KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLGY

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DEPARTMENT OF BIOCHEMISTRY AND BIOTECHNOLOGY

SCREENING OF SIX LOCAL SORGHUM VARIETIES FOR THEIR MALTING AND BREWING QUALITIES

Thesis Submitted as a Partial Fulfilment of the Requirement for the Award of Master of Science Degree in Food Science and Technology

BY

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DECLARATION

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

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Stephen Gu-Ayebeafo Okrah Prof. W.O.Ellis

DEDICATION

This work is dedicated to the Almighty God, my daughter, Akosua Boakyewa Okrah, my wife, Elna Okrah (Mrs) and my late Auntie, Beatrice Kane Bawah.

AKNOWLEDGEMENT

I wish to express my sincere thanks and appreciation to the Almighty God who has led me thus far in my educational career. My heartfelt appreciation goes to Prof. W.O. Ellis who professionally coached and guided me through this work. Prof, may the Good Lord bless you.

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Special thanks goes to Mr I. W. Ofosu, Lecturer at the Biochemistry Department of KNUST, for the indispensable role he played during the initial stages of my work. I must make special mention of Papa Toah Akunor, a course mate for his support in the cause of my work.

Finally, to the entire Food Science and Technology class of 2004, I say “Ayekoo” for all the support I enjoyed.

ABSTRACT

Six locally available sorghum varieties were assessed for their malting and brewing qualities. With the exception of Chere, all varieties had suitable 1000 corn weights (>25 g). The differences in corn weights were also significant ($p<0.05$). Analysis of Germination energy (GE) and Germinative capacity (GC) gave results of >90 % for each variety, signifying their viability. Nitrogen content ranging from1.67 %-2.03 % was obtained for the varieties studied in their unmalted state. Results obtained for malted samples ranged between 1.38 %-1.89 %. With the exception of Dorado (fat 0.96 %), other varieties studied gave high fat contents (i.e. >1.0 %) for the malts. Significant differences were obtained for the malting losses. This ranged between 15.75 % (for Kapaala) and 28.06 %(for Chere). Strong correlation existed between malting loss and Diastatic power (DP) ($r=0.803$). DP levels for varieties studied indicated that Kapaala (40.07 ^{o}Wk) was the least modified whiles Chere (60.3 ^{o}Wk) was the most modified. Differences in DP between varieties studied were significant ($p<0.05$). The mashing regime and results obtained for Cold water extract (CWE), hot water extract (HWE) and filtration rates showed that all the varieties studied need to be pre-gelatinised before being added to barley malt mash as none gelatinised within malt enzyme temperature range (i.e. 60 ^{O}C-70 ^{O}C). Results further showed that Kapaala had the highest potential extract (77.94 %) while Sample1 (71.01 %) had the least. In all, the HWE for all the varieties studied ranged between 71.01 % and 77.94 %. Response of mashes to starch check level showed that none of the varieties gelatinises at a temperature suitable for the endogenous amylases. Thus, use of exogenous enzymes or barley malt part mash is required to hydrolyse the sorghum malt starch during the brewing process.

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CHAPTER ONE

1.0 INTRODUCTION

Malts are produced when cereals are subjected sequentially to steeping, germination and kilning under controlled conditions. In spite of the availability of several cereal types, barley has traditionally been the grain of choice when it comes to malting. In tropical Africa, however, barley cultivation has not seen any success. Thus, industries that use barley malt as their major raw materials have had to rely on imports of this grain. This has not been a problem only to the industries concerned, mostly brewing industries, but also to the economies of mostly Tropical African countries. Therefore, some tropical cereals have been investigated for their malting properties (Beta, et al, 1995, Dufour and Melotte, 1992).

Sorghum, *Sorghum bicolour,* is the fifth most important cereal after rice, wheat, maize and barley, and it contributes significantly to the protein and energy requirements for millions of people, especially for the poor persons in Africa and Asia (Elkhier and Hamid, 2008). The importance of sorghum as a food crop in sub-Saharan Africa is mainly because it is adapted to a wide range of ecological conditions and can tolerate adverse conditions such as hot and dry, also in areas of high rainfall, water logging, drought, poor fertility and salinity soils.

In Ghana, sorghum is cultivated in all the three northern regions and part of Brong-Ahafo region where the annual rainfall is relatively low with relatively long periods of drought. Potentially, sorghum holds a high economic value for the people living in the above mentioned regions and the nation at large due to its low cost of cultivation and an emerging market for its use in the brewing industry as it constitutes cheaper source of extracts for that industry and therefore, offers potential employment to all

stakeholders along the supply value chain i.e. farmers, marketers, maltstars, brewers (for both alcoholic and non-alcoholic).

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In spite of all the potential advantages sorghum offers as discussed above, the full industrial utilization of sorghum has, however, not been realized due to the following reasons. Sorghum posses starch gelatinisation temperature higher than the temperature range for endogenous amylases. Therefore, additional processing equipments are required for pre-gelatinising the sorghum starch. Furthermore poor degradation of β-glucans during malting and poor hydrolysis of starch during mashing, leads to slow wort filtration, reduced extraction efficiency and haze formation in beers (Palmer, 1989).

In Ghana, sorghum has been malted for centuries and is used for the production of baby food and traditional alcoholic and non-alcoholic beverages. In recent times, the large body of work carried out on sorghum to understand the physiological behaviour of sorghum has led to the suggestion that malting temperature of 30^{o}C would produce commercially acceptable sorghum malt (Agu, *et al*. 1997). Malting has also been shown to reduce most of the quality and processing problems associated with sorghum use (Skinner, 1976, Taylor and Boyd, 1986, Dufuor and Mellote, 1992). Also, like barley, the development of sorghum varieties suitable for malting and brewing purposes has been a major area of research and development.

This work, therefore, has as it's main objective, the investigation of the performance of six different local varieties of sorghum (Chere, Dorado, Latuor, Kapaala, Sample1 and Sample 2), with the view to assessing their malting and brewing potential and subsequently, their suitability for use as brewing adjuncts.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1. Origin, Distribution and Production of Sorghum

Sorghum is a genus of about 30 species of grasses raised for grain, native to tropical and subtropical regions of Eastern Africa with one species native to Mexico. Although wild species of sorghum are attested as early as 8000 years ago in the Nilotic regions of southern Egypt and the Sudan, the location of its true domestication within East Africa is still speculative (Taylor, 1988). It is widely held that genetic separation of domesticated *S. bicolor* from its progenitor did not occur much earlier than 2000 years ago somewhere in East Africa, possibly the Ethiopian highlands, but more likely further west. However, the presence of true domesticated *S. bicolor* is claimed much earlier than this (3700-4900 years ago) in India, Oman, and Yemen, although the identity of the remains as full domesticates is still disputed (Kimber, 2000). According to ICRISAT, (1996), other names for sorghum include Durra, Egyptian Millet, Feterita, Guinea Corn (sweet sorghum), Jowar, Juwar, kaffir corn, Milo, Shallu and Sudan. The plant is cultivated in Southern Europe, Central America, Southern Asia and Africa. According to Ryden *et al*. (1993), grain sorghum is cultivated by farmers on subsistence level in the semi-arid tropics worldwide and consumed as food staple by humans. The continent of Africa is one of the largest producers of sorghum and produces approximately 18.5metric tonnes annually. Production levels, according to ICRISAT, (1996) for leading sorghum producers are Africa (16%), Asia (36%), Central and South America (21%) and USA (20%). Leading producers around the world are USA (11.9million metric tonnes), India (9.5million metric tonnes), Nigeria (7.5million metric tonnes), and Mexico (6.4million metric tonnes) (USDA, 2005).

2.1.1 Taxonomy and Varieties of sorghum

Sorghum makes up the genus *Sorghum* in the family *Poaceae* (or *Gramineae*).

The many subspecies are divided into four groups – grain sorghum (such as milo), grass sorghums (for pasture and hay), sweet sorghums (formerly called “Guinea corn", develop a sweet juice in their stalks and are grown for syrup production), and broom corn (for brooms and brushes), (Kimber, 2000). In Africa and Ghana, for that matter, the predominant subspecies form is grain sorghum and it is grown mainly for food (Demuyakor and Ohta. 1992). Grain sorghums are classified as *Sorghum bicolour* (with many cultivars). There are many varieties of sorghum bicolour, ranging in colour from white through red to brown and mixed classes in the grain standards. (Rooney and Waniska. 2000). Grain sorghum varieties may be grouped into three categories based on grain type (Rooney and Waniska. 2000)**:**

2.1.2 Bird-Resistant or Brown-Seeded Sorghum

The bird-resistant or brown-seeded sorghum contains tannin, which gives the unripe grain a bitter taste. Brown-seeded grain sorghums are more resistant to weathering than other grain types. The seeds have high dormancy, which keeps the head from sprouting when adverse weather occurs after grain is mature. This dormancy lasts for several months. Some seeds shattered in harvesting will survive the winter and germinate in the spring, creating a weed problem in the following crop (Rooney and Waniska, 2000).

2.1.3 Yellow Endosperm or Bronze-Seeded Sorghums

The yellow endosperm or bronze-seeded sorghum grain is a cross between a red-seeded sorghum and a yellow-seeded sorghum. Most varieties are of this type. They are classified as yellow in the market, and the producer is not docked as he might be for brown-seeded grain. Bronze- seeded sorghums do not weather as much as the brown-seeded type. However, you can let them field dry to 12 to 14 percent moisture content. Some bird damage and weather damage may occur. Seed dormancy is slight, and rarely do any volunteer plant problems exist. These sorghums can be used for green chop, silage, high moisture (35-40 percent) grain and can be harvested at 20 to 25 percent grain moisture and dried. Early harvest and drying reduces weather and bird damage (USDA, 2005).

2.1.4 Yellow-Seeded Sorghums

Yellow-seeded sorghum grain is produced when both parents of a hybrid are yellow-seeded. It makes excellent quality feed. The primary disadvantage of the yellow grain is weather damage. Grains of these varieties begin to wither earlier than other grain types. Grain will generally lose quality in a relatively short period of time after grain moisture reaches 12 to 14 percent. For consistently high grain quality, yellow-seeded sorghum must be harvested at 18 to 20 percent grain moisture, or higher, and dry. This sorghum type may be used in the same ways as the bronze-seeded sorghum (Serna and Rooney , 1995).

2.1.5 Composition of sorghum grain

The percentages of the seed components of sorghum are endosperm (82%), embryo (12%), and seed coat (5-6%) are similar to corn (Rooney and Waniska. 2000).

2.1.6 World trade

The United States is currently positioned as the number one exporter of sorghum to the world market. The US share of the market has not dropped below 70% in the last decade. Argentina, China, Sudan and Australia are the other significant exporters of the crop. Argentina exports approximately 500,000MT, whilst Australia exports roughly 250,000MT annually. Sudan has exported significant quantities in the past and in 1992/3 exported approximately 600,000MT. Mexico and Japan are the two most important importers of the crop accounting for 77% of total world importers in 1996/7. Other importers include the European Union, Morocco, Israel, Saudi Arabia, and Turkey (USDA, 2005). The table below shows the sorghum production levels by the world's leading producers.

Table 1: Major Sorghum producing countries

Country	Production in 2005 (million metric tonnes)
United States America	9.8
India	8.0
Nigeria	8.0
Mexico	6.3
Sudan	4.2
Argentina	2.9
China	2.6
Ethiopia	1.8
Australia	1.7
Brazil	1.5
World trade	**58.6**

Source: UN food and Agriculture organisation (FAO), 2005

2.1.7 Economic importance

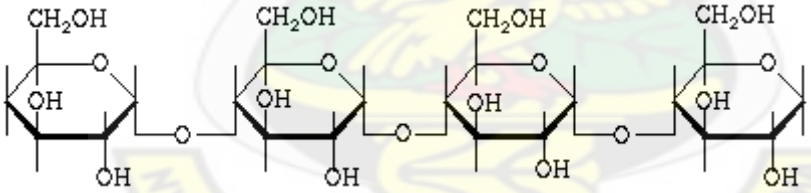
Sorghum is rated fifth in importance among the world's cereals, with 61,787 million tonnes being produced in 1988 (Zohary and Hopf, 2000). Sorghum is one of the leading cereal grains in Africa and is also important in India, China, Australia, various countries in Latin America and the United States. In Africa, where annual cereal production amounts to some 89 million tonnes, sorghum is second in importance only to maize, and accounts for 14% of the total cereal production, or 15,280 million tonnes per annum (ICRISAT, 1996).

2.2 NUTRITIONAL CONTENT

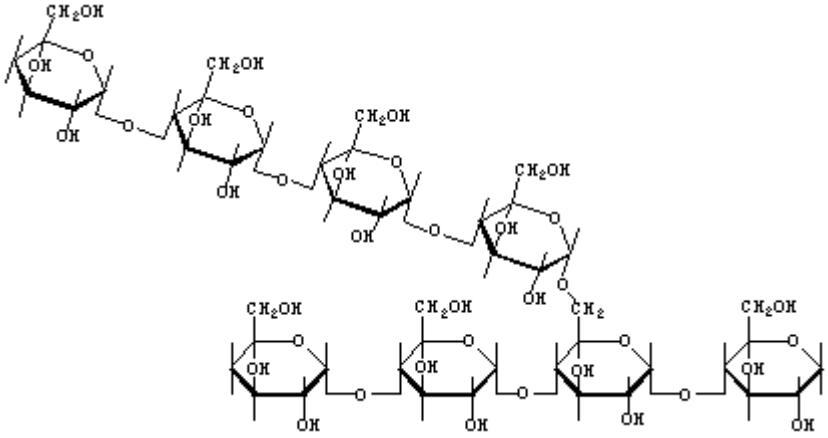
2.2.1 Carbohydrate

Starch is the major storage form of carbohydrate in sorghum and millets. It consists of amylopectin, a branched-chain polymer of glucose, and amylose, a straight-chain polymer.

Amylose



Amylopectin



With values ranging from 56 to 73 percent, the average starch content of sorghum is 69.5 percent (Jambunathan and Subramanian, 1988). About 70 to 80 percent of the sorghum starch is amylopectin and the remaining 20 to 30 percent is amylose (Deatherage *et al*, 1955). Both genetic and environmental factors affect the amylose content of sorghum (Ring, *et al*, 1982). Waxy or glutinous sorghum varieties are very low in amylose; their starch is practically 100 percent amylopectin (Ring et al, 1982; Deatherage *et al*, 1955). In sugary sorghum the amylose content of the starch is about 5 to 15 percent higher than in normal sorghum (Singh and Axtell, 1973b).

The digestibility of isolated starch of sorghum cultivars ranges from 33 to 48 percent as against 53 to 58 percent for corn starches (Sikabbubba, 1989). The digestibility of the starch, which depends on hydrolysis by pancreatic enzymes, determines the available energy content of cereal grain. Serna-Saldivar and Rooney, (1995), reported that the texture of the grain endosperm, the particle size of the flour and starch digestibility strongly correlated with each other. Starch in floury sorghum was found to be more digestible than that in corneous sorghum. Particles of ground floury sorghum were smaller than those of similarly ground corneous sorghum. The smaller particle size and correspondingly greater surface area facilitate the enzyme action and thus improve starch digestibility.

The chemical nature of the starch, particularly the amylose and amylopectin content, is yet another factor that affects its digestibility. The starch digestibility was reported to be higher in low-amylose, i.e. waxy sorghum than in normal sorghum, (Hibberd *et al*. 1982). Feeding trials in rats (Elmalik *et al*. 1986) and other animal species (Sherrod *et al*, 1969; Nishimuta *et al*, 1969) have confirmed the superiority of waxy sorghum over normal grain types in terms of dry matter and gross energy digestibility. The presence of tannins in the grain contributes to the poor digestibility of starch in some varieties of sorghum (Dreher *et al*, 1984). Tannins isolated from sorghum grain were shown to inhibit the enzyme α-amylase, and they also bind to grain starches to varying degrees (Davis and Hoseney, 1979).

Processing of the grain by methods such as steaming, pressure-cooking, flaking, puffing or micronization of the starch increases the digestibility of sorghum starch. This has been attributed to a release of starch granules from the protein matrix rendering them more susceptible to enzymatic digestion (McNeil *et al*., 1975; Harbers, 1975).

2.2.2 Gelatinization temperatures

The gelatinization temperature of isolated sorghum starch and that of finely ground flour of the corresponding endosperm has been reported to be the same (Iygor, 1987). On the other hand the pasting temperature, i.e. the temperature at which starch attains peak viscosity when heated with water to form a paste, was found to be about 10°C higher for the sorghum flour than for the isolated starch (Palmer *et al*. 1999).

