ABSTRACT

In this research, abridged infusion mashing trials were used to produce worts from three (3) sorghum varieties; SK5912, KSV8 and ICSV400 using a conglomerate of commercial enzymes to improve the wort properties as well as reduce the time for wort production. The grains were malted for six (6) days and several parameters related to malting were investigated. Thousand corn weight (TCW) was highest in ICSV400 (31.0g) while SK5912 and KSV 8 had (27.5g) and (25.0g) respectively. Moisture content (MC) was highest in KSV8 (10.5%) while SK5912 and ICSV400 had (9.4%) and (9.0%), respectively. Protein content was (10.4%) in ICSV400, (9.2%) in KSV8 and (8.5%) in SK5912. Germinative energy and germinative capacity ranged from 96% to 99% in all the varieties under this study. Malting loss was highest in KSV8 (18%) while SK95912 and ICSV 400 had (14%) and (16%) respectively, cold water extract (CWE) was highest in KSV8 (36%) while SK5912 and ICSV 400 had ((34%) and (32%), respectively. Hot water extract (HWE) was highest in ICS V400 233 $^{o}LKg^{-1}$, followed by SK5912, 230$^{o}LKg^{-1}$ and 228 $^{o}LKg^{-1}$ in KSV8. The Diastatic power (D.P.) was least in ICSV400 (18 ^{o}L) while SK5912 and ICSV 400 had (20^{o}L) and (22^{o}L), respectively. Worts were produced from malted and unmalted sorghum varieties using abridged infusion method, and subjected to various analyses according to IOB methods in order to determine their brewing properties. Such properties as carbohydrates, sugar analysis of various wort samples, total nitrogen, total soluble nitrogen (TSN), free alpha amino nitrogen (FAN) were determined. The worts from malted and unmated sorghum varieties were fermented for seven days and from the young beer fermented the following determinations were made: Percentage apparent extract, original extract, alcohol, apparent attenuation, and end degree of attenuation. In addition foam stability, P^{H}, viscosity (CP)/mPa.s) free amino nitrogen (mg/l) and bitterness (BU) were determined. Finally the effects of addition of Neutrase (a protease) on the quality of worts derived from malted and unmalted sorghum varieties were investigated. The parameters involved included: specific gravity. P^{H}, colour (EBC), carbohydrates (g%) and α-amino nitrogen FAN (mg/L). When the characteristics of fermented worts derived from malted sorghum varieties were compared with those from unmalted varieties it was discovered that worts from malted sorghum had higher values in both apparent and end degree of fermentation (%) – (80%) and (65.5%) against 79% and (64%), respectively. However worts from unmalted sorghum yielded more extract. Fermented worts

from malted sorghum varieties had lower values in alcohol content (3.38%) and foam stability 126 (EBC) as against (3.5%) and 129 (EBC). On the other hand fermented worts derived from unmalted sorghum had higher values in original gravity – (10.50%) – (10.65%) as well as higher viscosity values – (1.650 mPa.s) to (1.655 mPa.s). Other values were comparable except (FAN) which was higher in fermented wort derived from malted sorghum varieties. Significant differences were noted when the worts derived from malted and unmalted sorghum varieties were treated with additional neutrase (Protease). Worts derived from malted sorghum showed higher values in (FAN) which ranged from ((230 mg/L) to 260 mg/L as against 200mg/L to 230 mg/L in unmalted sorghum varieties. Carbohydrate values and specific gravity values, however, were lower in malted sorghum varieties than in unmalted varieties.

INTRODUCTION:

Beer is defined as an alcoholic beverage obtained from the fermentation by yeast culture of malt-wort from some cereals such as barley, wheat, sorghum and rice and flavoured with hop or hop preparations (Ogu, 2003). Beer is named according to the cereal from which it is produced hence we have all malt-beer and adjunct beer i.e. beer produced from malt and other suitable sources of carbohydrate. Apart from the above definition beer can also be named according to the particular cereal from which it is produced for example barley-beer, wheat-beer, rice-beer and sorghum-beer (Ogu, 2003).

Barley pioneered all the grains as brewing raw material and so acquired international acceptance in the brewing industries particularly in Europe and America. The reasons are obvious in that barley has high starch content, a strong husk which later serves as a filter-bed during wort separation and also rich in enzymes responsible for starch breakdown (Kunze, 1999). Wheat-beer is popular in Southern Germany where it is called "weissen-bier". Both barley and wheat can be used as adjuncts in their unmalted forms (Narziss, 1978).

In South Africa, Kafir-beer is popular, in Japan and China Rice-beer is native while in Nigeria and some other African countries sorghum-beer is very much available. The cereals can be used to produce either lager beer or ale that is bottom and top-fermented beers.

There has been a lot of innovations and modifications in the brewing industry. For instance the German Purity law-"Reinheitsgebot" is no longer observed except in Bayern (Southern Germany). The law states that "Beer should be brewed from barley malt, hops, yeast and water only. Presently, however, there have been some changes and modifications in the art of brewing the world over, for the following reasons:

1. There is no significant difference in the chemical composition of cereal grains.

2. Many countries would like to utilize what is locally available.

3. There is great need to conserve foreign exchange earnings, (Okolo and Ezeogu, 1995)

In 1987, Nigeria banned the importation of malted barley for the conservation of

foreign exchange. Sequel to this, local industries resorted to the use of sorghum in brewing assisted by the Universities and Research Institutes (Okoli, 2003, Aniche, et al. 1987). Nigeria presently uses both sorghum and maize in beer production and the beers have attained international standard. The use of sorghum for beer brewing in Nigeria has various limitations. Such limitations include inter alia the absence of husk which poses wort separation difficulties, low "Diastatic Power", that is, insufficient amount of α and β-amylases or their activities as compared to barley malt. Researchers are, however, trying hard to find solutions to some of these setbacks as to maximize the use of sorghum in Nigerian breweries (Agu and Palmer, 1988, Owuama, 1979).

Various enzymes are present in barley, sorghum and other grains used in brewing. These include α – amylase, β – amylase, proteinase eg neutrase, glucanases, pentosanases and phosphatases. Dicko et al,(2006) These enzymes play different roles during mashing. Eg. The amylases degrade starch, converting starch to sugar both fermentable and non- fermentable; the proteinases hydrolyse protein to amino – acids needed for yeast nutritiondressing fermentation. The glucanases and pentosanases breakdown glucans and pentosans which normally pose filtration problems. Apart from the enzymes inherent in the grains as mentioned above, exogenous enzymes are currently used in brewing. A starch degrading enzyme (an amylase) has been produced from Aspergillus carbomarius (Okolo et al, 2000) with optimum temperature of 40^oC at P^H 6. the enzymes retained 85% activity between 30^oC – 80^oC after 20 minuites incubation. Novo Nordisk, Denmark has produced and marketed microbial enzymes for brewing purposes. These include 'Cereflo', a blend of β – glucanase and α – amylase, Termamy – α termostable enzyme (75^o – 100^oC) and Fungamyl which is active at 60^oC – 65^oC. Thus the use of enzymes in brewing cannot be overemphasized.

The aim of this work therefore is to:

1. Develop a mashing process which shortens wort production time from malted and unmalted sorghum varieties.

2. Compare worts/beers derived from malted and unmalted sorghum varieties using exogenous enzymes.

3. Compare the cost analysis of conventional mashing programme and the newly developed mashing programme.

LITERATURE REVIEW

Brief History of Brewing

Beer brewing dates back to more than 5,000 years as the art of brewing was mentioned in Mesopotamia about 250BC. During the reign of King Hammurabi of Babylon, 1928 BC the production and distribution of beer was regulated in his laws (Kunze, 1999). Also Jasper and Philip, (1974) reported the brewing of alcoholic drink, beer, within the period. The middle Ages recorded brewing, mainly associated with the monasteries in Europe where beer was produced not only for their own consumption but also for supply to others. Kunze, (1999) reported the use of a mixture of flavouring plants called "grait" to replace modern hop but it was in the 14th century that the use of hops as the sole flavouring ingredient started. Northern Germany took the lead in brewing business whereas in the South only a few Princes were privileged to practise brewing. Such towns in the North like Bremen and Hamburg established large breweries in about 1300 AD. Brewing spread to the South of Germany, Munich taking the lead in Bayern (Bavaria). From Germany modern brewing spread to other areas in Western Europe especially Britain and Holland .As a result of such a development, Carlsberg Breweries (Denmark), Heineken Breweries (Amsterdam) and Brauerei Beck (Bremen) were established around 1873. The then largest brewery in the world, Bass-Brewery in Burton-on-Trent was established in 1876.

At about the same time European immigrants to the new world (America) established breweries first on the East Coasts in such places as Milwaukee, Wisconsin and Detroit. It is on record that Philip Schillinger, a German immigrant, founded the Birmingham Brewery which was the first to brew lager beer in Alabama in 1884. The first beer brewed by Shillinger's Birmingham Brewery was placed on the market on April 9. 1885. Eight thousand barrels were produced the first year and by 1886 the capacity of the brewery increased to 10,000 barrels ."The History of Brewing in Alabama".

Brewing trade and development spread to Japan and Australia between 1850 and 1865. Apart from ancient Egypt, the trend reached Africa not earlier than the 20th Century.

Nigerian Breweries plc was established in 1947 with the help of Heineken Brewery, Amsterdam. The first was in Iganmu, Lagos. Thereafter sprang up sister breweries in Aba, Ibadan and Kaduna (Kunze, 1999).

Though an old art, brewing has attracted a lot of interest in science, research and technology. Through exchange of ideas and interaction many countries have formed Guilds. Institutes and Conventions in order to solve technical problems and improve the quality of brewing.

In Germany for instance there exists the Doemen's Schule, for the training of young brewers, Techische University at Miinchen Weihenstephan and Versuchs und Lehranstalt fur Brauerei, the Institute of Brewing (IOB) in England while in America we have the American Society of Brewing Chemists (ASBC). There is in Europe, the European Brewing Convention (EBC).The establishments aforementioned have Scientific Journals and periodicals for teaching, learning and dissemination of information about brewing. Among these are: Brauwelt and Wochenschrift fűr Brauerei in German language, Journal of the American Society of Brewing Chemists and Master Brewers Association of the Americans (MBAA) journals.

Nigeria, with more than fifteen breweries can boast of self-sufficiency in brewing of beer. The country has as well many varieties of non-alcoholic beverages locally produced. Our pride in Africa as regards beer production is that the biggest single brewery, situated in Enugu, Ama Breweries PLC, is ours.

Raw materials in brewing

The main raw materials needed for beer production include water, malted grain e.g. barley, sorghum, rice and wheat, yeast and hops. All these materials have a vital role in determining the quality of the end product beer.

1. Water, which amounts to about 92% of beer is very important in that it is needed both for brewing and sanitation purposes (Narziss, 1978). Brewing water is normally treated to attain drinking water quality.

   By water quality, we understand a state of the water which it could be used without fear of contamination. To attain this desirable drinking water quality quality, the water is subjected to physical, chemical and biological examinations. This is necessary because the mineral, ionic content and P^H of water have profound effects on the type of beer produced (Okafor, 2007) calcium ion for instance is a very important mineral ion in the brewing process. It reacts with phosphates to form precipitates resulting in the releases of hydrogen ion (H^+) thereby lowering the P^H of the mash. This favours α – amylase, β – amylase and proteolytic enzyme activities. Such heavy metals as iron and cupper must be held at thresh hold levels – ion<0.2ppm, cupper <0.5 ppm; otherwise they may be toxic and at the same time affect beer stability. Zinc at concentration of 0.08 – 0.20 mg/l increases protein synthesis and yeast growth but beyond this level, there is the danger of fermentation problem and colloidal stability. Water treatment for brewing purposes may include use of chlorine, sterilizing filters, irradiation with ultraviolet rays and ion exchange resins, (Hough, 1983)

2. The most outstanding among the malted grains is barley. It was the first to be used and is still predominant. Other grains such as wheat, sorghum, rice, and oat are used as feed and food in many countries, apart from being used as adjuncts. Rice for example is the staple food of India and some other Asian countries. Not only are the grains used for brewing purposes they are also useful in the manufacture of non-alcoholic beverages in allied industries (Hough, 1985).

In order to reduce inevitable malting loss and to save starch and production cost such starch-containing substance as un-malted barley, rice maize and wheat are used in definite proportions for brewing. They are known as malt-replacement stuffs or adjuncts (Narziss 1980). These adjuncts must be converted to soluble form by the malt enzymes. For this reason as well as from the point of view of fermentation, yeast nutrition and lastly beer taste, adjuncts must be used in limited quantities. One of the most recent developments in brewing technology is the application of enzyme preparations to un-malted grains to reduce the need for malt. The use of adjuncts is not allowed in the Federal Republic of Germany.

Some exceptions may be made for export beers but again not in Bayern (Bavaria).

Of all the grains used in brewing, barley is preferred because it has husk, almost uniform size grains, rich in enzymes, high extract yield and less tannin and polyphenol (Hough, 1985).

3. **Hops**: Hops is inevitably added to wort. It lends to the wort a bitter taste, a definite aroma, and promotes wort clarification through protein precipitation. In addition, hops improve the quality of beer foam and serves as a natural preservative of beer.

The most important brewing technological portion of the hops is the female hops flower (Narziss 1980). It contains the bittering principles found in the lupulin glands.

4. **Yeast:** Yeasts are unicellular microorganisms wide spread in nature. They are divided into two groups depending on whether they form spore (Sporogenous) or not (Asporogenous). Beer yeasts belong to the sporogenous ones. Here again are two large groups to differentiate, which are basically different in behaviour: the top-fermenting yeasts (*Saccharomyces cerevisiae*) and the bottom fermenting yeast *Saccharomyces uvarum*). The top-fermenting yeasts, probably the stock, ferment at 15-25^{o}C, form spore clusters and rise to the top during intensive fermentation.

They are capable of fermenting the trisaccharide, raffinose to only one third ($^{1}/_{3}$).

The bottom fermenting yeasts on the other hand do not form spore-clusters but ferment at 5^{o}C to 10^{o}C and settle at the bottom at the end of fermentation. All the yeasts not belonging to the culture yeasts described above are called “Wild Yeasts”. They cause haze and off-flavour in beer.Yeasts can influence beer composition. Pure yeast culture is produced using the method of

Krűger and Bielig, (1976). In this method several single cells are isolated and allowed to multiply under sterile conditions in the laboratory. Yeast is rich in enzymes and these enzymes enable it perform the function of converting wort to beer through the metabolism of the sugar (manly glucose and maltose to ethanol and carbon dioxide and other by products (Narziss, 1980).

Table 1: Cereal Grains Percentage Average Composition (dry basis).

Grain composition (%)	Starch	Protein	Fat	Fibre	Ash
Sorghum	71.1	10.5	3.0	2.0	1.5
Corn	79	8.3	0.3	1.0	1.3
Wheat	69.8	13.2	1.9	2.6	1.8
Barley	63	10.5	2.5	5.4	2.7
Rice	85	8.5	0.6	0.75	1.0

Hahn 1966

Sorghum:

Sorghum is indigenous to Africa and thrives in semi-arid and sub-tropical conditions. It belongs to the grass family, the Graminae which includes barley, maize, rice, wheat, oat and rye.

The world production of sorghum is estimated at about 65 million tonnes yearly and amounts to about 11 – 12 % of the annual production of maize, wheat and rice together. Sorghum can tolerate very harsh environmental conditions such as drought as is the case in sub-Saharan Africa and so is usually cultivated where temperate cereals fail to grow (Palmer, 1992). There are more than 10,000 sorghum cultivars yet efforts are being made to cultivate sorghum grains of better quality. *Sorghum bicolor* (L) Moench and *Sorghum vulgare*, farafara are the most widely studied and rank fifth in the world after wheat, maize, rice and barley (Pomeranz, 1987). America (U.S.A) is the world's largest producer of sorghum. Sorghum is also produced in large quantities in India, Chain, Nigeria, Argentina and Mexico (Owuama, 1997). Sorghum is used as staple food in Nigeria and India as well as for the production of alcoholic and non-alcoholic beverage (Rooney *et al.*, 1988; Bello *et al.*, 1990). South Africa produces kafir beer from sorghum whereas Nigeria produces Pito and Burkutu in the Northern part of the country. Agu and Palmer (1998) reported the use of malted sorghum in the production of European type beer. This development has encouraged the mechanization of sorghum production within the last 10 – 15 years. (Palmer *et al*, 1989). Sorghum production in Nigeria by 1986 was about 4 – 5 million tonnes whereas in 1998, it stood at 12 million tones (Agu and Palmer, 1998).

Today, Nigeria is the third largest world producer of Sorghum. A lot of research is in progress in many countries for improving sorghum quantity and quality for both brewing ad food industries (Palmer, 1992; Okonkwo and Omoneyi,, 1998). Anyanwu *et al*, 2003 examined the influence of steeping temperature and germination time on the malting properties of red and white sorghum varieties. The authors discovered that high steeping and germination temperatures resulted in shorter period of malting but greater malting loss and less extract yield. Also, studies carried out by Anyanwu et al, 2004 revealed that the lautering performance of selected sorghum varieties was dependent on fine and coarse grist ratio as well as malt modification.

Upward infusion method of mashing was used by Anyanwu *et al*, 2006 in their studies on the use of *Azadirachta indica* (neem) as hop substitute in beer production using sorghum malt. The beeer

produced from 20% neem and 20% hops was tested organoleptically and found most acceptable to consumers because it compared favourably with beer brewed with 100% hops.

Table 2 shows the average composition and some properties of the sorghum grain.

Table 2: Sorghum grain Composition

Sorghum Grain Composition	% Dry weight
Embryo	8.0
Endosperm	86.0
Pericarp and testa-aleurone (Bran)	6.0
Starch	65.0
Protein	10.0
Lipid	3.5
β-D-Glucan	0.1
Pentosans	2.5
Lipid content (grits)	0.5 – 1.0
Gelatinization temperature	75^{o} – 80^{o}C

(Palmer, 1992b)

The Grain Morphology:

The sorghum seed is naked and the testa and pericarp contain high levels of polyphenol. There is a single-celled aleurone layer which is not responsive to gibberellic acid. (Hornsey 1999). The endosperm cell walls contain high levels of protein which can cause problems in the brewing of non opaque beer. The colour of sorghum varies from red to purple-brown, yellow and white to cream. Some varieties of sorghum do not retain their testa during development hence sorghum is a caryopsis (testa and seed coat are fused) (Palmer, 1992a). The phenolic materials in sorghum grain influence the properties causing astringency to the taste and desirable colour traits to develop in food products (Eneje et al, 2004).

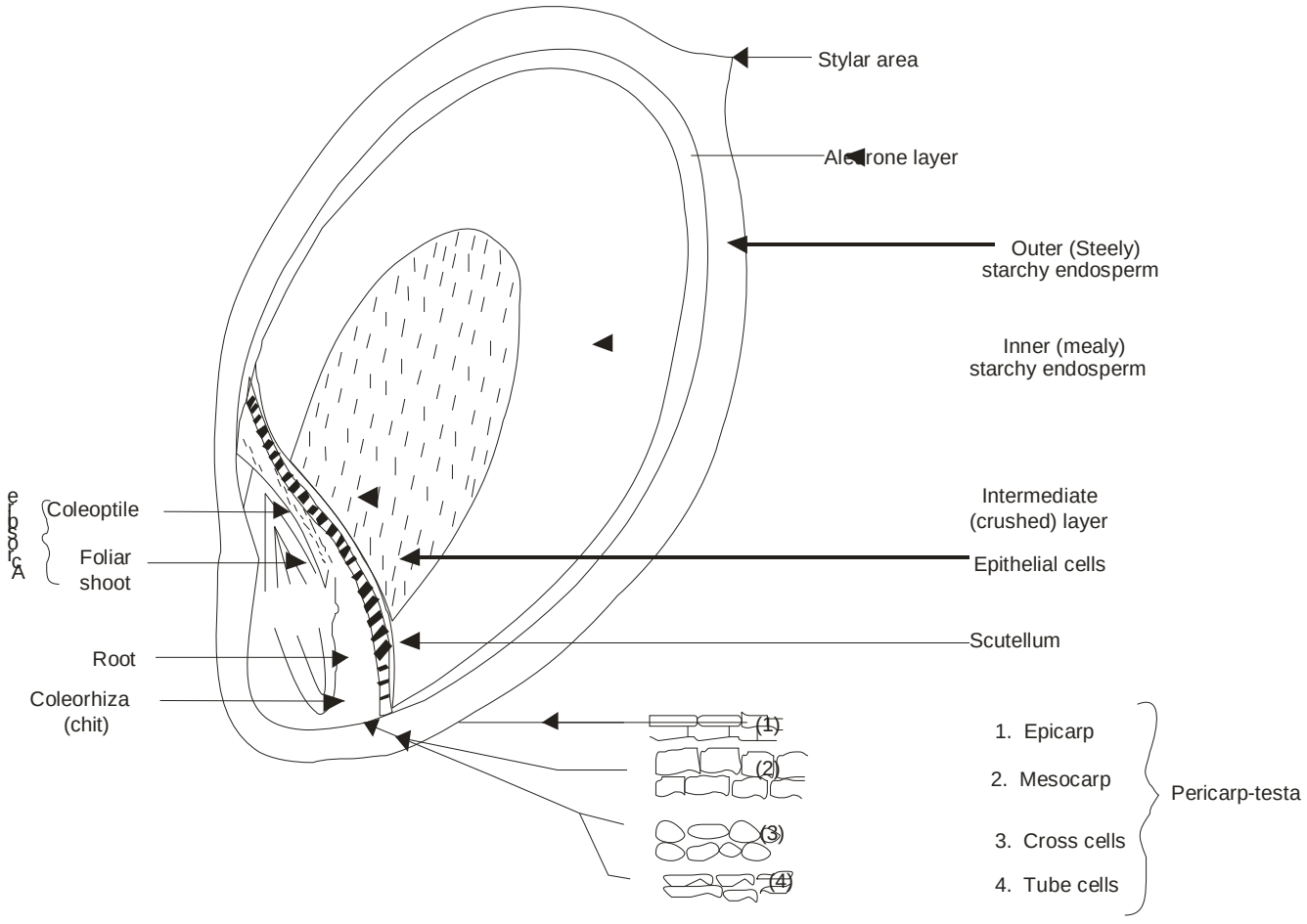

Figure 1: Longitudinal section of Sorghum Grain Etokaphan (1988)

