greatest yield of soluble nitrogenous materials	50 - 55	5.0 (wort)

2.8 SENSORY EVALUATION

Systematic sensory analysis began with wartime efforts to provide acceptable food to American Forces (Dove, 1946,1947) and with development of triangular test for beer in Sweden and Denmark (Bengtsson, 1943; Bengtsson and Helm, 1946; Helm and Trolle, 1946). Sensory Analysis treats the tasters as measuring instruments. As such they are high variable and very prone to bias, but they are the only instruments that measure what we need to measure so we must minimize the variability and control the bias by making full use of the best existing techniques in psychology and psychophysics (Peppard, and Meilgaard, 1986). Sensory evaluation has been defined by the Institute of Food Technologists (1975) as "the scientific discipline used to evoke, measure, analyze and interpret those reactions to characteristics of foods and materials as perceived through the senses of sight, smell, taste, touch and hearing. The complex sensation that results from the interaction of our senses is used to measure food quality in programs such as quality control and new product development (Poste *et al*. 1991). This evaluation may be carried out by panels of a small number of people or by several hundred depending on the type of information required.

In beers, flavours are derived from hop bitterness and aroma, malt component (before and after metabolism by yeast) the use of specified malts and adjuncts (Lewis and Young, 1995). Thus hopping rates, the use of dry hopping and or addition of specialized malts for colour and flavour generate specialized types of beer. Two extremes would be using starch in mashing to dilute overall flavour and mixing fruit pulp in the kettle boil to extract a specific flavour (Lewis and Young, 1995). There are certainly in excess 700 flavour components in beer (Lewis and Young, 1995). Most of these are present in levels just below those at which they are perceived. However acting both synergistically

and antagonistically and together with hops and malt constituents, they give the overall beery character and the specific flavour associated with the beverage (Lewis and Young, 1995).

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Sources of Raw Materials

Cassava starch obtained from Ayensu starch and Barley from Guinness Ghana Breweries Group – Kaase site, Kumasi

3.2 Proximate Analysis

3.2.1 Moisture

Five grams (5g) of each sample was transferred to previously dried and weighed dish. The dish was placed in an oven thermostatically controlled at 105°C for 5hrs.The dish was removed and placed in a desiccator to cool and weighed. The dished was re-placed in the oven and heated, cooled and weighed. This stage was repeated until constant weight was obtained. The loss in weight was reported as moisture. This was then expressed as the percentage

3.2.2 Crude Fat

The dried sample obtained from the moisture determination is transferred to 22 x 80mm thimble paper. A small ball of cotton wool or glass wool was placed into the thimble to prevent loss of the sample. Anti bumping granules was added to the previously dried (air oven at 100°C) 250mm round bottom flask and weighed accurately. 150ml of petroleum spirit B.P. 60 - 80°C was added to flask and assembled apparatus. A quick fit condenser was connected to the soxhlet extractor and refluxed for 4hrs on high heat on the heating mantle. The flask was removed and evaporated on a steam bath. The flask

and fat/ oil was heated for 30min in an oven (Memmert oven, Type:ULE 60, Germany) at 103°C. The flask and contents were cooled to room temperature in a desiccator. The flask was weighed accurately and the weight of oil/fat collected was determined. This was then expressed as the percentage of the original weight of sample.

3.2.3 Crude Fibre

Sample from the crude fat determination was transferred to a 750ml Erlenmeyer flask and approximately 0.5g of asbestos was added. 200ml of boiling 1.25% H_2SO_4 was added immediately and the flask was set on hot plate and a condenser was connected (cold finger type). The content came to a boil within 1min and frequently until sample was thoroughly wetted. Care was taken to keep material from remaining at sides of flask out of contact with the solution. At the end of 30mins, the flask was removed and immediately filtered through wetted linen cloth in a funnel and washed with boiling water until washings were no longer acid.

Sample and asbestos were washed back into flask with 200ml boiling 1.25% NaOH solutions using wash bottle calibrated to deliver 200ml. The flask was connected to condenser and boiled for exactly 30mins. It was filtered through line cloth and washed thoroughly with boiling water. The residue was transferred to a gooch crucible with water from wash bottle. It was then washed with approximately 15ml of alcohol. Crucible and contents were dried for 1hour at 100°C. It was then cooled in a desiccator and reweighed. The crucible was ignited in electric furnace for 30mins cooled and reweighed. Crude fibre was reported as the loss in weight and expressed as percentage.

3.2.4 Ash

2.00g of dried sample was transferred to a previously ignited and weighed crucible and placed in muffle furnace that is preheated to 600°C for 2hours. The crucible was removed and permitted to cool in a desiccator while still hot. Crucible was allowed to cool and then weighed. Weight was expressed as a percentage

3.2.1.5 Crude Protein

3.2.1.5.1 Digestion

2.00g of the dried sample and a half of selenium based catalyst tablet and a few anti-bumping agents was placed in a digestion flask. 25ml of concentrated H_2SO_4 was added and the flask shaken thoroughly to ensure the entire sample was wet. The flask was placed on a digestion burner and heated slowly until bubbling ceased and the resulting solution was clear. The flask was then made to cool to room temperature. The digested sample solution was transferred into a 100ml volumetric flask and made up to the mark.

3.2.1.5.2 Distillation

The distillation set up was flushed for 10min. The condenser is treated so as to carry over all liquid in the condenser. 25ml of 2% boric acid and 2drops of mixed indicator was put into a 250ml conical flask. Water was drain from the steam trap and the stopcock left open. The conical flask and its content were placed under the condenser in such a position that the tip of the condenser is completely immersed in solution. 10mls of the digested sample was pipetted into the steam jacket. 20ml of 40% NaOH was then added to the decomposition flask. The funnel stopcock was closed to allow the liberated ammonia into the collection flask. The stopcock on the steam trap was shut to force

steam through the decomposition chamber. There is a colour change of the boric acid to bluish green as soon as it comes into contact with ammonia.

Distillation was continued for 5min after which the receiving flask was lowered so that the condenser tip is just above the liquid. The end of the condenser was washed with a little distilled water and distillation was continue for 30sec and the process discontinued by removing the burner from the steam generator.

3.2.1.5.3 Titration

The distillate was then titrated with 0.1NHCl solution. The acid addition continued to run until the solution was colourless. A similar procedure was carried out on the blank. The % nitrogen (%N) was then calculated from the titre value obtained from the relation below:

%Nitrogen = [(VHCl * NHCl) – (VBK * NNaOH) – (VNaOH * NnaOH)]/1.4007 * W * Lab DM/100

The crude protein (CP) is then calculated from the relation below:

CP = %N * 6.25

Where:

VNaOH = Volume in milliliters of standard NaOH needed to titrate sample.

VHCl = Volume in milliliters of standard HCl pipetted into titrating flask for sample.

NNaOH = Normality of NaOH

NHCl = Normality of HCl

VBK = Volume in milliliters of standard NaOH needed to titrate 1ml standard HCl minus B.

B = Volume in milliliters of standard NaOH needed to titrate reagent blank carried through method and distilled into standard HCl

1.4007 = milliequivalent weight of nitrogen * 100

W = sample weight in grams.

3.3 Gelatinisation Temperature Determination

Gelatinization temperature of Cassava starch was determined with the Brabender Visco-amylograph (Brabender OHG Duisburg, Kulfurstrabe 51-55. D-4100 Duisburg 1). An aqueous solution was made by dissolving 30g of the cassava starch sample (moisture-free) in 500ml of distilled water. The suspension was heated at the rate of 1.5°C/min by means of a thermo regulator. Temperature was read and noted at the first point of inflexion and the peak of the curve just before a dip begun. Three determinations were obtained and the average calculated as the gelatinization temperature range.

3.4 Wort Generation

The malted Barley and Barley were milled separately using the Buhler – Miag Disc Attrition (Mill, Type DLFUW24050, Serial No.20352, Germany) to a near - floury consistency. The grist comprising of the adjunct, which is either barley or cassava used per brew, was 3000g. The various fractions of adjunct used ranged from 5%, 10%, 15%, 20%, and 25% to 30%. These fractions were weighed and made up with the corresponding grist of malted barley. For a whole malt brew, there is no adjunct added implying 0%.

3.4.1 Mashing

The 3000g-grist composite was placed in a previously cleaned aluminium-cooking utensil. Six litres of clean water at 68°C was added to the grist while stirring to prevent clotting or lump formation. The temperature of the mash was decreased to about 64°C

on mixing. This was then raised to between 66°C and 68°C and held for 1 hour while gently stirring, (Stand-on period). At the end of the stand-on period, a starch presence or absence test was done using iodine solution. This test involved taking a sample of the mash, cooling it to 20°C dropping it on a white tile and adding a few drops of iodine. A blue-black colouration indicates the presence of starch and the inverse the absence of starch.

3.4.2 Filtration

Generally all the starch is fully converted to fermentable sugars within the 1hour period. Filtration of the mash was done using sterilized cheese cloth.

After 5.5L of the strong filtrate (wort) has been collected, the residues (spent grains) are discarded.

3.4.3 Boiling

The filtrate was boiled over a gas burner for 60mins under atmospheric pressure.

The volume of wort after boiling decreased by 9%. The wort was then cooled by placing in a refrigerator to 20°C.

3.5 Wort Analyses

The following analysis were conducted on the wort

3.5.1 Specific and Present Gravities

The specific and present gravities were determined using the beer analyzer (**Anton Paar DSA 48 software version 411082, Austria)**. Samples are poured into vials that

have been pre-rinsed with the samples. The vials (three of them) are placed in specialized compartments which rotates and gets the samples in a position that allows the analyzer to suck the samples, analyse and provides results on a screen that can be printed.

3.5.2 pH

The pH was determined using the **INOLAB pH level 2 meter**.

3.5.3 Acidity

Thirty millilitres of the sample was pipetted into a conical flask and titrated against 0.1N NH_4OH solution. The end point is read at pH 7.1 and the acidity is calculated as follows: -

Acidity = Titre Volume X 0.2

3.5.4 Colour

Samples were filled into cuvettes and the absorbance of the samples read at 430nm wavelength (EBC, 1975) using the Unicam UV-Visible spectrometer (serial No. UV1 060521, England). The optical density (OD) was expressed in terms of European Brewing Convention colour units as follows,

Colour units = OD x 25(EBC, 1975)

3.5.5 Bitternesss

10ml of the samples were filled into test tubes, which had previously been filled with 20ml 2,2,4-trimethylpentane (iso-octane) and covered. The test tubes were placed in a water bath at 20°C for 30mins. At the end of the 30mins period the samples were

acidified with 1ml of 0.1N HCl and shaken vigorously for 60sec. The samples were then placed in the water bath at 20°C for another 30mins. The supernatant organic phase was carefully decanted into cuvette and the optical densities read at 275nm wavelength. The optical density was expressed in terms of European Brewing Convention bitterness units as follows,

Bitterness units = OD x 100. (EBC, 1975)

3.5.6. Perfect Primary

This is an assumption to determine extent of fermentation. Five milliliters of samples were filled into 20ml conical flask and each pitched with 0.5g of dry yeast. (Saccharomyces cerevisiae) which was harvested from actively fermenting yeast culture. The flask containing the samples were sealed using cotton wool to allow for some aeration and kept in an incubator at 20°C for 24hrs. A drop of the samples were gently placed on the surface of various sugar (sucrose) solutions of known specific gravities in the range of 7.0 - 17.0 degree saccharide(°S)

A sugar solution in which the drop hangs in the body of the solution gives the estimated specific gravity of the fermented wort at its attenuation.

3.6 Fermentation of Wort

3.6.1 Analysis of Fermentation Profile

Fermentation profile was studied by measuring the fall in wort sugar as fermentation progressed. This was done using the Beer Analyser. **(Anton Paar DSA 48 software)**

3.6.2 Green Beer Analysis

The Original gravity (OG), Present gravity (PG), and alcohol content (v/v) were measured using the Anton Paar Beer analyzer. The pH was measured with the **INOLAB pH level 2 meter** and acidity by titration as in wort analysis. The colour and bitterness were measured as directed in the European Brewing Convention method (EBC, 1975). All the analysis was carried on the filtered sample.

The Zahm – Nigel CO_2 Analyser

Fig. 2

3.6.3 Finished Product

After fermentation the sample was filtered and the finished beer blended with de-aerated water at 4°C to obtain an alcohol content of 6%. The resulting product was carbonated to CO2 content of about 3g/litre using the Zahm – Nigel apparatus. This method applies the principle of temperature and pressure. The piercer is lowered onto the crown and a force is exerted to pierce the crown as shown fig.2. The seal prevents

escape of any internal gas. The bottle under this locked condition is shaken vigorously and continuously until a constant pressure is read on the manometer. The sample is released and the temperature of the content of bottle is read with a thermometer. The pressure and temperature are read on a slide rule to give the carbondioxide reading of the content of bottle.

3.6.4 Analysis on Finished Product

The Original and Present gravities, Alcohol content, were determined with the beer analyzer. Acidity and pH using the INOLAB pH level 2 meter and then colour and bitterness were also determined based on the EBC method. The levels of CO2 were also determined using the Zahm – Nigel method and apparatus.

3.6.5 Head Retention by the Nibem Method.

Principle

It measures the time taken for the surface of foam to collapse by 10mm, 20mm and 30mm using conductivity.