The quality of cooked sorghum has been strongly associated with the total and soluble amylose content of the grain and also the soluble protein content (Cagampang and Kirleis, 1984). The swelling power of starch and its solubility

significantly influenced the cooking quality of sorghum (Subramanian *et al*. 1982). The percentage weight increase of cooked grain was negatively correlated with starch solubility at 60°C, a temperature at which most of the starch granules will have reached gelatinization stage. The swelling power of starch at 60° and 90°C and solubility at 25° and 50°C were inversely correlated with gruel solid content, which directly depended on the starch content of the grain. The starch gelatinization *temperature* did not show any significant effect on the cooking quality of sorghum.

2.2.3 Protein

Both genetic and environmental factors affect the protein content of sorghum. In sorghum the variability is large, probably because the crop is grown under diverse agroclimatic conditions which affect the grain composition (Burleson *et al*, 1956; Waggle *et al.*, 1967; and Deosthale *et al*, (1972))

Grain proteins are broadly classified into four fractions according to their solubility characteristics: albumin (water soluble), globulin (soluble in dilute salt solution), prolamin (soluble in alcohol) and glutelin (extractable in dilute alkali or acid solutions). In solubility fractionation studies with sorghum, five protein fractions were obtained (table 2 in page 12):

Wide variability has been observed in the essential amino acid composition of sorghum protein (Hulse *et al*, 1980; Jambunathan *et al*, 1984). Lysine content was reported to vary from 71 to 212 mg per gram of nitrogen and the corresponding chemical score varied from 21 to 62. Normal sorghum grown under similar conditions contained 12 percent protein and 2.1 g lysine per 100 g protein.

TABLE 2: Essential amino acid composition (mg/g) and chemical score of sorghum proteins

Amino Acid	Composition (mg/g)
Isoleucine	245
Leuccine	823
Lysine	126
Methionine	87
Cysteine	94
Phenylalanine	306
Tyrosine	167
Threonine	189
Tryptophan	63
Valine	313
CHEMICAL SCORE	37

Both in vitro and in vivo studies have demonstrated wide variability in protein digestibility of sorghum varieties (Axtell *et al*. 1981). Values ranging from 49.5 to 70 percent (Nawar *et al*. 1970) and from 30 to 70 percent (Silano, 1977) have been reported. The protein digestibility of sorghum grain was found to be extremely poor (45 percent) as compared to that previously observed for wheat (81 percent), maize (73 percent) and rice (66 percent) (Maclean *et al*. 1983).

However, in vitro studies conducted on extruded sorghum (Mertz *et al*. 1984) showed that extrusion processing of sorghum grain improved the protein digestibility and hence the nutritive value. Digestibility of sorghum protein was also improved after processing of the grain into nasha, a thin fermented porridge used as baby food in the Sudan (Graham and Carpenter, 1981). A decrease in the protein digestibility of sorghum on cooking was attributed to reduced solubility of prolamin and its reduced digestibility by pepsin (Hamaker *et al*. 1986).

2.3. Primary Uses of sorghum

While there are literally thousands of potential uses for this diverse grain, focus is being placed in food, fuel and feed, the primary growth area markets for grain sorghum (USDA, 2005).

2.3.1 Food

In many parts of the world sorghum has traditionally been used in food products and various food items, like porridge, unleavened bread, cookies, cakes, couscous, and malted beverages are made from this versatile grain. (Carter and Capenter, 1981)

Traditional food preparation of sorghum is quite varied. Boiled sorghums are one of the simplest uses and small, corneous grains are normally desired for this type of food product. The whole grain may be ground into flour or decorticated before grinding to produce a fine particle product or flour, which is then used in various traditional foods (Rooney *et al*. 2000).

Thick porridges are called ugali (Kenya, United Republic of Tanzania, Uganda), to (Burkina Faso, the Niger), tuwo (Nigeria),tuo zaafi (Ghana), aceda (the Sudan), bogobe, jwa ting (Botswana) and sadza (Zimbabwe).Thin porridges are called uji (Kenya, United Republic of Tanzania), ogi or koko (Nigeria, Ghana), edi (Uganda), rouye (the Niger, Senegal), nasha (the Sudan), rabri (India), bota or mahewu (Zimbabwe) and motogo we tiny (Botswana) (ICRISAT, 1992). Sorghum flour, sorghum malt, pigeon pea and groundnut are mixed in different proportions to improve the nutritional value of traditional porridges (Nout *et al*., 1988).

The palatability and the texture of foods can be changed and their shelf life can often be improved by fermenting them. Oniang'O and Alnwick (1988) described fermented

porridge made in Africa from sorghum, finger millet and pearl millet, which are commonly served to children. Fermented porridges are variously thought to promote lactation and to be unsuitable for young children. Ogi, a popular fermented porridge in Nigeria, is prepared using sorghum, millet and maize in various proportions (Steinkraus, 1983; Tomkins *et al*, 1988). Fermented breads called 'masa' and an unleavened bread called 'waina' are also produced in Ghana and Nigeria respectively (USDA, 2005).

Sorghum is a good source of lactobacilli, which is used in souring of foods used in traditional fermented foods and drinks (USDA, 2005). The predominant volatile and non-volatile acids in Ogi are lactic and acetic acids, respectively. Traces of formic acid have also been detected. These give Ogi its characteristic aroma and its sour taste (Usha *et al*, 1996).

In Kenya and South Africa, there is a small but growing market for pearled sorghum as an alternative to rice. In India, proposals have been made for use of dehulled sorghum within feeding regimes for infants and children (Hugo *et al*. 2003)

3.3.2 Weaning foods

Sorghum and millets are used in weaning foods in countries such as Ethiopia, India, the United Republic of Tanzania and Uganda. According to Seenappa, (1988) germinated sorghum flour, called "power flour" (kimea in the United Republic of Tanzania), reduces the viscosity of the food product. It is thus possible to use double the quantity of flour to make a product of similar consistency, so the energy density of weaning foods can be increased.

The quality of weaning foods prepared from cowpea and malted or roller-dried sorghum was evaluated by (Malleshi *et al*, 1989). The results showed that weaning food formulation based on malted sorghum and malted cowpea was nutritionally superior to roller-dried weaning food prepared using the unmalted raw materials. The available Iysine content was 3.85 percent in the malted food and 2.95 percent in roller-dried sorghum food. The protein efficiency ratio of malted food was 2.26, significantly higher than that of the roller-dried food (1.87). The cooked paste viscosity of malted sorghum was considerably lower than that of the roller-dried sorghum.

2.3.3 Traditional beers

Africa has a tradition of making opaque beers by the use of sorghum as the source of malt and the adjunct; though for commercial brewing maize may often be the source of the adjunct. Opaque beer is a product of a lactic and alcoholic fermentation, which is sold in a microbially active state, with a shelf life of only 5-7 days (Daiber and Taylor, 1995). This beer, for which sorghum and millets are valuable raw materials, is a popular beverage in several countries in Africa. It is called chibuku in Zimbabwe, impeke in Burundi, dolo in Mali and Burkina Faso and pito in Nigeria and Ghana. The main attributes of this product are short shelf life of about one week, low alcohol content, acidic nature, suspended solids and a characteristic taste and colour (Chitsika and Mudimbu, 1992).

Opaque beer is more than a beverage. It contains high proportions of starch and sugars, besides proteins, fat, vitamins and minerals. Malts are also sources of lactobacilli and essential nutrients.

Chevassus-Agnes *et al*, (1976) found that the traditional beer, amgba, and a wine, affouk, prepared from sorghum in Cameroon were found to be nutritionally superior to sorghum flour as they provide additional riboflavin, thiamine and lysine. Price and Butler (1980) also found that iron absorption from maize and sorghum beer was more than 12 times higher than that from the constituents that were used to prepare the beer. According to Taylor and Robbins, (1993) in traditional sorghum beer, most of the thiamine and about half of the riboflavin and niacin are associated with beer solids, which contain the yeast. The beer with the highest total solids contained the highest amounts of minerals and trace elements. Thus the beer is a source of vitamins, iron, manganese, magnesium, phosphorus and calcium. The beer contained 26.7 g starch and 5.9 g protein per litre.

In its manufacture white sorghum with less polyphenols is preferred, although red and brown sorghum varieties are also used. Red sorghum imparts a pinkish-brown colour to the beer. High tannin sorghum is not desirable for beer production. The malt used for the manufacture of beer should have high diastatic activity and solubility.

2.3.4 Feed

In the United States, sorghum is used primarily as a feed grain for livestock. Feed value of grain sorghum is similar to corn. The grain has more protein and fat than corn, but is lower in vitamin A. When compared with corn on a per pound basis, grain sorghum feeding value ranges from 90% to nearly equal to corn. The grain is highly palatable to livestock, and intake seldom limits livestock productivity. However, some sorghum varieties and hybrids, which were developed to deter birds,

are less palatable due to tannins and phenolic compounds in the seed. The grain should be cracked or rolled before feeding to cattle; this improves the portion digested (Carter and Capenter, 1981).

Pasturing cattle or sheep on sorghum stubble, after the grain has been harvested, is a common practice. Both roughage and dropped heads are utilized. Stubble with secondary growth must be pastured carefully because of the danger of prussic acid (HCN) poisoning.

Grain sorghum may also be used as whole-plant silage; however sorghum, sweet sorghum, was developed as a silage crop. Sweet sorghum produces much higher forage yields than grain sorghum, but feed quality will likely be lesser because there is no grain. Some growers mix grain sorghum with soybeans to produce a higher protein silage crop (USDA, 2005).

Sorghum has important animal feed used in countries like the US, Mexico, South America, and Australia. The nutritional value of good-quality sorghums has an average feeding value of 96 to 98% that of corn. Sorghum can be processed to further improve its feed value and techniques such as grinding; crushing, steaming, steam flaking, popping, and extruding have all been used to enhance the grain for feeding. The products are then fed to beef or dairy cattle, egg laying hens and poultry, pigs, and are used in pet foods.

From USDA report, (2005), there are four classes of certified sorghum for food and fed:

2.3.5 Certified Food Sorghum:

Includes sorghum varieties that provide for the highest and most consistent quality standards allowed. These varieties can be used for breads, cereals, salty snack foods, and pastas and popped snacks (USDA, 2005).

2.3.6 Certified Specialty Sorghum:

Includes sorghum varieties that offer functional food and nutraceutical benefits. These are the high anti-oxidant sorghum varieties. Many companies and research entities have focused on the antioxidant capabilities of sorghum. These verities are high in phytonutrients that offers benefits to the health conscious consumer. Varieties selected as Certified Specialty Sorghums are used to make breads, energy bars and can have the tannin extracted for nutraceutical uses. These varieties are only grown under contract identity preserved production (USDA, 2005)

2.3.7 Certified Sorghum Feed:

Includes sorghum varieties that benefit the animal feed industry. Sorghum feed represents one of the largest market segments in the US for example. Growth areas for these varieties include poultry and dairy feeds (USDA, 2005).

2.3.8 Certified Pet Food Sorghum:

Is used in both dog and cat foods. Mycotoxins like aflotoxin and vomitoxin do not readily occur in grain sorghum where they do occur in other grain sources. This eliminates a potential health risk for the family pet.

2.3.9 Poultry

Current demand for compounded poultry feed is 12 million tones per year in India, of which 30-35% is maize (Zea Mays). Projections suggest that by 2020 maize demand will rise from current levels of 3.5million to 31 million tones. In view of this the poultry industry recognizes the urgent need to develop alternative feed sources. Sorghum is already widely used, albeit at low inclusion rates. Thus, the industry anticipates that sorghum will need to replace most of maize currently used. However, access to reliable supplies of cheap sorghum of a consistent and adequate quality is identified as a problem in the poultry industry (Seshaiah, 2002).

2.4. Constraints to Sorghum use for human consumption

In Kenya and South Africa, many urban consumers consider sorghum to be a subsistence crop of low quality. This low social status for the grain constrains its desirability for inclusion in commercial products designed for urban consumers. In regions where the crop is not a staple, it may have low acceptability relative to maize due to its different organoleptic properties - unpleasant colour, aroma, mouthfeel, aftertaste and stomach-feel (ICRISAT, 1996).

2.5 Industrial uses

Industrial uses for sorghum include wallboard and biodegradable packaging materials. Millions of bushels of sorghum are processed into wallboards per year in central US alone. In addition to wallboard grain sorghum can be processed interchangeably with corn for the production of ethanol. (USDA, 2005). Many ethanol plants within the United States use grain sorghum as their principal grain due to their favourable geographic location. A very valuable co-product created from the

processing of grain sorghum for ethanol is dry distiller's grain. This high protein animal feed supplement generates additional revenues for the ethanol processing plant.

2.5.1 Brewing

Lager beers: Certain varieties of red sorghums contain active amylases at concentrations suitable for certain brewing applications (Okafor and Aniche, 1980). For the preparation of commercial lager beers, sorghum malt is not a direct replacement for barley malt since the diastatic power (measure of total amylase content of malt) of the sorghum malt is very low and variable compared to that for barley malt. Sorghum is milled for its endosperm grits as a starch source (adjunct) for hydrolysis by malt enzymes to fermentable sugars. Supplementary amylolytic and proteolytic enzymes are necessary to complete the fermentations. (O'Rouke, 2004)

2.5.2. Adjuncts

Adjuncts are nothing more than unmalted grains such as corn, rice, rye, oats, barley, wheat and sorghum. Although, adjuncts are used mainly because they provide extract at a lower cost (a cheaper form of carbohydrate) than is available from malted barley and because they are readily available, other definite advantages are also achieved (O'Rouke, 2004). Adjuncts are used to achieve some of these objectives:

- Adjuncts use results in beers with enhanced physical stability
- Adjuncts can be used to adjust fermentability of a wort
- Adjuncts are often used for their flavour contribution

- Adjuncts will also alter the carbohydrate and nitrogen ratio of the wort, thereby affecting formation of by-products, such as esters and higher alcohols.
- Adjuncts are used for colour adjustment, as in the case with dark sugars
- Some adjuncts are used for their chemical properties; e.g. raw barley and wheat, which contribute glycol-proteins to enhance foam stability (Bamforth and Barclay, 1993)
- Other adjuncts, low in protein, are used to improve colloidal stability since they will dilute the amount of potential haze-forming proteins.
- Finally, the use of adjuncts can result in increased brewing capacity, reduced labour cost, improved hot and cold break, and shorter brewing cycles

2.5.2.1 Classification of Adjuncts

According to O'Rouke (1996), adjuncts are classified according to the way they are used in brewing, i.e. whether they are added to the cooker, the mash tun, or the brew kettle.

2.5.2.2 Cooker Mash Adjuncts

The cooker mash adjuncts consist of non-gelatinised cereal products (meal, grits, flour, or dry starch) whose starches are in their native forms. A non-gelatinised adjunct needs to be heated in a separate cereal cooker to complete liquefaction since the starch gelatinisation temperature of the adjunct is higher than that used for the malt saccharification (starch hydrolysis) temperature.

2.5.2.3 Mash Tun Adjuncts

The adjunct can be mashed directly with the malt in either the mash tun or mash conversion vessel when the starch gelatinisation temperature of the adjunct is lower than the malt saccharification temperature required for the mashing or the adjunct has been pregelatinized (e.g. for flakes, torrified cereals or refined starches). Whole grains wheat and barley can be mashed this way after being milled.

2.5.2.4 Kettle Adjuncts

Kettle adjuncts consist of cereal grain syrups and sucrose sugar. These already contain the fermentable sugars. They are used predominantly to either deliberately alter the characteristics of the wort or to increase the brew size (O'Rourke, 2004)

2.5.2.2 Cereal adjuncts

Most of the Brewer's adjuncts are based on a limited range of cereal grains. The nonmalt brewing materials used in greatest quantity today are those derived from corn and rice, barley, wheat, and sorghum grains.

2.5.2.2.1 Grits

Grits consist of uncooked fragments of starchy endosperm derived from cereal grains. The starch of these adjunct products is in its native form, and is not readily attacked by the malt diastase enzymes during mashing. Consequently, these adjuncts must be processed by boiling in a cereal cooker to bring about solubilisation and gelatinisation of the starch granules and render them susceptible to diastase enzyme attack (Ogu et al., 2006).

2.5.2.2.2 Flakes

There are two different manufacturing processes used to produce brewing flakes. In the traditional process, corn and rice grits or whole barley are steam-cooked to soften the endosperm, which is then rolled flat and dried. Gelatinisation occurs during the steaming process. More recently, these materials are 'micronised' prior to flaking by subjecting the grain to internal heating by infrared heat (South and Ross, 1993)

2.5.2.2.3 Torrified Cereals

Torrified cereals are produced by heating the grain, which makes the endosperm expand and pop, thus, rendering the starch pregelatinised and easily milled. Torrified cereals can be added directly to the mash since starch granules have been gelatinised.