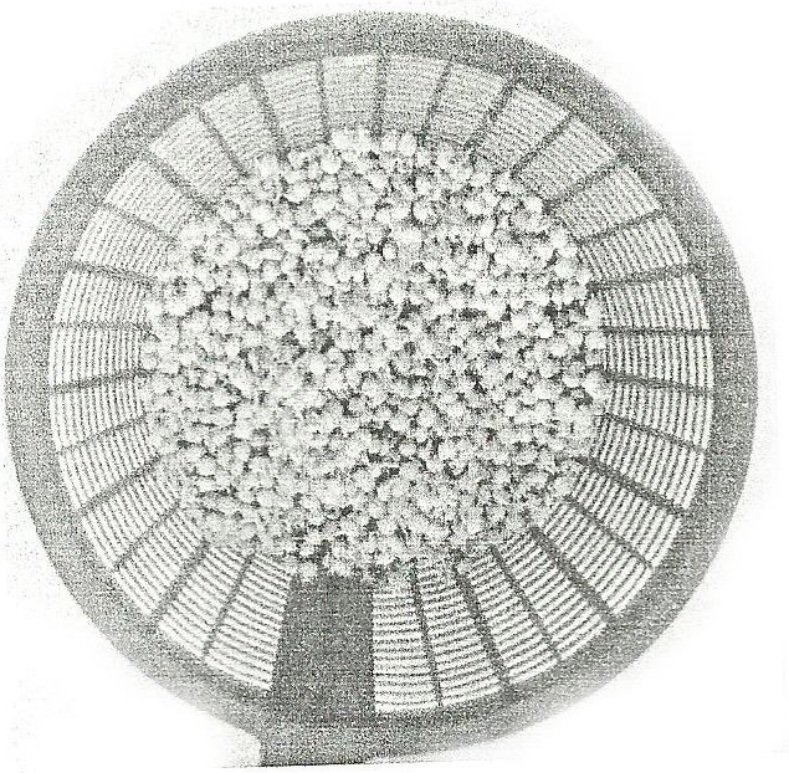

Fig. 2 : Sorghum grains after Steeping **(Anyanwu, 2004)**

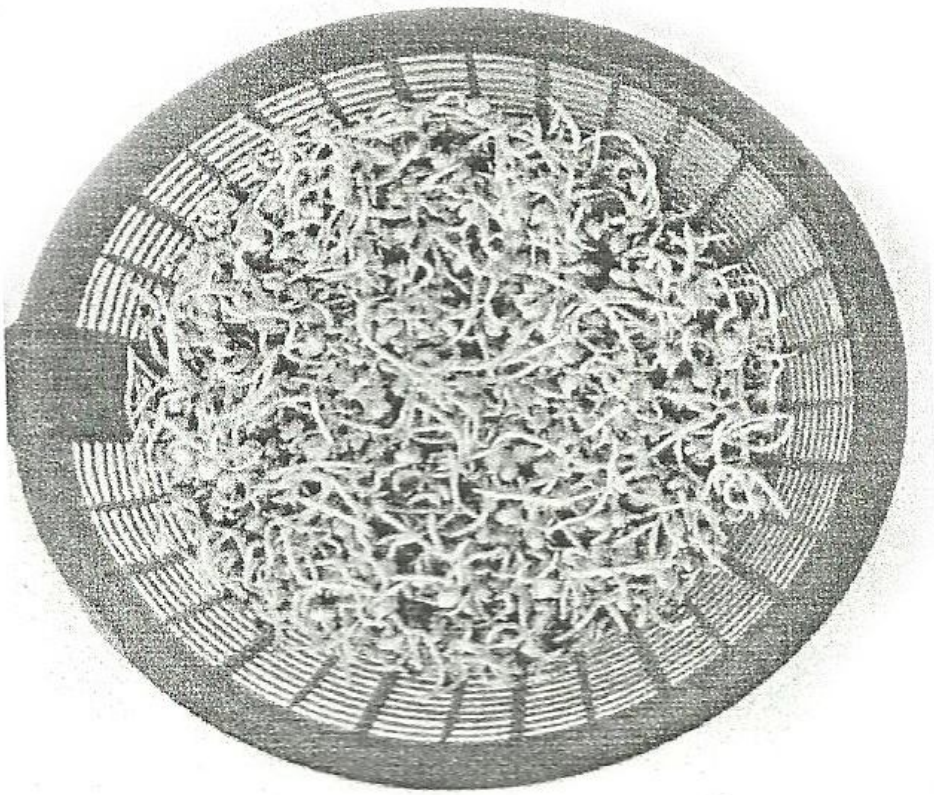

Fig 3 : Sorghum grain after Germination

(Anyanwu, 2003)

SORGHUM AS AN ADJUNCT

The importance of sorghum as adjunct was recognized in world war II. Adjucts in brewing are essentially starchy materials. They contribute to colour, taste, aroma, vitamins, and foam-head retention of beer. Unmalted maize, sorghum, soy beans, rice and barely flour have been added to grits as an adjunct. Sorghum grain contains soluble and insoluble fractions of amylases which are known to be active in some varieties of sorghum and are tenaciously attached to some other insoluble substances. The insoluble amylases may, however, be solubilized by breaking the link through a prolonged action of the grain protease during aqueous extraction. The activity of the amylases vary with the sorghum variety and apparently contributes towards hydrolysis during mashing optimal temperature for β- amylases, (60-65^{o}C) and α-amylases (72-75^{o}C). The optimal pH of the enzymes falls within 5 and 6 (Owuama 1972)

There are contradictory reports on the need to gelatinize starch adjuncts for the amylase to act. This is attributed to the differences in the finds of grinding, thickness of mash, or the quantity of enzymes present. Gelatinized sorghum grits, however, at different proportions have been mashed with barely malt to produce worts of varying sugar and protein contents. An industrial enzyme, termamyl used by Nigerian breweries for mashing unmalted sorghum, increased extract yield in wort when combined with malt. Moreover, the addition of external enzymes to 100% gelatinized sorghum malt during mashing produces lager beer which compares favourably with commercial brands produced from barely malt. (Owuama, 1997).

Embryo and Aleurone Layer Composition

When compared with barley sorghum embryo is larger and contains more unsaturated lipids (linoleic acid). The major sugar in un-germinated sorghum grain is sucrose, located in the embryo. In barley α –amylase is produced by the aleurone layer during malting. The embryo of sorghum has a single primary root whereas barley embryo has four. Sorghum embryo initiates food reserve mobilization directly by secreting large quantities of endosperm-degrading enzymes into the starchy endosperm (Palmer, 1989). Barley embryo on the other hand secretes gibberellins into the adjoining aleurone layer which is induced to produce and release endosperm-degrading enzymes into the starchy endosperm. Among these enzymes are α – amylase, protease, pentosanase and endo-β-1-3; 1:4 glucanase. Sorghum aleurone layer is

insensitive to gibberellic acid (plant hormone/growth factor) Hornsey, 1999, Aisien, 1983). The role of aleurone layer of sorghum in the mobilization of endosperm food reserves in sorghum is not yet understood.

The starchy Endosperm Composition

It is the largest tissue of the grain and as the name suggests, consists mainly of starch granules, storage proteins and cell wall which delineate the cells of the endosperm. Sorghum endosperm is made up of three parts viz floury, corneous and steely portions.

Viewed under electron microscope the floury and corneous areas of the endosperm show that the floury texture is due to loose packing of the starch granules; the corneous texture rather reveals the compact nature of the starch granules (Rooney and Miller, 1992); Palmer (1989) reported that protein deposits in the floury endosperm appear in matrix form whereas similar deposits in the corneous endosperm are recognized as protein bodies with some tiny indentations in the starch granules. It is observed that the structural differences between the floury and corneous endosperm influence the nature of flour or grits retained during milling. Fine flours are released from the floury areas of the endosperm whereas grits are produced from the hard corneous layer of the endosperm. Normal sorghum grain starch is composed of 75% amylopectin and 25% amylose; waxy starch from waxy sorghum on the other hand is made up of 100% amylopectin (Rooney and Miller, 1982). Considering starch hydrolysis, waxy starch is more easily hydrolysed than normal starch – a difference attributable to differences in chemical composition of these two starch types (Palmer, 1989). Sorghum has more concentration of β-D-glucans in the cell walls than in the endosperm. The same is applicable to barley. It is estimated that β-D-glucan concentration in barley by far out weighs that of sorghum in the ratio of 1:30. Since β-D-glucan causes viscosity problem and so delay in beer processing especially wort separation and beer filtration, it is obvious that sorghum has advantage over barley in this aspect. This is the case when sorghum is used as an adjunct in the form of grits. (Palmer, 1989)

Sorghum processing into adjuncts: Cereal grains such as maize, rice, wheat, raw barley can be used as adjuncts (malt replacement) in the breweries. In the USA about 60% replacement is obtainable while in the U.K about 20%. Germany does not allow the use of adjuncts in beer brewing (Narziss, 1978). It is possible to use these grains as adjuncts because of similarity in their chemical composition. Sorghum has corneous endosperm which has good gritting quality and is used in brewing lager beer. Palmer, (1992) suggested that because of its good gritting quality countries that brew with it should concentrate on producing sorghum cultivars with corneous endosperm layers. Sequel to this suggestion several cultivars of sorghum such as SK5912, KSV8-11, ICSV 400 and KSV4 have been developed for the beverage industries in Nigeria. White sorghum varietry is widely used for beer brewing because of its low polyphenol content. Coloured varieties are utilized by food industries because in this area the question of haze and astringency which would detract beverage quality does not arise (Palmer, 1992). Agu (2003) and Eneje et al.(2004) suggested that coloured sorghum would not cause brewing problems. When sorghum grits are used with barley malt to produce lager beer, it is observed that sorghum supplies a higher amount of α –amino nitrogen than other adjuncts as shown in Table 3. The importance of this that α –amino nitrogen is required by yeast during fermentation so that yeast can grow and produce alcohol and flavour compounds. It is recommended that breweries using only grits as the only source of fermentable sugars use a high quantity of a mixture of microbial enzymes during mashing.

Sorghum grits for beer brewing are so processed as to have less than 1% lipid content in order to avoid stale flavour, characteristic of oxidized lipids in beer.

Table 3: Average Wort Properties of Brewers wort Adjuncts:

Adjuncts	Extract yield oP	pH	Total (Nitrogen)	Amino N_2 (/ppm)
Sorghum grits	12.0	5.3	782	317
Corn Grits	12.0	5.35	820	259
Brewers Rice	12.1	5.35	812	239
Corn Syrup	12.0	5.30	785	181
Barley Malt	12.0	5.40	1345	415

Hahn, 1966

(Bajomo and Young, 1992) observed that the negative effect on the beer would be poor foaming quality since the enzymes would digest short peptide chains of protein that support foam-head as well as low free amino nitrogen (FAN) content.

Use of Sorghum Malt in Modern Beer Brewing.

Malting of sorghum: The aim of malting is to convert the cereal grain to malt. Before malt can be obtained, the grain has to be subjected to steeping, germination and kilning (drying). During these processes the grains develop hydrolytic enzymes which degrade them.

The malting of sorghum can therefore be defined as the controlled germination of sorghum grain during which enzymes are developed and the food reserves (endosperm) are sufficiently modified so that they can be further hydrolyzed during mashing (Narziss, 1977). Palmer et al. (1989) defined malting as the germination and growing of cereals during which the grains develop hydrolytic enzymes which degrade the substrates of the storage endosperm to sugars, protein amino acids and compounds which react to impart a malty taste and aroma after the kilning process. In Africa sorghum has been used in malted form to produce both alcoholic and non-alcoholic beverages. These include Tortilla produced from 100% sorghum grits. Tortillas are one of the best in mechanisms for testing sorghum grist quality. Otika is an alcoholic beverage which is traditionally brewed from sorghum malt and drunk along the West-coast of Africa especially in some parts of Nigeria. Kafir beer is also brewed from malted sorghum by a method in which the starch conversion to fermentable sugars is by a souring process (Ihekoronye and Ngoddy, 1985). Other beverages like Burukutu and Pito are very popular in the northern parts of Nigeria, Republic of Benin and Ghana. Others include Talla, an Ethiopian small-scale beer and Merissa which is popular in Sudan. (Novellie, 1977). During sorghum malting steeping takes between 36-50 hours in order to enable the grain absorb sufficient moisture to the tune of 33-38% as the pericarp is not so porous as that of barley (Evans and Taylor, 1990).

Barley on the other hand can be steeped out with 44-48% moisture within the same period (Baxter, (1978); Baxter and O'Ferrel, 1980). The steep water is usually changed at 6-8 hourly

interval. This helps clean the grain and maintain a good level of oxygen in the steep water. It is advisable to steep sorghum with 0.1% formaldhehyde per litre of water to avoid microbial contamination (Agu and Palmer, 1999).

At the end of steeping the grains are transferred to the germination compartment. Germination takes about 5-6 days at room temperature 28^{o} -30^{o}C. Within this time, humidified air is passed through the bed either from above or below (up draft or down draft). At designated intervals water is sprinkled on the germinating grains to avoid dehydration. At the same time the grains are turned gently to break up the rootlets and so avoid matting, aerate the bed and remove excess heat and so maintain appropriate germination temperature (Narziss, 1978).

At the end of germination the green malt is transferred to a kiln and kilned at 50^{o}-55^{o}C for 24h. This measure preserves some of the enzymes.

The essence of kilning is to arrest further growth of the grain, render the malt friable and provide malt flavour in the malted grain (Agu and Palmer, 1997). Barley on the other hand is malted at a lower temperature 15^{o}-18^{o}C and can be kilned at 60^{o}-80^{o}C or even 100^{o}C depending on the type of malt and beer to be produced. This is due to the physiological differences between barley and sorghum grain (Hough et al, 1982).

Advantages of Sorghum in Brewing

1. Sorghum is a cheap source of brewing raw material both locally and internationally as it is resistant to draught and thrives within the Savanna regions of Africa.
2. The high temperature (25°C-3O°C) required for sorghum malting is an advantage because the cost for refrigeration for barley malting is quite high.
3. High α-amino nitrogen level of sorghum malt will reduce or cancel the need to use additives during beer fermentation.

4. Serious viscosity problems will not be encountered during the processing of sorghum because of its low levels of β-glucan unless they result from incomplete saccharification (Agu and Palmer, 1998).

Malting qualities of Sorghum:

Malting loss: Malting loss is the loss encountered when losses arising from steeping, germination and kilning are taken together. It results in the volume and weight of the end-product, malt, being less than that of the cereal grain (Narziss, 1978). Malting loss does not include cleaning, sorting and storage losses because only cleaned and sorted grains are malted. Barley grain for instance has a moisture content of 12-16% before steeping but after kilning the malt has 2-4%, moisture. This is known as loss (dry basis), and lies between 16 and 25%.

Calculation of malting loss is of economic importance in malt production; it can be calculated from the difference in weight between the grain and the malt Narziss (1978) gave the formula for malting loss calculation as:

$$\text{Malting loss} = \frac{G - M}{G - 1} \times 100$$

Where G = Quantity of grain steeped.

M = Quantity of sproutless malt

Novellie (1992) and Malleshi and Desikacher (1986) defined malting loss as the summation of leaching/steeping, metabolic/respiration and vegetative/sprout losses i.e the loss in a given weight of grains after malting. The authors noted that malting loss in commercial Kaffir beer malts are due only to metabolic and leaching losses since the roots and shoots are not usually removed but are milled with the grain. Malting loss due to respiration and germination are influenced by the malting conditions. Respiration/metabolic loss arises from degradation of starch and oil to CO_2 and water.

Both are dependent of the prevailing germination conditions and occur or experienced during steeping, germination and kilning.

The entire loss is dependent on;

(a) Moisture level during germination, - the higher the level the more does the grain respire.

(b) The temperature of the grain bed - the higher the temperature the more the loss

(c) Composition of the air in the grain bed-the more CO_2 in the grain bed, the less the Respiration and growth.

(d) The character of the malt to be produced – malting loss increases with increasing modification and longer period of germination.

There are many possibilities to reduce malting loss factors and these include: shortening the period of germination, use of CO_2 in the ventilation of the grain bed, double steeping process, germination by falling temperatures eg. From 17-12^oC and finally use of growth factors and inhibitor e.g gibbrellin and potassium bromate. Formaldehyde and dilute H_2SO_4 would equally have the same effects (Narziss, 1987). Pathirana et al, (1983), Beta et al, (1995), however, noted that decreasing malting loss by reducing temperature or moisture level leads to a marked decrease in diastatic power, while Novellie (1962), suggested that a respiration/metabolic loss of 10-15% is recommendable for a well malted sorghum with good diastatic power.

Enzyme development during malting:

By choice of the changes which influence growth factors viz- moisture, temperature, oxygen and time, the biological processes of germination can be manipulated within certain limits. In barley the first appearance of the radicle is called "chitting" and this is followed by the first rootlets (fork) while the plumule grows out at the apex of the grain. Apart from these growth appearances, enzymes begin to metabolize the reserve stuffs of the endosperm and change them to soluble form. These activities lead either to the accumulation of energy or the formation of new tissues in the radicle and plumule. This is followed by the appearance of the growth factors (Gibberellic acid, Gibberellin A3) which follows a system of vessels until it reaches the aleurone layer near the schild. The growth factors which are formed throughout the period of germination cause the new formation of a series of enzymes in the aleurone layer. These enzymes include α-amylase, limit dextrinase and endopeptidase. Gibberellins also promote the development of endo-β-glucanase, endo-xylanase and phosphatase. Other interesting hydrolytic enzymes include the hemicellulases and proteolytic enzymes (Narziss, 1978). β-amylase in barley grain exists in

an “inert” form but is activated as soon as germination begins. Sorghum β-amylase on the other hand appears to be absent or very insignificant in vareities where it exists at all (Taylor and Robbins, 1993). In both barley and sorghum α-amylase develops during germination and increases with time in concentration, modifying/degrading the starch in the endosperm. Many hydrolytic enzymes are synthesized in the embryo in sorghum whereas they are synthesized in the aleurone layer in barley as afore-mentioned (Varner and Chandra, 1964; Ainsien et al; 1983; Aniche and Palmer, 1990). Since gibberellic acid is required for enzyme synthesis in barley whereas sorghum is insensitive to ($G.A_3$,) it is clear that these two brewing cereals show outstanding physiological differences. While phosphate is located in the embryo in sorghum it is an important mineral in barley aleurone layer.

(Palmer et al; (1989), and Ainsien et al: (1983), observed that such enzymes as endoproteases, and –glucosidase also develop during malting.

Mashing with Sorghum Malts:

Mashing is a portion of the brewing process in which malt particles are dissolved in water. The solution obtained at the end of mashing is called wort and the sum of the dissolved portion of the malt is the extract (Narziss, 1980). In order to dissolved the malt stuff a series of enzymes are needed which through their activities reduce the high molecular organic substances of the endosperm to lower molecular weight and so render them water soluble. Starch degradation is the most important enzymatic process in mashing. Malt starch which is made up of amylase and amylopectin is stored in form of starch grains. The dissolution embraces the bringing together of the starch grains with warm water in different stages and involves mechanical, chemical and enzymatic processes (Narziss, 1980). The aim of mashing is to obtain as much extract as possible and this involves enzymatic activities at different temperature and p^H optima (Kunze, 1999).

During mashing enzyme rests are held to enable the enzymes utilize their optimum temperature and p^H at different intervals:

a. Protein and β-glucanase rest $45^o - 50^oC$

b. Maltose rest $62^o - 65^oC$

c. Saccharification rest $70^o - 75^oC$

d. Mash-off temperature $78^o - 80^oC$

Types of Mashing:

There are two main types of mashing:

a. Decoction process and (b) Infusion process.

<u>Decoction Process</u>:

Decoction process involves boiling part of the mash and returning it to the main mash in mash vessel (mash tub). Usually about one third ($1^{1/3}$) of the whole mash volume is withdrawn and boiled for about 10-15 minutes and then returned to the mash tub and its temperature is raised. There may be three, two and single decoction mash processes but to save time and energy fewer part mash boils are encouraged.

Advantages of decoction process

a. Higher brew house exploit (yield)

b. Suitable for less modified malts

c. More extensive gelatinization and saccharification

d. Increased rate of extraction

e. Increased formation of melanoidines

f. Reduction of enzyme activity in the total mash

g. Less protein breakdown is obtained in the boiled mash because of the more rapid heating

h. Increased removal of dimethyl sulphide (DMS)

The disadvantages of decoction process include more energy consumption and longer time to complete a brew. It is estimated that a decoction mashing process takes about $5^1/_2$ hours to complete and about 20% more in energy consumption (Narziss 1980). Infusion process on the other hand can end within 3 hours with corresponding savings in energy.

For a three mash decoction process figure 6 the in-mash temperature lies between 39-40^{o}C. One third of the total mash is then pumped to the mash cooker, heated to 65^{o}C and held at this temperature for about 30minutes. It is then raised to boiling for over 40 minutes and held for 15 minutes for pale beer and 45 minutes for dark beer and then pumped back to the mash-mixing vessel. After the mixing the mash temperature rises to 50-52^{o}C the optimum temperature for both β-glucanase and proteolytic enzymes as pointed out earlier.

