AN INVESTIGATION INTO THE EFFECTS OF STORAGE PERIODS ON GERMINATION VIGOUR AND MALT QUALITY IN MALTING BARLEY (*HORDEUM VULGARE*), VARIETY "*HOPE*" :A CASE STUDY AT DELTA BEVERAGES, KWEKWE MALTINGS.

By

ASHLEY SIMISO SITHOLE

(R124000W)

A dissertation submitted in partial fulfilment of the requirements for the

Bachelor of Science Honours Degree in Biological Sciences

Department of Biological Sciences

Faculty of Science and Technology

Midlands State University

June 2016

ABSTRACT

Malts with high extract values, high enzymatic activities and good modification are fundamental to increase the brewing yield and efficiency. To produce malt that meets these requirements, the barley employed must have minimal post-harvest dormancy and be able to germinate rapidly and uniformly. The aims of this study were to assess the changes in seed germination vigour trends and the general storage stability of a Zimbabwean two-row malting barley variety "*Hope*" as the post-harvest storage time increased. Five samples of this commercially grown variety were obtained and stored under room temperature conditions. The samples had their germination capacity and energy determined using SAB Miller standards for malting barley analysis (controlled germination in an incubator at 18^0C – 21^0C for 72 hours). In addition other quality parameters (moisture content, water sensitivity, diastatic power, total extracts, free amino nitrogen and friability) necessary to assess the storage stability were analyzed using the same analytical standards as for germination tests for each sample. Results showed that "*Hope*" is better malted after a storage time of 2 years meaning that, the variety's germination vigour improves with time. However, at storage periods of more than 2 years, undesirable quality trends were observed and this could be because most of the grains are presumed dead. Malting of grains just after harvest also produced undesirable results probably because the grains were still dormant as dormancy breaks after 3 months of harvest. Dormancy is a common characteristic of seeds that retards rapid and uniform germination and thus it has the potential to affect malt quality adversely. Parameters measured before malting are moisture content, germination capacity, germination energy and water sensitivity. The results showed an increase with decrease into storage years, that is from 2011-2015. An exception was observed in water sensitivity as the results showed a decrease as years into storage decreased. Results after malting showed general increases as years into storage decreased, that is for, DP, FAN and extracts. However, for filtration speed, friability, PUGS, WUGS and TSN a decrease was observed with decreases in storage years. From the results obtained, it can also be concluded that "*Hope*" is more stable in storage of not more than two years. Thus after considering germination performance and general malt quality after storage it was concluded that "*Hope*" is a good malting barley variety. Since all malt quality parameters measured in this study significantly influence brewery efficiency, these findings have a significant commercial impact.

ACKNOWLEDGEMENTS

Above it all, I give glory and honor to the Almighty God, my source of strength and hope for his sufficient grace and enduring merciful love. My gratitude also extends to my academic supervisor Ms S.Mguni, together with the entire Biological Sciences lecturers for imparting their knowledge and for their encouragement throughout the course of writing this dissertation. Very special thanks go to Delta Beverages Kwekwe Maltings, especially the production and quality control departments for their unwavering support at every stage of this research. I also express my heartfelt gratitude to Nkosilathi Mlilo and Peter Dondo for continuously providing a helping hand during the course of writing this dissertation.

DEDICATION

I dedicate this fruit of my labor to my dearest mother and father, the most important people in my life, the source of inspiration and motivation. Without these people I would have never discovered my potential and may have never sought higher education. God bless them for me!

TABLE OF CONTENTS

PAGE

LIST OF APPENDICES

PAGE

LIST OF FIGURES PAGE NUMBER

PAGE

LIST OF TABLES

PAGE

CHAPTER 1

1.0 Introduction

1.1 Background of the Study

Malting is the directed manipulation of barley growth to attain a desirable extract and enzyme yield (Briggs, 1998). It includes the controlled germination of barley where hydrolytic enzymes and cell walls are synthesized whereas protein and starch of the endosperm is digested, thus, increasing grain friability (Enari and Sopanen 1986; Bamforth and Barclay 1993). In the manufacture of beer of excellent quality, the quality of the malt is of primary significance. Malts with high enzymatic activities, high extract values, and good modification are fundamental to increase brewing yield and efficiency. The barley employed must have minimal post-harvest dormancy and be able to germinate rapidly and uniformly so that it best meets the brewer's specifications can be produced (Riss and Bang-Olsen 1991; Woonton, Jacobsen, Sherkat and Stuart 2005). The criterion for selecting malting barley thus includes high germination index and germination capacity (Swanston and Taylor 1990; Larsenet 1994; Briggs 1995; Lu, O'Brien and Stuart 2000; Munck and Moller 2004). Barley which germinates rapidly and homogenously bests suites the malting process. Faster rates of germination shorten the time required to accomplish desirable modification during malting hence reducing the hours required for the germination stage. As a result the germination vigour which is the rate of germination determines the time required for malting and malting efficiencies. To ensure that sufficient barley is grown and in appropriate quality Kwekwe Maltings (KKM) engages vertical backward integration contractual farming with farmers across the farming regions of the country. Contracted farmers are supplied with farming inputs and loans in case they lack sufficient funds for a farming season guaranteeing Kwekwe Maltings of adequate raw materials for production throughout the year. As a result the company has the potential of receiving over 27 000 tons of barley (variety