2.5.2.2.4 Micronised Cereals

Micronised cereals are produced by heating the grain with infrared energy, causing the molecules in the grain to vibrate and heat up, which results in a rapid rise in water vapour. The grain becomes soft and turgid, thus, gelatinising the starch. Most of the nitrogen is denatured in the kernel and is not solubilised, thus, contributing to little or no nitrogen to the mash.

2.5.2.2.5 Refined Starches

Refined starches can be prepared from many cereal grains. In commercial practice, refined wheat starch, potato starch, and corn starch, have all been used in breweries; corn starches, in particular, are used in the preparation of glucose syrups. Wheat starch has been employed in breweries in Australia and Canada, where local conditions make it economical to use. However, the most important source of refined starch is corn (O'Rourke, 1996).

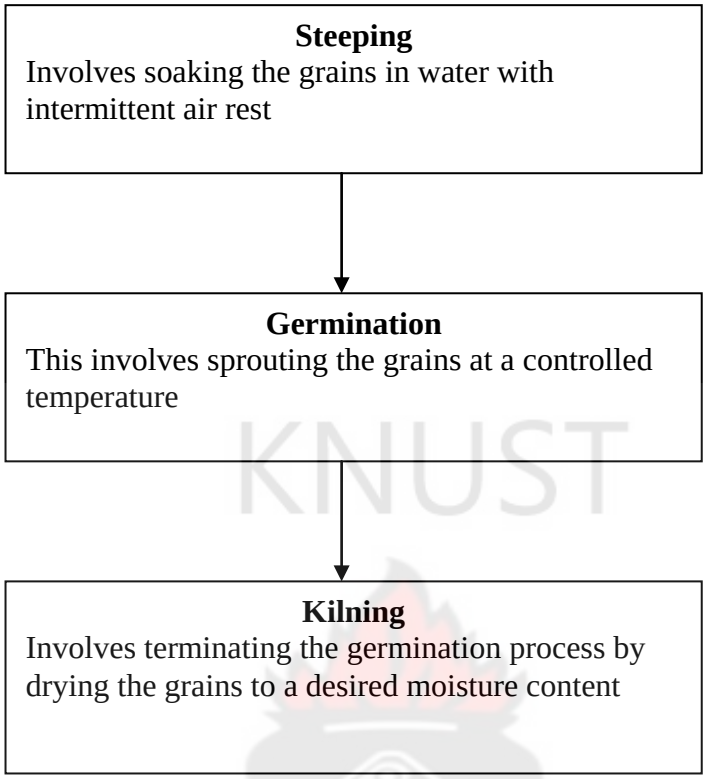
2.6 SORGHUM AS BREWING ADJUNCT

Sorghum use as brewing adjunct is either in its malted state or non-malted form. Gelatinisation temperatures is said to be higher than those required by starch-hydrolytic enzymes. It is, thus, pre-cooked before being added to the main mash (O'Rourke, 1996)

2.7 Malting

Taylor *et al* (1993) have identified malting as a traditional processing technology that could possibly be used to improve the nutritional quality of sorghum protein. Malting involves germination of the grain until the food store (endosperm), which is available to support the development of the germ of the grain, has suffered some degradation from enzymes (O'Rourke, 2004). A primary aim of malting is to develop and activate enzymes for the conversion of the insoluble reserved foods. The activities of these enzymes are terminated by drying (kilning) the young plant (green malt) so the endosperms are not completely depleted through respiration of the embryo and its growth. Hence, malt is the modified cereal grain resulting from induced germination under controlled conditions of moisture, aeration and temperature (O'Rourke**,** 2004)

Flow chart 1: The malting process



The kennel size and shape has been shown to affect the malting properties and water uptake of the grain (Rooney, 2000). Ilori (1989) also reported that the kennel size index correlated significantly with the germination energy and capacity. The reports of these workers indicate that there are varietal differences in the optimum malting conditions of sorghum. Varietal differences in sorghum indicate differences in kennel characteristics (Ilori, 1989), and these may be of importance in the malting of sorghum just as texture and size are important in barley malt.

2.7.1 Cereals for malting

Barely has been the preferred grain for malting in modern brewing. Other cereal grains such as rye, oat, sorghum and maize have been used for malting, though with

little success (Osmanzai, 1992). However, sorghum seems to be the most appropriate alternative to barley malt, on the basis of cost, utilization and production level. Indeed, the size of sorghum has been reported to be similar to that of barley, though more rounded in shape (Skinner, 1976).

2.7.2 Temperature effect on malting

Karababa *et al.,* (1993) reported that low temperature kilning schedules produce sorghum malts with greater diastatic power and α-amylase activity. A study by Iygor, (1987), on an improved Nigerian cultivar strengthened the observation that improvement in sorghum cultivar selection and manipulation of steeping and germination conditions will improve sorghum malt quality for lager brewing. The germination temperature range for sorghum and millet is reported to be 20-30 ^{o}C, however, higher germination temperatures resulted in higher malting losses. Demuyakor and Ohta (1992)

2.7.3 Malting Loss

Ogbonna, (2007) reported that enzyme activity is highest during the early stages of germination since the first 2-3 days coincide with the movement of the growth hormone (gibberellins). Ogundiwin and Ilori, (1991) observed that the malting loss increased with germination period. According to Novellie (1962) and Pathirana, *et al*. (1983), malting loss could be between 10.9% and 35%, depending on the malting conditions. Malting loss is incurred as a result of dry matter loss, mainly due to the growth and respiration of the embryo

2.7.4 Enzyme development (Diastatic power)

The diastatic power of sorghum malt has been shown to be variable and is usually less than those of barley malts (Taylor *et al*., 1993). According to Dewar (2001), a number of factors are known to have an effect on the development of enzymes synthesised during germination and thus on the quality of the malt produced.

- The moisture content of the grain at the end of steep (steep-out moisture) has been shown to be significantly correlated with the resulting malt quality.
- Recent research has shown that steeping sorghum in a dilute solution of alkali increases the rate of water uptake into the grain during steeping (probably as a consequence of disrupting the molecular structure of the non-starch polysaccharides which make up the structure of the sorghum cell walls) and also increases the quality of the malt produced at least in terms of brewing quality characteristics such as diastatic power (amylase activity) and free amino nitrogen (free amino acids and short peptides), (Dewar and Taylor. 2001).

Karababa *et al* (1993) reported that low-temperature kilning schedules produce sorghum malts with greater diastatic power and α-amylase activity.

2.7.5 α-Amylase Activity

The optimal α-amylase activity was determined and found to be between 72^{O}C and 75^{O}C for 6 sorghum varieties from Nigeria (Okafor and Aniche. 1980).

2.7.6 Effect of malting time on enzyme development

Analysis of malting and brewing characteristics of sorghum has shown that diastatic power, α-amylase, amyloglucosidase, and protease activities increased with malting time and malt modification (Nzelibe and Nwasike. 1995). It has also been reported that such parameters as Diastatic power, β-amylase activity, free amino nitrogen, hot water extract and malting loss, all increased with increasing time of germination (Pelember *et al*. 2002). However, Muoria and Bechtel (1998), contrary to the above, reported that there was no increase in diastatic power with more germination days.

2.7.7 Effect of germination moisture on malt quality

It has been reported by Pelember (2002) that increasing germination moisture improved sorghum, pearl millet and barley malt quality in terms of Diastatic power, free amino nitrogen (FAN), hot water extract and malting loss.

2.7.8 Effect of steeping water pH on malting quality

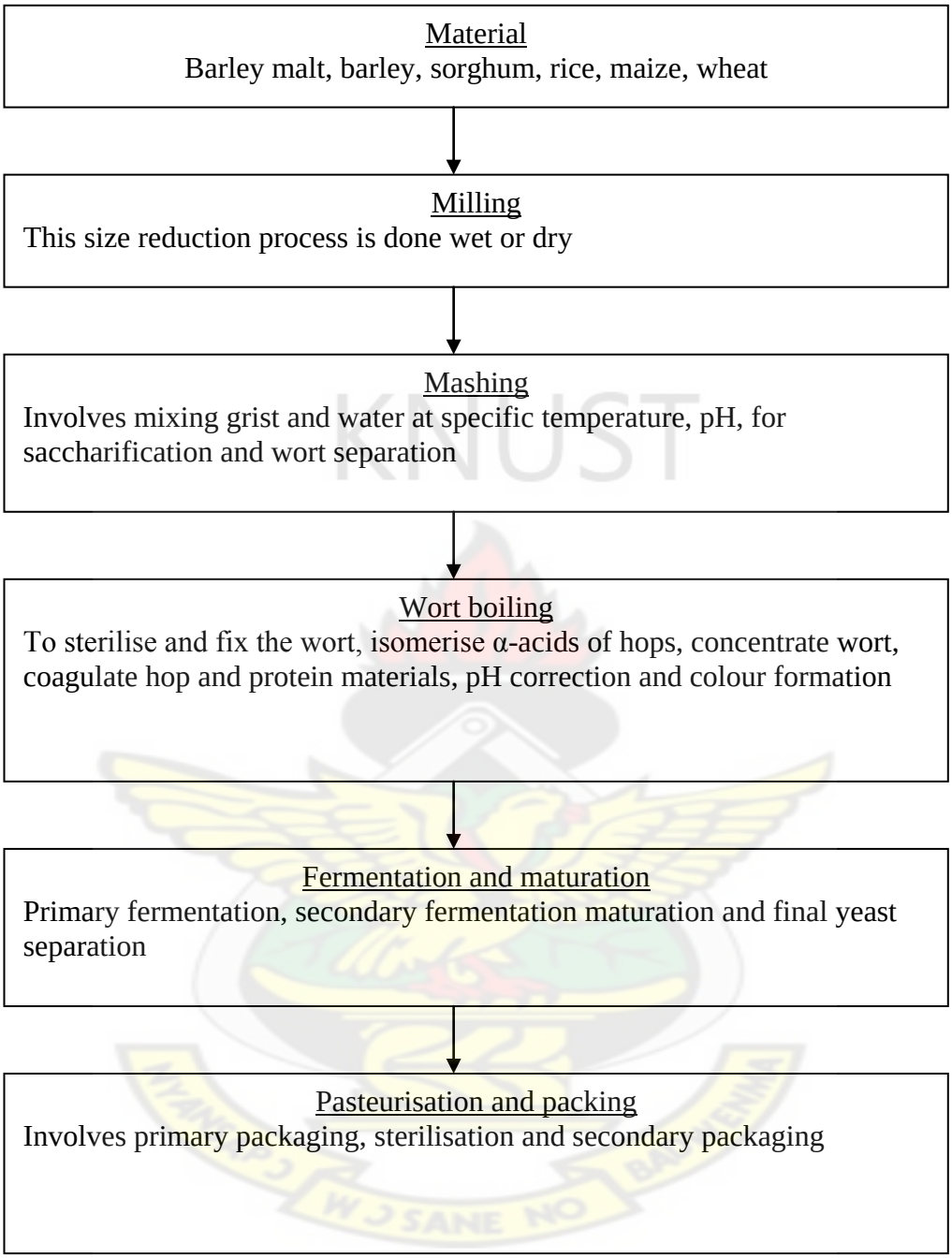
Okolo and Uguanyi (2004), found that soaking sorghum grains for a relatively short periods during the steeping process in a dilute solution of NaOH improved the beneficial effects of malting in terms of Diastatic power and free amino nitrogen and marginally enhanced the protein quality and digestibility. However, according to Dewar *et al.* (2001), optimum concentration at which alkaline treatment will yield the best results is variety and season dependant. He also demonstrated that steeping at higher NaOH concentrations actually reduced protein digestibility. MacGregor (1996) showed that microbial proliferation, especially yeast and fungi, during malting was reduced by the use of alkaline washes. Additionally, use of $Ca(OH)_2$, produced when calcium oxide is added to water and NaOH during barley steeping has been shown to inhibit microbial growth but prolonged exposure was harmful to

the grain. Furthermore, it was shown by Iygor (2001) that steeping in Ca $(OH)_2$ (2000ppm) prevented bacterial and mould growth without affecting malting loss, Diastatic power, cold and hot water extracts of sorghum malts.

2.8 Requirement for exogenous enzymes

EtokAkpan and Palmer (1990a) and Adejemilua (1995) reported that sorghum malts have lower Diastatic power than barley malt. Again sorghum has the disadvantage of a high gelatinisation temperature of the starch, coupled with the low temperature optimum for both α- and β-amylase. This, therefore, means that to use sorghum for brewing, one has to use heat-stable exogenous enzymes that can hydrolyse sorghum starch to the simple sugars. Most of the commercially available enzymes are obtained from fungi source and in most cases these are heat-stable. Other commercial enzymes used include β-bioglucanase, protease and the amylases.

Flow chart 2: The brewing process



2.9 Available local varieties of sorghums

There are several varieties of sorghum locally available in Ghana. These are grown mainly in the three northern regions and the northern part of the Brong Ahafo region. However, the most common varieties that are readily available on the market are Chere, Dorado, Latuor and Kapaala. These varieties are often chosen for the

production of local alcoholic beverage called “pito”. Latuor is also being used for the production of beer whilest kapaala and dorado are also being tried.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Source of Material

All sorghum varieties were supplied by Technoserve, Ghana Limited. They were all from the 2005 crop season. Dorado and Chere were from farms in the Upper West Region. Kapaala and Latuor were obtained from Tamale in the Northern region, while Samples 1 and 2 were obtained from Atebubu, in the Brong Ahafo Region.

All chemicals used were obtained from the Laboratories of the Biochemistry and Biotechnology and Chemistry Departments of the Kwame Nkrumah University of Science and Technology and Guinness Ghana Breweries Limited (Kaase and Ahensan sites).

Table 3: **Properties of Exogenous Enzymes used**

Parameter	**Name**		
	MPA1^{A}	**MPA1^{B}**	**Bioglucanase**
Source	Bacillus *lichenoformis*	Bacillus *amylolichefaciens*	Trichoderma *longibrachiatum*
Type	Amylase	Protease	Betaglucanase/ hemicellulose
Optimum pH	5.8-6.2	5.9-6.2	4.9-5.2
Optimum temp (^{O}C)	87-93	48-55	65-70
Deactivation temp. (^{O}C)	102	80	80
Activity	Hydrolyses α-1,4-glycosidic bonds in both amylase and amylopectin	Produces free amino nitrogen (FAN)	Degrades cellulose in plant cell walls

3.2 ANALYSES

3.2.1. 1000 Corn Weight

(EBC Method 3.4, 1997)

In determining the 1000 corn weight, 500g of cleaned samples of each variety was taken and divided into portions of 40g. Half corns and foreign materials were removed by hand. Each portion of the cleaned samples was weighed. The number of grains in each lot was countered by hand. The moisture content of each lot was also determined. The formula below was then used to calculate the 1000 corn weight:

$$G = W*1000\ (100-M)/N*100$$

Where:

G = weight of 1000 grains of sorghum (in g)

W = weight of "cleaned" lot of sorghum taken (in g)

M = moisture (in %)

N = number of grains in the "cleaned" lot taken

Average of two lots was taken for each variety of sorghum.

3.2.2. Moisture content of unmalted and malted Sorghum

(EBC Method 3.2, 1997)

Moisture content was determined by finely milling 20.0 g of unmalted sorghum using Disc Mill (Bühler, Braunschweig, Germany), which was set at 0.2 mm. The sample was mixed thoroughly and 5.0 g was immediately placed in a clean, dry moisture dish, which had been tarred to 0.001 g. The dish was covered and weighed to 0.001 g. The lid was then removed and together with the dish (which contained the sample) was placed in an oven, which had been preheated to 105 ^{o}C for 3 hrs. The dish was then covered with the lid and the set removed from the oven, placed in a dessicator and cooled to room temperature for 25 mins. The dish and content were then reweighed on an analytical balance. Triplicate determinations were carried out for each variety. The moisture content was calculated as a percentage of the initial

weight of sample. Moisture of the malted sorghum was also determined using the same method.

3.2.3 Germination Energy- Shonfield Method

(EBC Method 3.6.3, 1997)

Two lots of cleaned 500 sorghum grains were obtained. Each lot of 500 grains was transferred into a funnel standing in tap water (to ensure complete flooding of the grains) at 20 ^{o}C. The water was removed after steeping for 3 hours. The grains were covered with whatmans No. 4 filter papers and the funnel itself covered with a glass plate.

The steeping was repeated for 2 hours after 20 hours from the beginning of the test. The grains were again covered with filter paper and the funnel with glass plate. After 72 hours from the beginning of the test, the funnels were emptied and the number of non-germinated grains counted. Average result of the two counts (of the lots) after 72 hours was obtained. The formula below was used to calculate the Germination Energy (GE):

Germinative Energy (GE) = (500-N)/5

Where:

N = number of non-germinated grains after 3 consecutive days.

NB: results is reported as = a % (schonfield method 3 days).

This method was applied to each of the unmalted sorghum varieties.