Again the second 1/3 of the mash is withdrawn, pumped to the mash cooker and brought to boiling in 30 minutes and held at this temperature for 30 minutes and returned to the total mash for mixing. This brings the total mash temperature to 65.5^{o}C for β-amylase activity | for maltose production. The last $^1/_3$ portion of the mash is treated like the previous ones heated to boiling and mixed with the main mash whose temperature is now raised to 72-75^{o}C, a temperature suitable for α-amylase activities. Thereafter the enzymes are inactivated by raising the temperature of the entire mash to 78-80^{o}C. The last $^1/_3$ mash should not be “thick mash” because the 1st and 2nd part mashes after their treatments must have driven the enzymatic activities far enough. (Narziss, 1980)

Sorghum malt mashing depends on the higher gelatinization temperature of (75-80^{o}C) but barley gelatinizes at about 60-65^{o}C. When infusion mashing is used for sorghum malt, the resulting wort shows no saccharification as iodine test is always positive but decoction mashing process has some considerable success depending on the degree of gelatinization of the mash and the level of enzyme protected before gelatinization is reached (Agu and Palmer, 1998). Owuama and Okafor (1987) reported that in the three stage decoction process about 70% of the mash is boiled, thereby ensuring greatest gelatinization of starch and enough opportunity for proteolytic and amylolytic enzyme action. This process also affords minimum opportunity for the development of lactic acid bacteria (Skinner, 1976). Mashing of Sorghum malt at 65^{o}C and 70^{o}C for 30 minutes each at the second and third stage decoction process provides wort with complete hydrolysis (Okafor and Aniche, 1980; Solomon et al;1994). Maintaining the mash for a longer incubation time at the saccharification of 65^{o}C than the dextrinizing temperature of 70^{o}C gives wort with higher reducing sugar levels (Owuama and Okafor, 1987). The authors, however, recommended that maintaining the mash at 65^{o}C for 60 mins, in the second stage and third stage for 60 minutes, respectively for the three stage decoction process was the best in the results obtained by them (Owuama and Okafor, 1987).

Infusion Mashing Process:

This process involves the degradation and dissolution of malt particles in spite of the grist composition only through the effect/activities of the enzymes in the malt without the mechanical help of heating/boiling. Starting from an in-mash temperature of 35^{o}C a protein rest of about 30 minutes, a maltose rest by 62^{o}-65^{o}C

for 30 minutes and finally by 70-72^{o}C a saccharification rest is maintained until iodine normality is achieved. Prolongation of this resting period is neither necessary nor advantageous. Thereafter mash off is done at 78^{o}C. The process is cheap, saves time and energy but tends to loss of extract if malt quality and grist properties do not correspond. As the amylases could not be effective through boiling there could be danger of lower degree of fermentation by unsatisfactory malt quality. This problem, however, can be overcome by use of upward infusion process. Infusion process is also favourable for non-well modified malt, fine grist and wet-milling (Narziss, 1980). As a purely enzymatic process it takes into consideration wort

composition and at the end of mashing the enzymes are less inactivated compared to the boiling process that is decoction method. The remaining, some-how greater quantity of α-amylase is still effective during sparging and the peptidases could within a definite space of time degrade nitrogenous substances Narziss. (1980).

Infusion mashing process takes about 3 hours; which means that within 24 hours 8 brews can be produced in the absence of any interruption. Beers produced by infusion mashing tend to have a neutral and less pronounced taste on account of the absence of mash boiling, which is also responsible for less coagulable nitrogen in the wort, which must be precipitated during boiling. Consequently the beer shows bitter stuff loss. Upward infusion mashing process is suitable for strongly modified malts and here again for definite beer types for instance; Ale/Stout beer (Narziss, 1980).

The advantages of infusion process include:

1. It takes less time
2. It is cheaper (No adjunct cooker etc used)
3. It requires less energy (20% less than decoction)
4. Easy automation
5. Easy monitoring technique.

Less extract yield/brew house exploit is the major disadvantage of infusion mashing process. In spite of that, many brewing industries adopt infusion mashing process because it saves both time and cost. Narziss, (1980).

Extract Yield in Sorghum Malt during Mashing:

One of the most recent innovations in brewing technology is the decantation type of mashing. In this process all the enzymes active at various temperature regimes 45^{o}C, 55^{o}C and 72^{o}C are withdrawn before the rest of the mash is gelatinized at 100^{o}C and then mixing with the enzymes extract at about 65^{o}C or about 70^{o}C. It is discovered that the wort so produced is not only rich in free amino nitrogen (FAN) but with high extract yield (Agu and Palmer, 1996, 1997).

Mashing sorghum malt according to EBC procedure (1987) involving an enzymic malt extract with pre-cooked malt insoluble solids, produces wort with an approximate maltose to glucose ratio of 4:1 just like the ratio in the barley malt. Normal malt mashing without pre-cooking produces wort containing maltose to glucose ratio in about equal amount (Byrne et al; 1993; Taylor and Dewar, 1994). Again it is observed that mashing at pH4.0, which is close to the optional pH for α-glucosidase or maltase (maltase catalyses the hydrolysis of the terminal, non-reducing α- (1,4) linked glucose residues causing the release of glucose from oligosaccharides), produces wort with a relatively higher proportion of glucose than mashing at pH5 .0-5.5. EBC (1987) conventional method of mashing provides more glucose as sorghum α-glucosidase is highly insoluble in water and usually attached to the insoluble solids. But when the pre-cooked malt is employed, the α-glucosidase is inactivated hence the assumed conversion of starch to glucose is inhibited. The upward infusion method, produces more sugars and peptides and amino acids in the wort than the downward infusion method in sorghum malt. This could be attributed to the inactivation of the saccharifying and proteolytic enzymes at the initial high temperature of 70°C, (Owuama and Okafor, 1987).

Adding of external enzymes during mashing of sorghum malt, however, seems to increase the extract yields (Agu et al,1992). The activity of β-amylase (which catalyses the hydrolysis of the penultimate α,glycosidic bond at the non- reducing end of polysaccharides causing the release of maltose) in malted sorghum significantly increases when a combination of final warm water and air rest cycles are employed in malting sorghum. This results in β-amylase activity which is 27-49% of the total diastatic power/activity depending on the sorghum variety (Ezeogu and Okolo 1995). Novellie, (1962) had earlier noted that protein seems to play a minor role in determining the quality of sorghum malt because high protein content in sorghum malt causes no brewing problems. The author further stated that this is / attributed to the apparent degradation of most of the high molecular weight proteins into simpler compounds during mashing and the removal of coagulated protein sediment which results from wort boiling. (Owuama and Okafor,1987) reported that the amino acid content of worts obtained by mashing sorghum malt with the three step decoction, upward and down ward infusion mashing methods are apparently similar.

Approximately 30% free amino nitrogen (FAN) which is essential for yeast growth during fermentation is produced during mashing at *51°C* and pH4.6, while the remaining 70% is

preformed in the malt and adjunct (Taylor and Boyd, 1986). Sorghum beer contains a low percentage of proline, thus indicating that the free amino nitrogen is mostly metabolized during the fermentation (Taylor and Boyd 1986b). There is a slight advantage in using decantation mashing to produce wort in terms of FAN content (Agu and Palmer 1996) although the *65°C* conventional mash would always produce enough FAN to support the yeast metabolism during fermentation which is in the range of 100-l4Omg/l of FAN. Mashing of sorghum malt at conventional temperature of *65°C* also results in the production of high levels of peptides. Yeast metabolizes small peptides but its activities in (FAN) metabolism is of greater dimension compared to those of peptides (Agu and Palmer,1997; Bajomo and Young, 1992). It is therefore important to know that the conventional mashing system *(65°C)* for barley malt can only favour protein degradation by the protein enzymes when sorghum malt is used. Brewing with sorghum malt Table 4, would therefore require development of a suitable mashing regime which would be different from that of barley malt and would be capable of delivering high extract yields, high total soluble nitrogen (TSN) and high free amino nitrogen content respectively (Dufour et al; 1992; Agu and Palmer, 1998), since the gelatinization temperature of sorghum grain is high *(65-75°C). (*Okolo and Ezeogu 1995) showed that malting sorghum in slightly alkaline condition at a final steep temperature of 40°C with adequate air rest, favour reduction in malting loss and root length with increase in diastatic power (DP) and proteinase concentration.

Table 4: Properties of Sorghum, sorghum malt and Extract at Malting

Parameter	Sorghum	Sorghum Malt	Extract
Moisture%	9.4-13.0		
G.E (%4m1 test)	80.99		
Total Nitrogen	1.47 – 1.74		
Protein(N x *6.25)*	9.2 – 10.9		
Out of steep moisture (%)		33-36	
Malting loss %		21.3 – 28.5	
α- amylase		39 – 13.5	
β-amylase		80 – 168	
Wort Colour			5.5 – 12.0
HWE(%/Kg)			270 – 327 (89 – 196)
T.SN (%)			0.5 - 0.7
FAN(mg/L)(Ninhydrin)			(0.3 – 0.6) 135 – 316
FAN(mg/L TNBS assay.			(94 – 216) 164 – 412
TRS(mg/ml)			(172 – 350) 98 – 188
Glucose (mg/l)			24 – 220
Maltose (mg/l)			68 – 245

Key: HWE = Hot water extract; TSN = Total Soluble Nitrogen

FAN = Free Amino nitrogen TRS = Total reducing sugar

Values in parenthesis are from 65°C mashing regime

Agu and Palmer (1998)

NON- STARCH POLYSACCHARIDES:

The non- starch polysaccharides of sorghum are mainly located in the pericarp and endosperm cell walls and include cellulose and hemicelluloses (β-1,3)(1,4) D-glucan and arabinoxylans. Cellulose is (5-6%) and located exclusively in the cereal grain husks and acts as a structural substance (Kunze, 1999). It consists of long unbranched chains of 1,4 linked glucose residues bonded to one another with β-linkage arrangements ; unlike in starch where the bondage is in alpha position. Hemicelluloses are the main constituents of the endosperm cell walls. They form part of the cell wall and support their rigidity. Narziss, (1980) reported that cellulose in barley has neither taste nor odour, insoluble in water and is difficult to attack both chemically and enzymatically. It does not partake in metabolism but leaves the malting process unchanged. It is only during lautering process that it plays an important role as filter sheet. Analytically, it is regarded as fibre. The ones found in the pericarp are composed of mainly pentosan, a little bit of beta glucan and a small quantity of uronic acid. In solution, they contribute to viscosity. Endosperm hemicellulose has a high content of β-glucossan, a little pentosan and no acids. (Kunze, 1999). The β-glucan has a molecular weight of 200,000 and composed of glucose units which are bound to one another in β-1,4 (70%) and β-1,3 (30%) Palmer (1989).

β-glucans and petosans have different structures and very different effects on beer production and quality. Kunze (1999). The hemicelluloses when incompletely degraded produce the disaccharides cellobiose and laminari biose. The pentosans are composed of xylose units in β-1,4 linkages through which xylose and arabinose side-chains are attached. Though hemicelluloses are insoluble in water, they can be dissolved by dilute sodium hydroxide or enzymatic activities. (Narziss, 1980).

Gummy stuffs are water –soluble hemicelluloses of higher viscosity made up of β-glucan and pentosan and form with water colloidal solutions. Barley content of hemicellose and gummy stuffs vary greatly, it normally amount to 2%. Lignin is an incrusted substance of non-starch polysaccharides which is stored within the cell walls of the pericarp and lends rigidity to the cell wall Narziss(1980).

Soluble β-glucans and pentosans are very viscous and usually create filteraion problems in wort. They have also been detected in non-biological haze particles. Barley contains about 4% β-glucans and 9% pentosans by weight while sorghum, maize and wheat have about 9% pentosan content and about 0.9 β-D- glucan content.(Palmer, 1990). While barley cell walls are almost completely degraded during malting process, sorghum produces low levels of pentosanase and β-glucanases. This deficiency in cell wall degradation by enzymes is an important practical obstacle in brewing with sorghum and sorghum malt because good filterability of wort or the final beer is not guaranteed (Aisien and Mutts, 1987; Palmer, 1989).

Higher wort viscosities alone cannot be held responsible for poor wort filtration. Insufficient development of (1-3) (1-4)) β-glucanase in sorghum malt, release of (1-3), (14)β-D glucanase in wort which is not further degraded is also contributory (Dufour et a1 1992; Etokakpan, 1992). Even though the quantities of these glucans are too low to cause a high wort viscosity, it is believed that the aggregates can polymerize and become trapped in the filter pores causing filtration difficulties especially in the presence of un-degraded proteins and pentosan (Dufour et a11992). This is in spite of low β-D glucan content of sorghum when compared to that of barley. When sorghum adjuncts are used in lager beer brewing a complex mixture of exogenous enzymes are needed.

The use of malted sorghum would certainly reduce the quantities of these enzymes during mashing and wort filtration. As sorghum malt may release some β-D glucans into the wort, commercial β-glucanase can be added to eliminate any problems which these substances may cause during beer filtration (Palmer, 1989).

Cereal Eznymes (Carbohydrases):

Malt contains many enzymes which degrade malt carbohydrate. The important ones include α-amylase, β-amylase, β -glucosidase, β-glucanase (cytase) and α-dextrinase (debranching enzyme). The long starch chains are degraded to smaller dextrins containing *7* to 12 glucose residues by α-amy1ase. This enzyme is not present in

an unmalted grain but develops only during the germination of the grain (Narziss, 1980). It has an optimum temperature of 72-75°C, optimum pH of 5.6-5.8 but is rapidly destroyed at 80°C On starch hydrolysis its attack is internally as endo-enzyme at α -1,4 positions.

β -amylase: It attacks amylase or amylopectin molecule from outside and degrades single maltose units. β-amylase is present in resting grain, already, but is transformed from latent condition to active mainly during germination by appearance of activators through the degradation of inhibitors or releasing from protoplasmic linkages. The total activities of both α- and β-amylase are determined by the estimation of the diastatic power (DP (Narziss 1980).

α- and β-amylase produces both glucose and maltose, have an optimum temperature of 60-65°C, optimum pH of 5.4-5.5, very sensitive to higher temperatures and is rapidly inactivated at 70°C.

α- Glucosidase:

This is one of the enzymes involved in starch degradation during grain germination. The activities include catalysis of α- Glucosidase bonds in glucans and oligosaccharides yielding glucose. It is known to hydrolyse maltose *50* times faster than it can act on starch (Manners, 1971). α-Glucosidase is easily inactivated above *65°C.*

Limit_dextrinase: Pollock(1979), reported that limit dextrinase hydrolyses the *1,6* linkage in the dextrins which remain after amylolytic digestion of starch. The enzyme is also heat labile and can be easily inhibited above *65°C.* Limit dextrinase is one of the enzymes which are newly formed in the aleurone layer by the development of growth factors which are active throughout the period of germination. Other enzymes include α-amylase and endopeptidase (Narziss1980)

Endo – β-Glucanase: The development of endo- β-glucanase, endoxylanase and phosphatase is promoted by gibberellins. Apart from this, through the degradation of protoplasmic bond or release of activating groups eg. Sulfhydryl groups activation of exo-enzymes is established eg. β-amylase, various exopeptidases. The products of these various enzymes, namely lower molecular substances taken up by the shield and passed on to the seedling (Narziss, 1980). The above-mentioned enzymes attack non-starch polysaceharides usually referred to as gums and hemicellulose. Gums are β-glucans and pentosans which are soluble in hot water whereas hemicellulose refers to β-glucans and pentosans which are not soluble in hot water. β-Glucans

are degraded by endo-β-1,4 and endo-1,3 glucanase. The α-1,4-linked disaccharide is called cellobiose and is degraded by the enzyme cellobiase whereas the α-1,3- linked disaccharide is called laminaribiose.

Pentosan contains a polymer of β-1.4-linked xylose with side chains of single β- 1,3-linked arabinose units. They can be called arabo-xylans. Pentosans are sparingly degraded into pentose sugar by the enzyme pentosanase during malting. All these enzymes increase many folds as a result of the activities of giberrellic acid.

Other enzymes of malt include:

Proteolytic enzymes, especially carboxpeptidase and proteinases.

Carboxypeptidases degrade the ultimate amino acids. Phosphatases, esterases and glycosylases are enzymes that can degrade fats, phospholipids and glycolipids releasing sugars (glucose) and 1ipids (Hough,1985). Because of the importance of these enzymatic activities for the character of the beer later, protein degradation should neither be so short-lived nor unnecessarily prolonged. So are amino acids important for yeast nutrition whereas higher molecular polypeptides on the other hand are important for foam retention and mouthfulness of the beer.

The Debranching enzymes or R-enzymes. Among the debranching enzymes are

α- **Glucosidase:**

(a) Pullulanase *(E.C. 3:2.1.41)*

(c) Isoamylase(E.C.3.2.1.68)

(d) Glucoamylase(E.C.3.2.1.3)

α- **Glucosidase:** is a malt enzyme, not high temperature resistant but capable of reducing both α-1.4-and α-1.6 - glucosidic bonds of starch amy1opectin from the non – reducing ends.

α- **Glucosidases:** are widely distributed among microbes such as bacteria, fungi and yeast.

Those found to be active optimally at slightly acidic and neutral pH values and temperatures between 4O-55^{o}C have been produced from such aerobic bacteria as Bacillus subtilis, Bacillus amylo1iquefaciens, Pseudomonas and Myxobacter species. Urlaub and Weber (1978), reported that β- amyloliquefaciens and P.amyloderamosa are capable of hydrolyzing α-1.6 and α-1.4 linkages in short chain saccharides. Recently, α-glucosidases optimally active at a temperature range of 105-110°C have been identified in archea such as Pyroccus woesei and P.furiosus (Linke 1991). None of these enzymes, however, has been further investigated for use in brewing operations.

Pullulanase/lsoamylase are obtained from microorganisms. Pullulanase is obtained from Aerobacter aerogenes or Klebsiella aerogenes whereas isoamylase is obtained from yeast and moulds. In combination with α-amylase and β-amylase they can produce glucose and maltose mo1ecules very easily.

Glucoamylase also called amyloglucosidase belongs to the group of exo- α -1.4 glucan glucohydrolases. It attacks starch chains and releases glucose from the non-reducing end, at pH range of 3.0-6.0 and temperature *55-60°C.*

Commercial glucoamylase can be produced from Aspergillus awamori, A. niger and Rhizopus niveus. Amyloglucosidases can hydrolyse starch to give theotically 100% glucose yield (Gerhartz,1990). The principal application of glucoamylase is in the production of light beer. Table 5 represents the properties of amylases for commercial application.

Table 5: Properties of Amylases for Commercial Application.

Source	**Amylase Type**	**P^H Optimum Range**	**P^H Stability Range**	**Temprature Optimum range (^oC)**	**Effective Temprature Range (^oC)**
Aspergillus Oryzae	A- amylase	4.8 – 5.8	5.5 – 8.8	45 – 53	Up to 60
Bacillus Subtilis	A- Amylase	5.0 – 7.0	4.8 – 8.5	60 – 70	Up to 90
Barley malt	A-amylase	4.0 – 5.0	4.9-9.1	50-65	Up to 70
Porcine Pancreas	A-amylase	6.0-7.0	7.0 – 8.8	45-55	Up to 55
Barley malt	B-amylase	6.0 – 5.5	4.5 – 8.0	40 – 50	Up to 55
Aspergillus niger	Glucoamylase	4.0 – 4.5	3.5 – 5.0	55 – 60	Up to 70
Aerobacter aerogens	Pullulanase	5.0 – 6.0	5.0 – 7.0	45 – 55	Up to 60

Allen and Spradlin, 1974

Industrial Enzymes of Microbial Origin:

Some industrial enzymes of brewing interest are derived from microbes, hence they are described as microbial enzymes. These include: amy1ases, proteases, lipases, pectinases, invertases, cellulases and amyloglucosidases. They are obtained mainly from fungi, bacteria and yeast as aforementioned. These enzymes are used to save cost of malt in that they complement that which ought to be introduced from malt. They are therefore known as external or exogenous enzymes. The enzymes that convert starch first solubilize it, then liquefy it and finally saccharify it into fermentable sugars. (Narziss 1980). To obtain more fermentable sugars, addition of other carbohydrate enzymes such as glucoamylase and pullulanase have been suggested (Enevoldsen, 1975).

The characteristics of some starch degrading enzymes are shown in Table 6.

Table 6: Characteristics of some starch degrading enzymes

Amylase type	**Alpha – amylase**	**Beta – amylase**	**Glucoamylase**	**Pullulanase**
Specificity	α -1,4 glucosidic bond	x-1, 4-glucosidic bond	α-1,4 and α -1.6-glucosidic bonds	A -1,6-glucosidic bonds
Attack Mechanism	Endo-amylase	Exo-amylase	Exo-amylase -	Exo-amylase
Bond Limitation	Can by-pass but cannot hydrolyse α -1.6 glucosidic bond	Does not possess the ability to by- pass nor hydrolyse α -1-6-glucosidic bond	Possesses ability to hydrolyse and thus by-pass α -l,6-glucosidic bonds	Does not possess the ability to by pass but hydrolyse α -1-6-glucosidic bond
	Dextrins	**β -maltose**	**Glucose**	**Maltose**
Loss in viscosity Products	Fast	Slow	Slow	Slow
Loss in iodine colour	Fast	Slow	Slow	Slow
Glucose Production	Slow	None	Fast	None
Maltose production	Slow	Fast	None	Medium
Dextrin Production	Slow	Fast	Medium	Slow
Increase in reducing power	Slow	Fast	Medium	Slow

Allen and Spradlin (1974)

A close look at Table 5 reveals that alpha-amylase of Bacillus subtilis most resembles the malt alpha-amylase during mashing. The temperature optimum range and effective temperature range of the fungal (Aspergillus oryzae) and pancrease α -amylase are too low to be useful for the liquefaction of barley starch (Amos, 1955 and Miller et al, 1953). Most of the commercial α -amylases proposed for use in barley brewing originate from Bacillus subtilis. Since fungal α-amylase is inactivated before the starch gelatinizes, the addition of such alpha-amylase becomes ineffective. In this case the addition of bacterial alpha-amylase is most effective since it can hydrolyze the starch after the substrate has been rendered fully susceptible to enzyme action by gelatinization. Wieg (1968), noted that the heat stability of bacterial alpha-amylase is very well used in the enzymatic liquefaction of various starch products. On the other hand fungal alpha-amylase is applied in the baking industry (Bothma,1966,Wieg, 1968).