Method

Label

a - Stem
c and b - Electrode system needles

A - Foam

B - Beer

Nibem Appartus

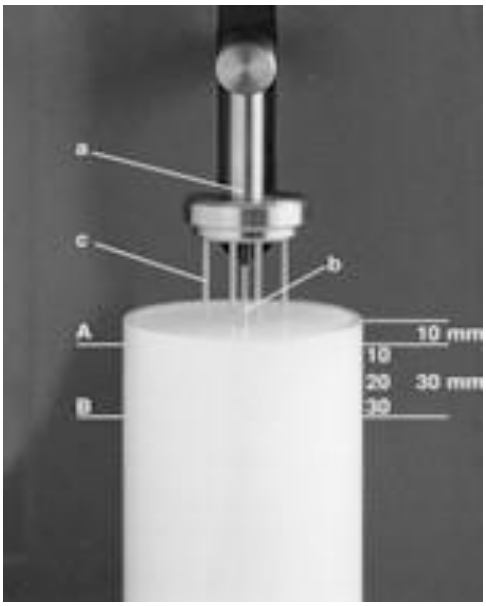


Fig. 3

A standard pour is used to pour the beer into a glass. A movable plate containing three electrodes (shown in fig.3) is lowered so that it just rests on the surface of the beer foam. As the foam collapses the electrodes reduce. The plate moves to maintain contact with the foam. The more rapidly the needle moves down to maintain contact, the less stable the foam.

Standard

A satisfactory head is one that lasts more than 260 – 280seconds.

3.6.6 Pasteurisation

The finished product were packaged in bottles to volumes of 330ml and pasteurized at 60°C for 60mins using the Sander Hansen bottles pasteurizer at a pasteurization unit of 18.

3.6.7 Sensory Evaluation

An acceptability and flavour profile (colour, tingly, estery and bitterness) tasting was conducted on four beer samples, two each from barley and cassava starch-substituted samples. The sensory analysis was conducted using 25 member-trained panelists using the sensory evaluation form in appendix 3. The panelists constituted 22 males who are staff and regular tasters in Guinness Ghana Breweries Group – Kaase site and 3 females also staff. The samples were served at 15°C so that panel members could easily pick flavour notes. Serving order was changed for every group of five (5) and samples were served in clean and odourless drinking glasses. Parameters for evaluation are shown in the questionnaire in appendix 3.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Gelatinisation Temperature.

The gelatinization temperature of the barley starch was determined from literature to lie between 59 and 70°C(O'Rourke, 2001), however that for Cassava Starch was between 62 and 71°C(appendix 4). This suggests that the milled malt and the cassava starch powder could be mixed into grist prior to mashing, since their gelatinization temperatures are close to similar.

4.2 WORT ANALYSIS

4.2.1. Specific Gravity (SG) and Present Gravity (PG)

Specific gravities obtained for wort from cassava starch substituted grist were generally higher than that with barley (Table.6). This suggests that a higher extract yield was obtained for cassava–starch substituted wort during mashing than for barley. This may be due to the fact that cassava gave a higher carbohydrate (83.00%) level than that of barley (77.93%) as shown in the results of the proximate analysis (appendix 2). The ANOVA results for samples and substitution levels confirmed that there was a significant difference in Specific and Present gravities between samples and no significant difference existed between substitution levels of the various samples at 5% significant level for the parameters under consideration. It should be noted that present gravities for various samples like that for specific gravities as a result of the above relation and hence ANOVA results were similar to that for specific gravity. Table.9 showed that present gravities obtained for cassava at various substitution levels were

higher than that for barley. This confirms the proximate analysis results (appendix 2), which showed that the starch content in cassava was about 5% higher than that in equal weight of the barley sample. Additionally the starches in cassava were in a state, which rendered them highly susceptible to enzyme attack during saccharification

Table. 6

Specific gravities of Cassava Starch and Barley at various substitution levels

*	5%	10%	15%	20%	25%	30%
CASSAVA (SG)	$1.09064_{(0.11)}$	$1.09228_{(0.05)}$	$1.09470_{(0.03)}$	$1.09161_{(0.04)}$	$1.09345_{(0.05)}$	$1.09436_{(0.12)}$
BARLEY (SG)	$1.08565_{(0.02)}$	$1.08593_{(0.01)}$	$1.08622_{(0.10)}$	$1.08504_{(0.04)}$	$1.08623_{(0.16)}$	$1.08602_{(0.09)}$

than that for barley. Kunze, (1996) has stated that the starch granules in barley are generally locked up in the endosperm cells and this makes normal attack of saccharification enzymes rather difficult. Schwarz and Hans, (1995) and Vietor *et al.,* (1991) have stated that due to its relatively inaccessible starchy endosperm, high inclusions of unmalted barley (>20%) without the aid of commercial enzymes can lead to problems such as low extract yields, high wort viscosities, decreased rates of lautering, fermentation problems and beer haze problems.

Kunze, (1996) had stated that up to 20% barley could be used as an adjunct with malt without addition of exogenous enzymes. It was therefore expected that there would be increase in extract yield and specific gravities for corresponding increase in substitution levels of adjuncts at least up to 20%. However this was not the case in both samples. For cassava starch, there was an increase in specific gravities until 15% substitution levels. At 20%, specific gravity was relatively low. This phenomenon may be due to the non-standard mashing temperatures associated with the equipments used. This is true as

there was a relatively higher extract yield and hence high specific gravity achieved for the 25% substitution level. Generally barley substitution was not very impactful as there were no marked differences in extract at the different levels of substitution.

Kunze, (1995) has blamed this phenomenon on factors, which cause the stretching out of the ß-glucan, which in turn results in the formation of gels, which adversely affect filterability. Goode and Arendt, (2003) have underpinned the above by stating that increases in barley adjunct can result in decreases in extract recovery as a result of increase in ß-glucans, which offer a lot of lautering problems and that the use of commercial (exogenous) enzymes can significantly improve this phenomenon.

4.2.2 pH

pH values obtained for cassava starch substituted samples were generally higher than that for barley (Table.7). The decrease in wort pH during mashing comes from the precipitation of phosphates and amino acids or polypeptides derived from the malts or barley (O'Rourke, 2002). The barleycorn comprises phosphate-containing compounds that constitute about 1% dry weight of the corn (O'Rourke, 2002).

The mean ash content (proximate analysis, appendix 2) for barley (2.09%) is 56% greater than that for cassava. This may suggest higher phosphate content in barley than in cassava. Hence the difference in measured wort pH for both samples. ANOVA results confirm a significant difference in pH between barley and cassava samples. However, no significant difference was detected for levels of substitution for the samples ($p > 0.05$.)

Table. 7

pH OF CASSAVA STARCH AND BARLEY AT VARIOUS SUBSTITUTION LEVELS

*	5%	10%	15%	20%	25%	30%
CASSAVA (pH)	$5.42_{(0.01)}$	$5.53_{(0.04)}$	$5.31_{(0.04)}$	$5.32_{(0.04)}$	$5.40_{(0.04)}$	$5.51_{(0.04)}$
BARLEY (pH)	$5.09_{(0.02)}$	$5.11_{(0.04)}$	$5.04_{(0.03)}$	$5.10_{(0.00)}$	$5.08_{(0.03)}$	$5.11_{(0.53)}$

4.2.3. Colour

Generally, higher colour values were obtained for barley-substituted samples in comparison to that for cassava (Fig.4). Light coloured adjuncts will dilute malt colours to produce lighter coloured beers (Kunze, 1995). Cassava starch fits into this observation and hence the observation in Fig.5. ANOVA results confirm that there is a significant difference ($p<0.05$) in colour between samples and between substitution levels. Comparing the difference in means within substitution level shows that there is a significant difference ($p<0.05$) in colour between at least two samples. The difference can be located between 5% and 20% substituting levels and then 20% and 30% substitution levels.

The Maillard's reaction is the principal cause of colouring during wort boiling (O'Rourke, 2002). The reducing sugars and α-amino acids present are the key factors for this reaction. Melanoidins, which are polymerized products of reductones, give rise to the colour(O'Rourke, 2002). Amino acid levels in the barley substituted samples could be higher than that from cassava and this inference is drawn from the proximate analysis results of the protein contents of both samples, barley and cassava.

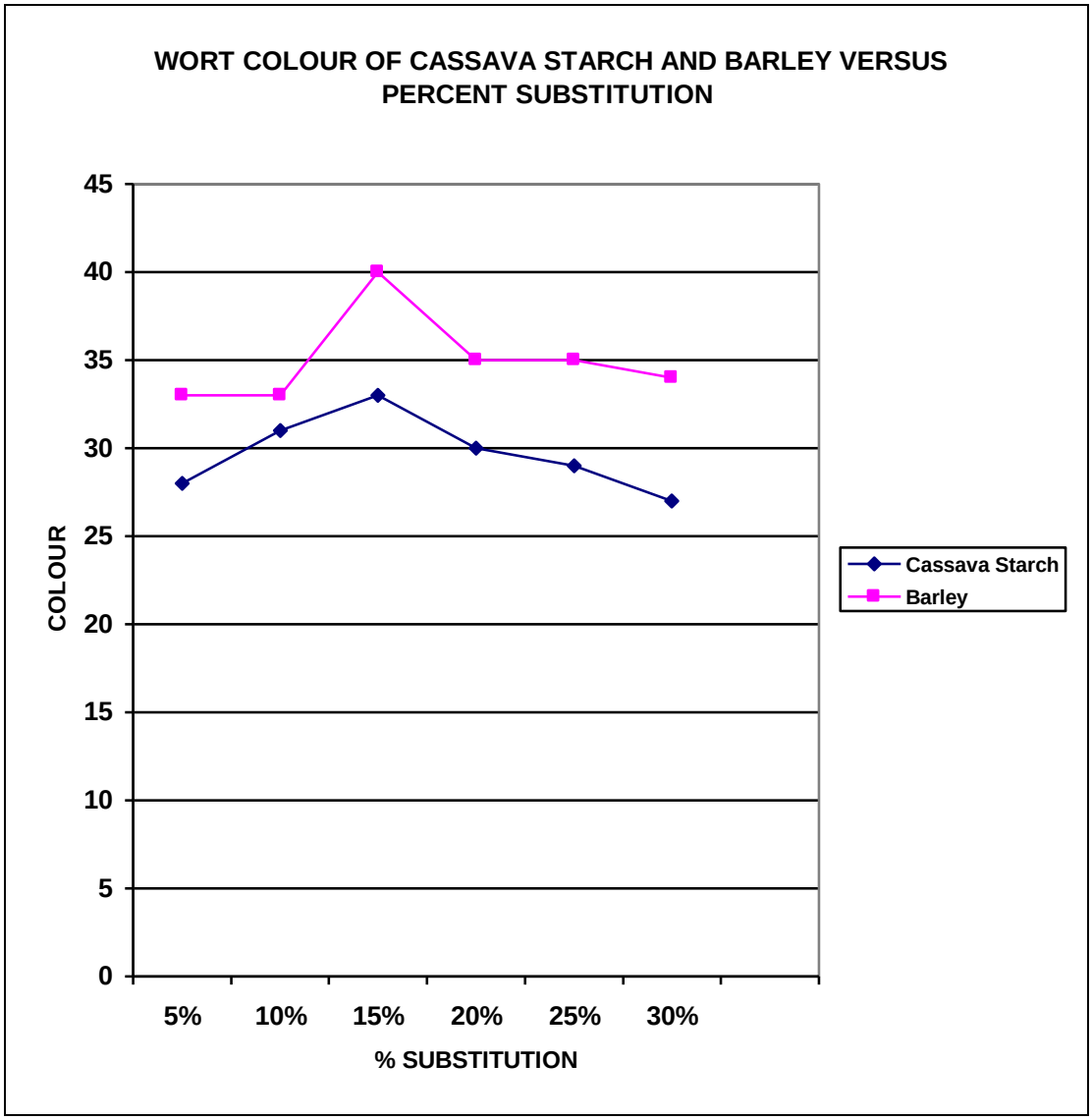


Fig. 4

4.2.4. Remainders (Perfect Primary)

Under favourable conditions of temperature, pressure and yeast vitality, fermentations will progress until the attenuation limit is attained (Bentley, 2006). Usually, there are still present some sugars which cannot be broken down by the yeast at this point. These sugars collectively form the remainders. Remainders give an idea of the extent to which wort fermentation may progress, how the principal enzymes of saccharification were manipulated during the mashing regime and sensory appeal of the beer (O'Rourke, 2002).