3.2.4 Germination Capacity

(EBC Method 3.5.2, 2004)

This is an attempt to quantify the percentage of viable grains within a sample. 200 uniform sized and clean grains were handpicked and steeped in 500ml beaker

containing 200ml of 0.75% hydrogen peroxide (H_2O_2) solution and incubated for two (2) days at a temperature of 21^{O}C. At the end of the 2 days, the grains were strained and steeped again in 200ml of H_2O_2 also at 21^{O}C for further 24 hours. The germinated grains were then counted and the germinative capacity calculated using the formula:

Germinative Capacity = (200-n)/2,

Where:

N = grains that did not show roots.

3.2.5 Total Nitrogen/ Protein: Kjeldahl Method (Malted Sorghum

(EBC Method 4.3.1, 2004)

Finely milled 0.1 g of sorghum was weighed in a small weighing disk, and transferred quantitatively into a dry kjeldahl flask. 10 g of catalyst mixture (a tablet) consisting of TiO_2, $CuSO_4.5H_2O$ and K_2SO_4 were added. The content was thoroughly mixed by gently adding 25 mls of conc. H_2SO_4 and some anti-bumping agent. The flask was then heated. The heating continued for 45 minutes after the solution turned bright green. The kjeldahl flask and contents were then cooled to room temperature.

Two drops of methyl red indicator was added to Erlenmayer flask into which had been pipetted 20ml of 0.1N H_2SO_4. The flask was connected to the distillation apparatus such that the outlet tube of the condenser dipped beneath the H_2SO_4 in the receiving Erlenmayer flask. After Kjeldahl flask and content had been cooled to room temperature, 150 ml of distilled water was cautiously added and mixed. The solution was cooled to below 25 ^{O}C. Anti-bumping agent was added to prevent bumping. 100 ml of 0.1N NaOH was then added slowly until two distinct layers

were formed. The Kjeldahl flask was connected to the distillation apparatus soon after the addition of NaOH solution.

The flask was heated smoothly for 5 minutes and then strongly until liquid began to distil. Distillation continued until Kjeldahl flask begun to bump. The excess H_2SO_4 in the receiving flask was titrated with standardised 0.1 N NaOH, using methyl red as indicator.

Simultaneously with the test, a blank determination on reagents was carried out in which 20ml instead of 25ml of 0.1N H_2SO_4 was added and without the addition of the test sample.

This method was used for all malted and unmalted sorghum varieties. The Total Nitrogen/Protein content in the dry samples were then calculated and expressed as a percentage.

3.3. MALTING

Malting was based on a modification of the method described by Ogu *et al* (2006). This was carried out as follows:

3.3.1 Steeping

The steeping involved the following steps:

- Steeping under water in a domestic plastic container for 20 hours
- Drain and air rest for 4 hours
- Steeping under water in a domestic container for 20 hours (this was a modification of the method as described by Agu *et al* (2006) in which the steeping was done under running tap water

- Within the 20 hour soak time, the water was regularly drained and immediately refilled.

0.1% formaldehyde was added to the steep water to reduce microbial load.

3.3.2. Germination

This was carried out at room temperature (approximately 25-27^{o}C) and involved the use of jute bags. The grains taken out of steep were spread on jute bags, which had been pre-wetted and well spread-out on the laboratory Bench. Another pre-wet jute bag was used to cover the grains. Germination was allowed to continue for 96 hours. The grains were turned at 24 hour intervals to avoid excessive matting. At the end of the period (i.e. 96 hours), the germinated grains were hand-rubbed to break-off the rootlets before kilning in an oven.

3.3.3. Malting Loss

In determining the malting loss, 1000 germinated grains were dried to a constant weight and the weight determined (M2). 1000grains of the unmalted sorghum were dried to a constant weight and the weight determined (M1). The malting loss was then calculated using the formula below:

Malting Loss = ((M1-M2)/M1)*100

Where,

M1 = weight of 1000 dried, sorghum grains

M2 = weight of dried, germinated sorghum grains

3.3.4. Kilning

Kilning of the germinated grains was carried out for 16 hours at 50^{O}C in an Oven (Memmert Celcius 2000, model: Beschickung-Loading model 100-800, Federal Republic of Germany).

3.4 ANALYSES OF MALTED SAMPLES

3.4.1. Extract Content: Congress Mash

(EBC Method 4.5.1, 2004)

3.4.1.1. Sample preparation and mashing

For the mashing process, 55g of malted sample were milled with a Disc Mill (Bühler, Braunschweig, Germany) set at 0.2mm. Each sample of ground 55g malt was transferred into a beaker and well mixed with spatula. 5g was taken for moisture content according to Method 3.3 above while the remaining 50g was measured into the mash beaker. 200ml of distilled water at a temperature of 45^{o}C was placed into each beaker and stirred with a glass rod to avoid formation of lumps. The mash bath before use had been pre-heated to 45^{o}C. The temperature of the mash bath was then maintained at this temperature for 30minutes. The temperature of the mash was then raised at 1^{o}C per minute for 20minutes to 65^{o}C (instead of 70, a modification). At 65^{o}C 100ml of distilled water at 65^{o}C was added. Also added to the mash were 20mg of MPA 1A (amylase), 20mg of MPA 1B (protease) and 20mg of Bioglucanase. MPA 1B was added at the beginning of mashing i.e. at mashing-in temperature of 45^{o}C. MPA 1A and Bioglucanase, on the other hand were added when mashing temperature reached 60^{o}C, during heating.

Complete hydrolysis of starch (saccharification) was determined from the time the 100ml distil water at 65^{o}C was added. This was done by transferring a drop of the mash unto a spot on a white porcelain plate and a drop of iodine solution added. This was done 10minutes after addition of the 100ml distilled water and repeated at 5minutes intervals for a maximum of 1hour. Complete saccharification or starch conversion was indicated by a clear yellow spot. The response to the iodine test was

then recorded as either positive or negative. After holding the mash for 1 hour at 65^{o}C for saccharification, the mash was cooled to room temperature with cold tap water in the mash bath. Stirrer was washed with small amount of distilled water and the outside of the beaker dried. The volume of the beaker (mash) was then adjusted to 450ml with distilled water.

The above procedure was repeated for different temperatures i.e.75^{o}C, 85^{O}C, and 95^{o}C (all with exogenous enzymes) and 65^{o}C (with no exogenous enzyme).

3.4.1.2 Filtration

The mash in the beaker was filtered by emptying into a pleated filter paper (whatmans No 4) in a funnel. The first 100ml of filtrate collected was returned into the funnel. A portion of the filtrate was taken for colour measurement using the UV/VIS Spectrometer (model Lambda 35, Australia). The cake did not dry up so the filtration was stopped after 2hours.

3.4.1.3 Determination of filtration speed

Filtration speed was described as “normal” if filtration was completed within 1hour. It was described as “slow” if it took longer than one hour to complete.

3.4.1.4 Determination of Specific gravity of the wort

This was determined using the Anton Paar gravity analyser (model GSA 48-Australia).

The specific gravity was converted to grams of extract in 100g of wort from a sugar conversion table. The formulae below were used to calculate the extract (on as is basis and on dry basis):

E1 = P (M+800)/(100-P)

E2 = E1*100/(100-M)

Where:

E1 = Malt extract in sorghum malt samples taken in % (m/m)

E2 = the malt extract content of dry malt, in % (m/m)

P = the extract content in wort, in g of extract per 100g of wort (= deg. Plato)

M = the moisture content of the sorghum malt sample, in % (m/m)

800 = the amount of distilled water added into the mash to 100g of the mash.

It must be emphasise that determinations were done in duplicates.

3.4.2 COLOUR

(Spectrophotometer Method-EBC Method 4.7.1)

Colour measurement was done based on European Brewery Convention method (4.7.1) (fifth edition, 1998) using the spectrophotometer. Fifty millilitre samples of the filtered wort produced was taken and re-filtered using whatmans number 4 filter paper. The first 20ml was discarded and remaining was collected. The spectrophotometer was set at 430nm wavelength. Blank test was first done with distilled water and used to adjust the absorbance to 0.000. After rinsing the cuvette with the bright malt wort, the absorbance of the sample was determined at 430nm.

The sample was diluted with distilled water to 1:5 wort: water ratio. The colour of the sample was then calculated using the equation below:

C= 25*A *f

Where,

C= the colour in EBC units

25=the multiplication factor

A= absorbance at 430nm in 10mm cuvette

F=dilution factor

3.4.3 DIASTATIC POWER

(EBC Method 4.12, 1997)

3.4.3.1 Sample Preparation

This was determined by weighing 52 g of sorghum malt which was finely ground using the Buhler Miag mill set at 0.2mm. This was done in duplicate. 40g of the grist were then weighed into each mashing beaker (two per variety).

3.4.3.2 Starch Solution

The starch solution was prepared by weighing 10 g of the dry matter of pure starch (Merck No 1252) into a mortar. It was stirred to a paste with the addition of a little cold distilled water. The paste was slowly added to 400 ml of boiling distilled water in a beaker. The mortar was washed with a little distilled water and added to the starch solution. Boiling continued for 5 minutes. The beaker was then placed in cold water and cooled under cover and with intermittent stirring to avoid skin formation. The solution was finally transferred into a 500 ml volumetric flask. The beaker was rinsed and added to the flask. The volume was then made up to 500 ml with distilled water. Fresh sample was prepared each day for analyses.

3.4.3.3 Extraction of the Enzyme

480ml of distilled water at ambient temperature was added to each beaker (which contained 52g of sorghum malt grist instead of 41g) and stirred with glass rod into a uniform mash. The beakers with their contents (mashes) were then placed in a

mashing bath, which had been pre-heated to 40^{o}C. The contents were then stirred for 60 minutes. The mashes were cooled to room temperature and the glass rods washed with small amounts of distilled water.

Each mash was immediately transferred to a 200 mm funnel containing 320 mm filter paper and filtered into 250ml conical flask. The first 200 ml filtrate (which is the extract) from each funnel was discarded and the next 50 ml was used for analysis.

3.4.3.4 Hydrolysis of starch

Into a 200 ml volumetric flask was pipetted 100 ml of starch solution. 5 ml of acetate buffer ($_{P}$H 5.6) was then added and the solution placed in water bath, which had been pre-heated to 200 ^{o}C and allowed to stand for 20 mins.

5 ml of test extract was then pipetted and mixed with the starch thoroughly by shaking.

The solution was then incubated at 20 ^{o}C for 30min from the time of addition of the enzyme. The enzyme was deactivated by the addition of 4 ml NaOH solution (1mol/lt) to the flask. The contents of the flask was then made up to 200 ml with distilled water and well mixed by shaking. The alkalinity of the solution was then tested with a drop of phenolpthalein solution. The colour was blue.

3.4.3.5 Blank test

For a blank test, 100 ml of starch solution prepared above was pipetted into a 200 ml volumetric flask. 4.0 ml of 1N sodium hydroxide solution was added and the content thoroughly shaken. 5 ml of the test extract was added and the solution made up to 200 ml with distilled water. After thoroughly mixing the solution, its alkalinity was

checked with the addition of a drop of phenolpthalein solution. The colour turned blue.

3.4.3.6 Iodometric determination of reducing sugars

Into a 150 ml conical flask was measured and transferred 50 ml aliquot of the digest (from 3.4.3.4 above). 25 ml of iodine solution and 3 ml of 1N NaOH solution were also added to the flask and stoppered to prevent loss of iodine. The solution was allowed to stand for 15 minutes after shaking, after which 4.5ml of 1.0N H_2SO_4 was added. The unreacted iodine in this solution was then titrated against thiosulphate solution until the blue colour disappeared. It must be emphasized that 52 g instead of 41g of malted sample was used for the extraction because the 41 g gave a titre value which was outside the 9-12 range specified by the methodology. Due to relatively low enzyme levels, extracts were not diluted before the determinations.

The Diastatic power (DP) expressed in Windish-a Kolback (WK) unit was calculated using the formula below:

DP1= F (Vb-Vt)

DP2=DP1*100/(100-M)

Where:

DP1= the Diastatic power of malted sample, unit WK.

DP2= the Diastatic power on dry matter of malted sample, in WK.

F = the correction factor to obtain the results per 100g of malt used for the extraction.

Vb = titre value of the unreacted iodine in the blank test, in ml

Vt = titre value of the unreacted iodine in the test portion in ml

M = the moisture content of the malt, in %(m/m)

3.4.4 Soluble Nitrogen Content of Wort (EBC Method 4.9.1, 1997)

40 ml of wort sample produced under section 3.4.1 (congress mash) was pipetted into a 500 ml kjeldahl flask 20 drops of Conc. H_2SO_4 and some anti-bumping agents were added. The solution was then gently evaporated to a syrupy consistency while ensuring minimal carbonization. 1.0 g portion of the syrup was then transferred quantitatively into a dry kjeldahl flask. 10 g of catalyst mixture (a tablet) consisting of TiO_2, $CUSO_4$.5H2O and K_2SO_4 were added.

The content was thoroughly mixed by gentle shaking. 25 mls of conc. H_2SO_4 and some anti-bumping agent were also added. The flask was then heated on the destruction apparatus. The heating continued for 45 minutes after the solution turned bright green. The kjeldahl flask and contents were then cooled to room temperature.

Two drops of methyl red indicator was added to Erlenmeyer flask into which had been pipetted 20 ml of 0.1 N H_2SO_4. The flask was then connected to the distillation apparatus such that the outlet tube of the condenser dipped beneath the H_2SO_4 in the receiving Erlenmeyer flask. After kjeldahl destruction flask and content had been cooled to room temperature, 150 ml of distilled water was cautiously added to prevent bumping. 100 ml 0.1N NaOH was then added slowly until two distinct layers were formed. The kjeldahl flask was connected to the distillation apparatus soon after the NaOH solution.

The flask was heated, smoothly for 5 minutes and then stirred until the liquid began to distil. Distillation continued until kjeldahl flask begun to bump.

The excess H_2SO_4 in the receiving flask was titrated with standardized 0.1 N NaOH, using methyl red indicator.

Simultaneously with the test, a blank determination on reagent was carried out in which 20 ml instead of 25 ml of H_2SO_4 was added and without the addition of the test sample. This method was used for wort of all the sorghum varieties. The soluble nitrogen content (Nsw) in 1.0 litre of wort was calculated using the formula:

$$\text{Nsw (mg/litre)} = \frac{14 * t * 1000 * (V2 - Vt)}{a}$$

Where:

Nsw= soluble nitrogen content in mg /litre wort

t = titre of the standard NaOH solution as meq/litre

V2 = ml of standard NaOH solution used in back titration of blank

Vt = ml of standard NaOH used in back titration for the test sample

a = volume of sample taken (ml)

14 = atomic weight of nitrogen

1000= conversion factor

The soluble nitrogen content as a % of dry malt was then calculated as follows:

Nsm=Nsw*E2/(10000*Ew)

Nsw =soluble nitrogen content in mg/litre work

Nsm =soluble content on dry malt in % (m/m)

E2 = extract content on dry malt in % (m/m)

Ew = g of extract in 100ml of wort at 20 ^{o}C (to convert g of extract per 100 g of wort)

10000 = conversion factor

3.5 **PROXIMATE ANALYSES UNMALTED AND MALTED SAMPLES**

3.5.1 Moisture

Five grams (5.0 g) of the sample (flour) was transferred to previously dried and weighed dish. The dish was placed in an oven thermostatically controlled at 105 ^{o}C for 5hrs. The dish was removed and placed in a desiccator to cool and weighed. The dished was re-placed in the oven and heated, cooled and weighed. This stage was repeated until constant weight was obtained. The loss in weight, which represents the moisture, was reported and expressed as a percentage. This was done for each variety in triplicates.

3.5.2 Crude Fat

The dried sample obtained from the moisture determination was transferred to 22 x 80 mm paper thimble. A small ball of cotton wool of glass wool was placed into the thimble to prevent loss of the sample. Anti bumping granules was added to the previously dried (air oven at 100 ^{o}C) 250 mm round bottom flask and weighed accurately. 150 ml of petroleum spirit B.P. 60-80 ^{o}C was added to the flask and apparatus assembled. A quick fit condenser was connected to the soxhlet extractor and refluxed and evaporated on a steam bath. The flask and fat/ oil was heated for 30min in an oven (Memmert oven, Type: ULE 60, Germany) at 103 ^{o}C. The flask and contents were cooled to room temperature in a desiccator. The flask was weighed accurately and the weight of oil/fat collected was determined. This was then expressed as the percentage.

3.5.3 Ash

In determining the ash content, 2.00g of dried sampled was transferred to a previously ignited and weighed crucible and placed in muffle furnace that is preheated to 600 ^{o}C for 2 hours. The crucible was removed and cooled in a

desiccator. Crucible was allowed to cool and then weighed. Weight was expressed as a percentage. This was repeated for all varieties and also for both malted and unmalted samples.