Commercial Enzymes for Protein Degradation.

The externally added proteolylic enzymes for barley brewing would be of microbial or plant origin. Culture filtrates of B. subtilis are probably the richest and most convenient sources of microbial protease for industrial use. Hagihara (1960), reported that a strain of B. subtilis produced two types of extracellular protease of optimum pH 7.0 and an alkali protease of optimum pH l0.5. Some proteolytic enzymes of commercial importance are those of the mold Aspergillus oryzae and Aspergillus niger, and from papaya a plant (papain). The neutral protease of Bacillus subtilis has its optimum activity at temperature range of 45- 50^{o}C while that of alkaline is at 65-70^{o}C.

Enzymes for the conversion of non-starch polysaccharides.

The externally added, β-glucanases can be of malt, fungal or bacterial origin. The specificity of these enzymes is as well as their temperature and pH optimum ranges are shown in (Table 7)

Table 7: Characteristics of some Typical Beta- glucanase

Source	Barley malt	B-subtilis	A. oryzae	A. niger
P^H Optimum range	4.5 – 5.3	4.5 – 7.0	4.0 – 6.0	3.0 – 6.0
Temprature optimum range	40 - 45°C	45 – 50°C	45 – 55°C	45 - 65°C
Degrades barley β-glucan	+	+	+	+
Laminaran	+	+	+	+
CMC	+	+	+	+
Lichena	+	+	+	+

Wieg, 1968.

The advent of microbial enzymes has revolutionized some industries. In the Netherlands for instance Naarden International manufactures enzymes for use in the breweries. Recently they have put in trade some more active and temperature stable enzymes including Brew-N-enzyme, AT, a bacterial thermostable alpha-amylase for starch liquefaction with a pH range of 5.0-8.0 and a temperature range of over (70°C-90°C). The enzyme is in non-viscous liquid form, brown in colour and miscible. Apart from Brew-N-enzyme AT, the company also produced two other types of enzymes namely: B.rew-N-enzyme GPGL. (alpha amylase), optimum temperature 70-75°C, which is active up to 90°C with calcium ion, pH range *5.5-8.9.* Others include Brew-N-enzyme GPG-L and LSH, proteolytic in nature, pH range 5-8 and temperature range 40-65°C. These also have endo and exo-glucanase activity and will break down β-1,3 and β-1,4 linkages of β-glucan even at pH range of 3.0-5.0 and temperature range of *65-85°C* (Wieg1969).

In 1984 the Novo industries A/S Denmark produced the following enzymes. Promozyme 200L and AMG 300L (debranching enzymes). Fungal 800L (fungal alpha-amylase) and Termamyl 1200L (bacterial-alpha-amylase and a thermostable enzyme). These enzymes are still very useful in most breweries including Nigerian breweries (Olsen, 1984).

Quest International, Ireland has produced a series of improved enzymes very useful to the brewing industries. They include Hitempase 2L, a heat-stable alpha amylase from a genetically modified strain of Bacillus licheniformis. The pH range is 5.0-8.0 and a temperature range of 75-92°C, but still effective at 100°C.

Bioform L, a fungal amylase from Aspergillus oryzae, P^H 4.0-6.0 and a temperature range of 55-60°C; Bioprotease N100L, a proteolytic enzyme from Bacillus subtilis, P^H range 4.0 -8.0 and temperature range 40-50°C and Bioglucanase ME 250, an endo betaglucanase active preparation for both β-1,3 and β-1.4 -linkages of β-glucan are among the enzymes currently in use.

Bioglucanase ME 250 is a product from a complex microbial mixture of Penicillium emersonni and Bacillus subtilis and has a pH range of 5.0-8.0 and a temperature range of 50-70°C. Others are Bioglucanase HS derived from Trichoderma reesei and Penicillium emersonni. It contains beta-glucanase, cellulase and hemicellulase activities with a pH range of 4.0-7.0 and temperature range of 50 - 75°C.

Biofase Plus L is a beta-glucanase with a pH range of 3.5-4.8 and temperature of 50-65°C. Profix

6500 is a papain from Carica papaya, used for chill-proofing. Majority of the enzymes are very useful to both breweries and syrup-producing industries in many countries of the world. They have helped to improve starch hydrolysis, reduce filtration problems and even haze- precursors to the barest minimum (Narziss 1980).

MATERIALS AND METHODS

MATERIALS

Sorghum Cultivars

The sorghum cultivars under investigation (SK5912, KSV8 and ICSV 400) were obtained from the Institute of Agricultural Research (I.A.R), Ahmadu Bello University Zaria, Nigeria.

Source of Enzymes

Termamyl 120L (α-amylase), AMG300L (amyloglucosidase) and neutrase (a protease), all products of Novo Nordisk, Denmark, were kindly supplied by Prof. F.J.C Odibo of the Department of Applied Microbiology and Brewing, Nnnamdi Azikiwe University, Awka , Nigeria.

Yeast . The bottom fermenting yeast, Saccharomyces uvarum was obtained from Life Breweries Limited, Onitsha, Nigeria.

Methods:

The sorghum samples were sorted by hand to remove broken kernels, damaged grains and other materials foreign to sorghum, which might cause microbial contamination during malting. Preliminary assessments such as moisture content, thousand corn weight, total nitrogen content, germinative capacity, germinative energy cold and hot water extracts and diastatic power were carried out.

Grain Analyses:

All the analyses done in this work were done according to the Institute of Brewing methods of analyses (lOB) 1979 and Palmer Laboratory methods.

Determination of Thousand Corn Weight.

Five hundred kernels of each sorghum cultivar were taken and shared into two portions of about 20g using a sample divider. Foreign matter such as (soil, silts and twigs) was removed and the samples weighed in tarred beakers of 50ml capacity to the third decimal place. The grains in each sample were counted by hands using a tally counter. The total weight taken – approximately 40g was divided by the total count of both samples and the calculated weight of 1000 corns reported one place of decimals

Determination of Moisture Content;

Twenty grams of the sorghum varieties were weighed out and finely ground with Bűhler Miag Mill (setting 2). Five grammes of each sample were placed in a moisture dish, covered and weighed to 0.001g accuracy. The cover was then removed and the dish placed in an oven preheated at a temperature of 105^{o}C for three hours. The dish was then covered with the lid and placed in a dessicator and allowed to cool for about 20min to room temperature. The dish was then reweighed to 0.001g and the moisture content (M.C) of the sample was then calculated as follows:

$$M.C. = \frac{W1-W2}{WI} \times 100$$

Where W1 = weight of sample before drying

W2= weight of sample after drying

Determination of Germinative Energy (G.E):

Germinative energy indicates the percentage of corns capable of germinating within 72h at 18°C in 4ml of water. Two filter papers were placed at the bottom of Petri dishes and wetted with 4ml of water. One hundred corns from each sample were separately added in the Petri dish and allowed to stand for 72h. The number of germinated corns were counted after 72h respectively.

G.E (%)= 100-n

where n = number of corns that did not germinate after 72 hrs.

Determination of Germinative Capacity:

A fresh solution of 0.75% solution of hydrogen peroxide was prepared by diluting 5m1 of 30% H_20_2 in l00ml of water. Two lots of corns were obtained. Each lot was steeped in 200m1 of fresh hydrogen peroxide solution for 48 hours in the first instance. The steep liquor was then changed with a fresh 200m1 H_20_2 solution for another 24hours. The solution was finally strained and the corns counted for those that have germinated or shown sign of germination.

Germinative capacity G.C(%) = $\frac{200 - n}{2}$

where n number of ungerminated corns.

Root Lengths:

At the end of Germinative Capacity determination, one hundred grains developed from each sample were randomly picked and their root lengths measured with the aid of a pair of veinier calipers and ruler and their average lengths recorded.

Malting of Sorghum grain:

The malting process started with sorting during which broken kernels, stones, sand and other foreign matter were removed by hand.

Steeping:

Two hundred and fifty gramms of each sorghum variety was steeped in 400ml tap water containing 0.1% formaldehyde. The formaldehyde was used to reduce microbial load and prevent foul odour. The first wet steeping lasted for 6 hours and was followed by another 18 hours air rest while the third wet steeping lasted for 4 hours. During the periods of air rest the grains were sprinkled with clean water after every 8 hours to avoid dehydration.

The total time for steeping was 50 hours and the steeping temperature was room temperature. (28-30^{o}C)

Germination:

Germination of grains was conducted in a shallow tray with fine mesh bottom in wooden boxes in a humidified environment for 6 days. Within this period, the germinating grains were turned every 8 hours with some water sprinkled on them to reduce matting of the roots and dehydration due to respiratory activity of the grains. Samples were taken from day 2 to monitor malting loss and diastatic activity.

Kilning

The germinated grains (moisture content, 48% green malt) were transferred to a hot air-oven preheated to 50°C and allowed to kiln for 24 hours at that temperature. After 24 hours, kilning was stopped and the samples were reweighed, the rootlets and shoots, removed by rubbing them between the palms to obtain malt. The difference in weight before malting and after malting gave the malting loss.

$$\text{Malting loss (\%)} = \frac{W1 - W2}{W1} \times 100$$

where W1 = wt. of grain sample before malting

W2 = wt of grain sample after malting

Malt analysis:

Moisture Content of Malt

The sorghum malt moisture content of the malt samples was determined as earlier described for the unmalted grains.

Determination of hot water extract.

This was carried out according to the methods of analysis of the American Society for Brewing Chemist (ASBC,1958) modified by Aniche (1982)

About 50.2g of each malt sample milled with Bűhler-Miag grinder at set *7* to obtain a good coarse/fine mixture was weighed into a one litre beaker containing 360m1 of disti1led water pre-heated for 10min in a water bath at 68°C. The temperature of the resulting mash was 65°C. The

beaker was covered with a clock glass and maintained at 65°C for exactly l hour; while stirring the mash at 10min. intervals. At the end of the period, the mash was quickly cooled to about 28°C in ice chips and then transferred to a 515ml flask. (Aniche 1982). The beaker was rinsed out into the 515ml flask with distilled water and thoroughly mixed for 5 minutes. After standing for 30 minutes, the entire mash was filtered in a 15cm diameter funnel through 32cm diameter Whatman No.1 filter paper. The specific gravity of the filtrate was then determined within one hour of filtration using a specific gravity bottle.

Further collections of the filtrate were kept for colour determination. The hot water extract (HWE) was calculated as follows:

HWE (as is)= Excess Gravity x 10.13=Wet extract in °L/Kg .

$$\text{HWE of Extract (dry)} = \frac{\text{Extract (as is)} \times 100}{100 - M}$$

where M=Moisture percentage of sample.

Determination of Cold Water Extract:

This was carried out according to the Recommended Methods of Analysis of the Institute of Brewing (1997). About 10g of ground sample were suspended in 200ml distilled water containing 12m1 of 0.1M ammonia solution. The mixture was incubated at 20°C for 3 hours with mixing at 30 minutes interval. The mixture was then filtered through Whatman No 1 filter paper. The specific gravity was then determined at 20°C

$$(\%)\ CWE = \frac{G \times 20}{3.86 \times 1}$$

where G= The excess degrees of gravity of the filtrate taking water at 20°C as 1000 ie G=1000 (SG -1).

Determination of diastatic power (DP) of malt.

The diastatic power of the malt samples was determined according to the recommended method of Analysis of the Institute of Brewing (1977). In principles, a malt infusion was obtained as described for the cold water extract (CWE). Ten millimeters of the aliquot were pipetted into 100ml of 2% buffered starch solution attemperated at 20^{o}C in a 200ml flask placed in a water bath for 1 hour. Thirty milliliters of a 0.1M sodium hydroxide (NaOH) solution was added to stop reaction and the solution was made up to 200ml at 20^{o}C with distilled water. Five milliliters of mixed Fehling's solution (solution A and B) were added into a 150ml narrow-necked boiling flask. The digested starch solution was titrated into the cold Fehling's solution to within 1ml to end point. The mixture was mixed in the flask and boiled with moderate ebulition for 2 min. Then 3 drops of methylene blue indicators were added and boiling continued until decolorisation of the boiled mixture. This was followed by the precipitation of Cu^{++} ions and the liquid became red with little titration.

Diastatic Power (DP) in (IOB) units was calculated as :

$$\frac{2000}{XY} = {}^{o}\text{Lintner}$$

Where X= ml malt extract used in conversion

Y = ml of conversion liquor used in titration.

A blank titre was determined by titrating the undiluted 2% starch solution against a mixture of 1ml of mixed Fehling's solution A and 2ml of Fehling's solution B using methylene blue as indicator.

Estimation of reducing sugars.

Reducing sugars were determined using the method of Miller (1959).One millilitre (1ml) of 3.5 dinitrosalicyclic acid (DNS) solution was added to 1 ml of supernatant of wort sample, in a test tube and the mixture heated in boiling water for 10 min. The test tube was cooled rapidly in tap

water and the volume adjusted to 12 ml with distilled water. A blank containing 1 ml of distilled water and 1 ml of DNS was also prepared. The optical density of each sample was read against the blank in a spectrophotometer at 540nm. The reducing sugar concentration in the samples was estimated from a standard glucose curve. Appendix 10.

Determination of Total Reducing Sugar.

This was determined using the revised recommended method of analysis of the Institute of Brewing (IOB) 1989. The results were calculated as the most appropriate sugar using the appropriate factor from the Lane and Eynon table. (appendix 6.)

Fehling's solution A & B in equal volumes, in addition to 1% methylene blue were prepared through a standard method described by (I0B) (appendix 5)

Twenty five (25ml) of the wort was used and diluted 10 times by making up the volume to 250 ml in a 500 ml conical flask. The flask was vigorously shaken to ensure thorough mixing. Twenty five (25 ml) of mixed Fehling's solution (equal volumes of Fehling's solution A and B) were pipetted into a 150ml narrow-necked boiling flask. The diluted solution was added from the burette to the cold Fehling's solution almost sufficient to effect reduction so that if possible not more than 1ml would be required to complete the titration. The flask content was mixed and heated over wire gauze under the oven. It was kept in a moderate ebullition for 2 min. and without removal from the flame, three drops of methylene blue indicator were added and the titration was completed in one minute with continuous ebullition. The end point was marked by the reaction liquid changing to red with some precipitations. The flask was then removed from the flame and placed against a white paper. The reducing sugar (total glucose and total maltose) could be obtained thus:

$$\% \text{ sugar } \frac{\text{glucose}}{\text{maltose}} = \frac{\text{Lane and Eynon factor X 10}}{\text{titre}}$$

Lane and Eynon factor represents the mg of sugar, which will reduce 25ml of Fehlhings solution. It is obtained from Lane and Eynon table. (Appendix 6)

Determination of Free α-amino nitrogen (FAN)

The Ninhydrin method of the AOAC, (1980) was used. Two millilitres (2ml) of clear wort sample (appendix 4) were mixed with 1ml of Ninhydrin colour reagent (appendix 3) stoppered and heated in a boiling water bath for 10 min. After cooling for 20 min to 20^{o}C, 5 ml of diluting reagent,(potassium iodate solution) (appendix 3) was added and thoroughly mixed. The absorbance was measured at 570 nm within 30 min. of mixing using a UV/visible spectrophotometer. A standard glycine solution was exactly treated as the test solution. Duplicate determinations were carried out and α-amino nitrogen in mg/litre was calculated from the reaction.

$$\text{Free amino nitrogen (FAN)} = \frac{\text{0D of sample x 2 x dilution factor}}{\text{mean 0D of standard solution}}$$

Determination of Fermentable sugars

The specific gravity of a sample was determined at 20^{o}C.

Two hundred ml wort was put in a 500ml conical flask and 12g pressed yeast added. The flask was then continuously shaken so as to keep the yeast in suspension. After 24 h shaking at 20^{o}C, half of the sample was filtered through a covered filter paper and the SG was measured at 20^{o}C. The shaking was continued with the remaining portion for a further 24h. and again the specific gravity determined. The lower value was taken for calculation.

Calculation:- The percentage fermentable sugar is given by:

$$\% \text{ F.S.} = \frac{OG - FG \times 100}{OG}$$

Where OG = Original specific gravity

FG = Final specific (Or Attenuation limit gravity).

Note: This formula gives the apparent fermentability. The real fermentability is obtained by multiplying the apparent fermentability by a factor which is 0.819 for wort, 0.857 for glucose or invert sugars.

Determination of total nitrogen/protein in wort. (Kjeldahl Method).

The Kjeldahl method described by AOAC (1980) was used. Ten (10)ml wort was quantitatively filled in a 500ml round bottom flask. This was followed by the addition of 10g seleinium reaction mixture and 20ml of conc H_2SO_4. The mixture was well shaken and allowed to react until foam formation lessened. Thereafter it was boiled until the brown colour disappeared. Finally it was boiled again for 10 min and the liquid became light-green colour. It was then cooled and step-wise diluted with 250 ml distilled water.

Then about 70ml sodium hydroxide was carefully poured into the round bottom flask so that a watery distinction of layers between the mixture and the sodium hydroxide could follow. There after Boric acid indicator solution (30ml) was put in a 250 ml Erlenmeyer flask and so connected to the distillation that the cooling pipe of the distillation apparatus dipped well in the liquid. The distillation was stopped when the volume of the Erlenmeyer flask reached 180ml. The cooling pipe was then taken out of the set up and rinsed inwards with distillate and outwards with distilled water. The boric acid was then titrated with sulfuric acid solution until the indicator colour changed.

A blank titration was carried out to determine the nitrogen present in the reagents or the nitrogenous substances there in.

$$\% \text{ Nitrogen content} = \frac{ml H_2S04 - ml H_2S0_4 Blank x F x 100}{\text{wt/volume of wort used.}}$$

F = Nitrogen factor in 1 ml 0.0715 H_2S0_4 solution corresponds to 1mg Nitrogen.

$$F = \frac{\text{molarity of used sulfuric acid solution}}{0.0715}$$

$$\% \text{ Protein content} = \text{Nitrogen X } 6.25.$$

Determination of soluble nitrogen in wort

This was done using the wort obtained from cold water extract (CWE) from malt samples. Seventy five grammes of fine malt grind was mashed in with 400ml distilled water at 20^{o}C in a mash beaker and stirred for 30 min. Then the mash beaker was made up to 500g with distilled water. The cold water extract was then filtered with filter paper whereby the first 100 ml filtrate was poured back.

Then, 10 ml of the filtrate was used for the determination of the soluble nitrogen according to the Kjeldahl method.

Calculation: Mg soluble nitrogen/100g wort

$$= \frac{\text{Mg N/100 ml wort x ml wort}}{100}$$

Measurement of protein content of sorghum wort samples (Bradford Method)

Protein in the samples was determined by the dye-binding method of Bradford (1976) as reported by Hammond and Krüger (1988) using bovine serum albumin (BSA) as standard. Bradford reagent was prepared by dissolving 0-1g Serva blue, (Coomassie Brilliant Blue G 250) in 50 ml of 95% v/v ethanol mixed with 100 ml of 85% v/v of phosphoric acid and made up to 1 L with deionized water. The solution was filtered with Whatman No 1 filter paper before storage in an amber bottle at room temperature.

To assay for protein, 0.5 ml of the Bradford reagent was added to 0.1ml of the enzyme sample (wort sample) and mixed gently.

A blank containing deionized water in place of the sample was prepared and the absorbance measured spectrophotometrically at 595 nm within 1 h of mixing.

A standard curve using (BSA) was prepared in the concentration range of 0 – 1 mg.

Mashing of sorghum malt samples with commercial Enzymes.

Fifty grams (50g) of each sample of milled sorghum malt were mixed with 300ml of distilled water at 62^{o}C . Then 1ml of neutrase (a protease) was added and the mash was allowed to stay for 20min before the temperature was raised to 85^{o}C within 20min. At 85^{o}C, 1 ml of termamyl, (thermostable amylase) was added to the mash and rested for another 20min. The temperature of the mash was then lowered to 72^{o}C within 20min and 1ml of amyloglucosidase (AMG) was added and rested for 1h. The iodine test was carried out and all the samples were iodne negative. The temperature was finally raised to 75^{o}C and the mashing ended (mash-off).

Mashing regime of malted sorghum samples without commercial enzymes

Fifty grams (50g) of each sample of milled sorghum malt were mixed with 300 ml distilled water of 62^{o}C in mash beakers placed in a water bath temperated at 62^{o}C.

The mashes were allowed to stay for 20min. The temperature was then raised to 85^{o}C within 20 min and allowed to rest for 20min. The temperature was lowered to 72^{o}C within 20min and incubated for 1 hr after which saccharification test was carried out. The temperature was again raised to 75^{o}C for mash-off.