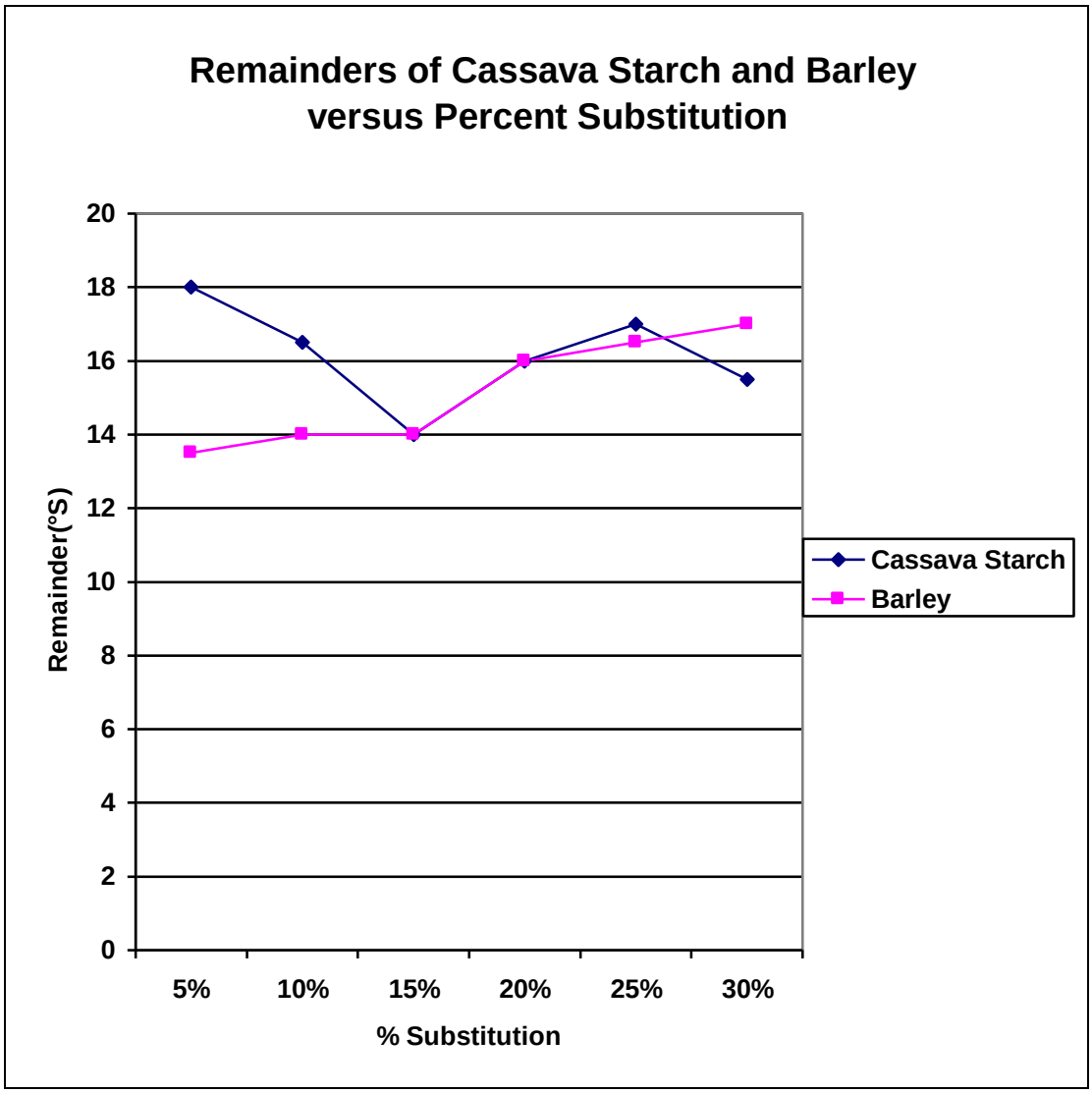


Fig. 5

Generally, the measured remainder values for the cassava starch substituted samples were relatively higher than that for the barley substituted ones (Fig.5). However, the ANOVA results suggest that, there were generally no significant difference ($p>0.05$) between remainders of samples and levels of substitution. There was significant difference ($p<0.05$) in some samples within the substitution levels. That is between 25% and 10%, 25% and 15% and 30% and 15%. The apparent higher value for the starch-

substituted samples may be due to the initial high levels of carbohydrate in the cassava starch samples (appendix 2).

4.2.5. Bitterness

There was no significant difference ($p>0.05$) in bitterness between samples and substitution levels. (Fig.6). The calculated least significant difference results compared to the difference of means of samples and substitution levels substantiates the above. The apparent equal levels of wort bitterness may suggest an almost standardized boiling temperature and time. Boiling isomerizes the α-acids to iso-α-acids, which is the principal bittering agent in hops (Lewis and Young, 1995). The results of hop utilization suggest no significant difference between samples and levels of substitution.

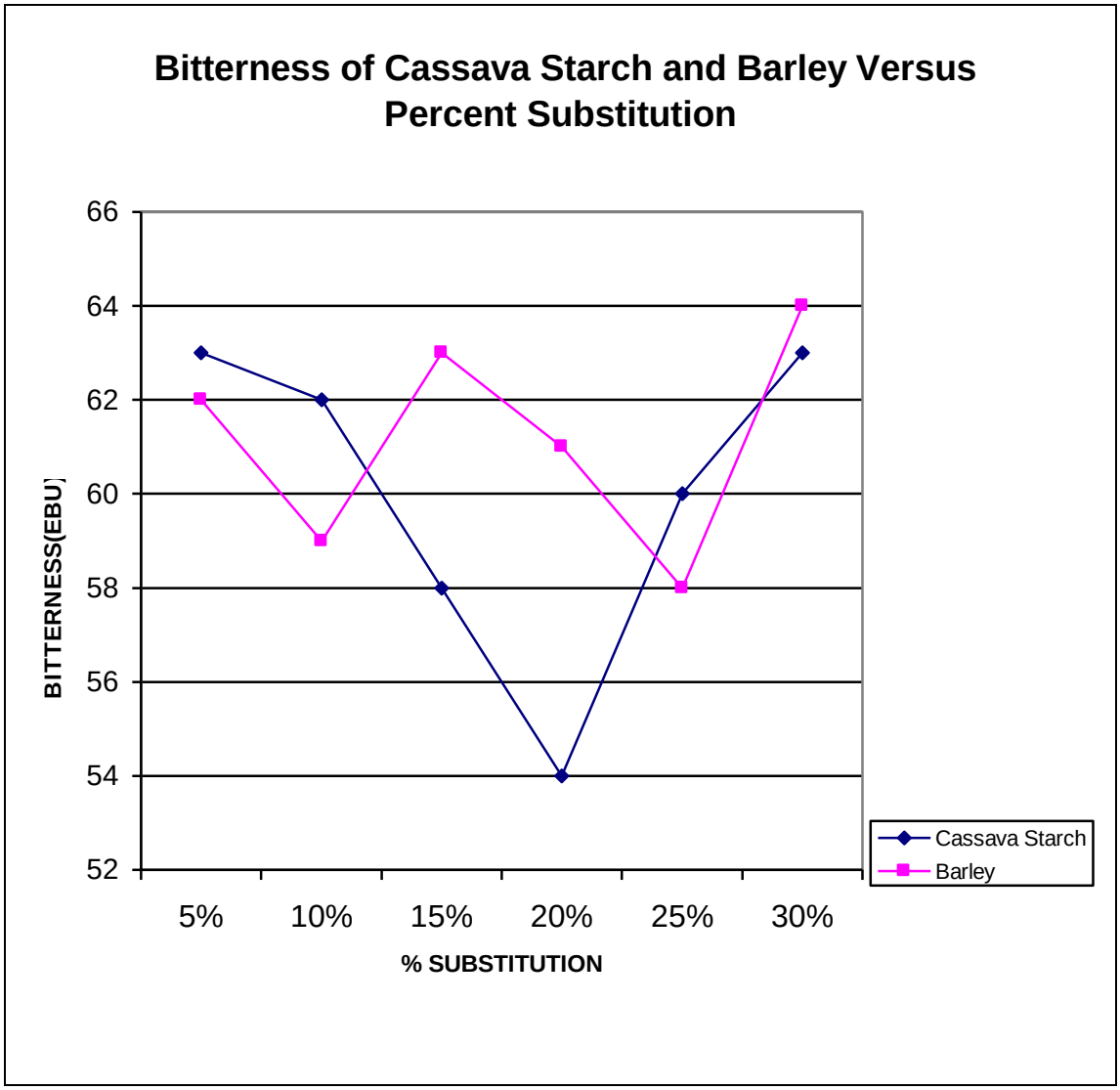


Fig. 6

4.3 FERMENTATION ANALYSIS

Fermentations were observed over a period of 68 hours at 20^{o}C in stainless steel cylindrical containers. Fermentation performances were measured as the rate of breakdown of the fermentable sugars such as maltotriose, sucrose, fructose, glucose and maltose into ethanol and carbon dioxide. Key parameters that were measured at the end of the fermentation were as follows: Original Extract or Gravity, Specific Gravity, Present Extract/Gravity, pH, Colour, Alcohol by Volume and Bitterness

4.3.1. Original Extract/Gravity

This is a calculated extract from a fermented sample, which gives an idea of the initial levels of sugars (Present gravity) prior to the commencement of fermentation. It should be noted that Original gravities (OG) are mostly not equal to the present gravity (Tables 8 and 9). O'Rourke (1984) had suggested that the apparent lower values of OGs are due to the following losses:

- Carbondioxide loss.
- Formation of certain acids
- Heat loss
- Alcohol formation

Analysis of the data showed that there was significant difference ($p<0.05$) in OG between samples and no significant difference between substitution levels.

Table. 8

Present gravities of Cassava Starch and Barley at various substitution levels

*	5%	10%	15%	20%	25%	30%
CASSAVA (PG)	$90.64_{(0.11)}$	$92.28_{(0.05)}$	$94.70_{(0.03)}$	$91.61_{(0.04)}$	$93.45_{(0.05)}$	$94.36_{(0.12)}$
BARLEY (PG)	$85.65_{(0.02)}$	$85.93_{(0.01)}$	$86.22_{(0.10)}$	$85.04_{(0.04)}$	$86.23_{(0.16)}$	$86.02_{(0.09)}$

Tables 8 and 9 show the present and original gravities of wort and green beer respectively. The results in table.14 shows that generally if any of the samples are blended to sales gravity, there will be more yield in terms of volume for cassava starch substituted samples than for barley. More volume means more profit.

Table .9

ORIGINAL GRAVITIES (OGs) FOR CASSAVA STARCH AND BARLEY AT VARIOUS SUBSTITUTION LEVELS

SAMPLE *	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**
Cassava Starch	$87.21_{(0.23)}$	$88.65_{(0.19)}$	$88.48_{(0.03)}$	$90.26_{(0.02)}$	$90.88_{(0.05)}$	$91.43_{(0.03)}$
Barley	$82.46_{(0.05)}$	$85.53_{(0.03)}$	$81.99_{(0.03)}$	$83.95_{(0.02)}$	$84.21_{(0.02)}$	$82.65_{(0.03)}$

4.3.1.1. Specific Gravity

Specific gravity figures measured post fermentation was very low compared to their initial values at the wort stage. This confirms that fermentation is catabolic in nature as the starting levels of fermentable sugars depleted as fermentation progressed (Fig.7). Though the remainder values obtained for Cassava starch and Barley at 5% (Fig.5) at the end of fermentation were not exactly the same as predicted by the Remainder test i.e.18°S and 13.5°S respectively, the results obtained for specific gravities and hence present gravities are in conformance to expectations at the end of fermentation (present gravities for cassava starch would be greater than that for barley at 5%). Analysis of the data showed that there was significant difference in specific gravities between samples and between substitution levels. The specific gravity value at end of fermentation is an index of the sensory quality and mouth feel (O'Rourke, 2002). A similar explanation holds for the present gravity. However, high specific gravity values obtained at end of fermentation may suggest poor yeast performance or questionable wort constituents which invariably results in poor yeast performance. It is worth stating

that the profile obtained for the 5% substitution sample (fig.7) is similar to that obtained for an all malt brew is seen in fig.7 at the same set of conditions.