3.5.5 Crude Protein

(EBC method 4.3.5, 2004)

3.5.5.1 Digestion

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

3.5.5.2 Distillation

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

colour change of the boric acid to bluish green as soon as it comes into contact with ammonia.

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

3.5.5.2 Titration

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

$$\% \text{ Nitrogen} = \frac{X}{W} \ (0.1N \text{ HCl})$$

Where,

x = mls of standard acid used

w = the amount of sample taken

Percentage Crude protein was then calculated as,

% Crude protein = % Nitrogen × 6.25

3.6 Statistical analysis

The general linear model (GLM) of SPSS statistical package (version 13) was used for the statistical analysis of results. Data obtained were examined statistically for variation among treatment conditions (ANOVA). Pearson correlation analysis was also used to determine the relationship between the various malt quality parameters

and physicochemical properties of the grains. The level of significant was $p<0.05$ for statistical methods, except as noted.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1. UNMALTED SORGHUM

4.1.1. 1000 Corn Weight

With the exception of Chere, which had the lowest unmalted corn weight (26.82 g), the rest of the varieties are all within specification. The higher the corn weight, the higher the expected brewers extract. From table 4, Kapaala variety had the highest 1000 corn weight (33 g). Similar results were reported by Ogu *et al*, (2006). The differences among the varieties were significant ($p< 0.05$). However, the difference between Dorado and Sample 1 (from Atebubu) was not significant ($p>0.05$). Generally, there was no significant correlation between corn weight and nitrogen contents of the unmalted grains ($r = 0.012$). Rooney (2000) reported that kernel size and shape of sorghum grains had effect on malting properties and water uptake of the grains. Additionally, there was no significant correlation between corn weight of unmalted samples and hot water extracts ($r = 0.052$), contrary to the assertion by Nso *et al* (2003), that unmalted grains with high thousand corn weights or grain density tend to have higher potential extract yields.

4.1.2 Germinative energy (GE)/Germinative Capacity (GC)

Two properties that have direct bearing on suitability of cereals for malting are germination energy (GE) and Germinative capacity (GC).

Table 4: Some properties of unmalted sorghum varieties

Property	Varieties					
	Chere	Dorado	Latuor	Kapaala	Sample 1	Sample 2
1000 corn weight (g)	26.86 (0.06)	32.10 (0.27)	27.37 (0.09)	33.42 (0.06)	32.28 (0.14)	28.35 (0.15)
GE (%) (4ml test)	96.90 (0.14)	95.80 (0.28)	95.50 (1.98)	94.00 (0.57)	95.50 (0.14)	94.7 (0.14)
GC (%)	98.00 (0.00)	97.30 (0.25)	98.50 (0.29)	98.30 (0.25)	98.80 (0.15)	97.8 (0.25)
Moisture (%)	8.11 (0.11)	9.08 (0.26)	8.39 (0.05)	9.34 (0.13)	11.16 (0.02)	10.67 (0.23)
TN (%)	1.82 (0.04)	1.87 (0.02)	1.67 (0.04)	1.68 (0.04)	2.03 (0.04)	1.96 (0.04)
Fat (%)	1.93 (0.05)	2.13 (0.01)	2.49 (0.06)	3.85 (0.10)	2.65 (0.20)	2.18 (0.18)
Protein (%)	11.38 (0.22)	11.68 (0.13)	10.43 (0.13)	10.51 (0.22)	12.70 (0.22)	12.26 (0.22)
Ash	1.62 (0.01)	1.51 (0.06)	1.43 (0.02)	1.43 (0.02)	0.59 (0.00)	2.18 (0.18)

KEY: Sample1 and Sample2 are new varieties under field test at Atebubu

GE: Germination energy, GC: germination capacity; TN: Total Nitrogen, Values in parenthesis ( ) are the standard deviation of triplicate results of each parameter.

From Table 4, the Germinative energy (GE) and the Germinative capacity (GC) of all the cultivars or varieties were high enough (>90%), compared with the recommended (i.e. not less than 90) (O'Rourke, 2004) for malting. This means that all the varieties are suitable for malting. Even Kapaala, which had a relatively lower GE, is high enough to allow for malting. Nso *et al*, (2003) reported similar results.

Muoria *et al*, (1998), reported that sorghum kernels having high germination energies were characterised by high Diastatic power. The germination energy of the unmalted samples were positively correlated (r= 0.828) to the Diastatic power (DP) of the malted samples. This is, however, at variance to findings by Beta *et al.* (1995) who stated that germination energy did not correlate with α-amylase and β-amylase activities.

4.1.3. Total Nitrogen content

All the varieties studied had suitable nitrogen content. The total nitrogen content ranged between (1.6-2.03%), for the unmalted grains (Table 3) and (1.38-1.89%), for the malted grains, (Table 5). For sorghum, the suitable range is 1.6-1.8 %. In spite of the ideal range stated above, it has been argued that Total Nitrogen (TN) values greater than 1.9% will usually give run-off problems during filtration and render the beer less stable, due to the formation of chill haze (O'Rourke, 2002). On the other hand, levels must be high enough to impart body to the beer, good head formation as well as healthy fermentation (O'Rourke, 2002). For the unmalted, Latuor had the least total nitrogen (1.67%), whiles sample 1 had the highest (2.03%). Interestingly, however, Dorado had the least Total nitrogen in the malted state and not Latuor. This may be explained on the basis that Dorado was better modified than Latuor, hence, the large difference. In general the mean difference among the varieties in terms of total nitrogen content was significant ($p<0.05$). However, differences between Chere and Dorado as well as between Latuor and Kapaala, in the unmalted state, were not significant ($p>0.05$). Even though significant differences existed between Chere and Latuor as well as Sample1 and Sample2 in the unmalted grains, the differences were not significant in the malted grains ($p>0.05$). This observation may mean that the

degree of modification was different in the different varieties or malting loss in terms of discarded rootlets may have been higher in one than the other. The possible influence of environmental conditions on nitrogen content was observed in samples 1 and 2 (both from Atebubu). Both had total nitrogen levels in the unmalted grains greater than 1.9%.

The effect of malting on nitrogen content (measured in terms of protein) of sorghum varieties was significant ($p<0.05$). The percentage nitrogen loss between unmalted and malted ranged between 4.1 – 26.2%, with Dorado having the highest loss (26.2%). Kapaala, on the other hand, had the least percentage loss in total nitrogen. This means that Kapaala was the least modified whereas Dorado was the most modified. Loss in total nitrogen (or protein) also correlated positively with Diastatic power ($r=0.759$). This observation agrees with the report by Palmer *et al*. (1989) that dry matter losses significantly correlated with Diastatic power.

4.1.4. Fat contents

Production of off-flavours due to the oxidation of fat in beer is a critical issue which brewing industries try to tackle by reducing its content in cereal raw material. The effects of malting on fat content of the sorghum varieties were significant ($p<0.05$) table 4. The malting process reduced the fat content in the grains. The effects of varietal differences in respect of the fat contents were significant ($p<0.05$). However, with the exception of Dorado, the levels of fat obtained in the sorghum malts were higher than was reported by Serna (2004). The crude fat content ranged between 1.93 – 3.85% in the unmalted samples (Table 4) and 0.96 – 2.57% in the malted samples (Table 5). Dorado had the least fat content in both the unmalted and malted. The level of fat after malting in the Dorado variety is within the specification set by

the brewing industry. According to Serna *et al.* (2004), the maximum fat content as set by the brewing industry is less than 1.0%. All the other varieties had fat levels in the malted state higher than the specification. Thus, a degermination step before milling into grits may have to be used to further reduce crude fat content in refined products before using them for brewing. On the other hand, since germination generally reduced the fat content, the germination period for the other varieties could be extended in order to further reduce the fat content. It must, however, be stated that this second option will lead to an increase in malting loss and hence a reduction in potential extract.

4.1.5. Moisture content

Moisture contents of grains are important so far as keeping quality is concerned. Tables 4 and 5 depict the moisture content of unmalted and malted varieties respectively. The values for the unmalted ranged between 8.11-11.16% whereas, the malted ranged between 5.50 – 6.21%. In both states, the values obtained were higher than that reported by Ogu *et al.* (2006). Ogu *et al.* (2006) reported moisture content of unmalted grains to be in the range rang 8.5- 10.5% and that of the malted between 5.2-5.7%. In both the unmalted and malted, there were significant differences ($p<0.05$) in the moisture content which may be due to differences in variety (Okokon 2004). High moisture content encourages microbial growth and allows increase in metabolic rate, which depletes the extract content. According to Okon and Uwaifo, (1985), the moisture content specification for sorghum malt is less or equal to 5.4% whiles that of barley malt is less or equal to 4.2%. This problem of high moisture obtained in the study, however, could be overcome by optimising the kilning regime.

4.2. MALTED SORGHUM

Efficient grain modification during germination is mainly due to the combined action of cell wall degradation enzymes such as β-glucanase and proteases, which help to break down the protein matrix. These enzymes expose the starch granules to amylase attack during mashing (Palmer, 1989). Table 5 shows the results of the properties of the malted grains.

4.2.1. Malting Loss

The malting loss was measured in terms of dry matter loss. From Tables 4 and 5, there were clearly losses in dry matter content (1000 corn weight) from unmalted to malted grains. According to Pollock (1962), malting loss is incurred as a result of dry matter loss, mainly due to the growth and respiration of the embryo. It has been reported that, malting loss could be between 10.9%-35% depending on the malting conditions (Novellie, 1962; Pathirana *et al*. 1983). Palmer *et al* (1989) reported malting losses of 15-20% in sorghum, compared to 7% in barley. From Table 5, the malting loss ranged between (15.75 – 28.06%), with Kapaala, having the least malting loss (15.75%). Chere, on the other hand, had the highest malting loss (28.06%). These results are in agreement with the findings of Ogu *et al.* (2006). The relatively high malting losses in sorghum, according to Agu *et al.,* (1996) are caused by the rather high steeping and germination temperatures.

Table 5: Some properties of malted sorghum varieties

Property	**Varieties**					
	Chere	Dorado	Latuor	Kapaala	Sample 1	Sample 2
1000 corn weight (g)	$19.32_{(0.40)}$	$24.17_{(0.26)}$	$21.429_{(0.19)}$	$28.15_{(0.08)}$	$25.75_{(0.13)}$	$22.13_{(0.46)}$
Malting loss (%)	$28.05_{(1.56)}$	$22.84_{(1.36)}$	$21.72_{(0.94)}$	$15.75_{(0.14)}$	20.22(0.44)	$21.94_{(2.01)}$

PH	5.78 (0.00)	5.39 (0.01)	5.80 (0.00)	4.75 (0.01)	6.04 (0.00)	6.09 (0.00)
Colour (^{o}Lovibond)	5.25 (0.00)	3.39 (0.00)	6.78 (0.00)	6.68 (0.01)	11.40 (0.00)	9.88 (0.00)
Moisture (%)	5.55 (0.10)	5.50 (0.18)	6.14 (0.19)	6.06 (0.02)	5.61 (0.06)	6.21 (0.15)
Total nitrogen (%)	1.42 (0.16)	1.38 (0.13)	1.53 (0.02)	1.61 (0.04)	1.89 (0.23)	1.79 (0.23)
TSN (%)	0.89 (0.03)	0.87 (0.02)	0.76 (0.01)	0.43 (0.02)	0.92 (0.01)	0.85 (0.04)
TSN/TN (%)	48.9	46.7	45.5	25.33	45.2	43.5
Fat (%)	1.44 (0.05)	0.96 (0.02)	1.76 (0.22)	2.04 (0.08)	2.57 (0.23)	2.03 (0.23)
Protein (%)	8.32 (0.99)	9.08 (0.83)	9.52 (0.13)	10.07 (0.22)	11.82 (0.22)	11.16 (0.12)
Ash (%)	1.70 (0.04)	0.99 (0.08)	1.17 (0.67)	1.06 (0.03)	0.28 (0.01)	0.64 (0.02)
CWE at 20^{o}C	38.93 (0.01)	35.16 (1.30)	31.68 (1.29)	25.72 (0.00)	33.71 (1.29)	34.71 (2.26)
HWE at 95^{O}C (%)	75.82 (1.14)	76.61 (0.00)	73.09 (1.42)	77.94 (1.43)	71.01 (1.40)	71.51 (1.41)
DP (^{o}wk)	60.31 (0.49)	51.07 (0.43)	40.93 (0.49)	40.07 (0.49)	42.06 (0.85)	43.49 (0.49)
Filtrate vol. (ml)*	120	115	105	83	102	122

DP: Diastatic power, HWE: Hot water extract; TSN: Total Soluble Nitrogen; CWE: Cold Water Extract

* Filtrate volume obtained after 60mins at 65^{O}C(with enzymes), ( )is the standard deviation of triplicate results of each parameter.

The high malting temperatures used during the germination of sorghum. These high malting temperatures are used (greater than 28^{o}C in sorghum, compared with 17^{o}C for barley malt), because of the tropical nature of sorghum (Palmer, *et al*, 1989).

Malting loss is an index of the extent of modification; this indicates that whereas Kapaala was the least modified, Chere was the most modified. Furthermore, the differences between the varieties were significant ($p<0.05$). There was a significant

difference between dry matter content of the unmalted and that of the malted grains. There was generally a significant reduction in dry matter content of the varieties.

Analysis of the data obtained showed a significant correlation between Diastatic power and malting loss (r=0.803). From Table 5, Chere, which had the greatest malting loss, also had the highest Diastatic power (DP). Beta *et al.* (1995) in their studies on sorghum reported that the highest dry matter losses were found in sorghum varieties with the highest α-amylase activity. This is quite understandable, since the greater the DP, the greater the extent of modification and therefore, the higher the amount of endosperm content used for respiration and other metabolic activities by the germinating embryo.

4.2.2. Diastatic Power (DP)

Subramanian *et al.* (1995) stated that the most important characteristics of good malt are high enzyme levels to degrade starch and obtain high extract yield. The Diastatic power (DP) of the malted samples were determined and compared. The correlations between DP and some other parameters such as cold water extract (CWE), hot water extract (HWE), thousand corn weight, and malting loss were also determined.

From Table 5, the DP obtained ranged between 40.07-60.3^{O}wk, with Chere, having the highest and Kapaala, the least. The differences among the varieties were significant ($p<0.05$), with the exception of that between Latuor and Kapaala. This demonstrates that the varieties are physiologically different (Ogu *et al*. 2006)

It must be emphasised that, generally, the DP for sorghum was lower than what has been reported for barley malt. EtokAkpan and Palmer, (1990) and Adejemilua (1995)

reported that sorghum malts have lower Diastatic power than barley malts. Taylor and Robbins (1993) also reported that Diastatic power of sorghum malt was less than 25% of that of barley malt. Muoria and Bechtel (1998), on the other hand, reported that sorghum malt had only less than 4% of barley malt Diastatic power. These relatively low levels of DP in sorghum may indicate the necessity of addition of exogenous α- and β-amylases. The DP results obtained (40.07 – 60.31 ^{o}Wk) were lower than was reported by Ogu *et al*. (2006), (59.5 -65.0 ^{o}Wk). It was, however, higher than that reported by Beta *et al.* (1995), which ranged between 12.6 – 43.8 ^{o}Wk.

Analysis of the data showed that DP is positively correlated with malting loss(r=0.803), loss in total nitrogen (r=0.759), total soluble nitrogen (r = 0.867), Cold Water Extract (r=0.819), Hot Water Extract (r=0.304) and Germinative Energy (r = 0.828). However, the degree of correlation was low with hot water extract (HWE). It also showed a low but negative correlation with loss in fat (r = -0.220). The cause of this negative correlation is still not fully understood. Further more, the DP and 1000-corn weight of the unmalted samples were found to be negatively correlated (r= -0.409). The degree of correlation is however not very high. This is contrary to reports by Beta *et al*. (1995) that a positive correlation existed between Diastatic power and 1000 corn weight.

4.2.3 Total Nitrogen and Total Soluble Nitrogen

The total soluble nitrogen (TSN) or total nitrogen in wort, in relation to the total nitrogen in the malted grain (TN) is an indicative of the extent of modification and the total proteolytic activity of the malt (Okokon *et al*, 2004). From table 4, Chere

had the highest (48.9%), indicating that it was the most modified. Kapaala had the least (25.33%), which means that it was the least modified of all the varieties studied.

4.3. EXTRACT

4.3.1 Cold Water Extract (CWE)

All the varieties, except Kapaala, had cold water extract of above 30% (Table 4). Results obtained for CWE ranged between 25.72 -40.10 %. Ogu *et al.* (2006) reported similar figures. Indirectly, CWE indicates the extent of modification of the grain, i.e. amount of soluble sugars present in the grain due to germination. The CWE correlated positively to the Diastatic power (r=0.819). The differences between the CWE of the different varieties were significant ($p<0.05$). However, the difference between Latuor and sample1 was not significant.