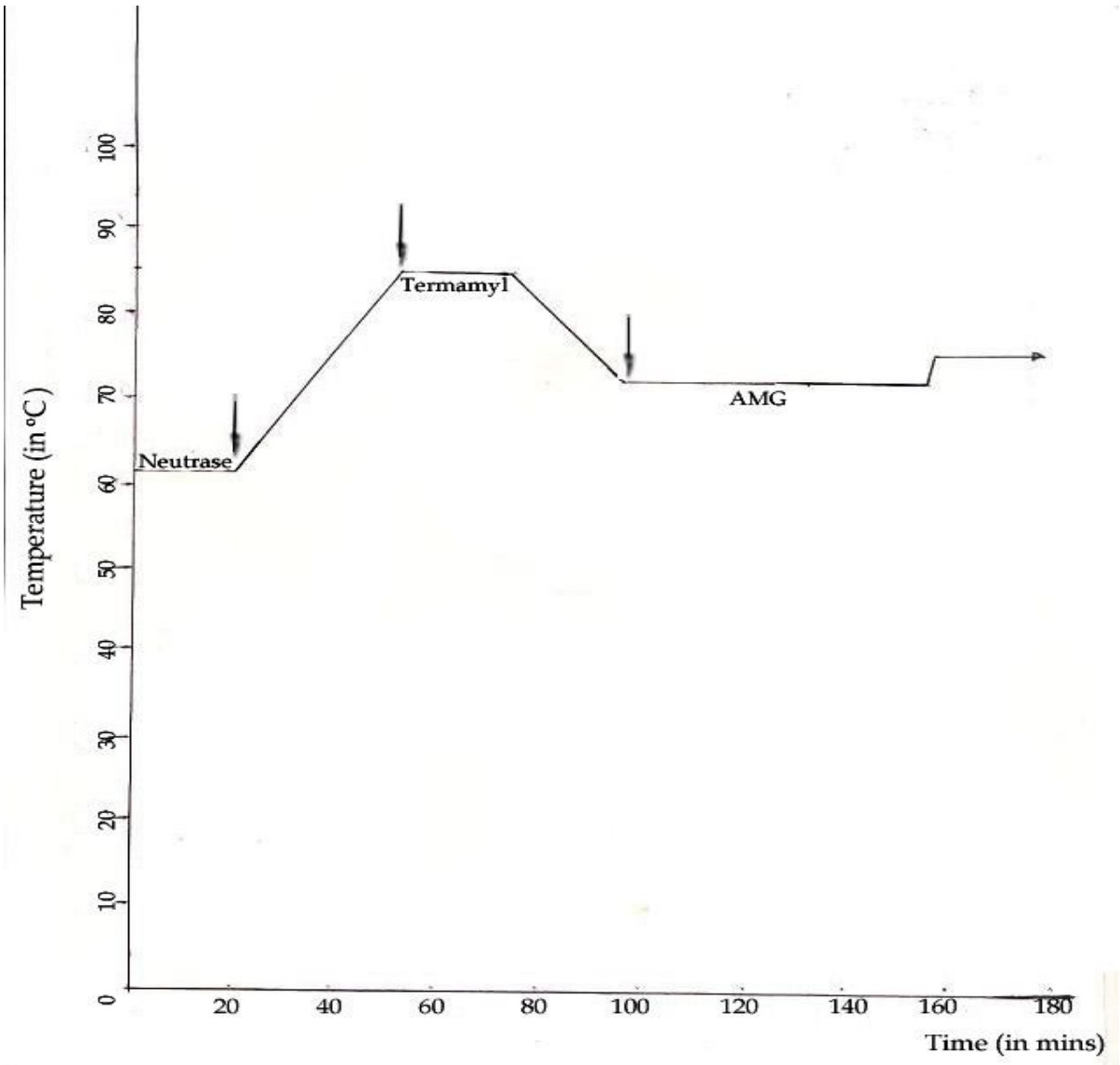

Fig 8; Mashing regime : Abridged infusion for SK5912, KSV8, ICSV400

Conventional Infusion mashing process.

Two hundred and fifty grams (250g) of each of the sorghum cultivars were mashed in at 50^{o}C with 1 litre of warm water in a water bath – ratio grist to water was (1:4). The mash was rested for 30 min, protein rest. The temperature was then gradually raised to 62^{o}-65^{o}Cand a maltose rest of 30min was allowed. There after the temperature was gradually raised to 72^{o}-75^{o}C. The mash was held at this temperature until the requisite iodine normality was achieved. This was followed by mash-off temperature at 78^{o}C. The mashing process lasted for 4 hours. The newly developed mashing programme on the other hand lasted for 2 and half hours thereby shortening the mashing process by $1^{1/2}$ hours. Translated into cost, one sees that a shorter mashing time of $2^{1/2}$ hours gives a better cost per unit of operations compared to the conventional mashing programme.

Conventional two stage decoction mashing programme.

Two hundred and fifty grams grist (250g) from each sorghum cultivar were mashed in at 50^{o}C in 1 litre of warm water contained in mash beaker placed in a water bath and rested for 20 minutes and before being returned to the main mash. This raised the temperature of the main mash to 65^{o}C. The second part (1/3) was withdrawn and boiled for 15 minutes and returned to the main mash and this raised the temperature to 75^{o}C. In each case the portion that was boiled was allowed to rest for 60 minutes-amylase rest. At last, the saccharification test was carried out and as soon as it was found to be iodine negative, the mashing was ended. This decoction process lasted for $4^{1/2}$ hours. Of these mashing trials viz abridged mashing conventional infusion and conventional decoction mashing, the abridged mashing was the shortest in wort production. Consequently the cost per unit operation would be the least and so the abridged infusion programme is a much better alternative in wort production using exogenous enzymes.

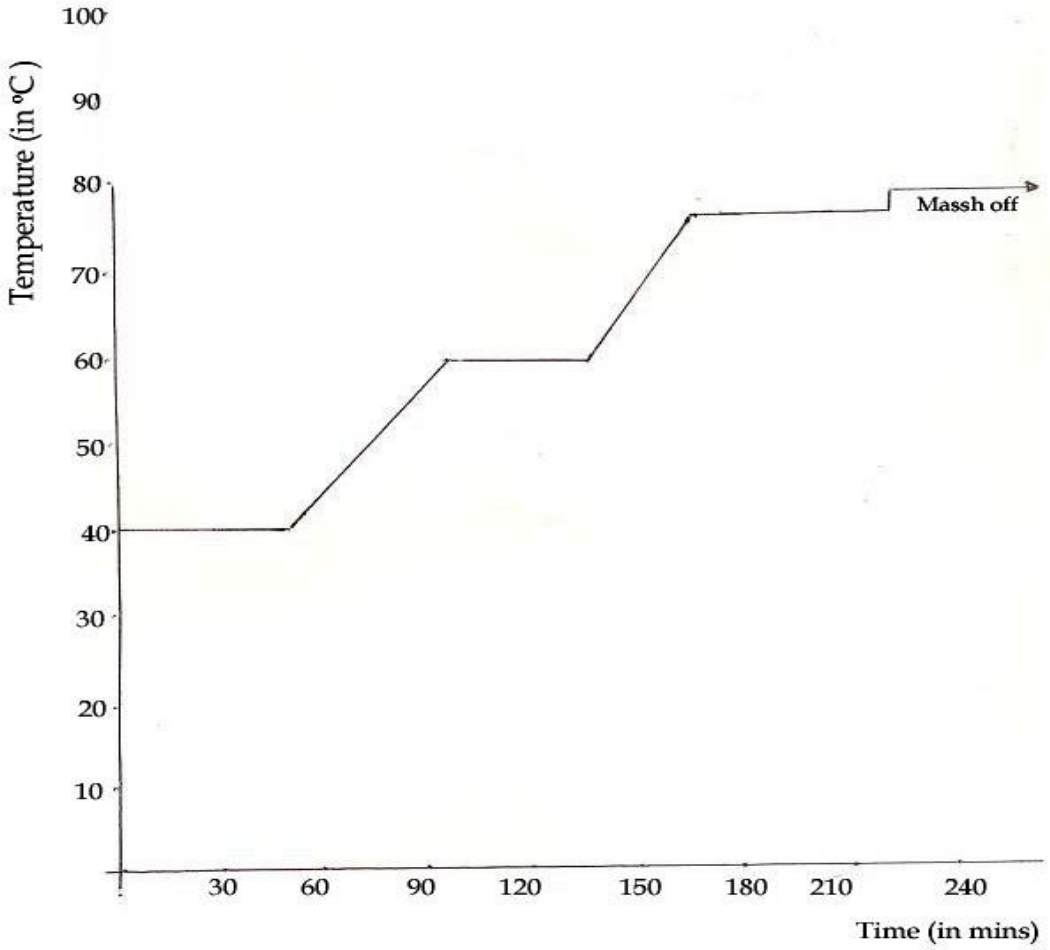

Fig 9: Conventional Infusion Mashing Process

Mashing regime of unmalted sorghum samples with commercial enzyme preparations.

Fifty grammes (50g) of each sample of milled sorghum malt were mixed with 300 ml distilled water at 62^{o}C in mash beakers placed in a water bath temperated at 62^{o}C. The mashes were allowed to rest for 20 min after the addition of 1 ml neutrase, a protease and the temperature was raised to 85^{o}C within 20 min. The mashes rested for 20 min at 85^{o}C after the addition of 1 ml termamyl. The temperature was then lowered to 72^{o}C within 10 min, 1 ml of amyloglucosidase (AMG) was added and the mashes were allowed to rest for 1h. At the end of the rest, saccharification test was carried out. The temperature was then raised to 75^{o}C before mash-off.

The original gravity of the worts obtained from the sorghum cultivars was determined using the method of I0B (1977). Abridged infusion method was used for the production of various wort samples as described earlier. The wort samples were filtered using Whatman No1 filter paper and allowed to cool to 20^{o}C. One hundred (100 ml) millilitres of each sample was collected in a 100 ml measuring cylinder and a hydrometer was used to determine the original gravity, given in degrees Plato (^{o}P).

Determination of specific gravity of Worts

This was determined according to the official Methods of analyses of AOAC (1980).

A ten milliliter (10 ml) or (50 ml) specific gravity bottle was thoroughly cleaned with distilled water, dried in an oven for $1^{1}/_{2}$ h at 50^{o}C and then cooled in a desicator. The weight of the cooled, dried bottle (W_1) was determined to three decimal places. The dried bottle was filled with deionized water and allowed to stand for 20 min at 20^{o}C in attemperated water bath. The surface of the bottle was dried with Whatman No1. filter paper. The bottle with water was weighed (W_2) correct to three places of decimal. The bottle was emptied and cleaned twice with 10 ml of sample extract; after which the bottle was filled to the brim with sample and immersed in water bath at 20^{o}C for 20 minutes. The bottle was dried with Whatman No 1 filter paper and weighed (W_3). The procedure was repeated for each sample.

Iodine Test.

Iodine test was carried out by putting together and mixing with a glass rod one drop of the mash with 1 drop of 0.5 M iodine solution on a white ceramic tile. This happened 10 minutes before mach off at 75^{o}C mash temperature. The appearance of a clean yellow fleck on the plate showed complete saccharification that is iodine negative

Wort boiling.

At the end of mashing, that is when the different wort samples were confirmed iodine negative, they were filtered into 1 litre capacity beakers using Whatman No 1 filter paper. The beakers were placed in a water-bath and the worts were heated to boiling. Wort boiling lasted for ninety (90) min. One hundred ml was taken from each of the samples and cooled to 20^{o}C in a measuring cylinder. The extract (original gravity) of each wort sample was determined by means of a hydrometer (spindle) graduated in degrees Plato (^{o}P). Other measures involved in wort treatment were taken before the samples were allowed to ferment.

Determination of Original Gravity. (OG)

At the end of sample of the hot wort was taken and filled in a 100 ml measuring cylinder and quickly cooled to 20^{o}C. Then a saccharomrter (spindle) was put in making sure that there was no air bubble in the wort and as soon as the spindle stopped swinging, the level to which it sank was read at eye-level and noted. The (O.G.) was reported in (^{o}P.).

Determination of Wort Viscosity

The wort viscosity was determined using wort from the O.G determination. Oswald-Viscometer was used while the standard liquid was distilled water with a specific gravity S.G taken as 1000. The discharge time for 50 ml water and an equal volume of wort was noted and from there the viscosities of the wort samples were obtained using the following formular:

$$\frac{C^1}{C_2} = \frac{t^1}{t_2} \_ \frac{d^1}{d_2}$$

Where C^1 = viscosity coefficient of water = 1.00 cP

C_2 = Viscosity coefficient of wort

t^I = flow time of water

t_2 = flow time of wort

d^1 = density of water (1000)

d_2 = density of wort

Determination of the percentage end degree of fermented wort.

Thirty (30 ml) of each of the samples were filtered and boiled for 10mins in reflux cooler. Then the quantity lost by importation as results of the boiling was made up with distilled water. The extract contents of the samples were determined with a hydrometer. Thereafter 200ml of each sample was put in a conical flask and 15g pressed yeast was added and the flasks closed.

Care was taken to ensure that the yeast was completely suspended and the flasks placed in the shaker.

The apparent extract was checked for at least twice daily with a hydrometer. As soon as the apparent extract did not reduce any more, the fermentation was stopped.

Apparent end degree of fermentation

$$= \frac{O.G - \text{apparent extract}}{OG - 1000} \times \frac{1000}{}$$

Real end degree of fermentation ≈

Apparent and degree of fermentation x 0.81.

Determination of Apparent degree of fermentation.

For the determination of apparent degree of fermentation, 300 ml of wort sample was boiled in a 1000 ml flask for 2 min, cooled and diluted to the original weight with water. Each wort sample was diluted to an original gravity of 1025^{o}P with distilled water. Thereafter, specific gravity of each wort sample was determined at 20^{o}C. Two hundred milliliters portions were put in a 500 ml flask and 12g pressed yeast added and continuously shaken so as to keep the yeast in suspension.

After 24 h shaking at 20°C, half the sample was filtered through a covered filter paper and the specific gravity measured at 20°C.

The shaking of the remaining was continued for a further 24 h and then again the specific gravity determined. The lower value was taken for calculation.

Calculation

The percentage fermentable sugars was given by

$$\% \text{ F-S} = \frac{OG - FG \times 100}{OG}$$

Where OG = Original specific gravity 1000.

F.G = Final specific (or attenuation limit) gravity 1000.

The beer was temperated to room temperature and filled in a bottle . The bottle was closed with a bung bearing an open tube and shaken and by removing the thumb from the opening pressure balance was ensured.

The process was repeated several times. Finally the beer was filtered through a dry folded filter paper (Whatmann No 1).

Determination of the apparent Extract (EA)

From the beer freed from CO_2 and using the specific gravity bottle, a weight relationship $20^o/20^oC$ was measured and the corresponding extract values taken from the sugar table.

Determination of the alcohol content (A)

In a weighed 500 ml flask 100 g CO_2-free beer was weighed in. It was diluted with 50 ml distilled water and connected to a cooler. Five milliliter of distilled water was put in a weighed 100 ml measuring cylinder and connected to the cooler end.

The beer in the flask was slowly heated so that extract material did not touch the walls of the flask (use of asbestos tunder the bottle). As soon as the neck of the bottle was filled with condensate, the distillation was stopped. The measuring cylinder was then weighed off with

distilled water at exactly 100g. After mixing very well, the weight relationship of alcohol – water experiment was determined and the corresponding alcohol content read off from the sugar table.

Determination of the real extract (RE)

The 500 ml flask with beer residue was cooled and the content weighed off again at 100 g.
After shaking thoroughly the weight relationship was determined with specific gravity bottle at $20^o/20^o$ and the corresponding extract content read off from the sugar table of (VLB) Versuchs Lehranstalt der Brauerei in Berlin

Determination of foam retention/stability

Carbon dioxide (CO_2) was introduced into the beer sample in order to produce a certain quantity of foam. At definite time intervals, the quantity of beer formed from the foam was measured on the assumption that the speed with which the foam fell was proportional to the quantity of foam at any given time. The result was given as foam count or sigma value.
If the time between formation and collapse is under 110 seconds, the foam stability is bad. If it lies between 110 – 119 it is poor, our measurement lay between 120 – 129 seconds and so was satisfactory.

N.B. 130 - 140 seconds is good
Above - 140 is very good.

Determination of Bitterness.

The wort/beer samples were centrifuged in plastic test tubes. Ten milliliter of each sample were pipetted in test tube attemperated at 20^{o}C. One half milliliter (0.5 ml) hydrochloric acid (HCL) 6n and (20 ml) isooctane were added, and after putting three glass balls into each of the test tubes, they were closed and shaken for 15 mins on a shaker.

Finally they were centrifuged for 3 min at 3000 rpm. The optical density (OD) of the isooctane extract was measured in 1 cm cuvette against iso-octane at 275 nm.

Bitterness unit = (OD) x 50

The answer was given in bitter unit (BU) per litre.

Determination of beer colur.

The beer samples were filtered with 0.1% kieselguhr and filter paper. The first portion of the filtrate was discarded while the next filtrate was used and measured with a 25 mm cuvette. The samples were put in Hellige-Neo-Komparator, compared with itscolor discs and noted.

Calculation = $\frac{M \times 25,}{\text{Mm thickness of cuvette}}$

Where M= measurement

The answer is given in EBC (European Brewing Convention.) units.

Beer colour (EBC) = $\frac{\text{measured colour} \times 25}{\text{mm cuvette thickness}}$

Estimation of Carbohydrates by the anthrone method.

Principle

The anthrone reaction is the basis of a rapid and convenient method for the determination of hexoses, aldopentoses, and hexuronic acids, either free or present in polysaccharides. The blue-

green solution shows an absorption maximum at 620 nm, although some carbohydrates may give other colours.

The extinction depends on the compound investigated, but is constant for a particular molecule.
Four ml of the anthrone reagent was added to 1 ml of a protein free carbohydrate solution and rapidly mixed (care strong acid). The tubes were placed in a boiling water bath for 10 min with a marble on top to prevent loss of water by evaporation. The tubes were then cooled and the extinction read at 260 nm against a reagent blank which contained 1ml of distilled water.
Then standard curves for glucose were prepared and compared.

RESULTS

Some properties of the sorghum grains/Malt varieties.

Thousand corn weight, was the highest in ICSV 400, (31g). This was followed by SK5912, (27.5): the lowest was (25g) in KSV8. The moisture content was highest in KSV8,(10.5%). There was no significant difference in values for SK5912 and ICSV 400 which had (9.4%) respectively.

The protein contents ranged from (10.4) in ICSV 400 to (8.5%) in SK5912. There were no significant differences in percentage (%) germinative energy which ranged from (99%) in ICSV400 to (96%) in KSV8. These values are quite good when compared to values for barley but much better than values for local varieties. Cold water extract (CWE) was highest in KSV8 (34%), followed by SK5912, (32%) whereas ICSV400 had (30%). KSV8 which had the highest value of (34%)(CWE) had the lowest value of $200^{o}LKg^{-1}$ were recorded for ICSV400 and SK5912 respectively. Diastic power (DP) ranged from ($22^{o}L$) inKSV8 to ($18^{o}L$) in ICSV400. These values are characteristic of sorghum but much lower than the values obtainable in barley, namely ($49^{o}L$) – ($94^{o}L$). The malting losses ranged from (18%) in SK5912 to (22%) in ICSV400. High (%) malting loss in sorghum is attributed to high malting temperature.

Table. 8:

Some properties of the sorghum grains/Malt varieties.

Parameters	**SK5912**	**KSV8**	**ICSV400**
Thousand corn weight (g)	27.5	25.0	31.0
Moisture content (%)	9.4	10.5	9.0
Protein content (%)	8.5	9.2	10.4
Germinative energy (%)	98	97	99
Germinative capacity (%)	97	96	98
Cold water (%)	32	34	30
Hot water extract $^{o}LK_{g}^{-1}$	233	200	217
Diastatic power (^{o}L)	20	22	18
Malting loss (%)	18	20	22

Table 8b: Some Properties of worts obtained by normal infusion Process.

The original gravity values were the same in KSV8 and ICSV400, 1015^{o}.P respectively where as it was 1010^{o}P in SK5912. ICSV400 has the highest P^{H} value, 5.8; followed by SK5912, 5.5 and then KSV8, 5.4. The viscosity values were very low and ranged from 1.010 (m.Pas) in SK5912 to 1.040 (m.Pas) in KSV8 as compared to the values of 1.550 m.Pas) to 1.655 (m.Pas) obtained in worts mashed according to the abridged infusion mashing process. (Tables 12 and 13**).**

In all the cases under consideration, the worts were iodine negative. The flow rates ranged from 1.40 (ml.Sec) in SK5912 to 1.11 (ml/s in ICSV 400. ICSV400 has α- amino nitrogen (FAN) value of 76(mg/L) followed by KSV8, 70(mg/L) and 65.0(mg/L) in SK5912, the least. These values were low compared to the values obtained in abridged mashing process which showed higher values – ranging from 220 to 250 (mg/L). The values in reducing sugar as glucose ranged from 114 (mg/L) in SK5912 to 117 (mg/L) in KSV8. Reducing sugar as maltose ranged from 185mg/L in KSV8 to 190 (mg/L) in both SK5912 and ICSV400. In general, one can conclude that worts from abridged infusion mashing process had better qualities than that from ordinary infusion mashing process.

Table 8b:

Some Properties of Wort samples Obtained with the normal infusion process

	Samples		
Parameters	**SK5912**	**KSV8**	**ICSV 400**
Original gravity (^{o}P)	1010	1015	1015
P^{H}	5.5	5.4	5.8
Viscosity (cp)	1.010	1.040	1.030
Iodine Test	-ve	-ve	-ve
Flow rate (ml/sec.)	1.40	1.20	1.11
Free amino nitrogen FAN (mg/1	65	70	78
Reducing sugar as glucose (mg/L)	114	117	115
Reducing sugar as glucose (mg/L)	190	185	190
Reducing sugar glucose + maltose (mg/L)	304	302	305
Glucose /Maltose ratio	1:1.6	1:1.6	1:1.6

Characteristics of worts obtained by the new mashing regime using commercial enzyme preparations (1ml). (Table 9)

The original gravities ranged from 1020^{o}P in SK5912 to 1025^{o}P and ICSV400respectively. The pH values ranged from 5.4 to 5.8 with ICSV400recording the highest value 5.8. KSV8 had a wort viscosity of 1.05mP.s while SK5912 and ICSV400had 1.02 and 1.03mPa.s respectively. These values were rather low compared to the barely worts values. (Krűger Bielig, 1976) reported values between 1.5mPa.s and 1.7mPa.s respectively. In all the wort samples, iodine test was negative. This was not surprising because at 85^{o}C, the starch had already been gelatinized, liquefied and saccharified. The flow rate was fastest in SK5912, 1.43mm/sec. in KSV8, it was 1.3ml per sec), while the slowest rate was in SCSV400 (1.13ml/sec). The FAN values ranged from 220mmg/litre in SK5912 and KSV8 respectively; 230ml/litre in ICSV400. The above named values reflected good proteolysis. Ogu et al(2005) reported lower values of 185ml per litre in SK5912, 104 ml per litre in KSV8 and 211 ml per litre in ISV400. The new mashing method definitely had better results. Barley wort on the other hand could reach 250 ml per litre (Kruger and Bielig, 1976). Reducing sugars as glucose and maltose were adequate because within the temperatures of 62^{o} – 65^{o}C, β-amylase is most active and releases sufficient sugar in the wort. The quantity of maltose, however, is more than that of glucose (Kunze, 1999). The ratio of glucose to maltose was 1:1.6, a factor which tends to remain unchanged no matter the manipulations adopted during mashing.