"*Hope*") from a winter growing season. There is therefore a great need to have proper storage facilities that will ensure that barley retains its desirable malting qualities such as germination vigour and capacity during the prolonged storage prior to malting. There are two Zimbabwean based varieties that KKM use as raw material for production of malt and these are "*Hope*" and "*Sierra"*. Malt exported to Zambia is under tolling agreement. This is a contract whereby Zambian Breweries' barley, variety "*Dawn"*, is processed into malt and exported to Zambia. The variety "*Hope*" is the most malted variety because it produces the desired quality of malt with the regimes that are employed by the company. It is also has a better storage stability than Sierra. In accordance to the findings of the research that was done at the Carlsberg Research Laboratory, during prolonged storage, the barley grain will slowly lose its vitality. This causes a slower germination rate or even grain death, and will therefore be of less value for the maltster (European Brewery Convention Congress / EBC 1989 and 1991).The rate at which barley loses its vitality is dependent on the storage conditions.

Kwekwe Maltings (KKM) is a subsidiary of Delta Corporation. The company is located at 5 Bessemer Road in the heavy industrial sites of Kwekwe. Kwekwe Maltings is the only barley malting plant in Zimbabwe and the third largest in the Southern Africa Region, thus making it monopoly in Zimbabwe. It processes barley into malt which is the major raw material in the production of clear lager and opaque beer by contributing 70% input of clear beer manufacture. Kwekwe Maltings boasts as the sole producers of barley malt which is the major by product in beer brewing. Malt is the main product that is produced by the company although other by-products are produced and sold to other customers. These by-products are sold on the domestic markets normally farmers who need them for use as stock feed. These by-products however have an insignificant percentage of total sales volumes.

1.2 Problem statement

The storage time of malting barley at KKM is determined by the quantity of barley received from farmers for a particular growing season. Aging of barley is the chief factor that contributes in compromising the quality of the malt. Storage can either reduce barley quality (Woods *et al*., 1994; White et al. 1999), or increase maltability (Woonton *et al*., 2002). Just as in other varieties there are great possibilities for the germination vigour to vary with time in the variety "*Hope*". Consequently because of the same reasons, stability of other quality parameters will vary during storage. No scientific study has been done to determine trends of changes in seed germination vigour with increase in storage time for the variety "*Hope*" and to further analyze their storage quality stability. As a result KKM lacks adequate experimentally proved information to justify whether or not the barley variety they malt retain its desirable germination attributes for the same length of storage time. Due to this knowledge gap, the organization cannot explain whether or not the compromised germination performances evident in some instances when barley is malted, are due to prolonged periods of storage. As a result the researcher has found a necessity to carry out: An investigation into the effects of storage periods on germination vigour and malt quality in malting barley (*Hordeum vulgare*), variety "*Hope*"), a case study at Delta Beverages Kwekwe Maltings.

1.3 Justification of the study

The findings of this study will benefit the maltster in a number of ways. A knowledge gap exists in regard to the trends of seed germination vigour for the malting barley of the "*Hope*" variety, therefore a successful completion of this research will seal this gap. Germination vigour determines the time and conditions required for malting (thus has a direct effect on malting efficiencies) and hence the information comparing the trends of germination vigor of the variety

is necessary for effectively determining processing cycles and conditions at various storage stages. Information obtained from the research findings will be essential to determine the quantities of barley to be grown each year by the contractual farmers which Delta Beverages Kwekwe Maltings engages with. This is done so as to avoid growing of amounts of barley which cannot be malted in the time frame which the barley will be retaining high germination vigour.

1.4 Objectives of the study

Main Objective

To assess the effects of storage periods on seed germination vigour and malt quality of barley, variety "*Hope"* during malting.

Specific objectives

- To determine the effect of storage time on germination vigour and malt quality by measuring, germination energy, germination capacity, moisture content and water sensitivity in barley.
- To determine the effect of storage time on germination vigour and malt quality by measuring, friability, free amino nitrogen, diastatic power, total extracts, filtration speed and total soluble nitrogen in malt.
- To compare quality of the barley kernels with storage time.

1.5 Hypothesis

- H_0: There are no significant differences in seed germination vigour and malt quality with prolonged storage time in Hope barley variety.

- H_1: There are significant differences in seed germination vigour and malt quality with prolonged storage time in Hope barley variety.