4.3.2 Hot water Extract (HWE)

4.3.2.1 HWE at various Mashing Temperatures

The hot water extracts were studied at four different temperatures, i.e. $65^{O}C$, $75^{O}C$, $85^{o}C$ and $95^{o}C$. Table 6 depicts the results (extracts) obtained and table 8 shows the response of the resulting wort to the iodine test, which indicates the presence or absence of starch.

At $65^{O}C$, all the varieties tested positive to iodine, suggesting the incomplete hydrolysis of starch into sugars. This means that at this temperature, the starch contents are not gelatinised and therefore, not available for enzymatic action (Palmer

et al. 1999). In addition the filterability was very low with none of the varieties producing 100ml of wort within the first 60minutes (see Figs 1 and 2).

Table 6: Extracts at different mashing temperatures

Variety	Mashing Temperatures (^{O}C)					
	20 (Cwe)	65 (no enzyme)	65 (enzyme)	75 (enzyme)	85 (enzyme)	95 (enzyme)
Chere	38.93 (0.01)	62.13 (0.02)	62.92 (1.36)	67.72 (1.40)	75.01 (1.43)	75.82 (1.40)
Dorado	34.41 (1.30)	57.37 (0.00)	58.16 (1.36)	67.69 (1.39)	74.98 (1.41)	76.61 (0.00)
Latuor	32.43 (1.29)	55.44 (0.00)	56.23 (1.37)	67.40 (0.00)	73.09 (1.42)	73.09 (1.42)
Kapaala	25.72 (0.00)	49.92 (1.34)	50.69 (0.00)	71.39 (1.41)	77.94 (1.43)	77.94 (1.43)
Sample1	34.46 (1.29)	54.31 (1.35)	56.66 (1.36)	65.37 (1.39)	72.63 (1.41)	71.01 (1.40)
Sample2	35.84 (2.26)	55.49 (0.00)	56.28 (1.37)	56.28 (1.40)	71.51 (1.41)	71.51 (1.41)

(..) Brackets indicate the standard deviation

When the mashing was repeated at 65^{O}C but with the addition of exogenous enzymes; heat-stable amylase, glucanase and protease (Table 7), the resulting wort still tested positive to iodine; confirming incomplete starch hydrolysis. This is an indication that none of the varieties has gelatinisation temperatures around this temperature. Thus, the starch granules were not available to the amylases to hydrolyse into sugars.

Further more, there was no appreciable increase in extract obtained from mashes with the exogenous enzymes compared to the mash without exogenous enzyme at the same mashing temperature (i.e. 65^{O}C). The difference in extract was not significant.

Nonetheless, there was a significant increase in extract at 65^{o}C, compared to the cold water extract. The differences in extracts between these two conditions were highly

significant ($p<0.000$). This shows that some limited starch granules might have been available for enzymatic attack during the malting phase.

When the mashing was carried out at 75^{o}C and with the addition of the exogenous enzymes, Chere and Sample 2 showed only traces of the blue-black colouration. The rest were all positive to iodine test (Table 8). It therefore implies that the starches of Chere and sample 2 are more susceptible to enzyme hydrolysis during mashing at 75^{o}C.

Table 8: Response of wort produced at different Mashing temperatures to iodine test

Variety	**Mashing Temperatures (^{O}C)**					
	20 (Cwe)	65 (no enzyme)	65 (enzyme)	75 (enzyme)	85 (enzyme)	95 (enzyme)
Chere	Positive	Positive	Positive	Trace	Negative	Negative
Dorado	Positive	Positive	Positive	Positive	Negative	Negative
Latuor	Positive	Positive	Positive	Positive	Negative	Negative
Kapaala	Positive	Positive	Positive	Positive	Negative	Negative
Sample1	Positive	Positive	Positive	Positive	Negative	Negative
Sample2	Positive	Positive	Positive	Trace	Negative	Negative

Again, this is an indication that these two varieties (Chere and Sample2) have gelatinisation temperature at 75^{o}C or below. This property of Chere agrees with findings by Eneje *et al.* (2001) that sorghum gelatinises at 75^{o}C. It is, however, contrary to the assertion that sorghum gelatinises at temperatures above 80^{O}C

(Palmer, 1989). Furthermore, this difference in characteristics indicates that Sample2 and Chere are physiologically different from the rest.

Interestingly, all the varieties tested negative to iodine when mashing was carried out at 85^{O}c with commercial enzymes. This seems to confirm, to a greater extent, the assertion that sorghum gelatinises at temperatures above 80^{o}C (Palmer, 1989). One remarkable observation is that, with the exception of Chere, which had highest increase in extract yields at 75^{O}C, the rest of the varieties had their highest increment in extract when mashed at 85^{O}C. It was also surprising to note that even though sample2 was almost fully gelatinised at 75^{o}C (as it tested negative to iodine); nevertheless, it also had it greatest increment in extract yield at 85^{o}C. Kapaala had a rather unusual characteristic. Even though it gelatinised at 85^{o}C, the saccharification time was unusually high. It took about twice the average time for it to saccharify (i.e. for complete hydrolyses of starch to sugars).

Again at 95^{o}C, all tested negative to iodine. There was no significant increase in extract obtained at this temperature, with the exception of Dorado.. However, extract for Sample1 actually declined marginally (72.63% at 85^{o}C and 71.01% at 95^{o}C).

On the whole, Kapaala had the greatest extract yield, followed by Dorado, and Sample 1, the least. It must be emphasised, however, that Kapaala, irrespective of the high extract, was the most difficult to process.

In general, the difference in extract when mashing was carried out at 75^{o}C and 85^{o}C was statistically significant ($p<0.05$) for each variety. On the contrary, the differences in extracts were not significant ($p>0.05$) between 85^{o}C and 95^{o}C for all the varieties studied.

This means that the starch contents of all the varieties were fully gelatinised around 80^{o}C and thus became available for amylases to completely hydrolyse them.

Industrially, this means that heating sorghum mashes to 95^{o}C is not necessary and therefore, breweries can limit themselves to 85^{o}C and thereby save on energy and reduce operational cost.

Hot water extract at 95^{o}C, however, negatively correlated to total Nitrogen (protein) content of the unmalted samples (r= -0.553).

4.4. Wort pH and Colour

pH is very important during mashing since mashing is entirely an enzymatic process and therefore it plays an important role. It was interesting to note (Table 5), that the mash pH of all the varieties studied (except Kapaala) were within the range (5.39-6.09), which fall within the required mash pH (5.6±0.4), even though 6.09 is slightly on the higher side. According to Agu, *et al.* (1998), recommended sorghum mash pH ranged between 5.3-6.0. Kapaala, on the other hand, had a pH of 4.75, which is outside the required mash pH. Therefore, the pH of Kapaala had to be adjusted to 5.3 with 10ml of 0.5N KOH, at all the mashing temperatures. Therefore, in brewing with Kapaala, there must always be correction for pH. On large scale industrial processing, this could be quite a challenge and expensive.

With the exception of Dorado (which had a colour of 3.39 EBC), all the varieties studied had their wort colours ranging between 5.25 – 11.40. Johnson, (1991) and Seward, (1986), reported sorghum wort colours of 3 EBC and below. Okonkon *et al.* (2004) also reported that malt wort colour could vary between 6 – 12 EBC, depending on storage time and conditions. He further indicated that colour of sorghum malt worts dropped by 10EBC units over six-month storage period.

4.5. Wort filterability

Poor degradation of β-glucans during malting and poor hydrolysis of starch during mashing can lead to slow wort filtration, reduced extraction efficiency and haze development of the finished beer (Palmer, 1989). Wort filterability (which is an indirect measurement of wort viscosity) was determined by measuring filtrate volume over a period of time (i.e. after returning first 100ml). Figures1-10, show the plots of filtrate volume versus time at various mashing temperatures.

When the mashing was carried out at 65 ^{o}C, without exogenous enzymes (Figure.1), the filterability for all the varieties were low, as none produced 100ml filtrate volume in 60 minutes. Prolonged filtration of sorghum wort has been reported by Aisien and Muts. (1987). This observation has been attributed by EtokAkpan (1992), to lack of β-D-Glucan degrading endo 1-3, 1-4 β-glucanase enzymes in sorghum malts. From graph1, Kapaala was the least filterable. This may suggest that it was the most poorly modified. In other wards, wort produce by Kapaala at this temperature contained the least amount of soluble sugars.

There was tremendous increase in filterability for all varieties when mashing was again carried out at 65 ^{o}C (Figure 2), but this time with the incorporation of exogenous enzymes (Protease, amylase and betaglucanase). This means that, the β-glucans underwent extensive hydrolysis with the application of exogenous β-glucanase. The extent of hydrolysis may thus have been facilitated by the inherent β-glucanase content of the sorghum malt.

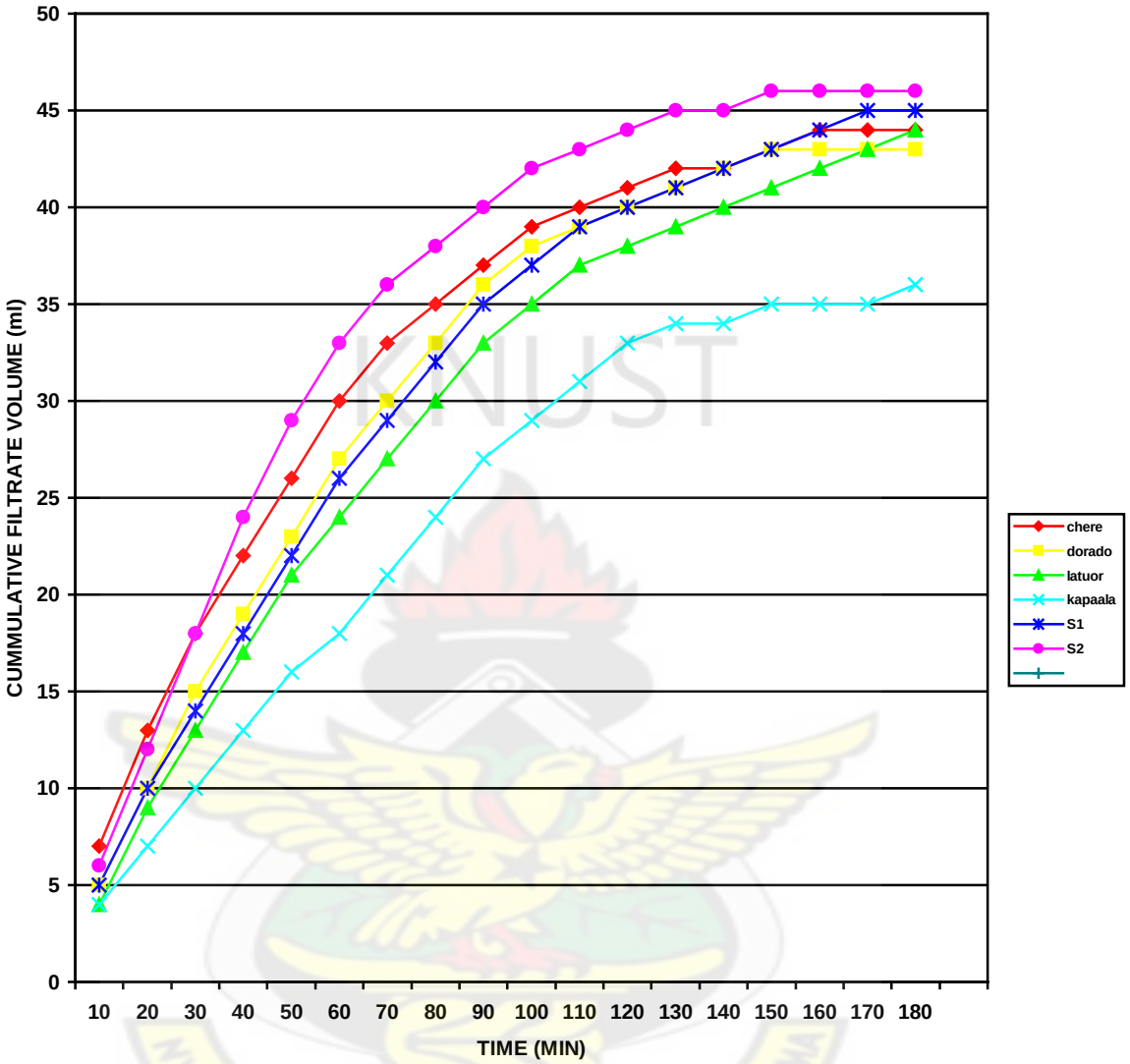


Figure1. A Chart comparing filtrability at 65oC (No enzyme)

In fact, low β-glucanase potential of sorghum has been reported by Duffuor *et al.* (1992), EtokAkpan *et al.* (1990) and Palmer (1989). Thus, either the β-glucanase contents in the malts were too low, or as was explained by Palmer *et al***,** (1999), β-glucanase in sorghum malts, like in barley malt are not active during mashing.

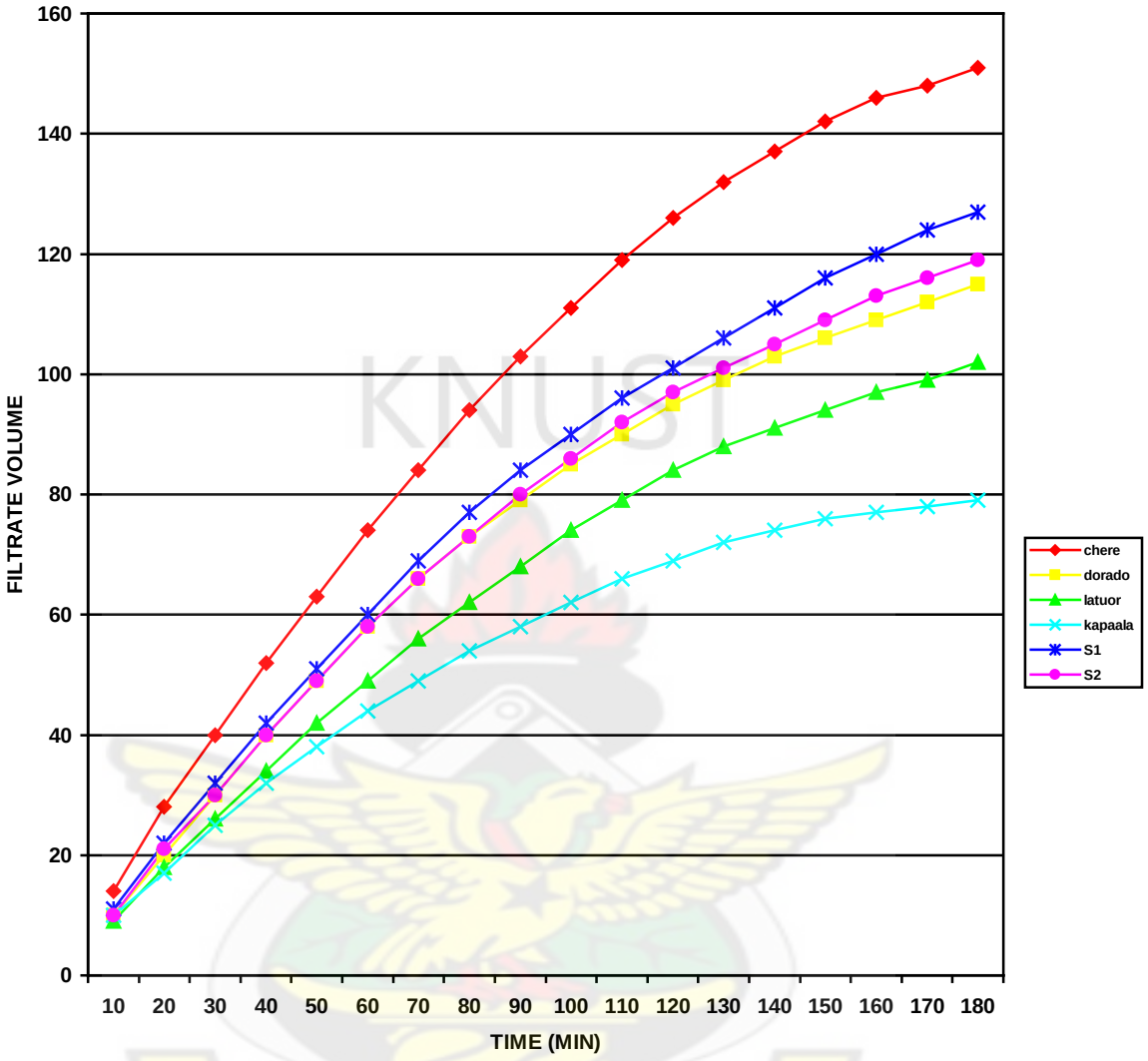
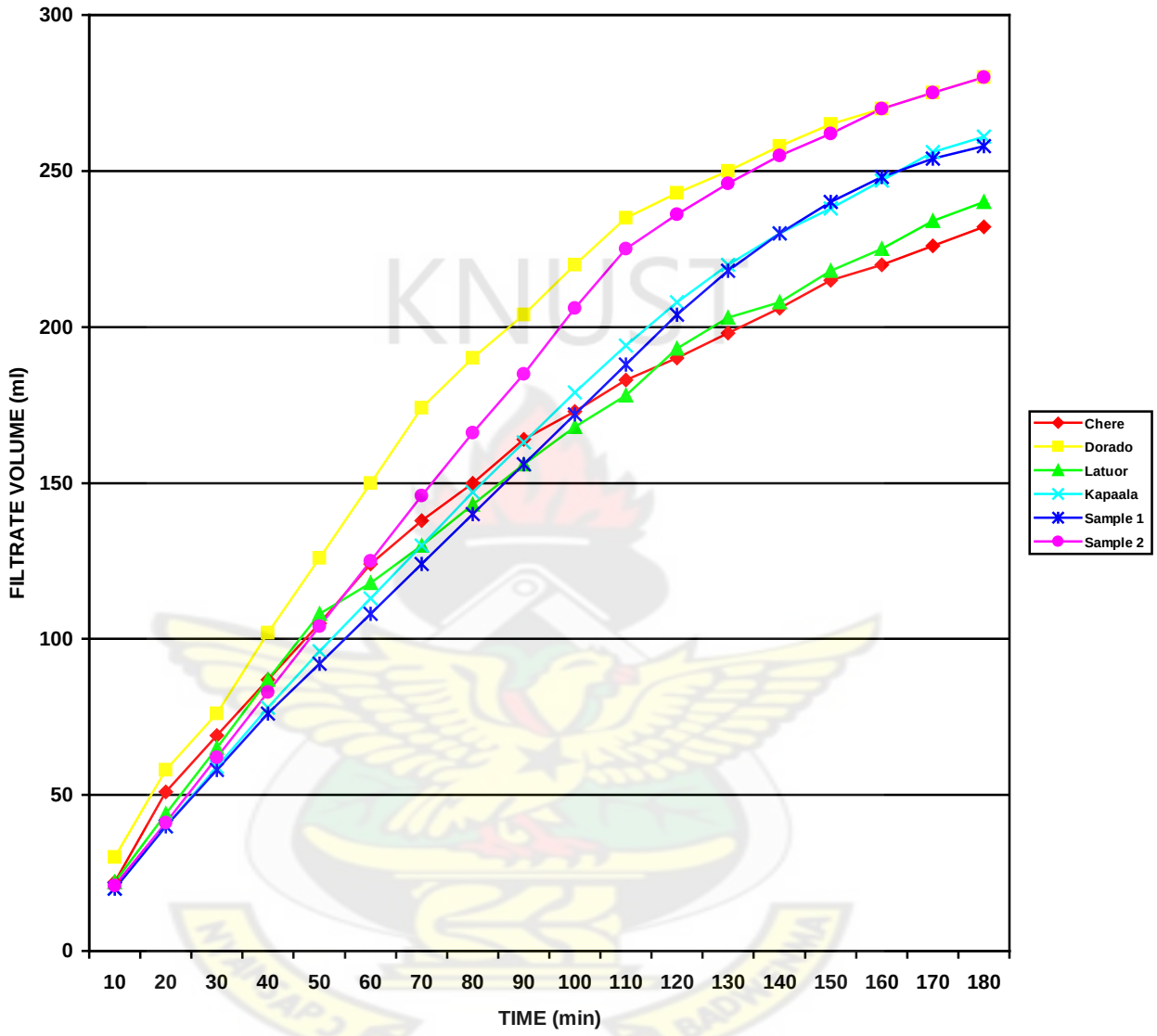