Tables 9:

Some properties of the wort samples obtained by abridged infusion mashing with mixtures of commercial enzymes.

	Samples		
Parameters	**SK5912**	**KSV8**	**ICSV 400**
Original gravity (^{o}P)	1020	1025	1025
P^{H}	5.6	5.4	5.8
Viscosity (cp)	1.02	1.05	1.03
Iodine Test	-ve	-ve	-ve
Flow rate (ml/sec.)	1.43	1.3	1.13
Free amino nitrogen FAN (mg/1	70	80	100
Reducing sugar as glucose (mg/L)	116	118	115
Reducing sugar as glucose (mg/L)	192	190	190
Reducing sugar glucose + maltose (mg/L)	308	318	305
Glucose /Maltose ratio	1:1.6	1:1.6	1:1.6

Table 10:

Some properties of unmalted sorghum grain varieties

Malting loses ranged from 18% to 22% (table 11). It was a rather high level of loss though it could reach 24% Hornsey (1990) as sorghum is associated with high malting loss. Barley shows a lower level of malting loss – 17 – 20% Narziss (1980). The differences could be due to physiology of cereal and malting activities Agu and Palmer(1988). Cold water extract ranging from 30% - 34% could be regarded as normal. SK5912 had the highest value in hot water extract 223.0^{o}L Kg^{-1} while KSV8 had the least value 200^{o}L Kg^{-1} SK5917 had the highest malting colour 5.8 EB while KSV8 and ICSV400 had comparable values.

The diastatic power of the sorghum malts were rather low as it ranged from (22-18^{o}L) compared to barely whose diastatic power ranges from 34^{o} – 104^{o} (Krűger and Bielig 1976) Brewers with sorghum must of necessity resort to the use of exogenous enzymes for liquefaction and saccharification of mashes. The color of the wort obtained from the sorghum malts ranged from 4.2 to 5.8 EBC. This value seems to fall in line with Vienar wort color which ranges from 4.4 – 8.1 EBC (Krűger and Bielig 1796).

Table 10: Some properties of sorghum malt varieties

Parameters	SK5912	KSV8	ICSV00
1000 corn weight (TWC)(g)	25	26	24
Moisture Content (%)	10.5	11.3	11.0
Germinative energy (%)	96	97	96
Germinative capacity(%)	98	98	96
Root Length (cm)	1.3	1.2	1.4
Colour (natural)	Cream	White	White
Total Nitrogen (%)	1.62	1.60	1.65

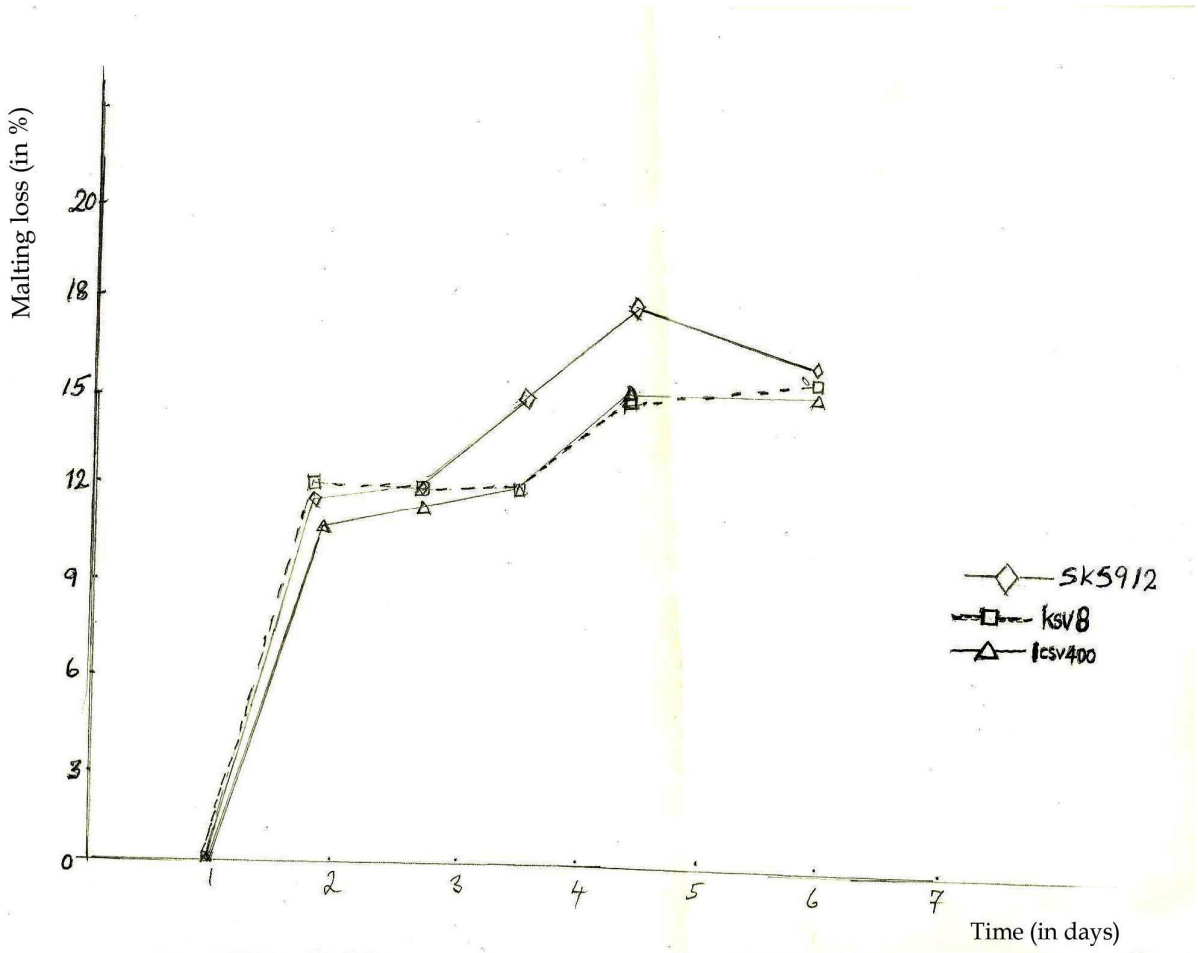

Fig. 10: Effects of germination on the malting loss of sorghum malts

GRAIN ANALYSIS FOR SORGHUM VARIETIES (SK5912, KSV8, ICSV400)

The results of the grain analysis of the sorghum varieties under investigation are shown in Table 10. The thousand corn weights are almost in the same pedestal – SK5912 (28%), KSV8(26g) though ICSV400 (24g) was the lowest. As regards moisture content, germinative energy and germinative capacity, the three varieties gave comparative values. They ranged from 11.50% - 11.3% for moisture and 96 – 98% for germinative energy and germinative capacity respectively. The root lengths and total nitrogen were within close ranges but ICSV400, gave the highest values compared to the other counterparts. Sorghum is noted for vigorous growth during germination Kunze (1999).

Some properties of Sorghum malt varieties

Malting losses ranged from 18% to 22% (table 11). It was a rather high level of loss though it could reach 24% Hornsey (1990) as sorghum is associated with high malting loss. Barley shows lower level of malting loss – 17 – 20%, Narziss (1980). The differences could be due to physiology of cereal and malting activities, Agu and Palmer (1988). Cold water extract ranging from 30% - 34% could be regarded as normal. SK5912 had the highesr value of hot water extract 223.0^{o}L Kg^{-1} while KSV8 had the least value 200^{o}L Kg^{-1} SK5917 had the highest malting colour 5.8 EB while KSV8 and ICSV400 had comparable values.

The diastatic power of the sorghum malts were rather low as it ranged from (22 – 18^{o}L) compared to barley whose diastatic power ranges from 34^{o}-104^{o} (Kruger and Bielig 1976). Brewers with sorghum must of necessity resort to the use of exogenous enzymes for liquefaction and saccharification of mashes. The color wort combinedfrom the sorghum malts ranged from 4.2 to 5.8 EBC. This value seems to fall in line with Vienar wort color which ranges from 4.4 – 8.1 EBC (Kruger and Bielig 1796)

Table 11. MALT ANALYSIS

Parameters	SK5912	KSV8	KSV400
Malting Loss (%)	18	20	22
Cold water Extract (%)	32	34	30
Hot water Extract (°L Kg^{-1})	223	200	217
Malt colour (EBC)	5.8	4.2	4.2
Diastatic Power (°L)	22	18	20

Table 12: Characteristics of Fermented Worts derived from malted sorghum varieties 5K5912, KSV8 and ICSV400using enzymes preparation mixtures (1ml)

Apparent extract values were almost the same for all the cultivars see (table 12), original Extract (%) values were same in 5K5912 and KSV8 (10.15%) but (10.0%) in ICSV400. These values considered above were indicative of high extract yield. The percentage apparent attenuation (80%) from all the varieties as well as the values for percentage end degree of fermentation, 65.50% for all the varieties, above witness to the intensive fermentation due to yeast of high viability and very good wort composition. The values in foam stability (129 EBC) in all the cases was quite satisfactory since hopped barely wort with a value of 129 – 140 EBC was regarded as very good.

The P^H values were similar 4.5 to 4.6, the viscosities were rather high 1.550 to 1.554 (mPa.s) and the bitterness units (BU) (12.5BU) for an unhopped wort were quite normal. The α- amino nitrogen (FAN) was highest in ICSV400230(mg/l) but the same values in SK5912 and KSV8 220(mg/l) respectively.

Table 12:

Characteristics of Fermented Worts derived from malted sorghum varieties 5K5912, KSV8 and ICSV400using enzymes preparation mixtures (1ml)

Parameters	5K5912	KSV8	ICSV400
Apparent Extracts (%)	2.03	2.03	2.02
Original Extracts (%)	10.5	10.15	10.10
Alcohol (%)	3.38	3.38	3.36
Apparent attenuation (%)	80	80	80
End Attenuation (%)	65.5	65.5	65.5
Foam Stability (EBC)	129	129	129
pH	4.5	4.5	4.6
Viscosity (cP)/(mPa.s)	1.550	1.554	1.554
α- amino nitrogen (mg/l)	220	220	230
Bitterness units (BU)	12.5	12.5	12.5

Table 13: Some properties of Fermented Worts Derived from Unmalted Sorghum Varieties using Enzymes Preparations (1ml).

Apparent extract (%) was highest (2.5%) in KSV8 and ICSV400 (2.3%) while the value for SK5912 (2.2%). Original extract (%) was highest in KSV8 (10.65%) while ICSV400 and SK5912 had (10.55) and (10.50) (%) respectively. These values indicate high extract yield as a result of the exogenous enzyme used during mashing. KSV8 had an `alcohol content of 3.6%, the highest followed by ICSV400, 3.52(%) and then SK5912, 3.5(%). SK5912 had apparent attenuation of 79(%) percentage end degree of fermentation was almost the same values in SK5912 and ICSV400, 64(%) and 63.9(%) respectively, KSV8 had 62.3(%), the least. All the values obtained in this analysis so far indicate high extract yield as well as good fermentation profile. The values in foam stability 126(EBC) were also satisfactory. The P^H value was 5 for all the varieties. The wort viscosity was high as well. The same values 1.650(mPa.s) in SK5912 and KSV8 but a little bit higher 1.655 (mPa.s) in ICSV400. High viscosity is their case can be attributed to high in mash temperature 62^{o}C as pentosanase and glucanase activity optima were skipped in line with the abridged infusion mashing principles. Though it is claimed that protease has residual activities. The level of FAN was highest in KSV8, 133 (mg/l) but lowest in ICSV400 124 (mg/l). These values are quite high enough to provide yeast with sufficient nutrition since a level of 85(mg/l) – 120 (mg/l) in barley wort is already acceptable. The bitterness units 12(BU) were quite normal for an uhopped wort as was the case under investigation.

TABLE 13:

Some properties of Fermented Worts Derived from Unmalted Sorghum Varieties using Enzymes Preparations (1ml).

PARAMETERS	WORT SAMPLES		
	SK5912	KSV8	ICSV 400
Apparent Extract (%)	2.2	2.5	2.3
Original Extract (%)	10.50	10.65	10.55
Alcohol (%)	3.50	3.53	3.52
Apparent Attenuation	79	76	78
End Degree of attenuation	64	62	63.9
Foam Stability (EBC)	126	126	126
α – Amino Nitrogen (FAN) mg/l	128	133	124
Viscosity (cP)	1.650	1.650	1.655
PH	4.5	4.6	4.4
Bitterness units (BU)	12	12	12

In table 14,

KSV8 had the lowest value for specific gravity 1.025 followed by SK5912, 1.02 while ICSV400 had the highest value 1.030. In all the cultivars there was no difference in PH values, 6 in color, SK5912 had (3.5 EBC) while KSV8 had 3.8 (EBC) where as ICSV400 had 3.7 (EBC). One would have expected SK5912 to have the highest color profile by virtue of the grain being creamy before malting; the other cultivars were white before malting. The kilning oven may be held responsible. KSV8 had the highest value in carbohydrate (5.0%). This was followed by ICSV400, (4.5%) while SK5912 had (3.5%), the least of all. ICSV400 had the highest value for (FAN) 260(mg/L); this was followed by KSV8, 250(mg/L), the least was SK5912, 230 (mg/L). All these values are quite high and reflect very good proteolysis due to neutrase activities at 62^{o} – 65^{o}C during wort production.

Table 14:

Effect of Addition of neutrase (Protease) on the quality of Worts Derived from Malted Sorghum Varieties.

Parameters	**SK5912**	**KSV8**	ICSV**400**
Specific Gravity	1.026	1.025	1.030
PH	6	6	6
Colour EBC	3.5	3.8	3.7
Carbohydrates (g%)	3.5	5.0	4.5
α – amino Nitrogen (mg/l)	230	250	260

Table 15: Some Property of Fermented Worts Derived from unmalted Sorghum varieties using enzyme Preparations (1ml)

The percentage apparent extracts of the various fermented worts, table 15 ranged from (2.2%) in SK5912 to (2.5%) in ICSV400. the original gravity, extract (%) was highest in KSV8, (10.65%) followed by ICSV400, (3.55%) and SK5912, (10.50%). The alcohol content was highest in KSV8, (3.63%) followed by ICSV400, (3.52) while SK5912 had (3.50%). The percentage apparent attenuation ranged from (79%) in SK5912 to (76.0%) in KSV8. The percentage end degree of attenuatiuon was also highest in SK5912, (64.0%) followed by ICSV400 (63.9%); the least was 62.3% in KSV8. there were no differences in that all the worts had 126(EBC). All the worts had a P^H value of 5. The viscosity values in all the worts were rather high. ICSV 400 had 1.650 while SK5912 and KSV 8 had 1.650 (mP.as) respectively. These high values may be due to the predominance in protease of neutrase over glucanase and pentosanase which reduce wort viscosity during mashing (Ian Hornsey (1999). KSV8 had the highest value in (FAN) 133 (mg/L), followed by SK5912, 128 (mg/l) and then ICSV 400 124 (mg/L). The bitterness values (BU) were the same for all the fermented worts, 12.0 (BU). This value is quite normal for larger beer.

TABLE 15:

Some Property of Fermented Worts Derived from unmalted Sorghum varieties using enzyme Preparations (1ml)

Parameters	SK5912	KSV8	KSV400
Apparent Extracts (%)	2.2	2.5	2.3
Extracts (%)	10.5	10.65	10.55
Alcohol (%)	3.50	3.63	3.52
Apparent attenuation (%)	79.0	76.0	78.0
End Attenuation (%)	64.0	62.3	63.9
Foam Stability (EBC)	126	126	126
pH	5	5	5
Viscosity (cP)	1.650	1.650	1,655
α- amino nitrogen (mg/l)	128	133	124
Bitterness units (BU)	12.0	12.0	12.0

<u>Table 16:</u> Effects of Addition of neutrase (Proteases) on the quality of Worts Derived from unmalted Sorghum Varieties.

The specific gravity of the worts ranged from 1.033 to 1.035 Table 16. KSV8 and ICSV400 had equal values, (1.033). The P^H values of the worts were similar in SK5912 and KSV8, 5.8 while ICSV400 had 5.0 (EBC) in color, SK5912 and KSCV8 had 4.5 (EBC) respectively, ICSV400 had Carbohydrate levels of the worts were SK5912, (6.3%), KSV8 (6.0%). ICSV400, (4.6%). Compared to worts derived from malted sorghum varieties, table 14, the values were higher. This may be as a result of the grains in table 14 having been malted and so subjected to malting loss. The FAN values were highest in KSV8, 230(mg/L) followed by ICSV400, 220(mg/L), the least was 200mg/L in SK5912. These values are high enough to justify the activities of neutrase at $62^oC - 65^oC$.

Table 16:

Effects of Addition of neutrase (Proteases) on the quality of Worts Derived from unmalted Sorghum Varieties.

Parameters	SK5912	KSV8	ICSV400
Specific Gravity	1.035	1.033	1.033
pH	5.8	5.8	5.9
Colour(EBC)	4.5	4.5	5.0
Carbohydrates (g%)	6.3	6.0	4.6
α – amino Nitrogen (mg/l)	200	230	220

DISCUSSIONS

Grain Analysis

The grain analysis of the sorghum varieties under investigation viz KSV8, SK5912 and ICSV400 indicated that the grain weights were comparable. (Table 10) ICSV400 when compared with others as regards germinative energy and germinative capacity has values differing with only two percent from SK5912 and KSV8. This shows that all the grains were almost at the same pedestal when considering good germinative quality. The moisture contents ranging from 7.8% to 10.0% in all grains, suggested not only durable dormancy but also long storage and tendency to resist microbial spoilage. Higher percentage of moisture would encourage microbial attack (Agu and Palmer, 1999). It could equally affect extract yield, ICSV400 had the longest root length (1.8cm), SK5912 and KSV8 had comparable lengths. 1.5 and 1.6cm respectively. It could be that ICSV400 had faster rate of metabolism than the rest but at the same time, it could lead to a situation known as Husaria ie overgrowth of the roots at the expense of the extract yield of that cultivar.

The protein contents ranged from10.2% in the SK5912 to 8.5% in KSV8. It is disadvantageous for malting grains to have more than 12% protein, the best range being 9-11.5% (Narziss, 1980). Less values would mean less yeast nutrition while higher values would mean less extract yield and haze problem during beer filtration later.

Malt analysis

Malt moisture content of 6.0%, 8.0% and 7% for KSV8, SK5912 and ICSV400, respectively are normal for sorghum but rather high for barley. (Narziss, 1980). This is so because though both are cereals, climatic conditions, soil type and malting temperatures and other parameters are not exactly the same. In Germany, for example, barley malt of more than 5% moisture content would not be acceptable for the production of light beer. Malting loss which occurs as a result of respiratory activities during germination, removal of dried roots and shoots after kilning and duration and condition of kilning are generally high in sorghum.

SK5912 had 22% whereas ICSV400 and KSV had 21%, respectively. Malting loss in barley ranges from 17 – 20% while in sorghum the loss can reach 24% Agu *et al* (2005) reported that SK5912 had the longest root length while ICSV400had the shortest. They concluded that the

longer the root and short length, the higher the malting loss. It would be rash to draw any conclusion here. It is probable that the tropical nature of sorghum and the temperate nature of barley could be held responsible for the differences in malting losses (Agu and Palmer 1998). High malting temperature (25-30^{o}C) and low malting temperature (16 – 18^{o}C) for optimum malt quality for sorghum and barley, respectively have a major role to play with regard to malting loss.

Cold water extract (C.W.E.)

Cold water extract is the amount of extract obtained when malt is ground and dissolved in water in the presence of ammonium solution to inhibit enzyme activity. This is done at 20^{o}C and usually calculated as a percentage of the original weight. In this investigation, the values obtained appeared to be similar though ICSV400had the highest value followed by SK5912 and KSV8 in the order of 37%, 35% and 32%, respectively (table 14). There is some relationship between protein content and cold water extract (Narziss, 1980). For instance, barley which has 10% nitrogen and shows a cold water extract of 38% to 42% is said to have a favourable value. In the same vein our sorghum cultivars with protein contents of 10.2% - 8.5% which gave cold water extract values between 33% and 37% could be regarded as having a good C.W.E value. The highest value of 37% was recorded for ICSV400. Ogu *et al* (2005) recorded very high value of 51% C.W.E but could not reconcile this value with the corresponding nitrogen contents of their own sorghum varieties. It should be noted, however, that the nitrogen content of the cereal and eventually the C.W.E could be influenced by the climatic condition and soil on which it was grown (Narziss 1980)

Diastatic Power (DP)

Inspite of this study being based on improved sorghum varieties, the diastatic power of the cultivars could not be compared with values obtained in the barley malt. SK5912 had 28^{o}L, KSV8 had 26^{o}L while ICSV400 had 30^{o}L. It is generally believed that sorghum malt has low diastatic power, this time only when compared with barley malt. Light barely malt for instance has daistatic power ranging from 49^{o}L to 94^{o}L whereas dark barley malt has a range of 19^{o}L to $49^{o}Lkg^{-1}$ (Krűger and Bielig, 1975). When compared to the values obtained during this study, the difference is wide and clear. Low diastatic power of sorghum probably swings sorghum beer brewers to resort to exogenous amylolytic enzymes during mashing. (Ogu et al., 2005).