1.6 Delimitations

The scope of the research was restricted to only one of the malting barley varieties malted at Delta Beverages Kwekwe Maltings. Only the malting barley harvested from the 2011, 2012, 2013, 2014 and 2015 winter growing seasons were used for the research.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Definition and purpose of malting

Malting is defined as the limited germination of cereal grains or, occasionally, the seeds of pulses, under controlled conditions (Briggs, 1998). More specifically relating to barley malt, malting is the controlled germination of cereals, to ensure a given physical and biochemical change within the grain, which is then stabilized by grain drying (Abu-Ghannam, and Gallaghar, 2010).

2.2 Malting barley

Barley (*Hordeum vulgare*) is a type of cereal that belongs to the grass family (*Gramineae*) usually grown in winter and one of the ancient domesticated crops (Naidu, 2008). Barley is used worldwide for malting purposes in the brewing industry despite that in recent years, there has been a growing interest in incorporating barley into the human diet because of its wholesomeness and that it is cheap and readily available (Keenan, Coulson, Shamliyan, Knutson, Kolberg and Curry, 2007). Barley has historically been preferred for malting purposes because it has desirable characteristics that are lacking or deficient in other grains. One such characteristic is that the grain retains its husk during malting. This is important at the end of the brewing process because the husks collect at the bottom of the mashing tank forming a filter bed through which the wort (sugar water extracted from the grain) passes before going on to the brew kettle (Simpson and Ogorzaly, 2001). Another desirable characteristic is the quantity of enzymes produced within a germinating barley grain. These enzymes sufficiently convert starches into sugars making them available for fermentation. Other grains produce the same enzymes but not to the extent of barley and often require the addition of supplementary enzymes during fermentation (Simpson and

Ogorzaly, 2001). Although barley is the most malted cereal, Briggs (1998) notes that other cereals such as wheat (*Triticum aestivum*) and sorghum (*Sorghum vulgare*) also malted in significant quantities (the latter in Africa). In other countries small amounts of rye (*Secale cereale*), oats (*Avena sativum*) and various species of millets are also employed. Malting barley must meet special quality specifications which are essential to ensure homogenous modification and finished malt whose properties lie within the brewer's specifications (Kunze, 1999). The malt quality of a given barley variety is determined by its genetic background and the physical conditions during growth, harvest and storage (Kunze, 1999).