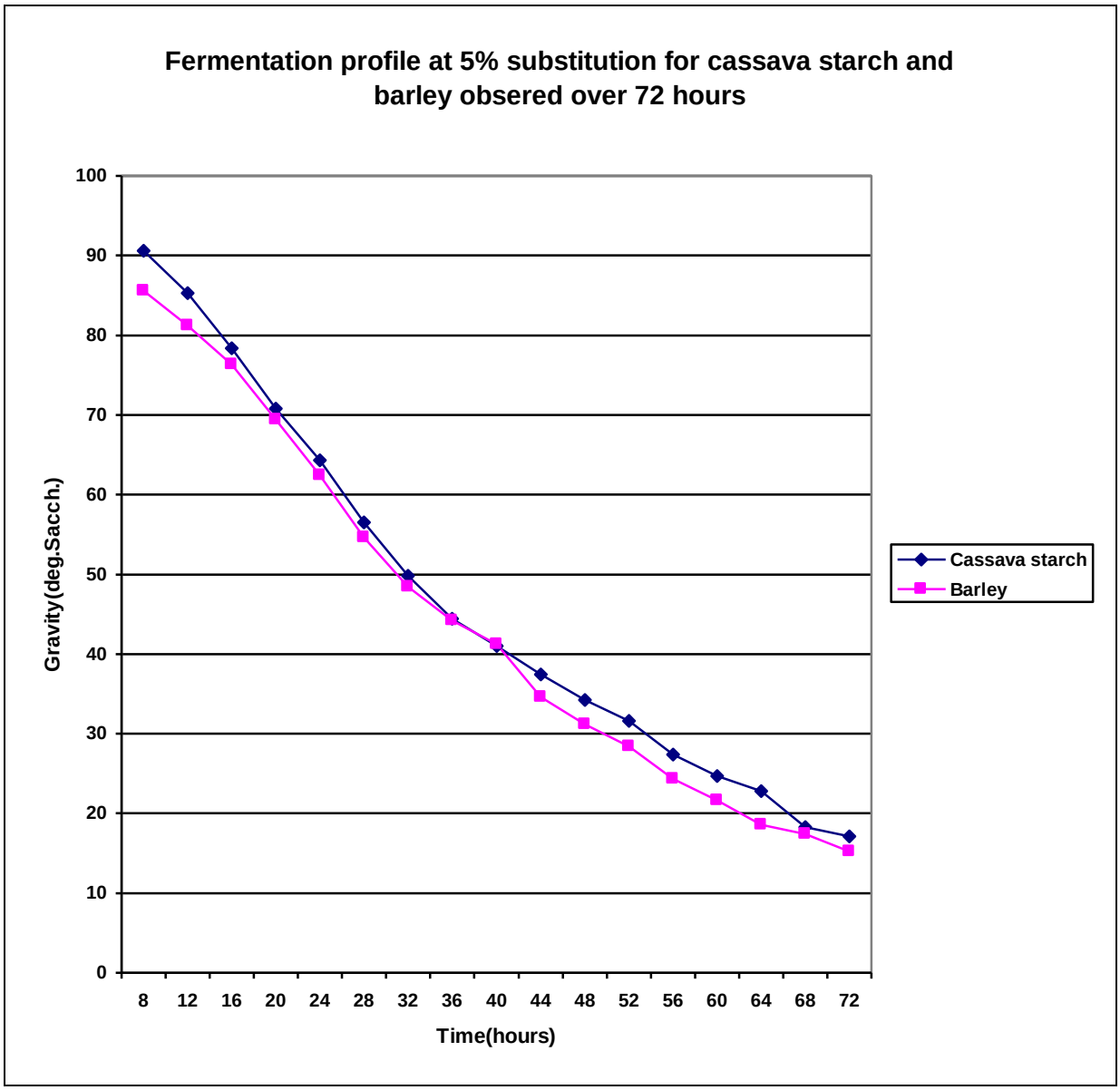


Fig.7

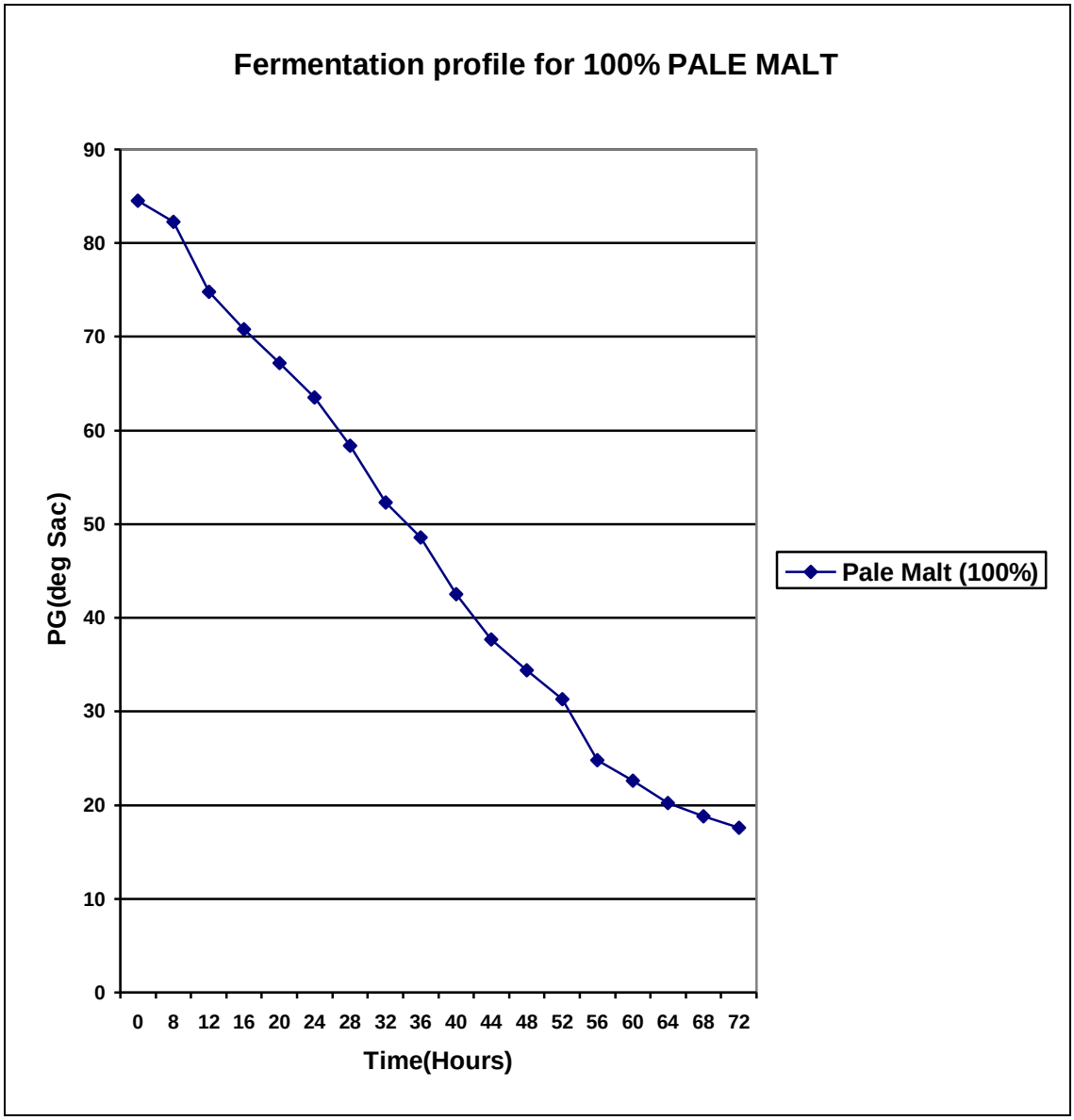


Fig.8

4.3.1.2 pH

pH values as shown in fig.8 shows the general drop in pH as wort ferments. It was observed that the mean pH of cassava starch and barley substituted wort at start of fermentation were 5.3 and 5.1 respectively. However pH trends changed as fermentation progressed to the end yielding final pHs of 4.26 and 4.25 for cassava starch and barley samples respectively. During wort sugar metabolism, several organic

acids, which do not only impart flavour but also give rise to a drop in beer pH, are released into the beer.

Lewis and Young (1995) have stated that the fall in pH is a result of the consumption of ammonium ions, potassium ions and amino acids by yeast and the consequent release of hydrogen ions and secretion of organic acids. The high levels of ethanol and drop in pH render beers a poor substrate for most microorganisms. Kunze, (1995) has stated that pH has a considerable effect on the quality of beer. A beer pH of less than 4.4 would have among other effects the following.

- Accelerates precipitation of colloidal unstable protein-polyphenol complexes,
- Produces faster maturation,
- Refines the beer taste and
- Is an essential requirement for a good biological stability of the beer.

A pH value of less than 4.1 causes beer to taste acidic and is a probable indication of acidification by microbial infections. (Kunze, 1995)

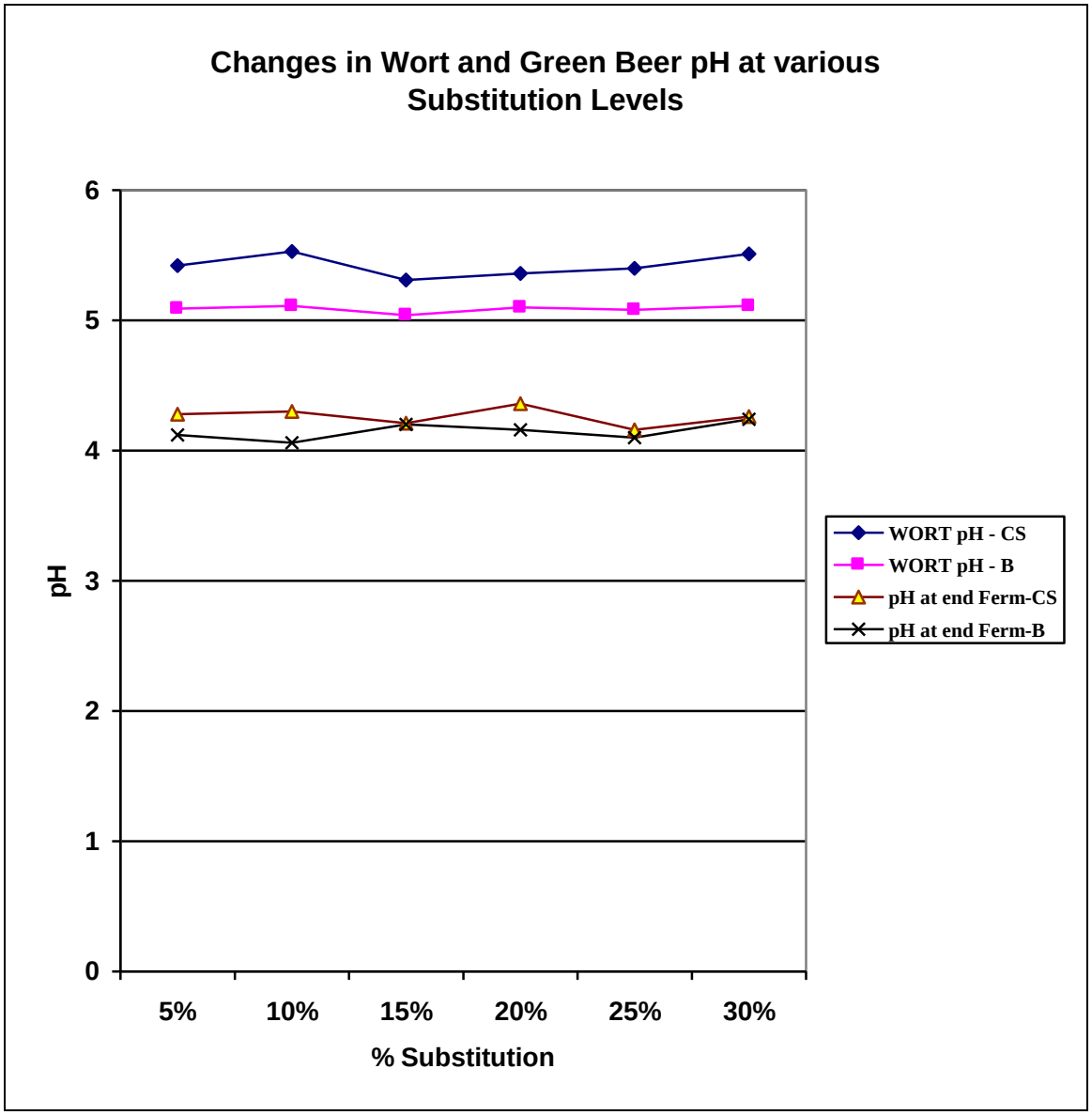


Fig.9

Analysis of the results shows there is a significant difference in pH between samples and no significant difference in pH exist between substitution levels (Fpr 0.524). Comparing the least significant difference values obtained for samples (0.1028), the difference in sample means (0.115) showed a significant difference in pH between samples and no significant difference in pH at substitution levels.

4.3.1.3. Alcohol (Alcohol By Volume)

Ethanol is a major end product of beer. It forms part of the end by products of the glycolytic pathway of wort fermentation. Ethanol levels were generally very high, a result of high gravity brewing. Analysis of results showed that there was a significant difference ($p<0.05$) in ethanol levels between samples. However substitution levels did not impact much as regards ethanol generation. High gravity beers are usually blended with deaerated water to sales gravity.