(Krűger and Bielig, 1975). When compared with the values obtained during this study, the difference is wide an clear. (Agu and Palmer, 1998) argued that the wide spread difference in the values could be from the chemicals used in the analysis. Odibo et al,(2006) had values similar to the ones obtained during this study namely SK5912, 29^{o}L, KSV4, 30^{o}L, KSV8, 27^{o}L and ICSV400, 28^{o}L. On the other hand, (Ogu et al, 2005) had very low values ranging from 8^{o}L to

10^{o}L, and suggested that low diastatic power of sorghum probably swings sorghum beer brewers to resort to exogenous amylolytic enzymes during mashing.

HOT WATER EXTRACT (HWE): Hot water extract is the extract yield obtained when mashing is carried out at 65^{o}C and 30 mins given for amylase enzymes to act. It is expressed in degrees Lintner per kilogram ($^{o}LKg^{-1}$). The values obtained for the sorghum cultivars under study were satisfactory. SK5912 had the highest value (28 $^{o}LKg^{-1}$) followed by ICSV400 (223 $^{o}LKg^{-1}$) while KSV8 had (212$^{o}LKg^{-1}$). These values commend the varieties as suitable brewing raw materials. SK5912 was among the first variety to be recommended for brewing purposes (Aniche and Okafor, 1980) because it showed good malting properties.

MALT COLOUR.

The malt colours ranged from 5EBC to 8EBC, ICSV400 showing the highest 8.0EBC (Kunze, 1999) showed that pale malt colour range was from 3,5EBC to 4.0EBC while dark malt ranged from 9EBC to 16EBC. This seems to suggest that our malt is neither pale nor dark. Having kilned at 50^{o}C for 24 hrs shows that the temperature was both mild and the time short. On industrial bases, kilning takes much longer and a higher temperature of about 80^{o}C or more. Such a procedure gave room for melanoidine formation and so a darker malt colour profile. This is the European method. Even in some cases, sulphur may be used to give the malt a lighter color profile as in the case of pale malt. (Narziss 1980)

Characteristics Of Worts Derived From Unmalted Sorghum Varieties Using Enzymes Preparations (Table 13)

the worts obtained by the new mashing regime showed very high levels of extract yield. SK5912 had 1050^{o}P, ICSV400 had 1055^{o}P while KSV8 had 1065^{o}P. these values were much higher than the values obtained from worts derived from malted sorghum varieties described earlier in table 12. many breweries in Nigeria today brew with unmalted sorghum and maize as their main raw material. The also utilize mixtures of exogenous enzymes for their mashing. Though the breweries produce acceptable beer, they hardly reach the levels of extracts which have been achieved in this study. Life breweries Onitsha, for instance does not exceed an (OG) of 1030 ^{o}P. That not withstanding they spend more hours in mashing – a minimum of 3 hours. This new mashing regime would therefore yield more extracts and at the same time, save cost by shortening the time spent on mashing. Ogu *et al* (2005) showed that the use of exogenous enzyme mixtures would increase extract yield in malted sorghum worts. The authors reported and recorded extract yields of 1040^{o}P in SK5912, 1045^{o}P in KSV8, 1056^{o}P in ISV400 and 1035^{o}P in in KSV4. Archibong et al,(2009) also produced worts of reasonably high levels of original gravity (OG) using mixtures of exogenous enzymes. The authors however, malted new improved varieties of sorghum namely SCSV III, which had an (OG) of 1039oP, SSV 200504, (OG) 1037oP and SSV 200503, (OG) 1036 ^{o}P. all the aforementioned authors including our group of researchers have shown that extract yield could be improved upon by the use of exogenous enzymes. The distinction however, is that it has been shown that in addition, time and cost could equally be saved using the new mashing regime.

The economics of the new mashing regime: its benefit to the Nigerian brewers:

1. There is no need to construct a malting factory which would gulp millions of naira for purchase of land and labour.
2. The money needed for the equipment would have been saved as well.
3. The employment of labor force would have been a welcome idea but then the workers would equally be paid.
4. As soon as this new method of worth production is put into practice, nationwide, the price of beer would be reduced and the money saved for beer purchase would be used for other necessities.

Characteristics of fermented worts obtained from malted sorghum varieties using commercial enzymes.

The apparent extracts were higher in KSV8, (2.5%) while both SK5912 and ICSV400had equal values, (2.04%). The extracts, O.G were also the same in SK5912 and ICSV400viz (10.2%) KSV8 once again had a higher value (10.25%). These values were in line with the values obtained by (Ogu et al, 2005). Fermented wort obtained from barley would have a higher value ranging from (11.5%) to (12%), Kunze (1999). The alcohol content of the fermented worts were (3.41%) for KSV8, (3.40%) for SK5912 and ICSV400 respectively. The percentage attenuation for these worts were the same for both KSV8 and ICSV400, (73.2%) but SK5912 recorded (76%) of attenuation Percentages were also the same for KSV8 and ICSV400, (64.3%) while SK5912 had (73.0%). These values indicated a rather vigorous primary fermentation due probably to viable yeast activities. In fermented barely wort for instance, the percentage apparent attenuation could reach 80 – 85%.

The values recorded for foam stability were the same for the three varieties under investigation 129 (EBC). These values were good because the fermented worts in question were not hopped; they were also not carbonated. The best foam stability value for hopped carbonated beer from fermented barley wort ranges from 130 – 140 EBC Nazis (1980). The P^H values were the same for all the fermented worts, 5.2. This values was rather high and would encourage microbial spoilage. PH4.3 to 4.6 is regarded as ideal for barley beer. The wort viscosity was the same for KSV8 and ICSV400 (1.579 mPa.s). SK5912 recorded (1.557 mPa.s). These values were rather high but not surprising because during mashing at 62^oC both the pH and temperature optimum of the glucanases and pentosanases were skipped. The problems of wort run off and filtration would be anticipated.

The α- amio nitrogen (FAN) values were the same for KSV8 and SK5912, 220mg/l. The value was higher in ICSV400 (230mg/L). These bitter unit values were 18BU for SK5912 and ICSV400, while KSV8 had 20BU. These values were normal for unhopped fermented worts from sorghum and could have originated from dissolved hemicellulosic materials of the cereal and perhaps brewing water. Compared to the hopped pilsener worts 30 – 40 (BU) one would admit that the values we obtained were encouraging.

[Table 12]: **Characteristics of fermentated worts derived from sorghum malts from malted sorghum varieties using commmercial enzymes (1ml).**

The percentage apparent extract of the worts were equal in SK5912 and KSV8 (2.03%) respectively; ICSV400had 2.02%). The differences in these values were certainly insignificant. The original extract values were also the same in SK5912 and KSV8, (10.15%) ICSV400 on the other hand (10.10%). The alcohol (%) contents of both SK5912 and KSV8 were once again the same. While ICSV400 had (3.6%), the other two had (3.38%) respectively. There seems to be some affinity and similarity in the properties of these two cultivars as the above discussion portrays as regards apparent attenuation and end attenuation, the values were the same for all the wort samples (80%) for apparent attenuation and (65.5%) for percentage end degree of attenuation, respectively.

The foam stability value was 126 (EBC) for all the samples while the P^H values were also similar, 4.5 EBC to 4.6(EBC). The viscosity was higher in SK5912, 1.550(mPa.s) while KSV8 and ICSV400 had 1.554 (mPa.s) each. The values for α – amino nitrogen (FAN) were the same for SK5912 and KSV8, 220(mg/l) while ICSV400 had a higher value. 230 (mg/l). High (FAN) levels indicate good proteolysis.

Bitterness units (BU) were the same for all the samples under investigation – 12.5(BU). Though there is no standard wort, Findlay (1977) our investigation has shown that the worts we studied will end up producing good quality beer based on the high levels of all the properties we have studied with particular reference to (FAN) whose high values > 220mg/l was indicative of sufficient yeast nutritional materials. The worts so obtained would encourage vigorous fermentation, leading to high apparent and end attenuation percentages and finally good alcohol content for good flavour and security against in – bottle fermentation.

[Table 13]: **Characteristics Of Fermented Worts Derived From Sorghum Malts From Unmalted Sorghum Varieties Using Commercial Enzymes (1ml).**

KSV8 had the highest percentage extract (2.5%) followed by ICSV400(2.3%) and SK5912 (2.2%). The extract % was also highest in KSV8 (10.65%) followed by ICSV400, (10.55%) and SK5912 (10.50%). These values were normal and reflected proper amylolytic activities during mashing. The alcohol contents of all varieties were similar and ranged from (3.5%) in SK5912 to (3.6%) in KSV8. All the parameters so far considered seem to give KSV8 an edge over all others. As regards percentage apparent attenuation, SK5912 had the highest percentage and degree of fermentation (64%) followed by ICSV400(63.9%) and lastly KSV8 (62.3%). Both the foam stability and PH values were similar in all the varieties. The Viscosity values were rather low and ranged from (1.033 mPa.s) in both SK5912 and ICSV400to (1.050 mPa.s) in KSV8. The values for α–amino nitrogen (FAN) were 128 (mg/l) in SK5912, 133 (mg/l) in KSV8 and 124 (mg/l) in KSV 400. the bitterness with (BU), 12.0(BU) were the same for all samples. These values when compared with hopped worts would not be accessed as good but under the prevailing circumstances, they were acceptable.

CONCLUSION

This research has shown that malts from sorghum varieties SK5912, KSV8 and ICSV400 have improved qualities which will enable brewers to use them effectively in beer production. These qualities include high extract yield, whether malted or unmalted sorghum, a good spectrum of reducing sugars and appreciable FAN levels. To achieve all these needed a well – designed mashing programme. Our Mashing regime could be described as partly infusion and partly decoction but had nothing to do with decantation mashing advocated by (Agu and Palmer 1997). In the mashing design, the aim was to shorten the time by starting at a rather high temperature (62^{o}C) thereby emphasizing β- amylase activities. This measure enabled sufficient reducing sugars to be produced and all the sugars would be easily fermented. The addition of termamyl at 85^{o}C ensured both liquefaction and saccharification and thereby promoted wort run-off at the end of mashing.

The introduction of amyloglucosidase, a debranching enzyme at 72-75^{o}C was to ensure that the more glucose was formed during the amylase rest which takes about 1 hour or more. The application of neutrase, a protease at 62^{o}C was to have as much of free amino nitrogen (FAN) as possible to enable the yeast obtain sufficient nutrition so as to perform optimally during fermentation. Nigerian brewers are advised by these research findings to embark on abridged infusion mashing system which we used using improved sorghum varieties and commercial enzymes. This system definitely saves time and cost and produces a high level of extract as well as beer of acceptable organoleptic properties.

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APPENDIX 1: PREPARATION OF IODINE SOLUTION

About 0.5g of potassium iodide was dissolved in 10ml of distilled water, few pellets of iodine were added to the solution and mixed.

APPENDIX 2: PREPARATION OF FEHLINGS SOLUTIONS A & B

Fehlings' solution A: About 69.28g of pure $CuSo_4.5H_20$ oos dissolved in distilled water containing a few drops of (about 0.5ml) of concentrated H_2So_4 and the solution is made up to 1000ml(1 litre) at 20^oC with distilled water.

Fehlings Solution B: About 100g and pure NaOH and 346g of pure salt of sodium potassium tatarate ($NaKC_4H_6O_6H$) are dissolved in distilled water. It is filtered (if necessary) through a sintered glass funnel and the filtered is made up on washing to 1000 and 20^oC with distilled water. The solution is kept in tightly stoppered.

APPENDIX 3: DETERMINATION OF α – AMINO NITROGEN (FAN) IN THE WORT SAMPLES. NINHYDRIN METHOD.

Preparation of Reagents For FAN determination (Ninhydrin method)

(a) Colour Reagents: Exactly 100g of sodium hydrogen orthophosphate ($NA_2HPO_412H_2O$) 60g potassium dihydrogen orthophosphate (KH_2PO_4), 5g Ninhydrin (A.R.gradesigned) and 3g of fructose (BDH) were dissolved in 1 litre of distilled water.

(b) Dillution Solution: this was obtained by dissolved 2g potassium iodate in 600ml 96% ethanol

(c) Glycerine Standard Stock Solution: 107.2mg of glycerine was dissolved in 100ml of water.

(d) Glycderine Standard working Solution: This was obtained by diluting 1ml of glycerine stock solution to 100ml with distilled water so that the diluted solution contains 2mg α-amino nitrogen per litre.

(e) Wort: Hot water Extract wort (HWE) obtained according to 10B methods, was diluted 100 times with distilled water.

APPENDIX 4. PREPARATION OF 3,5 DINITROSALICYCLIC ACID REAGENT (DNS)

Dissolved 2.5g of 3,5 dinitrosalicyclic acid in 50ml of 2M NaOH (Solution does not mix easily, use stirrer).

Dissolve 75g sodium potassium tartarate ($NaKC_4H_6O_6.4H_2O$) in 125 ml of distilled water.

Mix the two properly and make up to 250ml water

Filter through whatever No1 filter paper before storage and subsequent use.

APPENDIX 5: LANE AND EYNON FACTORS FOR 25ml FEHLINGS SOLUTION

1ml of sugar Solution used5	Invert sugar (Sucrose absent)	Glucose	Anhydrous Maltose
15	123.6	120.2	197.8
16	123.6	120.2	197.4
17	123.6	120.2	197.0
18	123.7	120.2	196.7
19	123.7	120.3	196.5
20	123.8	120.3	196.2
21	123.8	120.3	195.8
22	123.9	120.4	195.5
23	123.9	120.4	195.1
24	124.0	120.5	194.6
25	124.0	120.5	194.5
26	124.1	120.6	194.2
27	124.1	120.6	193.9
28	124.2	120.7	193.6
29	124.2	120.7	193.3
30	124.3	120.8	193.0
31	124.3	120.8	192.8
32	124.4	120.8	192.5
33	124.4	120.9	192.2
34	124.5	120.9	191.9
35	124.5	121.0	191.7
36	124.6	121.0	191.4
37	124.6	121.1	191.2
38	124.7	121.2	191.0
39	124.7	121.2	190.8
40	124.8	121.2	190.5
41	124.8	121.3	190.3
42	124.9	121.4	190.1
43	124.9	121.4	189.8
44	124.0	121.5	189.6
45	125.0	121.5	189.4
46	125.1	121.6	189.2
47	125.1	121.6	189.0
48	125.2	121.7	188.9
49	125.2	121.7	188.8
50	125.3	121.8	188.7

APPENDIX 6: PREPARATION OF BUFFER.

Citrate Buffer

Stock Solutions:

A: 0.1M solution of citric acid (21.0kg in 100ml)

B: 0.1M solution of sodium citrate (29.41g $C_6H_5O_7Na3.2H_2O$ I n 1000ml) xml of AxYml of B, diluted to a total of 100ml.

X	Y	PH
46.5	3.5	3.0
47.3	6.3	3.2
40.0	10.0	3.4
37.0	13.0	3.6
35.0	15.0	3.8
33.0	17.0	4.0
31.5	18.5	4.2
28.0	22.0	4.4
25.5	24.5	4.6
23.0	27.0	4.8
20.5	29.5	5.0
18.0	32.0	5.2
16.0	34.0	5.4
13.7	36.3	5.6
11.8	32.2	5.8
9.5	41.5	6.0
7.2	42.8	6.2

APPENDIX 7: PHOSPHATE BUFFER

Stock Solutions.

A. 0.2M solution of monobasic sodium phosphate (27.8g in 10ml)

B. 0.2M solution of diabasic phosphate (53.65g of $Na_2HPO_47H_2O$) or 71.7g $Na_2HPO_4.12H_2O$ in 1000ml)

Xml of A+Yml and of B, diluted to a of 200ml

X	Y	PH
93.5	6.5	5.7
92.0	8.0	5.8
90.0	10.0	5.9
87.7	12.3	6.0
85.0	15.0	6.0
81.5	18.5	6.2
75.5	22.5	6.3
73.5	26.5	6.4
68.5	31.5	6.5
62.5	37.5	6.5
56.5	43.5	6.9
51.0	49.0	6.9
47.0	55.0	7.0
39.0	61.0	7.11
33.0	67.0	7.2
28.0	72.0	7.3
23.0	77.0	7.4
19.0	81.0	7.5
16.0	84.0	7.6
12.0	87.0	

APPENDIX 8: ACETATE BUFFER .

Stock Solution

A: 0.2M solution of acetatic acid (11.55ml in 100ml)

B: 0.2M solution of sodium acetate (16.4g of $C_2H_3O_2Na$ or 27.20 of $C_2H_3ONa.3H_2O$ in 100ml

Xml of A + Yml of B diluted to a total of 100ml

X	Y	PH
46.3	3.7	3.6
44.0	6.0	3.8
41.0	9.0	4.0
36.8	13.2	4.2
30.5	19.5	4.4
25.5	24.5	4.6
14.8	35.2	5.0
10.4	39.5	5.2
8.8	41.2	5.4
4.8	45.2	5.6

APPENDIX 9: CITRATE – PHOSPAHATE BUFFER

Stock Solutions

A. 0.1M Solution of citric acid (19.21g in 1000 ml)

B. 0.2M solution in diabasic sodium Phosphate (53.65g of Na2HPO4.7H2O in 1000ml xml of A+ O yml of B, diluted to a total of 100ml

X	Y	PH
44.6	5.4	2.6
44.2	7.8	2.8
39.8	10.2	3.0
37.7	12.3	3.2
35.9	14.1	3.4
33.9	16.1	3.6
32.3	17.7	3.8
30.7	19.3	4.0
29.4	20.6	4.2
27.8	22.2	4.4
26.7	23.3	4.6
25.2	24.8	4.8
24.3	25.7	5.0
23.3	26.7	5.2
22.2	27.8	5.4
21.0	29.0	5.6
19.7	30.3	5.8
17.9	32.1	6.0
16.9	33.1	6.2
15.4	34.6	6.4
13.6	36.4	6.6
9.1	42.9	6.8
6.5	43.6	7.0