Figure 2: A Chart comparing filtration rates of varieties at 65^{O}C (with Enzymes)

Again, the fact that there were no appreciable increases in filtration rate at higher temperatures may also mean that β-glucan levels in the malts were low. A study conducted by Demuyakor and Ohta. (1994) reported that β-glucan level in the sorghum malts were very low compared with barley malt.

Figure 3: Graph comparing the wort filtrability of varieties at 85^{o}C



When mashing was carried out at 75 ^{o}C (Figure 4), 85 ^{o}C (Figure 3) and 95 ^{o}C, all with addition of exogenous enzymes, the increases in filterability were very significant for all varieties. However, at 65 ^{o}C (both with and without exogenous enzymes), Kapaala was the least filterable, even though there was significant increase when enzymes were added (Figure 8).

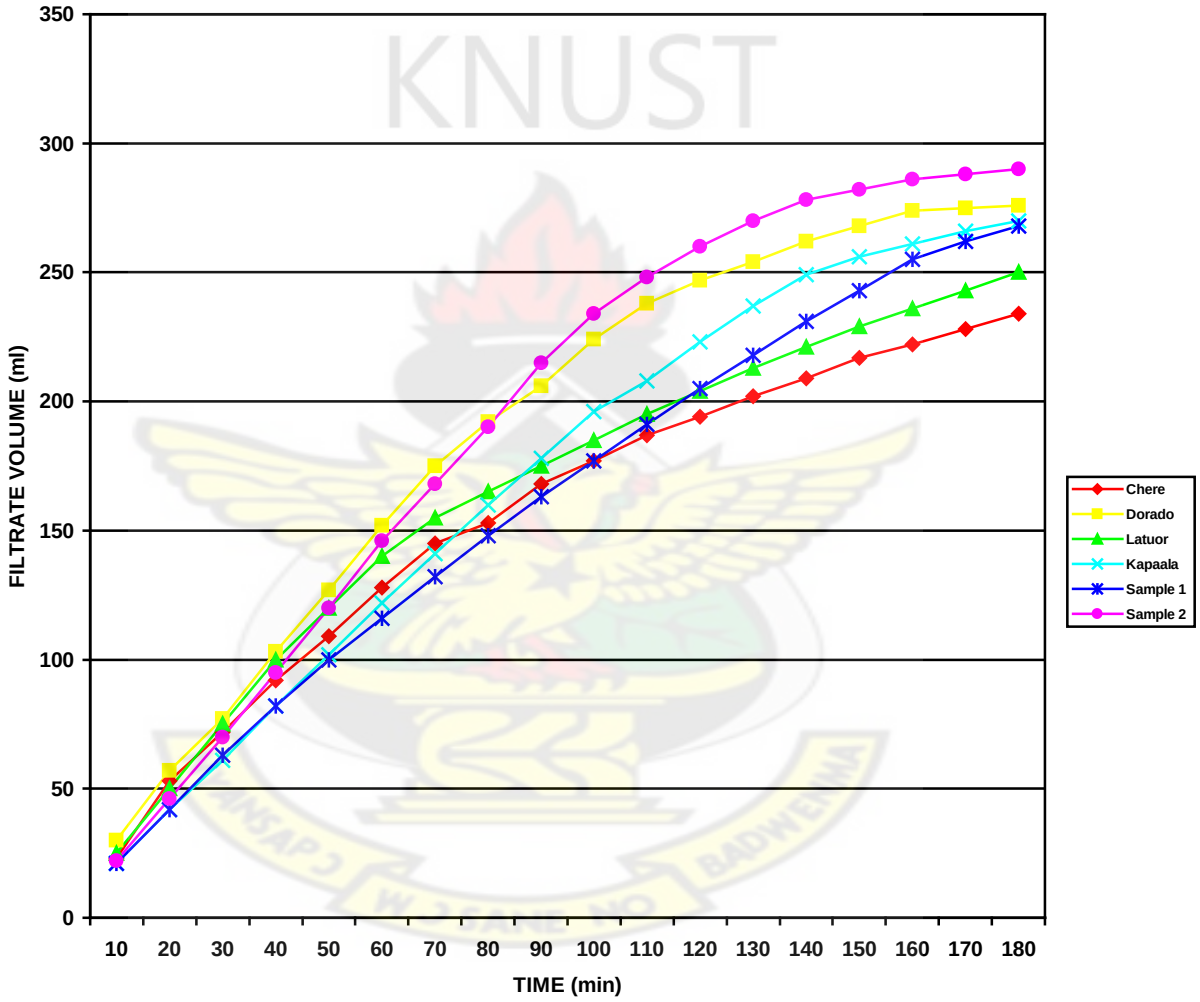


Figure4: A Chart comparing filtrability of varieties at 75^{o}C

The highest increases in wort filterability were obtained when mashings were carried out at 75 ^{o}C for all varieties. With the exception of Kapaala, Dorado, and to some extent

Sample2, which saw significant improvement in their filterability between 75 ^{o}C and 85 ^{o}C (Figs. 6, 8 and 10), the remaining three varieties were not. This may be an indication that the starches of these three varieties gelatinise around 80 ^{o}C.

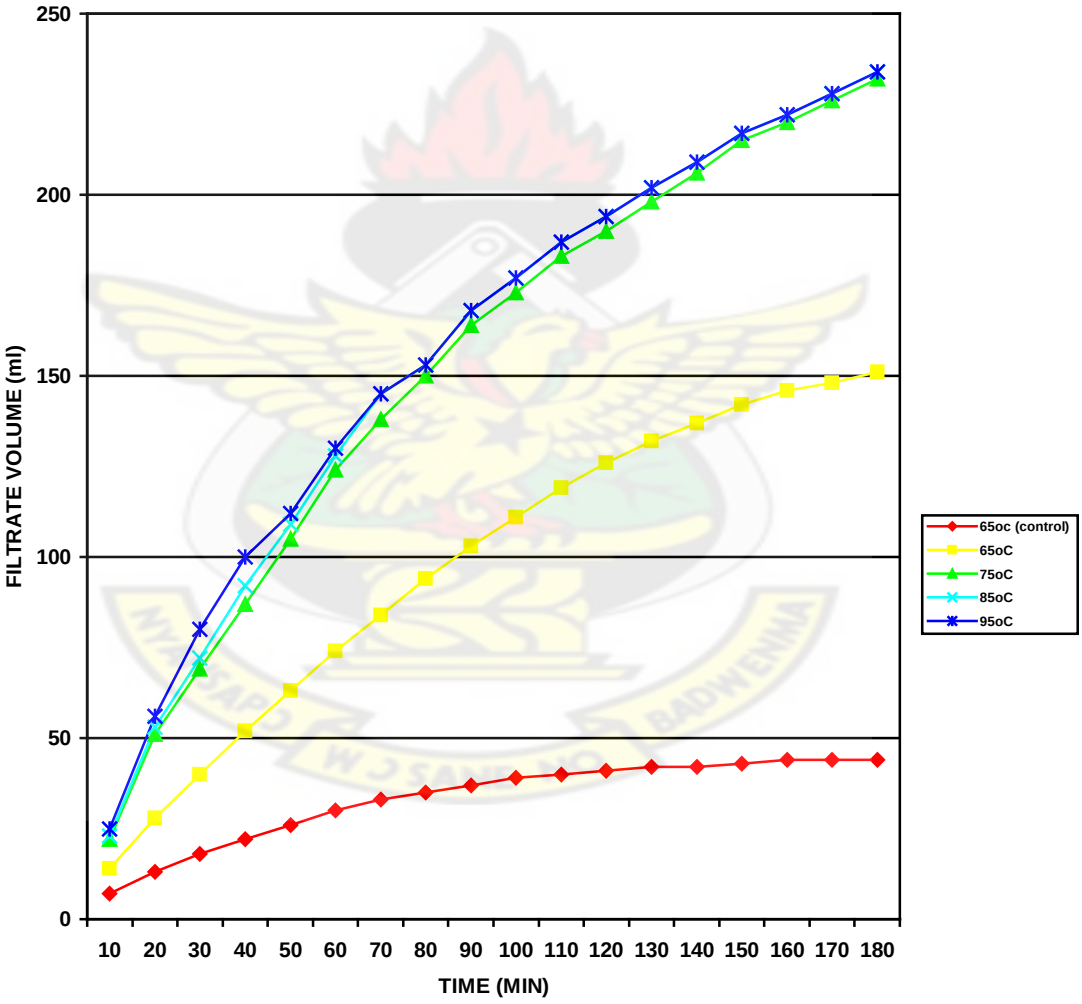


Figure 5: Variation of wort filtration rate of Chere at different mashing temperatures

It is worth mentioning that mashes at 95 ^{o}C for all varieties did not show further improvement in wort filterability. This observation could be explained on the basis that the starch contents of all varieties were fully gelatinised around 80 ^{o}C.

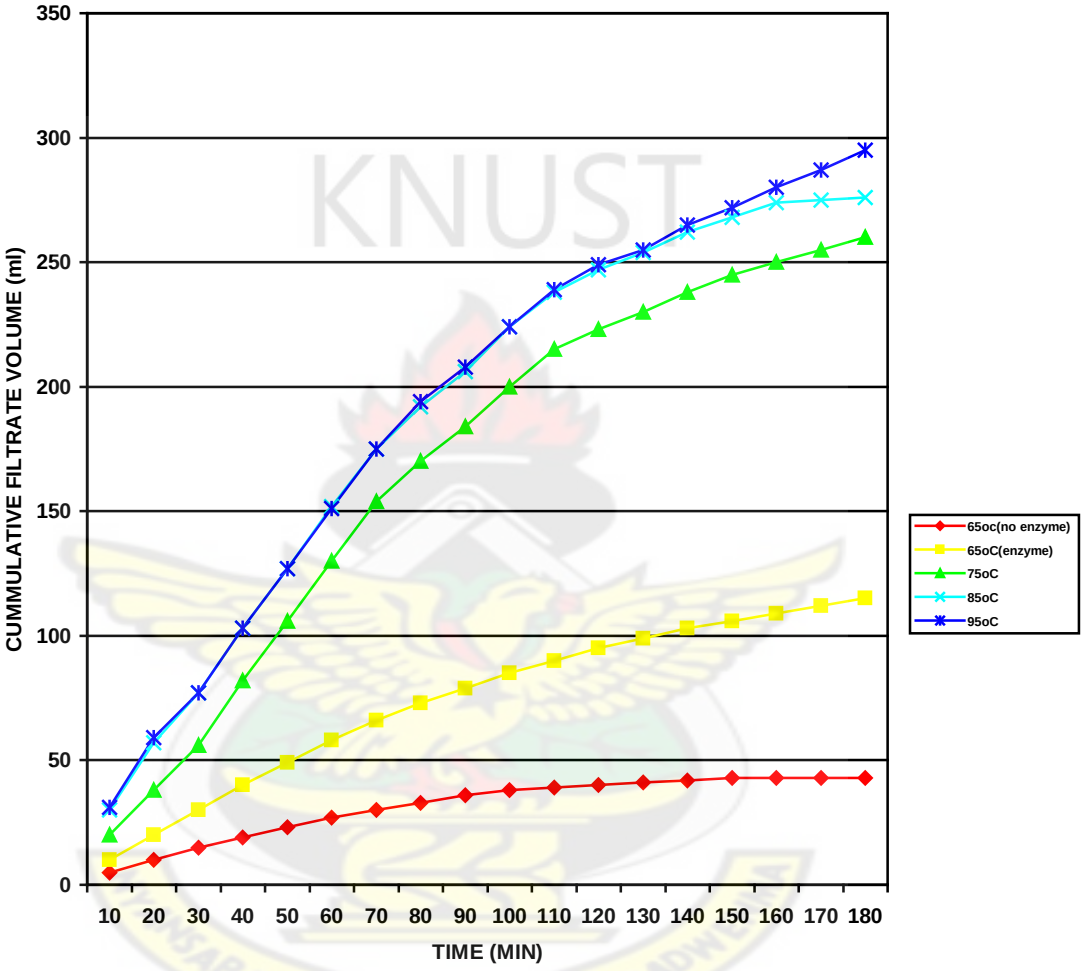


Figure 6: A Chart comparing wort filtrability of Dorado at different mashing temperatures