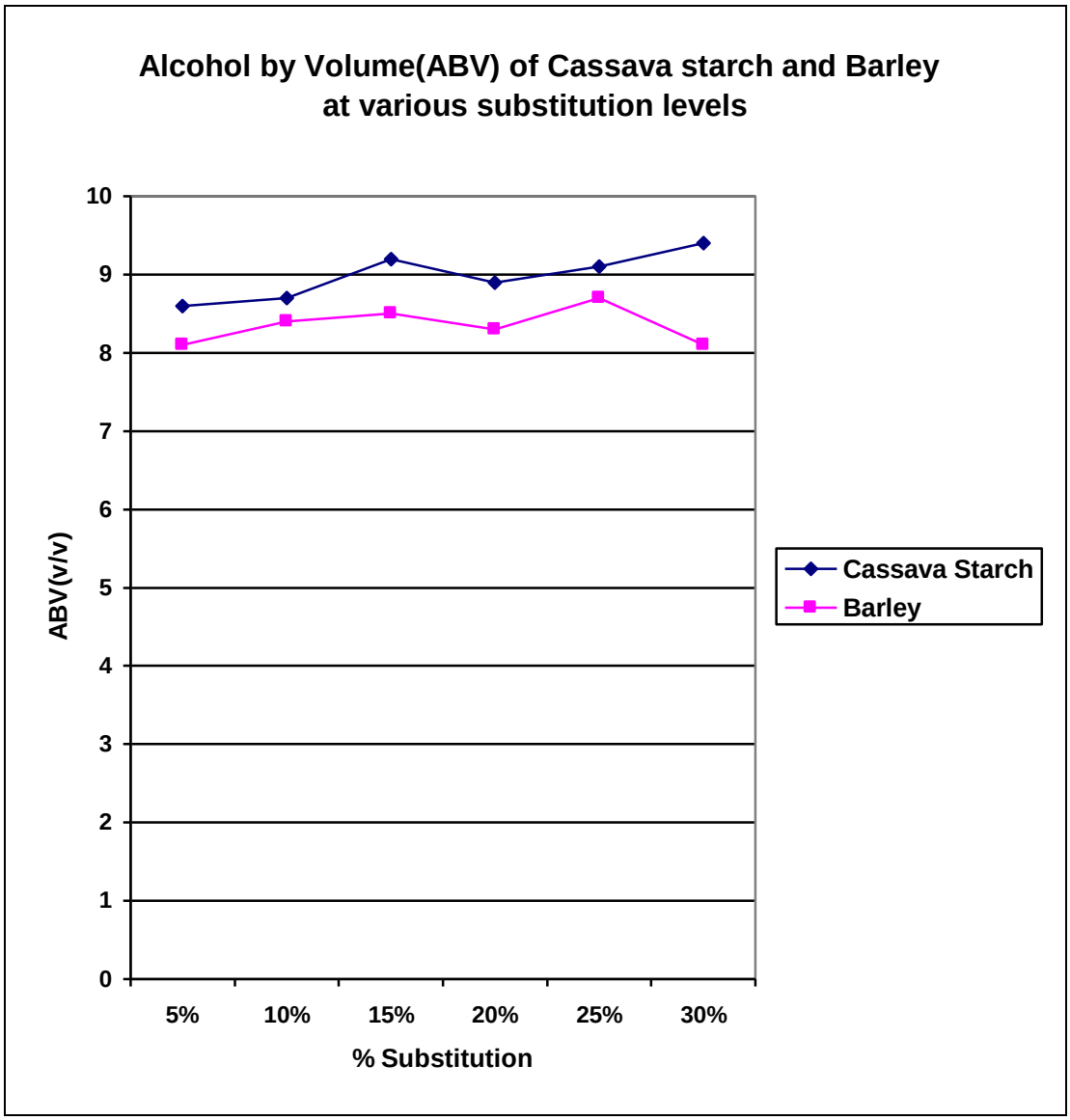


Fig.10

Fig.10 shows that the levels of alcohol obtained for cassava starch at end of fermentation were generally higher than that for barley. The initial substrate levels for cassava starch samples were generally higher than that for barley as shown in table 4 and therefore the above is expected. It was observed that the highest alcohol yield was obtained for cassava starch at 30% substitution and was therefore expected that a similar observation be made for barley. However alcohol obtained for barley at 30% was the least as that of 5% levels.

The remainder test as depicted by fig.6 showed that there would be high remainders in barley at the end of fermentation at 30% substitution. High remainders mean high levels of both fermentable and unfermentable sugars. The fermentables, under favourable conditions should ferment further to yield more alcohols. However the remainder test results suggest that the nature of the wort prior to fermentation had much influence on the end results for barley at 30% than for fermentation conditions. Hence the wort generated for cassava starch at 30% from its mash determined its fate during and at the end of fermentation.

4.3.1.4 Bitterness

Bitterness levels generally dropped during and at the end of fermentation. O'Rourke, (2000) mentions the following factors as culminating in the loss of bitterness during fermentation.

a. Excessive fobbing resulting in beer loss and hence bitterness
b. CO_2 evolution with volatile hop components
c. Adsorption of hops components on cell surfaces of yeast during and at the end of fermentation.

Kunze, (1995) had explained that as a result of the decrease in pH during fermentation, a number of colloidally dissolved bitter substances and polyphenols are brought into their isoelectric point range and are precipitated as surface active compounds on the CO_2 bubbles in the foam head or as a result of adsorption on the yeast cells. Kunze, (1995) further adds that in conventional fermentation and maturation, starting with the amount of bittering substances in the cold wort as 100%, 25 to 30% is lost and 70 to 80 percent of this by the end of primary fermentation. Results obtained are in accordance with the above statement as there was an average of 19.2% loss in bitterness noted for cassava starch and 21.6% for barley. ANOVA results however showed that there were differences ($p<0.05$) in levels of bitterness between samples and between substitution levels.

Poor bitterness value obtained for cassava starch at 20%(Fig.11) substitution may be attributed to less vigorous boiling leading to poor isomerisation of alpha acids to iso-alpha acids. It was noted that cassava starch sample at 15% suffered the least loss in levels of bitterness by end of fermentation. This was about 2 international bitterness units (IBUs) loss. This is usually a rare observation and may be attributed to the poor regeneration of the 2,2,4-trimethylpentane solvent, which was used to extract the bittering compound. This may have carried over some levels of iso alpha acids and hence augmenting the results.

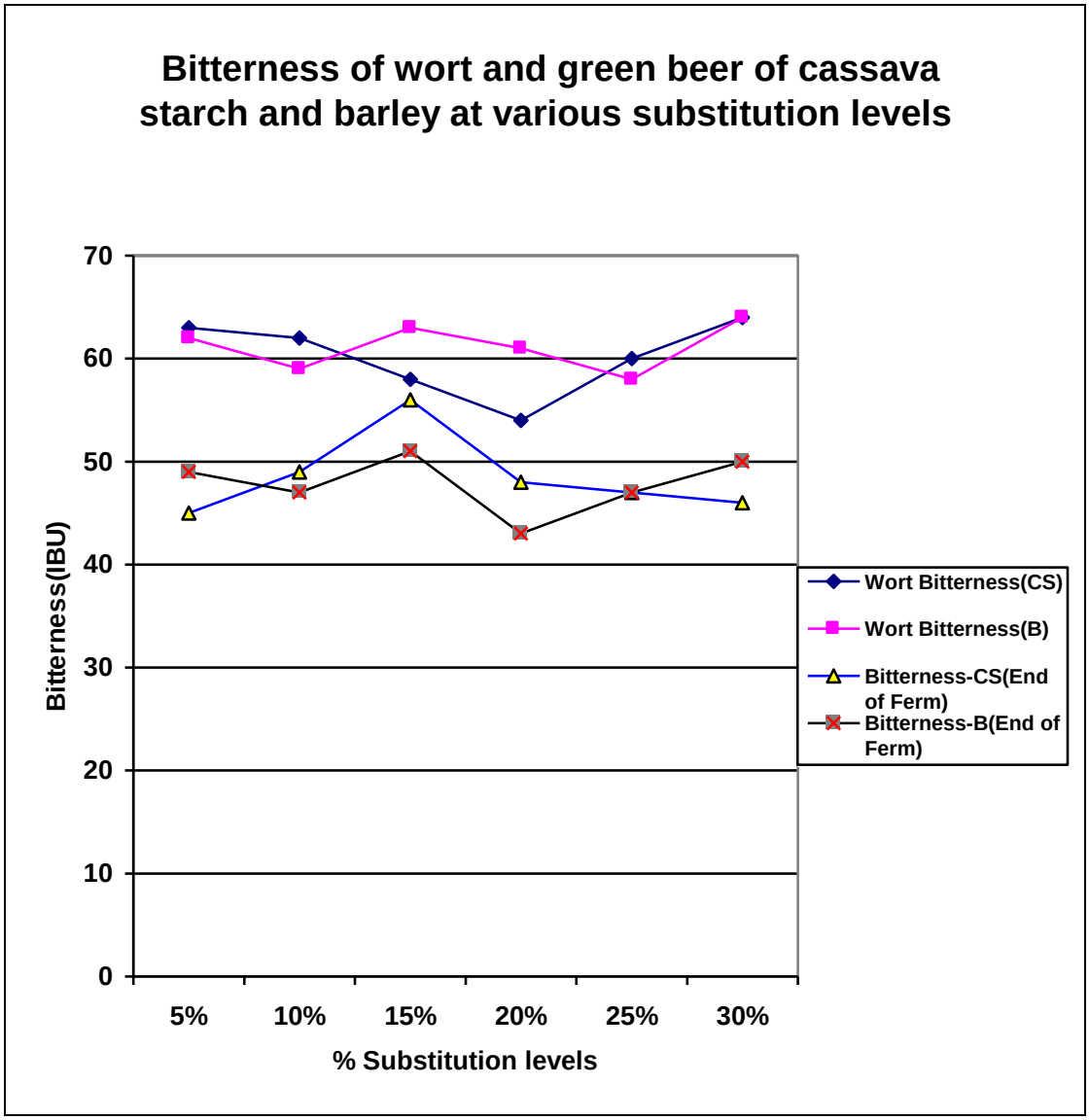


Fig.11

4.4 BLEND TO SALES GRAVITY AND CARBONATION

4.4.1 pH

Blending hardly impacted on the pH as water used for blending is a weak electrolyte and will not ionize in solution under blending conditions to impact on pH. ANOVA

results showed no significant difference ($p>0.05$) in pH between samples and between substitution levels.

Table. 10

pH of one litre blended samples of cassava starch and barley at various substitution levels

SAMPLE *	5%	10%	15%	20%	25%	30%
Cassava Starch	$4.13_{(0.02)}$	$3.87_{(0.02)}$	$4.06_{(0.03)}$	$3.89_{(0.03)}$	$3.92_{(0.02)}$	$3.95_{(0.01)}$
Barley	$3.96_{(0.02)}$	$3.92_{(0.00)}$	$3.97_{(0.01)}$	$3.90_{(0.02)}$	$3.87_{(0.00)}$	$3.92_{(0.03)}$

Carbon dioxide (CO2)

Table. 11

CO_2 levels of one litre blended samples of cassava starch and barley at various substitution levels

SAMPLE *	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**
Cassava starch	$\mathbf{3.43_{(0.04)}}$	$\mathbf{3.21_{(0.02)}}$	$\mathbf{3.30_{(0.02)}}$	**3.08(0.02)**	$\mathbf{3.16_{(0.01)}}$	$\mathbf{3.10_{(0.03)}}$
Barley	$\mathbf{3.21_{(0.00)}}$	$\mathbf{3.22_{(0.02)}}$	$\mathbf{3.41_{(0.02)}}$	$\mathbf{3.52_{(0.04)}}$	$\mathbf{3.10_{(0.02)}}$	$\mathbf{3.33_{(0.01)}}$

Though there were relatively high levels of CO_2 in the green beer at end of fermentation, post fermentation treatment such as filtration resulted in huge losses of

CO_2. Physical incorporation of CO_2 generally resulted in amounts that could be measured from bottled samples. The mean CO_2 of cassava-substituted samples was 3.2g/L and 3.3g/L for the barley substituted samples. Even though these levels were generally lower than the specification in good beers (4.7g/L – 5.2g/) (Kunze, 1995), there was a high level of consistency in CO_2 retention in all samples. ANOVA results showed that there was significant difference ($p<0.05$) in CO_2 retention between samples and substitution levels. Since CO_2 dissolution in beers principally depends on temperature, it shows that temperature of sample prior to carbonation were generally the same at about 2°C.

4.5 Head Retention

Beers are usually judged by their clarity, colour and foam. O'Rourke, (2002) said that beer is a supersaturated solution of gas; when poured out, the bubbles break out from

Table 12

Head retention times for cassava starch and barley at various substitutions levels

Sample *	5%	10%	15%	20%	25%	30%
Cassava starch	$83_{(0.6)}$	$81_{(0.6)}$	$79_{(0.6)}$	$76_{(1.0)}$	$77_{(0.0)}$	$79_{(1.2)}$
Barley	$82_{(0.6)}$	$83_{(1.7)}$	$84_{(0.6)}$	$84_{(0.0)}$	$80_{(1.0)}$	$82_{(0.0)}$

solution and rise to the top of the glass. The effect is called "tracing". The foam in beer is generally considered to be the head on the top of the glass. Two complementary conditions must be met in order to ensure satisfactory foam performance (O'Rourke, 2002).

They are head formation and retention. Beer head is formed from the bubble formation. They require a minimum level of dissolved carbon dioxide or mixed gas.

Measured times (seconds)(table 12) as head retention was generally below standard times [Rudin Method, ≥ 90 secs and Nibem Method, 260 – 280 secs] (O'Rourke, 2002). This may be due to the method used and generally low levels of CO_2 dissolution. A mean head retention time of 76.2 sec was measured for the cassava starch-substituted samples and 82.5 sec for the barley substituted samples. ANOVA results showed that there was significant difference in head retention values between samples. Since all other conditions such as dissolved CO_2 and dispensed temperature were generally the same, the presence of hydrophobic proteins, which were present in greater amount in barley than in the cassava starch-substituted samples accounted for the higher observation. All malt grist with low malt modification with the addition of wheat or barley will increase the level of hydrophobic proteins (O'Rourke, 2002). There was no significant difference in head retention between substitution levels at $p < 0.05$. Higher alcohol products (those with more than 7 or 8% alcohol by volume) tend to have poorer foam performance (O'Rourke, 2002). Though alcohol levels were generally high for both samples 9.0%abv and 8.4%abv for cassava starch and barley samples respectively a good level of head retention may have been achieved if adequate CO2 could have been incorporated into the samples.

4.6 Blended Beer Volume

The original gravity values measured showed that higher original gravity were generally obtained for the cassava-substituted sample than that for the barley substituted samples. This represented a 7.1% increase in original gravity for cassava starch-substituted samples.