Appendix 10: Standard Glucose Curve

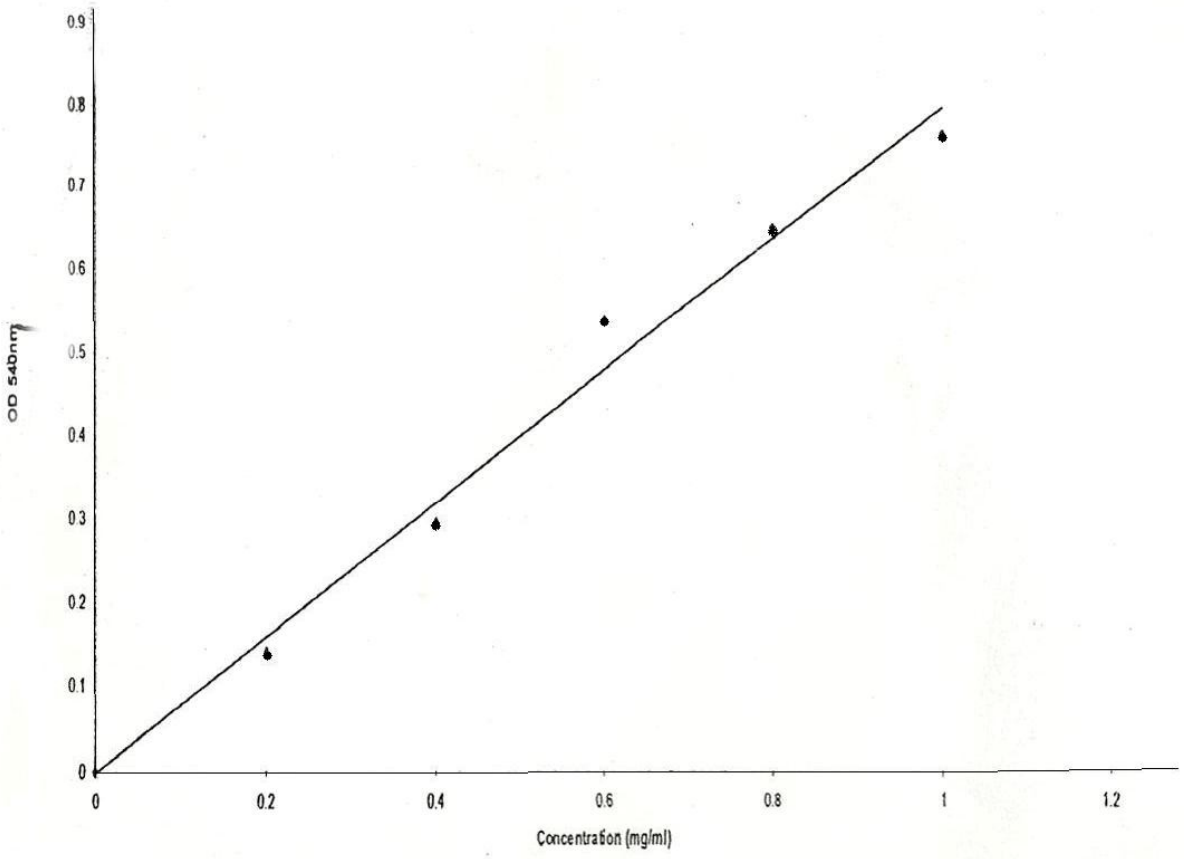

Fig. 9 : Standard Glucose Curve

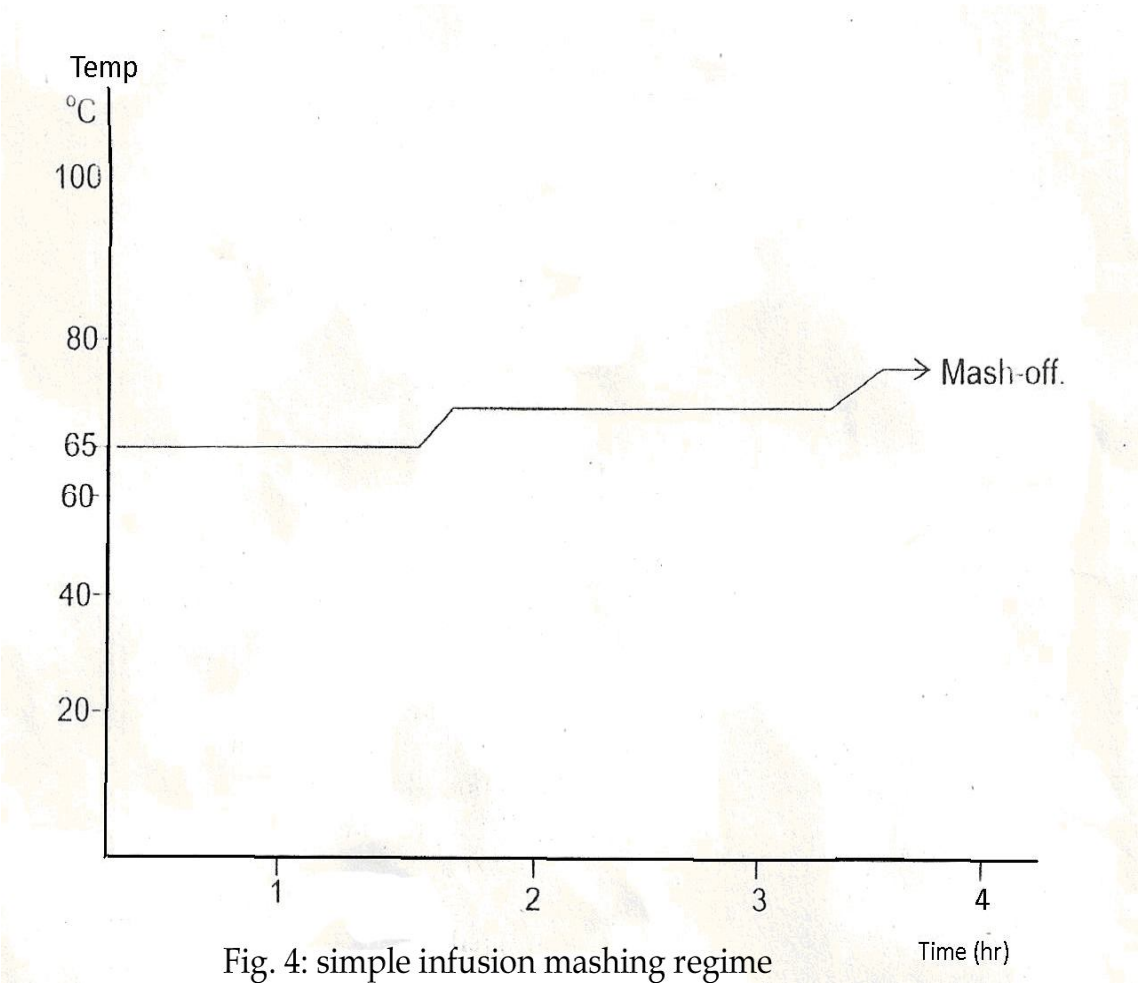

Fig. 4: simple infusion mashing regime

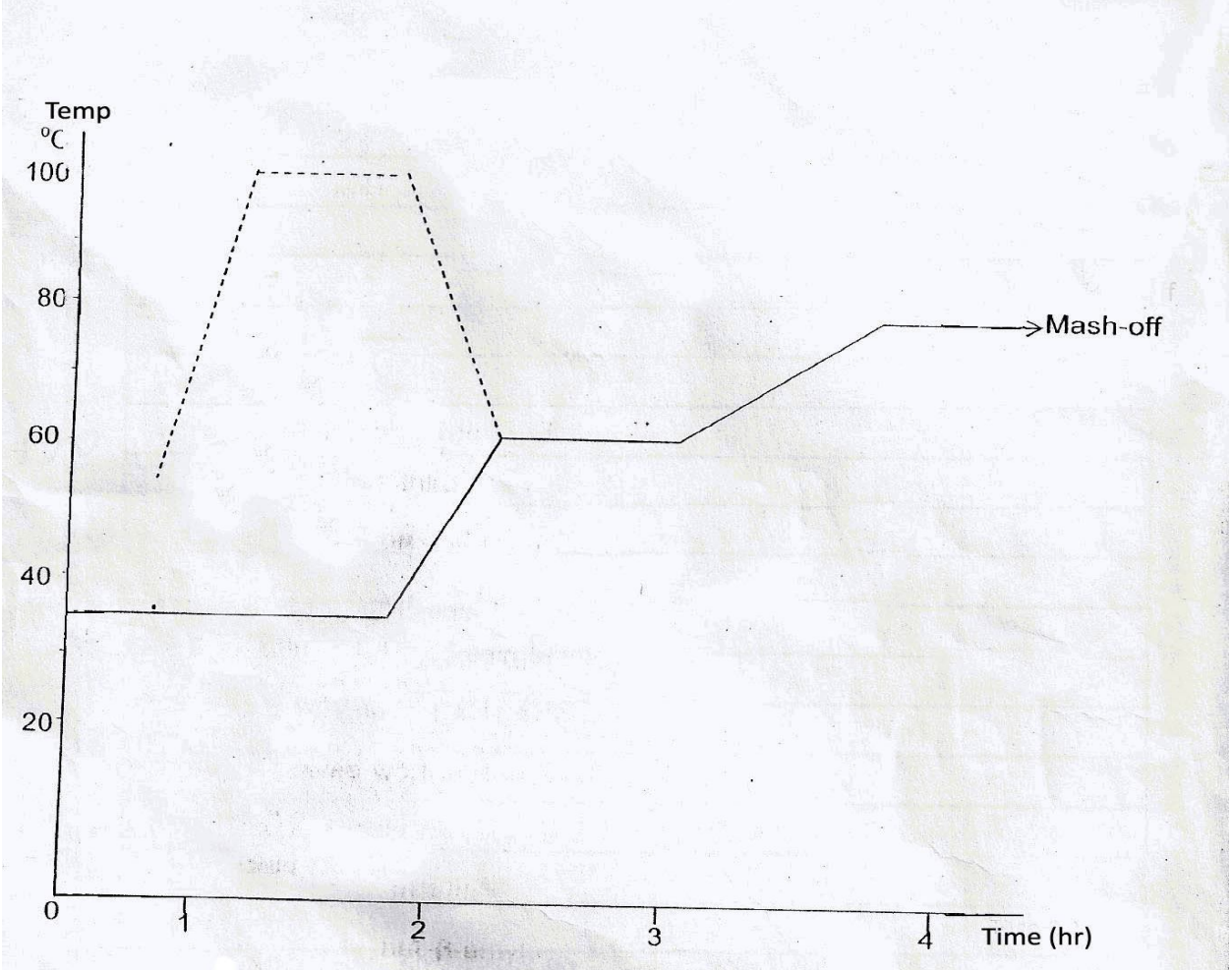

Fig 5: A double Mash Process (Okafor, 2007)

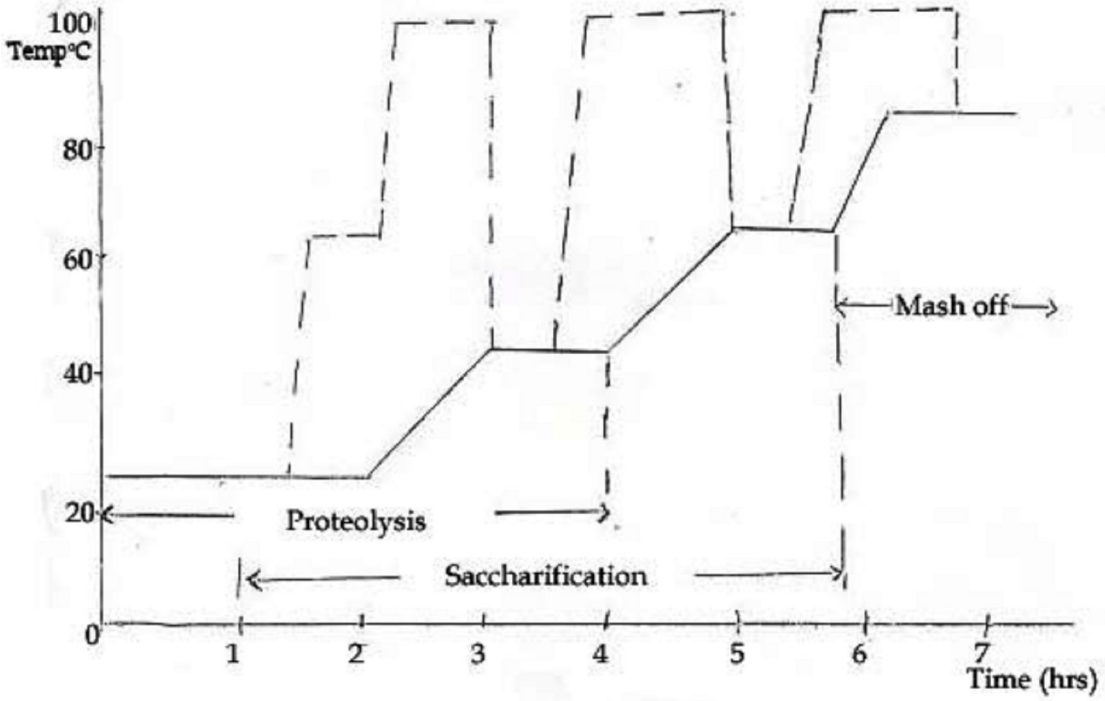

Fig. 6. Three- mash decoction process. Ogu (2003)

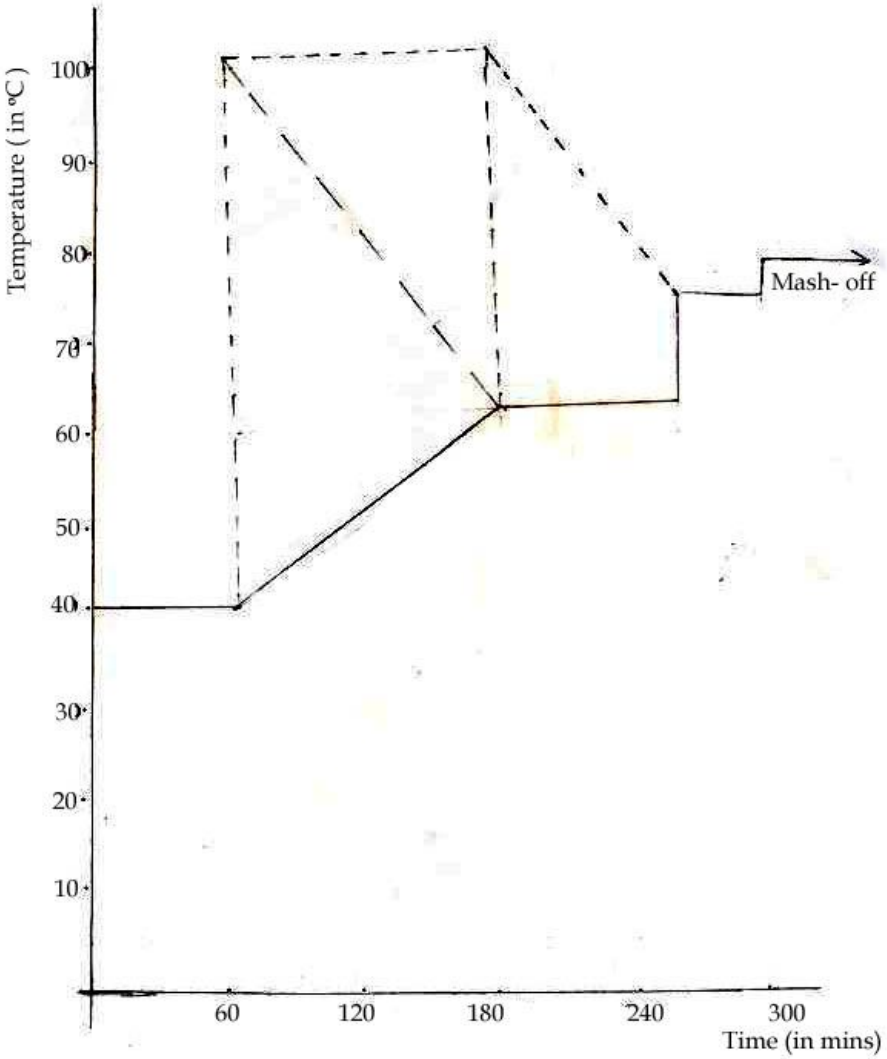

Fig. 7: Two Mash Decoction Mashing

APPENDIX 11: STATISTICAL ANALYSIS OF VARIABLES REPRESENTED IN VARIOUS TABLES.

Two – Way analysis of Variance

Between – Subjects Factors

		Value Label	N
Preparation Process	1.00	Malted	27
	2.00	Unmalted	30
	3.00	Normal Infusion	30
Sorghum Varieties	1.00	SK5912	29
	2.00	KSV8	29
	3.00	ICSV400	29

Descriptive Statistics

Dependent Variable: Response

Preparation Processes	Sorghum Varieties	Mean	Std. Deviation	N
Malted	SK5912	58.2344	75.40492	9
	KSV8	58.2349	75.40454	9
	ICSV400	59.3371	78.11791	9
	Total	58.6021	73.32764	27
Unmalted	SK5912	43.1350	51.94552	10
	KSV8	43.1930	52.52371	10
	ICSV400	42.6325	51.14141	10
	Total	42.9868	50.05328	30
Normal Infusion	SK5912	169.1535	312.64846	10
	KSV8	169.7265	313.79474	10
	ICSV400	171.1565	313.71428	10
	Total	170.0122	302.38802	30

Descriptive Statistics

Preparation Process	Sorghum Varieties	Mean	Std. Deviation	N
Total	SK5912	91.2757	193.01777	29
	KSV8	91.4934	193.74212	29
	ICSV400	92.1353	194.08562	29
	Total	91.6348	191.35145	87

Tests of Between-Subjects Effects

Dependent Variable:Response

Source	Type II Sum of Squares	Df	Mean Square	F	Sig.
Model	1.015E6	5	203059.088	5.814	.000
Preparation.Processes	284750.302	2	142375.151	4.076	.021
Sorghum.Varities	11.584	2	5.792	.000	1.000
Error	2864160.443	82	34928.786		
Total	3879455.885	87			

a. R Squared = .262 (Adjusted R Squared = .217)

Estimated Marginal Means

1. Preparation Processes

Dependent Variable:Response

Preparation Processes			95% Confidence Interval	
	Mean	Std. Error	Lower Bound	Upper Bound
Malted	58.602	35.967	-12.949	130.153
Unmalted	42.987	34.122	-24.892	110.866
Normal Infusion	170.012	34.122	102.133	237.891

2. Sorghum Varities

Dependent Variable:Response

Sorghum Varities			95% Confidence Interval	
	Mean	Std. Error	Lower Bound	Upper Bound
SK5912	90.175	34.719	21.107	159.242
KSV8	90.392	34.719	21.325	159.460
ICSV400	91.034	34.719	21.966	160.102

Post Hoc Tests
Preparation Processes

Multiple Comparisons

Response

LSD

(I) Preparation Processes	(J) Preparation Processes	Mean Difference (I-J)	Std. Error	Sig.
Malted	Unmalted	15.6153	49.57773	.754
	Normal Infusion	-111.4100*	49.57773	.027
Unmalted	Malted	-15.6153	49.57773	.754
	Normal Infusion	-127.0253*	48.25542	.010
Normal Infusion	Malted	111.4100*	49.57773	.027
	Unmalted	127.0253*	48.25542	.010

Based on observed means.

The error term is Mean Square(Error) = 34928.786.

*. The mean difference is significant at the .05 level.

Multiple Comparisons

Response

LSD

(I) Preparation Processes	(J) Preparation Processes	95% Confidence Interval	
		Lower Bound	Upper Bound
Malted	Unmalted	-83.0106	114.2412
	Normal Infusion	-210.0359	-12.7841
Unmalted	Malted	-114.2412	83.0106
	Normal Infusion	-223.0207	-31.0299
Normal Infusion	Malted	12.7841	210.0359
	Unmalted	31.0299	223.0207

Based on observed means.

The error term is Mean Square(Error) = 34928.786.

Two-Sample T-Test and CI: SK592, Unmalted and Malted

```
Two-sample T for SK592

Unmalted
and                          SE
Malted    N  Mean  StDev  Mean
1        10  43.1   51.9    16
2         9  58.2   75.4    25


Difference = mu (1) - mu (2)
Estimate for difference:  -15.0994
95% CI for difference:  (-79.5002, 49.3013)
T-Test of difference = 0 (vs not =): T-Value = -0.50  P-Value = 0.623
DF = 14
```

Two-Sample T-Test and CI: SK592, Unmalted and Normal Infusion

```
Two-sample T for SK592

Umalted
and
Normal                       SE
Infusion   N  Mean  StDev  Mean
1         10  43.1   51.9    16
2         10   169    313    99


Difference = mu (1) - mu (2)
Estimate for difference:  -126.019
95% CI for difference:  (-352.740, 100.703)
T-Test of difference = 0 (vs not =): T-Value = -1.26  P-Value = 0.240
DF = 9
```

Two-Sample T-Test and CI: KSV8, Umalted and Normal Infusion

```
Two-sample T for KSV8

Umalted
and
Normal                       SE
Infusion   N  Mean  StDev  Mean
1         10  43.2   52.5    17
2         10   170    314    99


Difference = mu (1) - mu (2)
Estimate for difference:  -126.534
```

```
95% CI for difference:  (-354.132, 101.065)
T-Test of difference = 0 (vs not =): T-Value = -1.26  P-Value = 0.240
DF = 9
```

Two-Sample T-Test and CI: KSV8, Unmalted and Malted

```
Two-sample T for KSV8

Unmalted
and                          SE
Malted    N  Mean  StDev  Mean
1        10  43.2   52.5    17
2         9  58.2   75.4    25


Difference = mu (1) - mu (2)
Estimate for difference:  -15.0419
95% CI for difference:  (-79.6578, 49.5740)
T-Test of difference = 0 (vs not =): T-Value = -0.50  P-Value = 0.625
DF = 14
```

Two-Sample T-Test and CI: ICSV400, Umalted and Malted

```
Two-sample T for ICSV400

Unmalted
and                          SE
Malted    N  Mean  StDev  Mean
1        10  42.6   51.1    16
2         9  59.3   78.1    26


Difference = mu (1) - mu (2)
Estimate for difference:  -16.7046
95% CI for difference:  (-82.9258, 49.5166)
T-Test of difference = 0 (vs not =): T-Value = -0.54  P-Value = 0.595
DF = 13
```

Two-Sample T-Test and CI: ICSV400, Umalted and Normal Infusion

```
Two-sample T for ICSV400

Unmalted
and
Normal                        SE
Infusion   N  Mean  StDev  Mean
1         10  42.6   51.1    16
2         10   171    314    99


Difference = mu (1) - mu (2)
Estimate for difference:  -128.524
95% CI for difference:  (-355.904, 98.856)
T-Test of difference = 0 (vs not =): T-Value = -1.28  P-Value = 0.233
DF = 9
```

Two-Sample T-Test and CI: SK5912, malted and Normal Infusion

```
Two-sample T for SK5912

malted
and
Normal                        SE
Infusion   N  Mean  StDev  Mean
1          9  58.2   75.4    25
2         10   169    313    99


Difference = mu (1) - mu (2)
Estimate for difference:  -110.919
95% CI for difference:  (-338.218, 116.380)
T-Test of difference = 0 (vs not =): T-Value = -1.09  P-Value = 0.302
DF = 10
```

Two-Sample T-Test and CI: KSV8, malted and Normal Infusion

```
Two-sample T for KSV8

malted
and
Normal                        SE
Infusion   N  Mean  StDev  Mean
1          9  58.2   75.4    25
2         10   170    314    99


Difference = mu (1) - mu (2)
Estimate for difference:  -111.492
95% CI for difference:  (-339.574, 116.591)
T-Test of difference = 0 (vs not =): T-Value = -1.09  P-Value = 0.302
DF = 10
```

Two-Sample T-Test and CI: ICSV400, malted and Normal Infusion

```
Two-sample T for ICSV400

malted
and
Normal                        SE
Infusion   N  Mean  StDev  Mean
1          9  59.3   78.1    26
2         10   171    314    99


Difference = mu (1) - mu (2)
Estimate for difference:  -111.819
95% CI for difference:  (-340.350, 116.711)
T-Test of difference = 0 (vs not =): T-Value = -1.09  P-Value = 0.301
DF = 10
```

From the table of the two-way analysis of variance, we observed that the three preparation processes (malted, unmalted, and normal infusion) are not same since the P value is equal to 0.021 is less than 0.05. Hence, we can say that there is a significant difference between the three preparation processes thereby making a new hypothesis to be rejected. Now, since the new hypothesis is rejected, we subjected the test again to multiple comparism (LSD) in order to find out which of three preparation processes actually contributed to the rejection. From the multiple comparison, we observe that normal infusion differ significantly from malted and unmalted which conforms with earlier conclusion that malted and unmalted processes take lesser time to prepare than normal infusion. Also, from the ANOVA table, we observed that the sorghum varieties (SK5912, KSV8 and ICSV400) from the three processes are the same since the P value =1.00 which is greater than 0.05