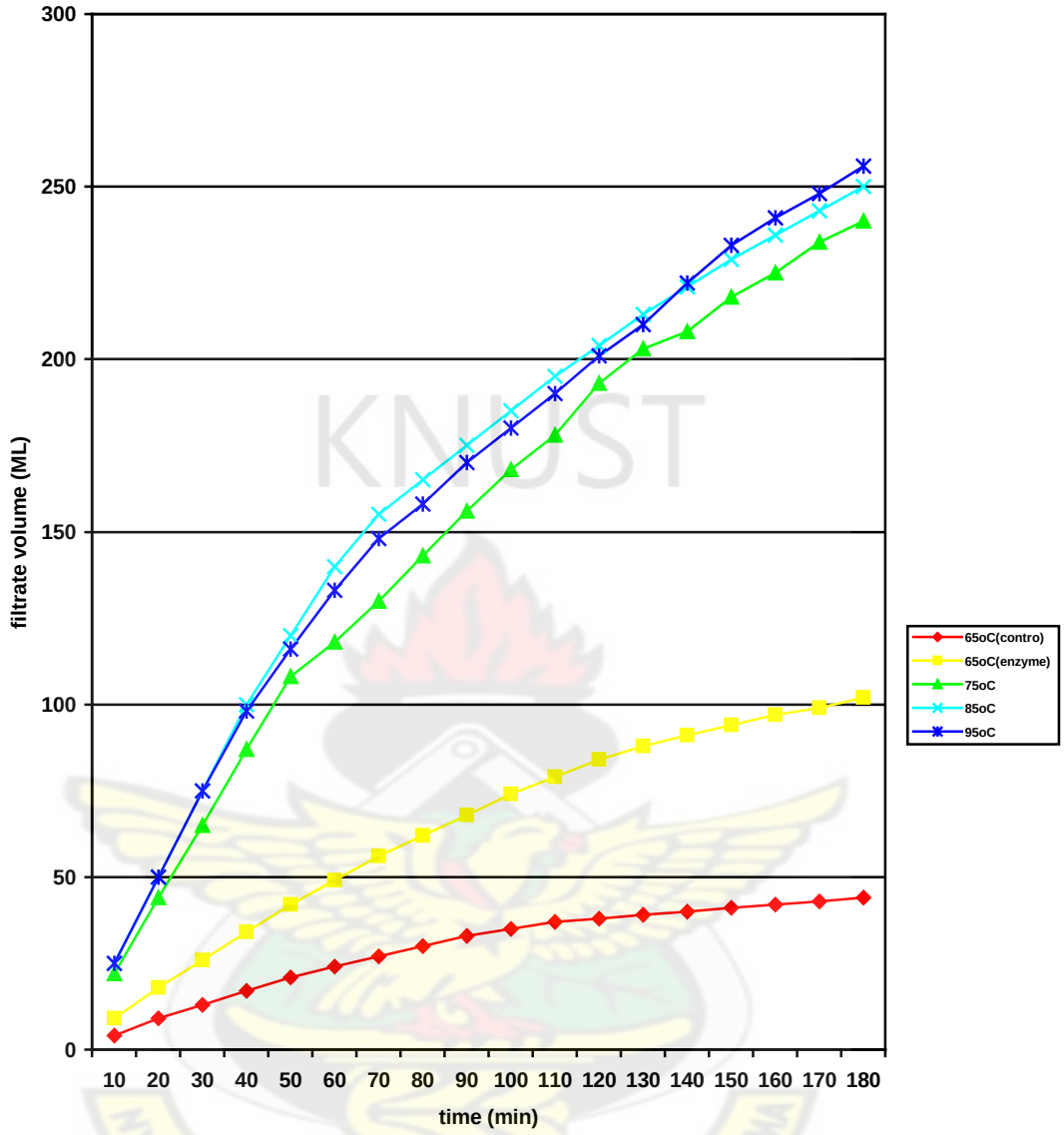


Figure 7: A Chart comparing wort filtrability at different mashing temperatures for Latuor

For Chere (Fig. 5), there were no significant increases in filterability when mashings were carried out at 75^{o}C, 85 ^{O}C and 95 ^{O}C. This may be the indication that Chere was extensively gelatinised around 75 ^{o}C.

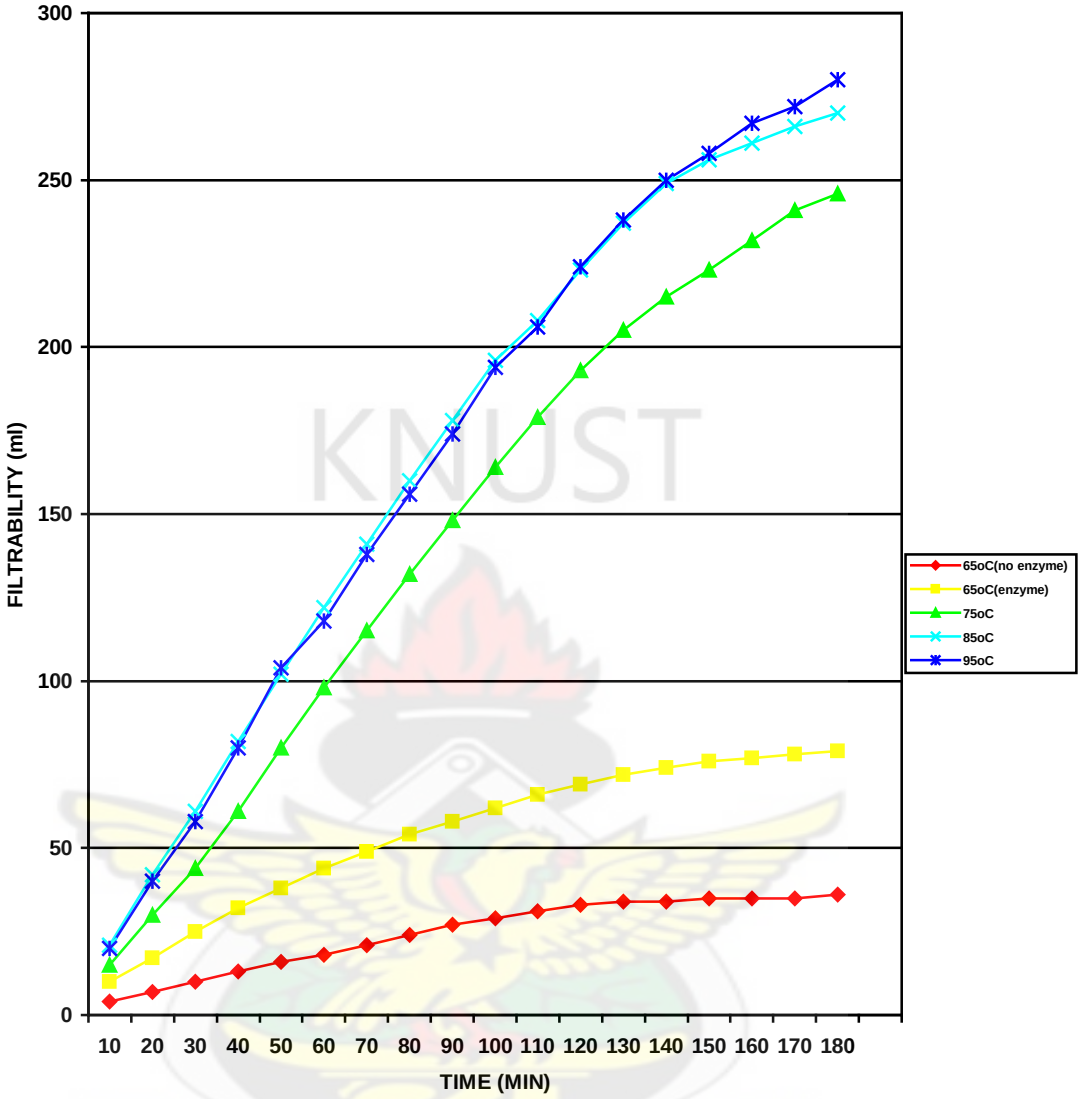


Figure 8:A Chart of wort filtrability of Kapaala at different mashing temperatures

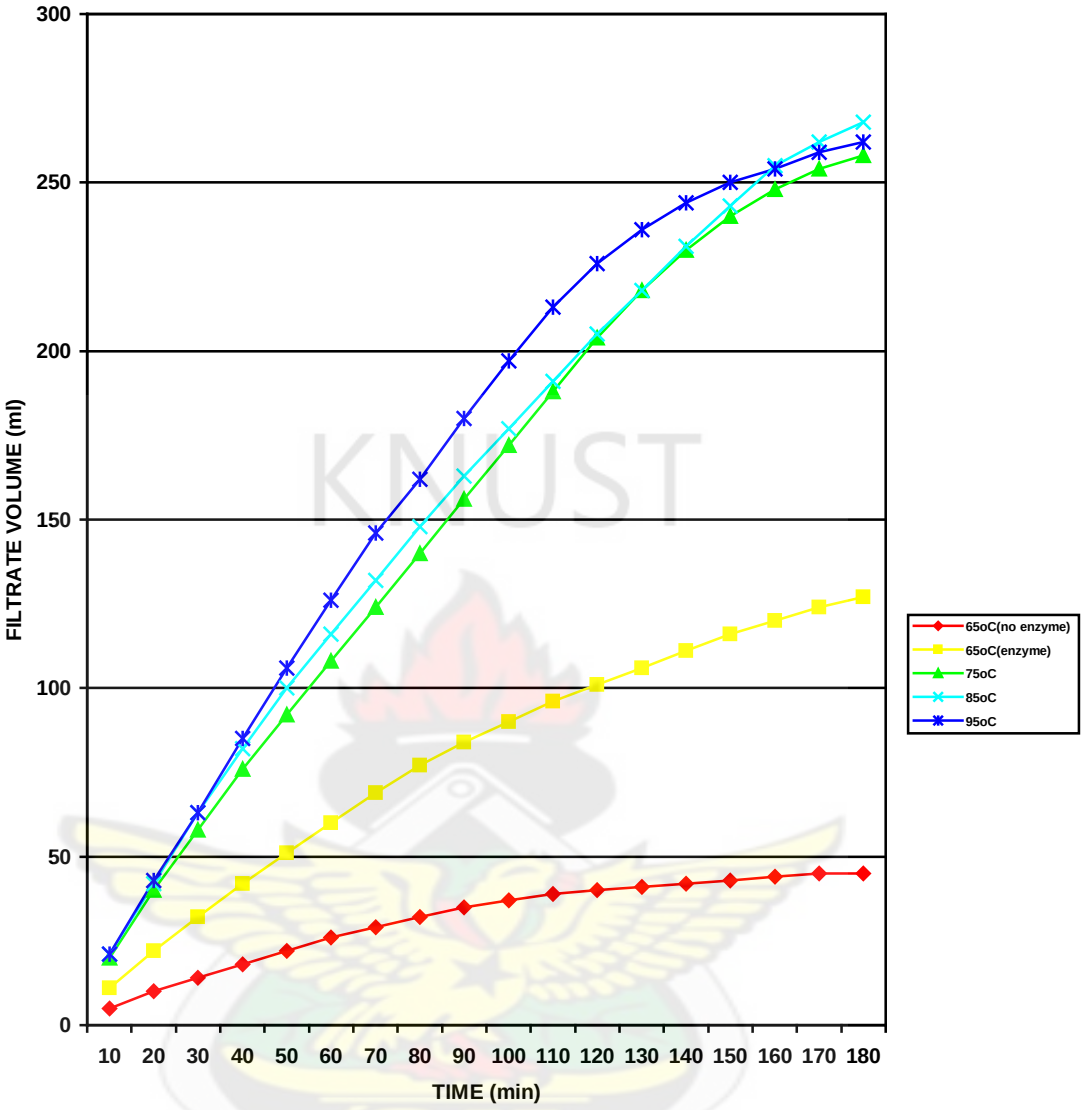


Figure 9: A Chart showing wort filtrability at different mashing temperatures for Sample 1

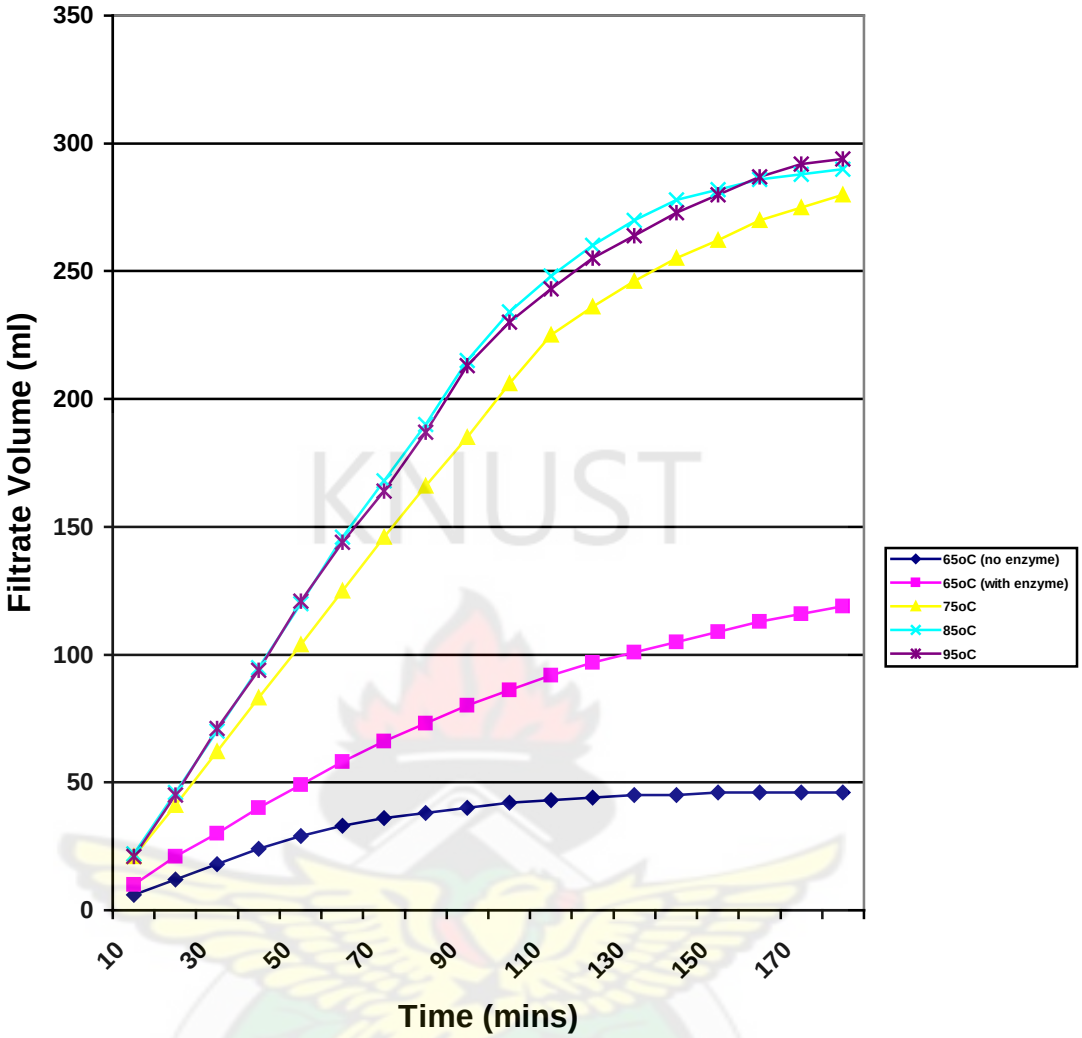


Figure 10: A Chart showing wort filtrability of at different mashing temperatures for Sample 2

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

The work showed that all the varieties studied varied with respect to their ability to malt. This was indicated by their varied but acceptable germination energy and germination capacity values. The suitability of the varieties for malting was further shown by the suitable nitrogen contents. Equally important determinant of suitability for malting was the 1000 corn weight. With the exception of chere having corn weight lower than the minimum required for sorghum, the remaining varieties were suitable for malting.

Varietal differences with respect to the effect of the malting process were evident. All varieties, even though subjected to the same conditions of malting, varied with respect to malting loss, diastatic power, cold water extract and loss in fat. Dorado malt was the only variety with suitable fat content. Physiological differences between varieties were clearly demonstrated with the differences in diastatic power, hot water extract, and gelatinisation temperatures.

The work also brought to the fore the importance of the temperature employed in mashing sorghum as a brewing material. At the temperature of 65 ^{O}C, which is usually used for mashing barley malt, none of the varieties had its starch completely hydrolysed, as they were not gelatinised. Addition of exogenous enzymes at this temperature did not give any appreciable improvement. However, at the mashing temperature of 85 ^{O}C and above, the starches were effectively hydrolysed. This was shown by the negative response to the iodine test and increased in filterability of wort of all varieties. Since the temperature at which sorghum starch granules become available for enzyme attack is higher than the range required by the endogenous

amylases, as this work has shown, exogenous enzyme is required for higher sorghum substitution as brewing adjunct.

The work has highlighted Dorado as the most suitable variety for brewing and Kapaala, the least suitable, in spite of having the highest hot water extract. However, Chere is the most suitable for malting, provided the objective is to produce malt with high diastatic power.

5.2 RECOMMENDATIONS

From the study, the recommendations below can be made:

1. Samples 1 and 2 need to be further processed to reduce their nitrogen content in other for them to be suitable for brewing
2. Further studies are required on Kapaala to reduce its processing constraints before it can be used for commercial brewing
3. Future studies on any of the varieties must consider measurement of total soluble nitrogen (TSN) and free amino nitrogen (FAN).
4. Further studies on the selected variety should be extended to cover fermentation process in future work in order to completely assess the quality of the final beer produced.

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