Fig.11 shows a similar trend as noted in levels in alcohol by volume achieved at end of fermentation. Alcohol by volume among other key parameters form the principal basis for blending high gravity beers to sales gravity as it is top on the list in terms of legal requirements. A blend of a litre of each sample to 6% ABV (Alcohol by Volume) yielded various volumes as shown by Figure 11. Analysis of the results showed clearly that there were significant differences ($p<0.05$) between volumes obtained from a litre blend of each sample to alcohol strength of 6% ABV. No significant difference ($p>0.05$) was noticed for samples at various substitution levels. Comparing blended volumes in figure 12, the mean volume obtained for a litre blend sample of cassava starch substituted sample was 1481.2L at 6% ABV and that for the barley substituted sample was 1378.1L at 6% ABV. This is a 7% increase over the latter and suggests that generally a lot more volume may be obtained from cassava adjunct brews compared to barley.

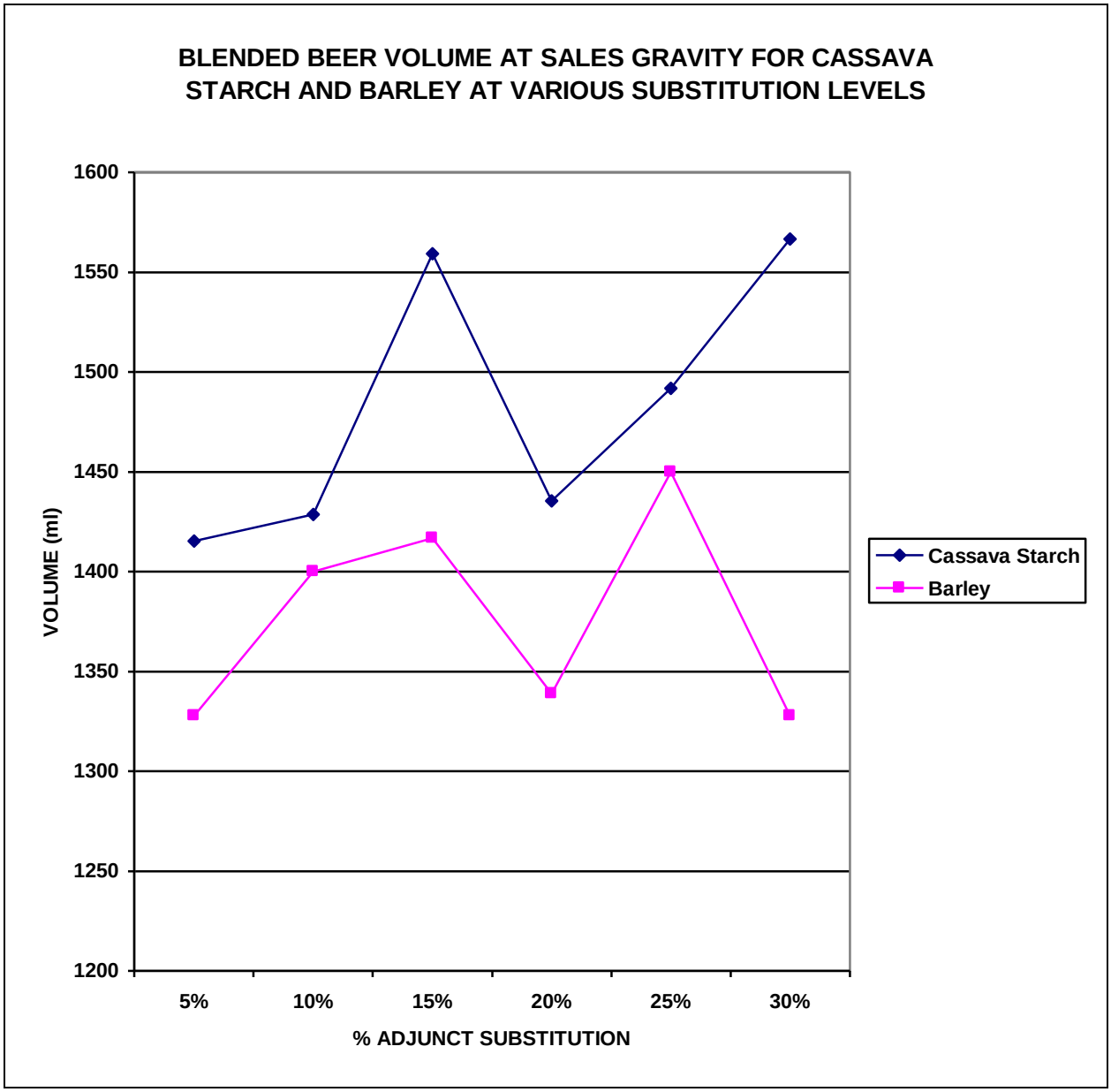


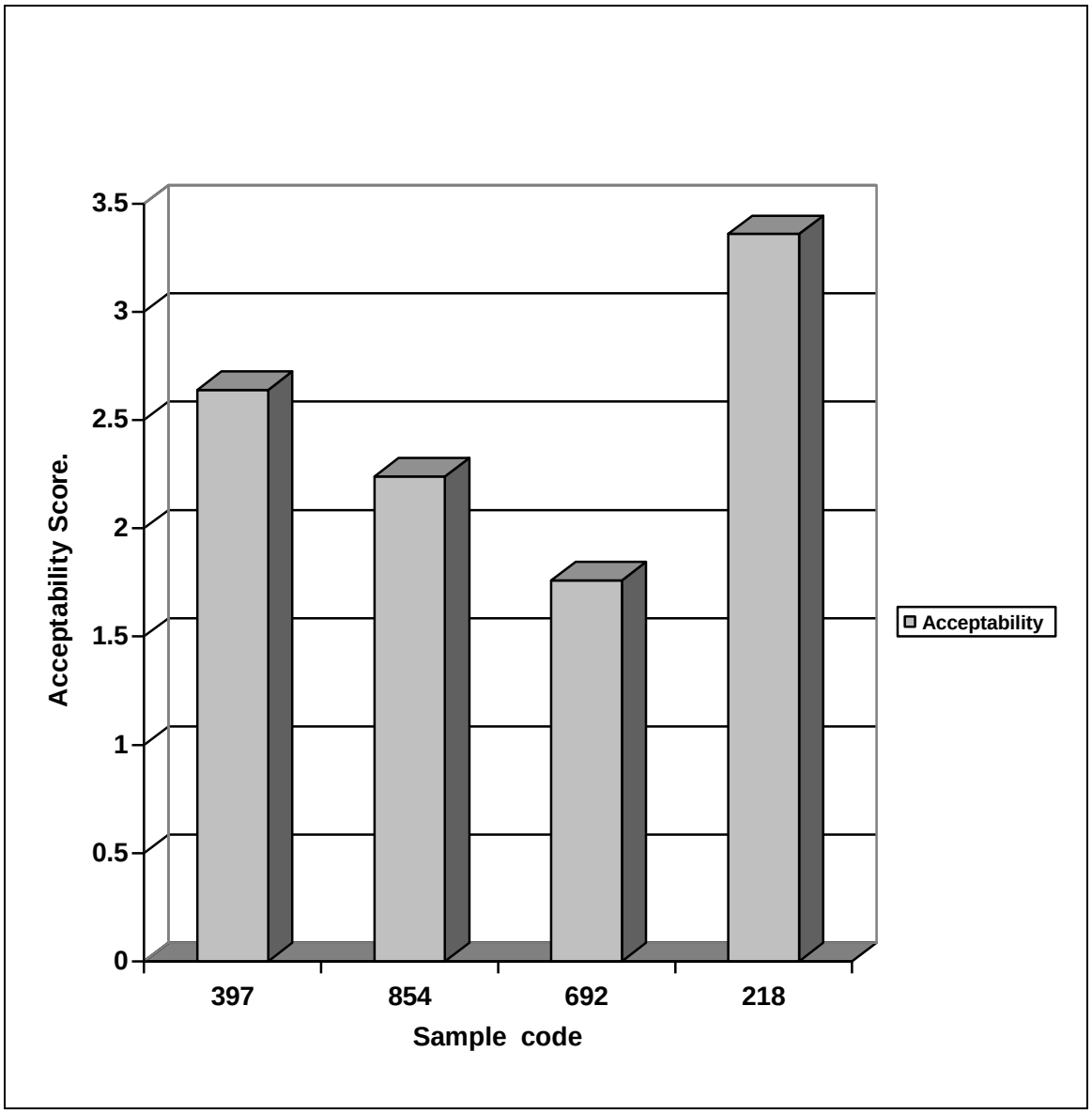
Fig. 12

Since alcohol was the basis for blending or dilution, it is expected that figures 10 and 12 would display the same trend.

4.7 Sensory Evaluation

4.7.1. Acceptability tasting

The overall acceptability tasting gave the results shown in the figure14 below.



Acceptability taste score

Fig. 13

KEY: - **Code**

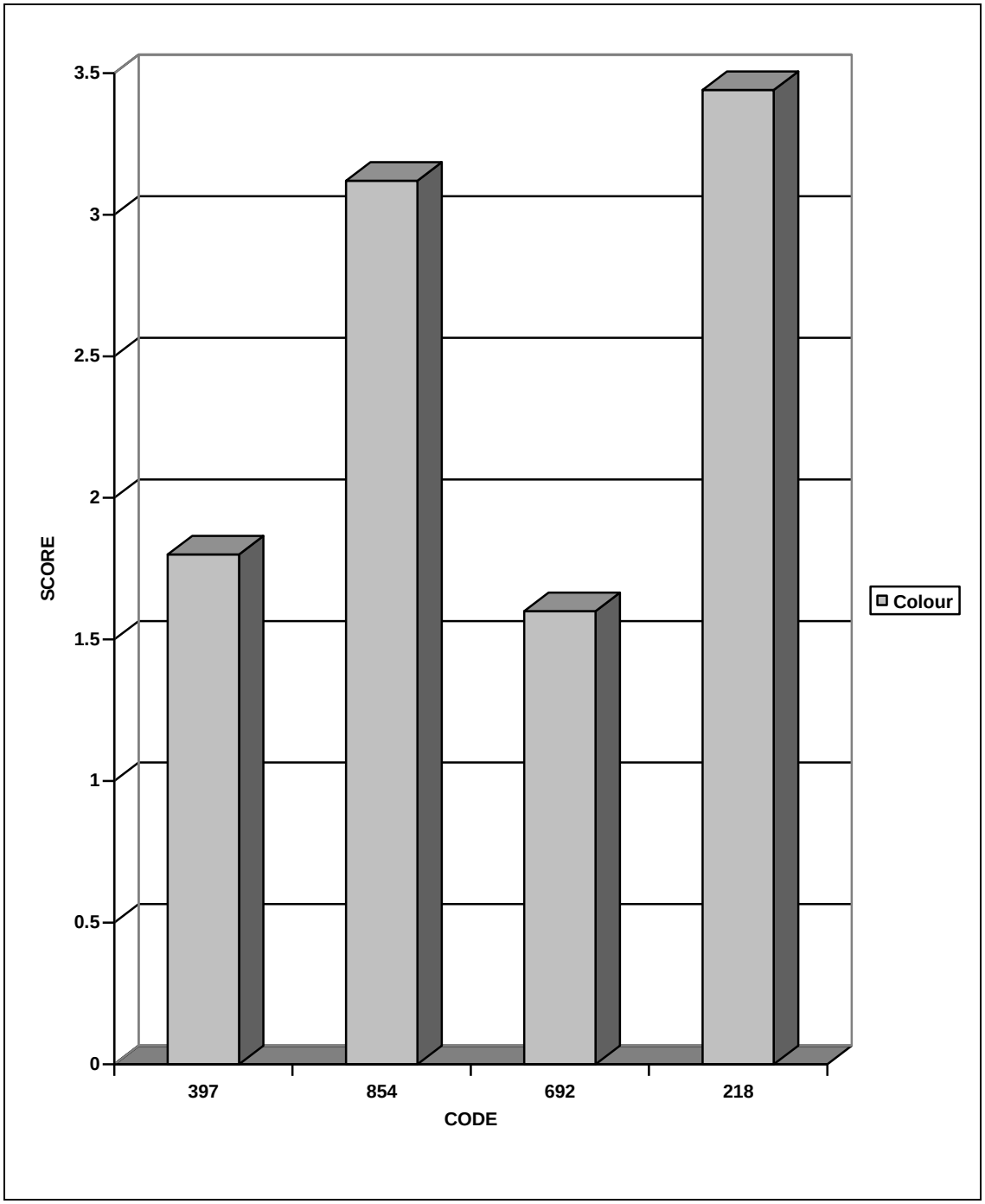
397 - 5**%** barley substitution

854 - **10%** cassava substitution

692 - **10%** barley substitution

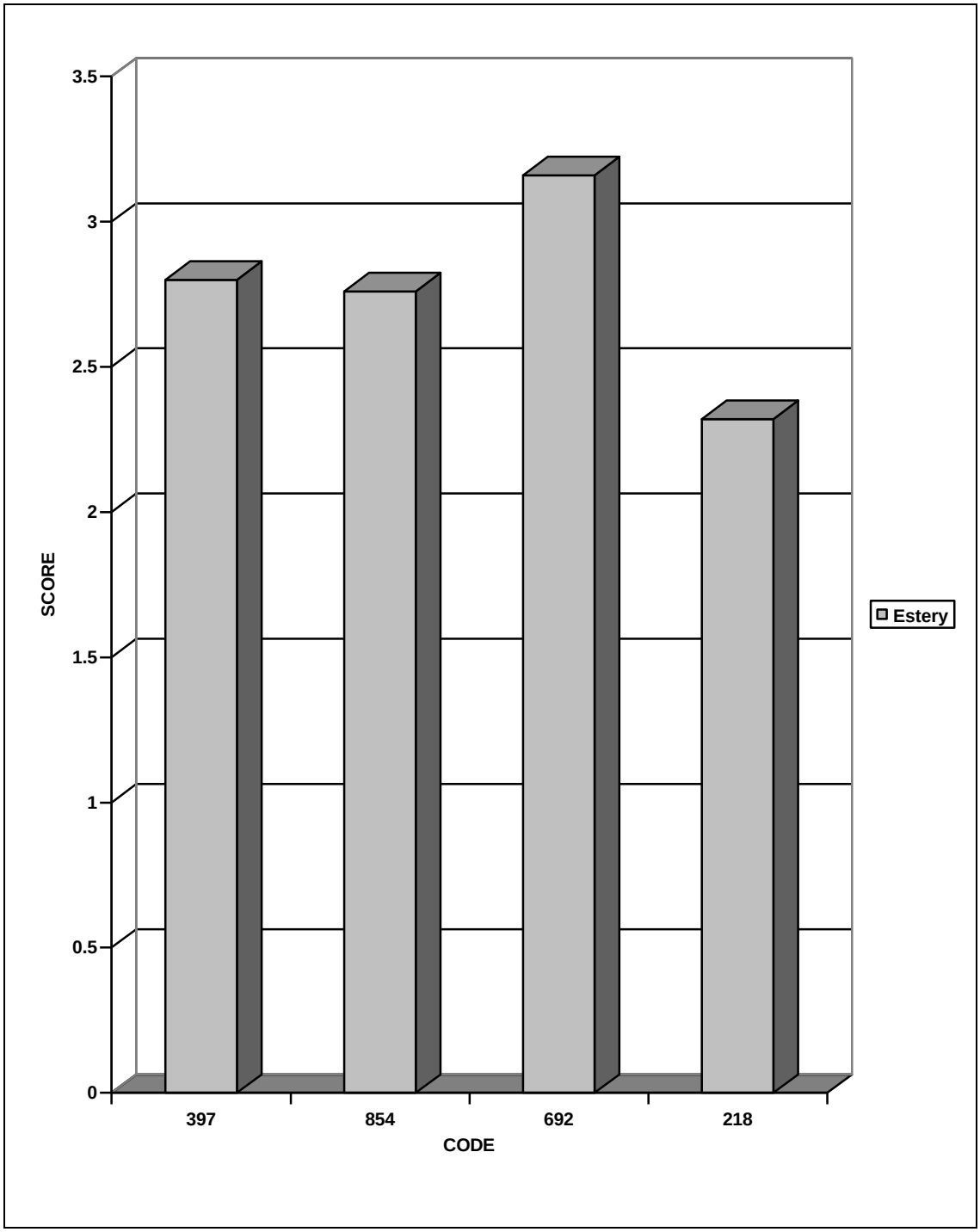
218 - **25%** cassava substitution

The above shows that the preferred samples, ranging from the most preferred to the least preferred were in the order, **218, 397, 854 and 692.** This judgment may be arrived at based on the combined flavour attributes of colour, tingly, estery and bitterness exhibited by each sample. From fig.15 above, buttressed with the ANOVA results, bitterness and tingly flavour attributes were not significantly different ($p>0.05$) between samples and may hardly influence the panel's ratings. However ANOVA results showed a significant difference in colour and estery flavour attributes between the samples ($p<0.05$). These two flavour attributes invariably, may be the determining factors for the panel's rating in the acceptability test. The 25% cassava starch-substituted beer was the best-rated sample. The fermentation profile (appendix 2) of the 25% cassava starch substituted sample even suggested that its beer when given the right post-fermentation treatment could give beer of good quality from sensory perspective. From Fig.10, colour tends to be the only flavour that puts sample code 218 ahead on the others. Panelist therefore judged and rated the samples with their colours as the most influencing flavour attribute. First impression counts. Most consumers drink with their eyes and appearance is often more important than taste (O'Rourke, 2002).



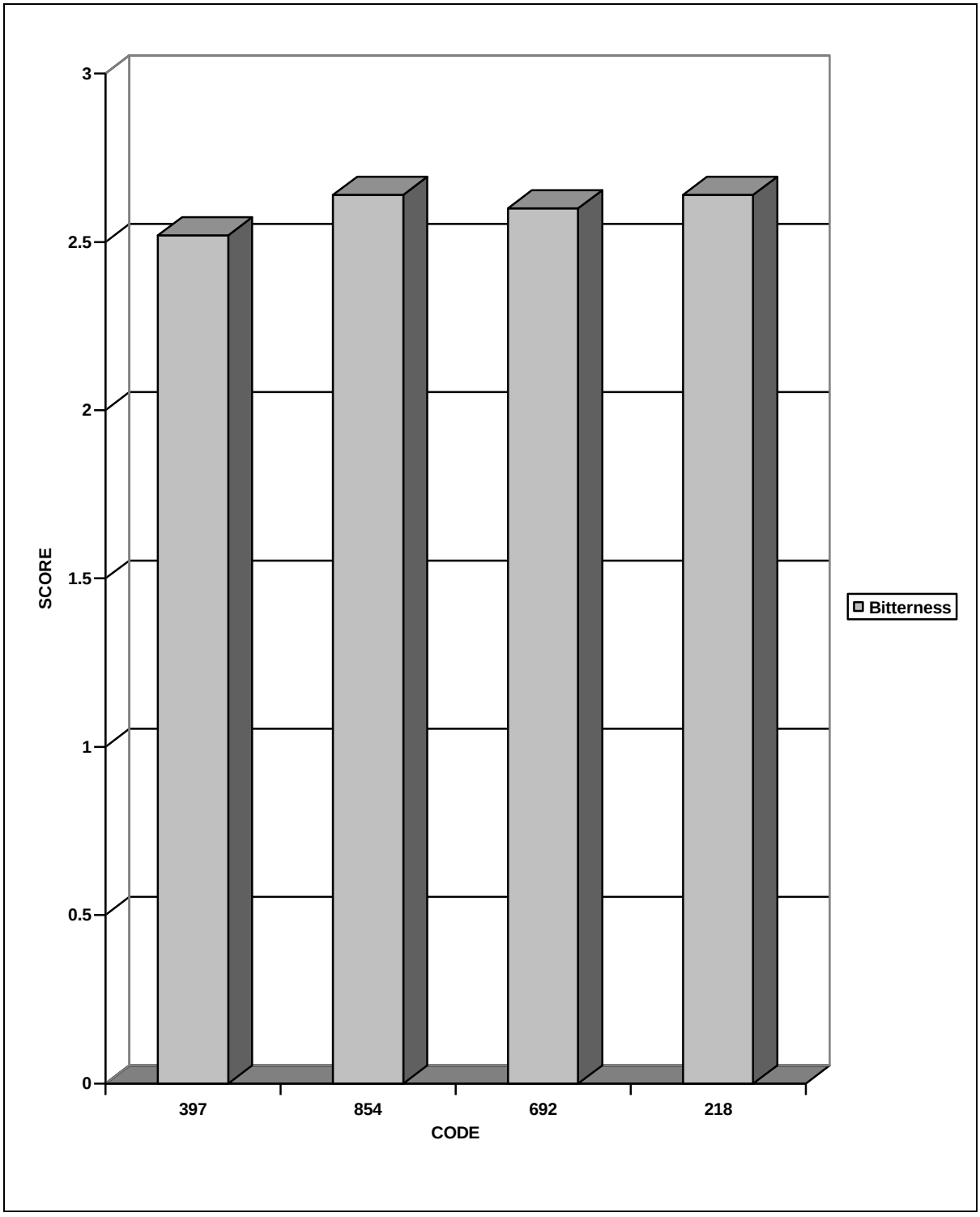
SCORE ON COLOUR

Fig. 14



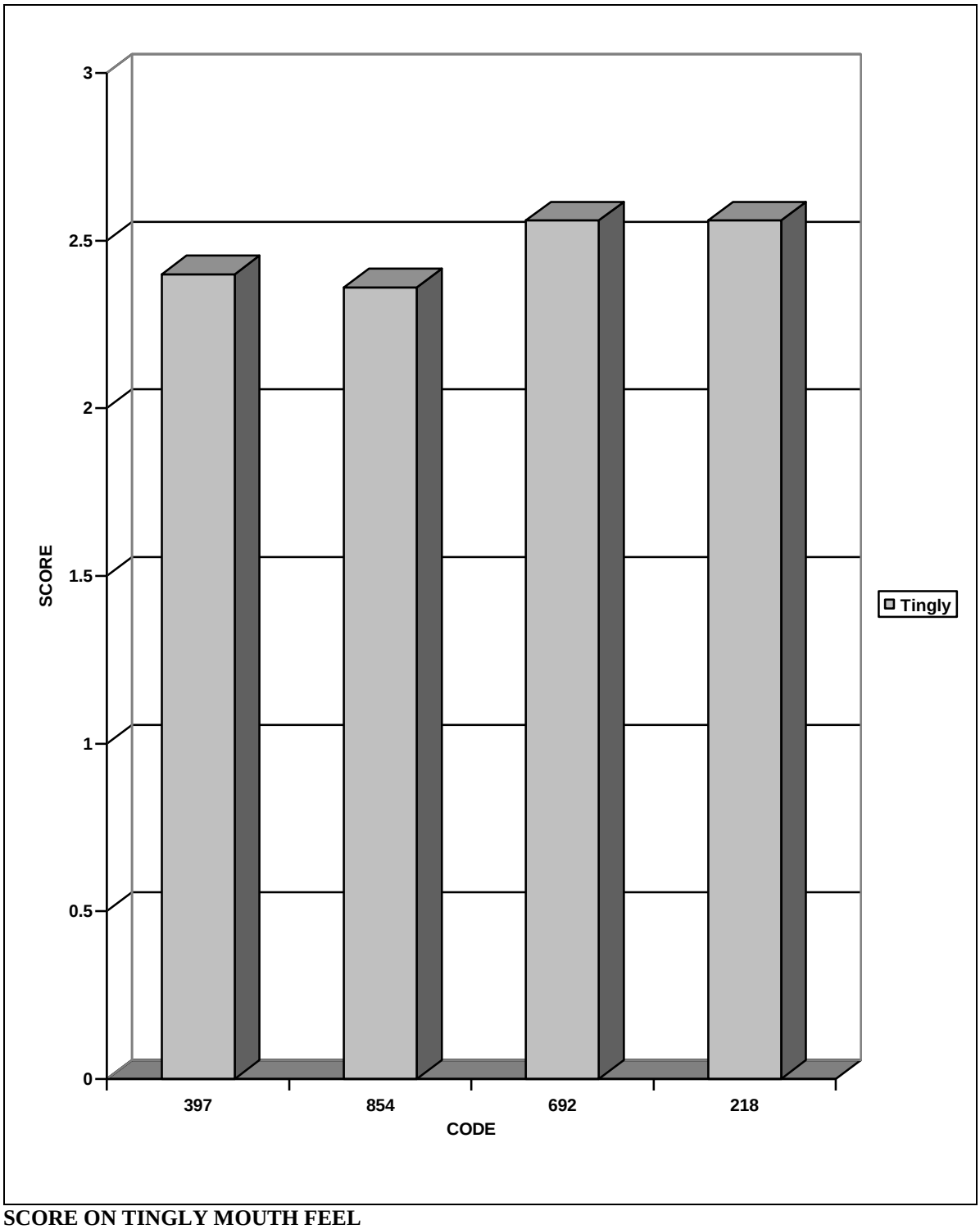
SCORE ON ESTERY AROMA

Fig. 15



SCORE ON BITTERSS

Fig. 16



SCORE ON TINGLY MOUTH FEEL

Fig. 16

***** Each value in table (Results and Discussion) is the average of triplicates. The values in bracket are standard deviations about the mean.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

Wort generation from both samples (cassava starch and barley) at various substitution levels was successful as both samples at various substitution levels succumbed to the action of saccharification enzymes on their starches into fermentable sugars. Generally there were significant differences between samples for parameters determined for the wort samples such as specific gravity, pH and colour but no significant difference existed between remainders and bitterness ($p>0.05$). For fermented samples (green beer), there were significant differences between samples for parameters such as original gravity, specific gravity, pH, bitterness and alcohol. Such differences also existed in substitution levels for specific gravity, pH and bitterness ($p<0.05$). For the blended sample (sales gravity), no significant differences existed between samples and substitution levels for pH. However, significant differences existed between samples and substitution levels for the carbon dioxide parameter. Significant difference also existed between samples for head retention but not for substitution levels. From yields point of view, the cassava starch substituted samples were better than that for barley. O'Rourke, (2002) has said that in the purchase of adjuncts, the brewery looks for a product that will yield the most economic extract (wort) and will operate satisfactorily under brewhouse condition and throughout the brewing process.

RECOMMENDATIONS

- Starches from various cassava breeds can be obtained and analyzed as a means to identifying breeds with higher extracts.

- Higher substitution levels can be ventured with the view to establishing the optimum levels of cassava starch adjuncts that could be used without the addition of exogenous enzymes, managing cost but not compromising on quality.

- Composite mixture of cassava starch and barley adjunct substitute may be used and production challenges such as wort generation, fermentability, green beer processing and final product quality assessed.

REFERENCES

1. Asiedu J. J., 1989. Processing Tropical Crops – A Technological Approach, pp.1and 3
2. Bamforth, C. (2001). The Brewer International (A Brewer's Biochemistry – Lipids), Vol. 1, Issue 7; pp 12 – 15
3. Bamforth C. W. (2003) Opportunities for newer technologies in the oldest technology brewing, Applied Biotechnology, Food science and policy, 1: 213 – 222.
4. Bamforth, C. W. (2006)(Ed). Brewing new technologies. Pp 30, 31 and 34
5. Bentley I. (2006). The Brewer International (Enzymes in Brewing), Vol.2, Issue 7, p 36)
6. Briggs D. E. (1988). Malts and Malting, 1st Ed, Blackie Academic and Professional, pp35 and 535
7. Brauer J, Walker C. and Booer C. (2005), of pseudo – cereals and roasted rice. The quest for gluten-free brewing materials, Brewer and Distiller, 1: 24-26
8. Briggs D. E., Hough J. S., Stevens R., Young T. W., (1971), Malting and Brewing Science, Vol. 1, pages 306 and 307
9. Canales A. M. (1979). Food Science and Technology (A Series of Monographs) Pollock J. R. A. (Ed) Reading England; pages 238, 239
10. Candy E. (2003). The Brewer International (Water – Unlimited), Vol. 3, Issue 6; pp 12 – 18.
11. Cege P, Shah S and Kubai E (1999), Kenyan beer brewed with unmalted barley, Ferment, 12: 41 – 45.

12. Coors J.,(1976). Technical Quarterly. Master Brewers Association of America. 13(2) p 78.
13. Delvaux F, Gys W, Michiels J, Delvaux F R and Delcour J A (2003), Contribution of wheat and wheat protein fractions to the colloidal haze of wheat beers, Journal of the American Society of Brewing Chemists, 59: 135 – 140.
14. Glienke, J. and Edward, W. R.(1965), Brewers' Digest p.115
15. Goode, D. L. and Arendt, E. K (2005), Brewing, New technologies, Bamforth C. W. (Ed). Woodhead, Cambridge England, p.57.
16. Grujic O (1999), Application of uncoventionnal raw materials and procedures in wort production, Journal of Institute of Brewing, 105: 275 – 278.
17. Hallegren L (1995), Lager beers from sorghum in Dendy D A V, Sorghum and millets, Chemistry and Technology, American association of cereal chemist, St. Paul, MN, USA, 283 –298.
18. Hong, K.; Ma, Y. and Li, M. (2001) Solid-state fermentation of phytase from cassava dregs.pplied biochemistry and biotechnology. vol. 91 –93, p. 777-785
19. Hough J. S. (1985). The Biotechnology of Malting and Brewing. Baddiley J., Carey N. H., Davidson J. F., Higgins I. J., Potter W. G., (Eds) Cambridge University Press, pages 58, 59, 85 and 86
20. Kosaric, N., Vardar – Sukan, F. and Pieper, H. J. (2001). The Biotechnology of Ethanol, Roehr M. (Ed). WILEY – VCH Verlag GmbH. D-69469 Weinheim (Federal Republic of Germany), 2001, p78.
21. Kunze, W. (1995) Technology Brewing and Malting; pp19, 30,66,84, 192,285,334 and 5
22. Lewis, M., J. and Young, T., W. (1995). Brewing. Published by Chapman and Hall, 2 – 6 Boundary Roll, London SE1 8HN, UK, pp 89,142,160-2, 236 and 7.

23. Mac Fadden D P and Clayton M (1989), Brewing with sorghum – Use of exogenous enzymes, Brewing and Beverage Industry International, 1: 71 – 81.
24. Masschelein, C. A. (1989). Biotechnology Applications in Beverage Production Cantarelli C. and Langzarini, G. (Eds) Elsevier, New York, USA; pp 77 and 78
25. Nduele,M.; Ludwig, A. and Van Ooteghem, M.(1993) The use of Cassava starch in the formulation of gelatin capsules. *Journal de Pharmacie de Belgique*. vol. 48, no. 5, p. 325 –334
26. Nicphiarais, B. P, Wijngaard H.H and Arendt E. K (2005), 'Kilning of buckwheat', *Journal of the Institute of Brewing, in review*
27. Okrah, S. (2004). Annual brewhouse report, Guinness Ghana Breweries Limited, Kaase site, Kumasi; p 1.
28. O'Rourke, T., (1999) Back to the Basics – Brewers Guardian; pp 23 – 5
29. O'Rourke T. (2002). The Brewer International (Getting A-Head), Vol. 2, Issue 7; pp 10
30. O'Rourke, T. (2002). The Brewer International (Malt Specifications and Brewing Performance), Vol. 2, Issue 10; pp 27 – 29
31. O'Rourke T. (2002). The Brewer International (Predicting Colloidal Stability in Beer), Vol. 2, Issue 4; pp 41 – 42
32. O'Rourke T. (2002). The Brewer International (The Function of Wort Boiling), Vol. 2, Issue 2; pp19
33. O'Rourke T. (2002). The Brewer International (The Role of pH in Brewing), Vol. 2, Issue 8; pp 21 – 2
34. O'Rourke T (2002). The Brewer International (Malt specification and brewing performance) Volume 3; Issue 10, page 28.

35. O'Rourke T, (2003). The Brewer International (The Role of Oxygen in Brewing), Vol. 2, Issue 3; pp 45 – 47

36. Peppard, T. L., Meilgaard, M., C. (1986). The Flavour of Beers, Food Flavours Part B. Morton I. D and Macleod, A. J (Eds), Elsevier, York, USA, page 101, 112 and 113

37. Poste, L. M.; Mackie, D A; Butler, G and Larmond, E. (1991), Laboratory methods for sensory analysis of food pp1 and 52.

38. Rajeshwarisivaraj,Sivakumar, S,; Senthilkumar, P. and Subburam,V.(2001) carbon from cassava peel, an agricultural waste, as an adsorbent in the removal of dyes and metal ions from aqueous solution. Bioresource Technology, vol. 80, no. 3, p. 233-235.

39. Roble, N.D.; Ogbonna, J.C. and Tanaka, H. (2003) L-Lactic acid production from raw cassava starch in a circulating loop bioreactor with cells immobilized in loofa (Luffa cylindrica). Biotechnology Letters.vol. 25, no. 13, p. 1093 - 1098

40. Shimizu C, Ohno M, Araki S, Furusho S, Watari J and Takashio M (2002). Effective reduction of carbonyl compounds by yeast on flavour stability of Happoshu, Journal of the American Society of Brewing Chemist, 60: 122 – 129.

41. Steward G. G., (1996). The Brewer (Yeast Performance and Management), Vol. 82, No. 979; pp 211 – 214

42. Tan, K. H.; Ferguson, L.B. and Carlton, C. Conversion of cassava starch to biomass, carbohydrate, and acids by *Aspergillus niger*. Journal of applied Biochemistry, 1984,vol. 6, no. 1-2, p. 80 – 90.

43. Vaclavik, V. A., and Christian E. W. (2003). Essentials of Food Science; 2nd edition. Heldman, D. R., (ED) Kluwar Academic/Plenum Publishers, New York, USA, pages 50 and 51

44. Vuilleumier, S. (1993) Worldwide production of high-fructose syrup and crystalline fructose. American Journal of Clinical Nutrition. vol. 58, no. 5, p 733S-736S.

45. www.foodmarketexchange.com/datacenter/feedstuff/tapioca/detail/dc_pi_ft , 17/04/2008, 14:34GMT.

APPENDICES

Appendix 1

WORT PRODUCTION, FERMENTATION AND BLENDING

TOTAL GRIST WEIGHT 3000g
GRIST TO LIQUOR RATIO 1 to 6

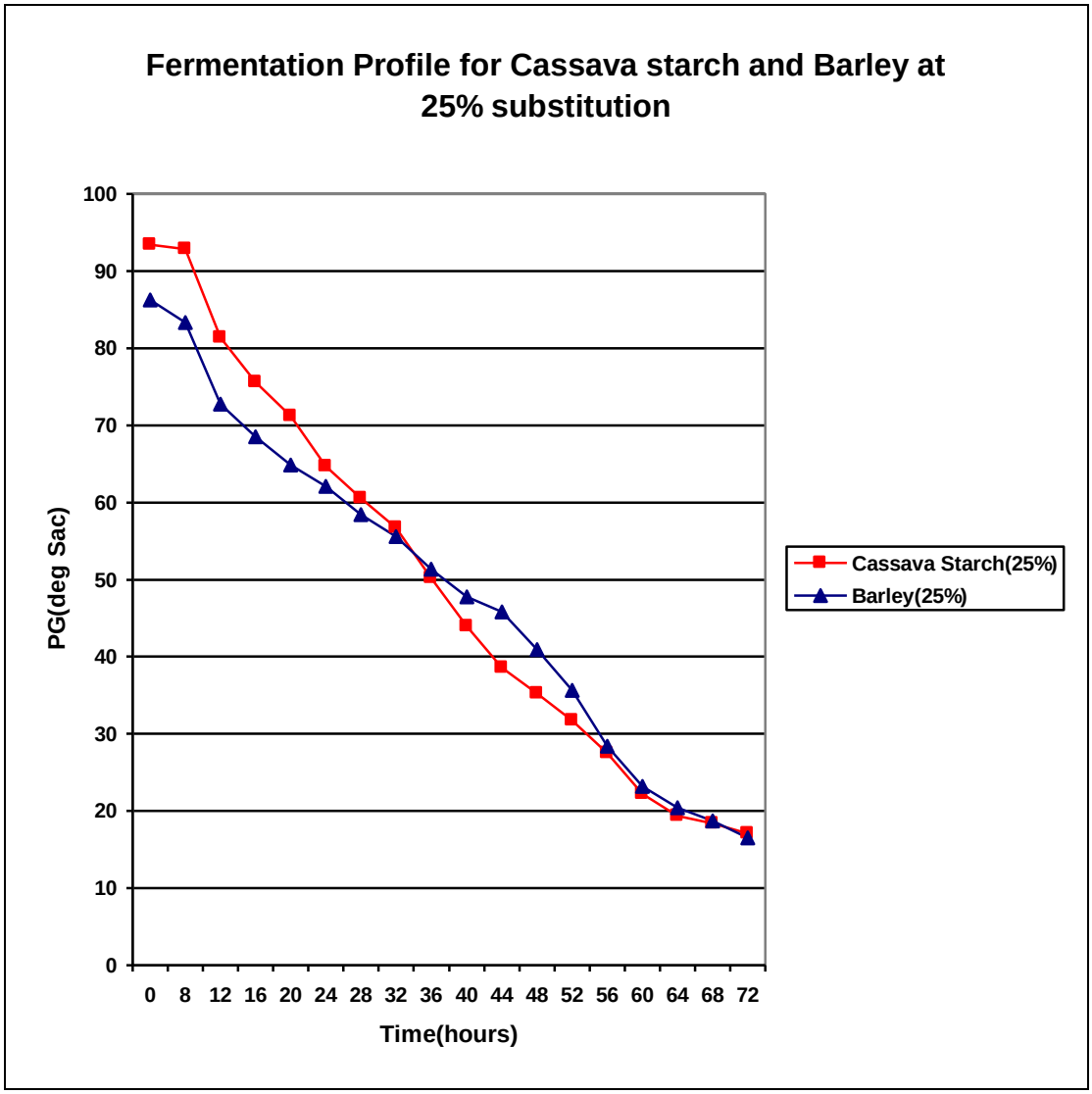
PERCENTAGE OF SUBSTITUTION			
%	GRIST (g)	BARLEY (g)	CASSAVA (g)
5%	2850	150	150
10%	2700	300	300
15%	2550	450	450
20%	2400	600	600
25%	2250	750	750
30%	2100	900	900
100%	3000	0	0

PROXIMATE ANALYSIS OF CASSAVA STARCH AND BARLEY

	BARLEY (SAMPLE A)(%)	CASSAVA STARCH(SAMPLE B)(%)
MOISTURE	$11.2_{(0.17)}$	$11.8_{(0.08)}$
ASH	$2.09_{(0.01)}$	$0.60_{(0.02)}$
PROTEIN	$4.43_{(0.04)}$	$0.43_{(0.01)}$

FIBRE	$3.75_{(0.04)}$	$3.67_{(0.05)}$
FAT	$0.60_{(0.00)}$	$0.50_{(0.02)}$
CARBOHYDRATE	$77.93_{(0.14)}$	$83.00_{(0.09)}$

Appendix 2



Appendix 3

QUESTIONNAIRE FOR OVERALL ACCEPTABILITY RANKING TEST

DEPARTMENT OF BIOCHEMISTRY

KNUST., KUMASI

NAME:……..

PRODUCT: ALE BEER

INSTRUCTIONS:

Using the numbers 1,2,3,4(as shown below) please indicate the intensity of the various characteristics of the coded products below.

1 – None

2 - Slight

3 – Moderate

4 – Strong

CODE	COLOUR	BITTERNESS	TINGLY	ESTERY	OVERALL ACCEPTABILITY
218	……	……	……..	…….	……
397	……	……	…….	…….	…….

692 …… …… ……. …… …….

854 …… …… ……. ……. ……

COMMENTS(OPTIONAL)

……

……

Thank you.