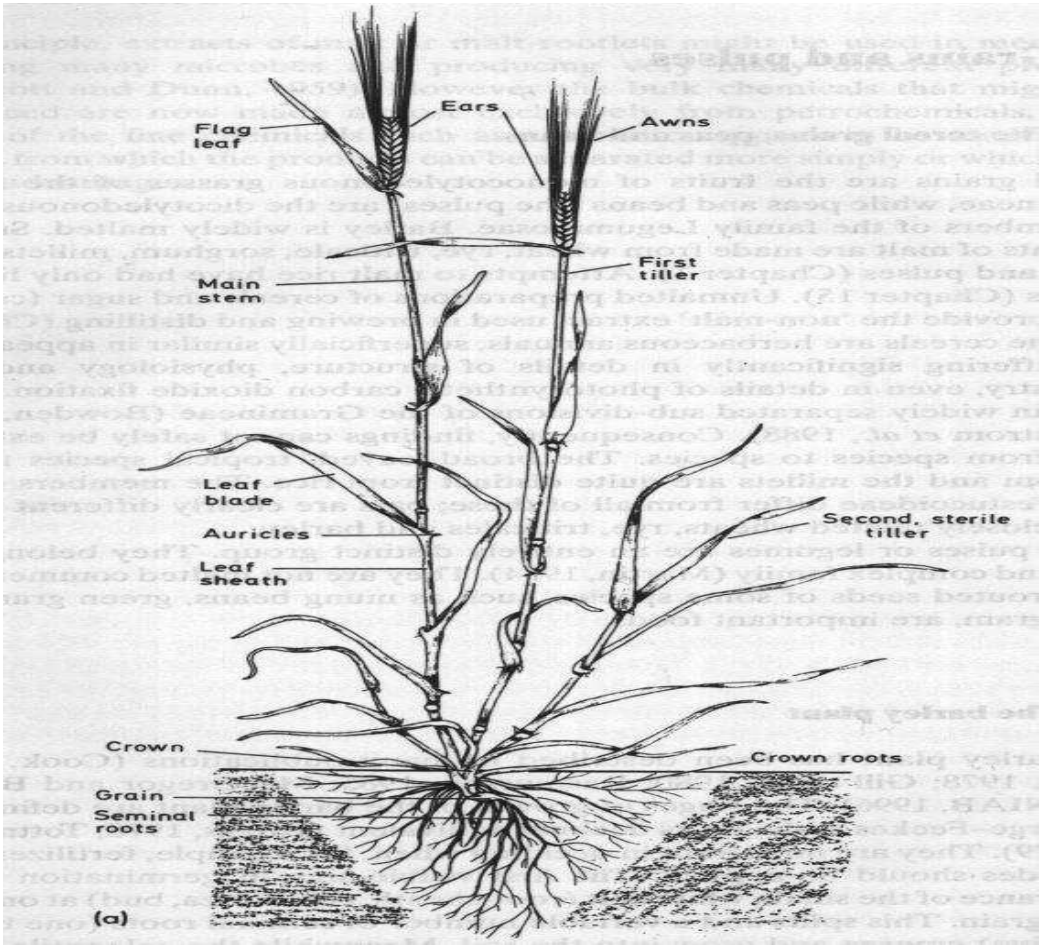

Figure 2.1: A general view of a fully grown barley plant (extracted from Briggs; 1998, Page 39)

2.2.1 Characteristics of the barley grain

To fully understand the process of malting, grain physiology is an essential tool. Longitudinal and transverse cross sections diagrammatic representations of the barley grain and description of the barley structure are given below.

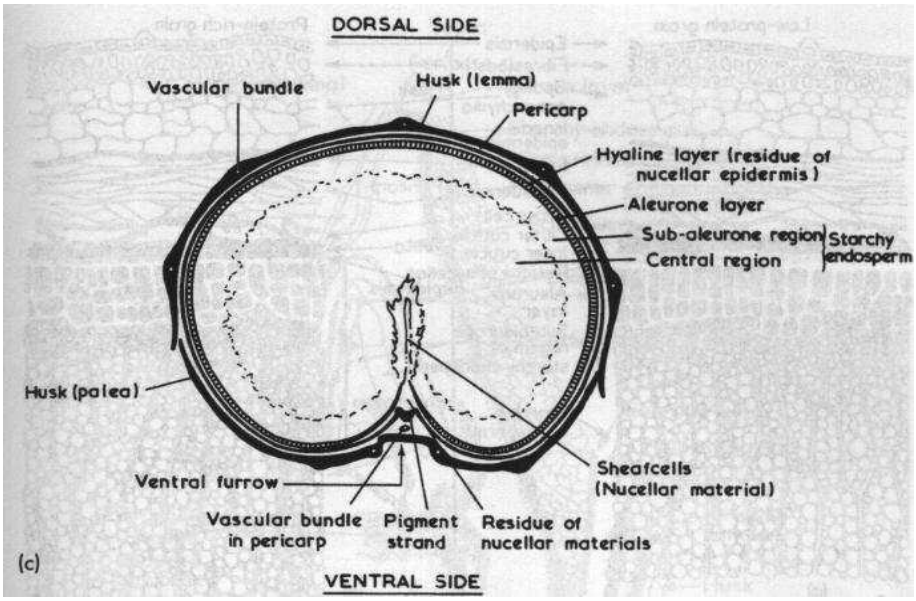

Figure 2.2: A longitudinal cross section of the barley grain (Briggs, 1998, Page 43).

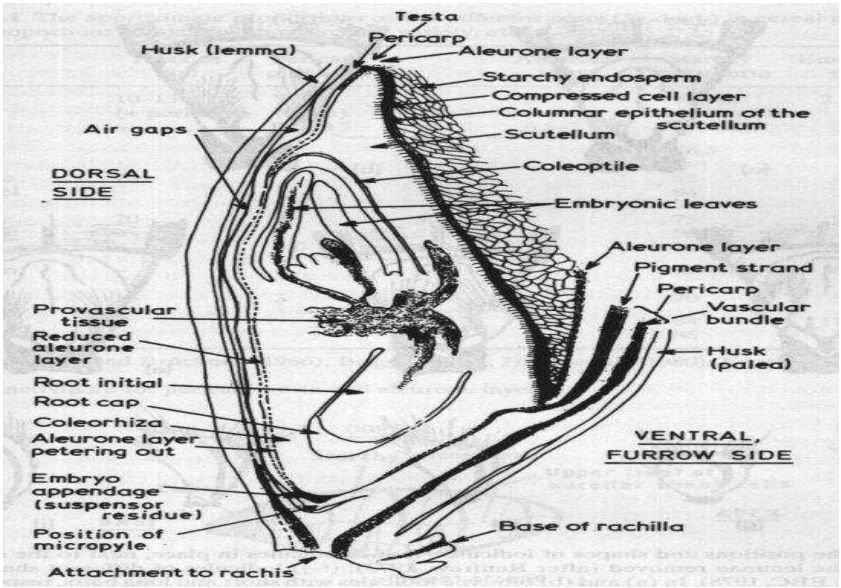

Figure: 2.3 Transverse cross section of a barley grain (Briggs, 1998, Page 45).

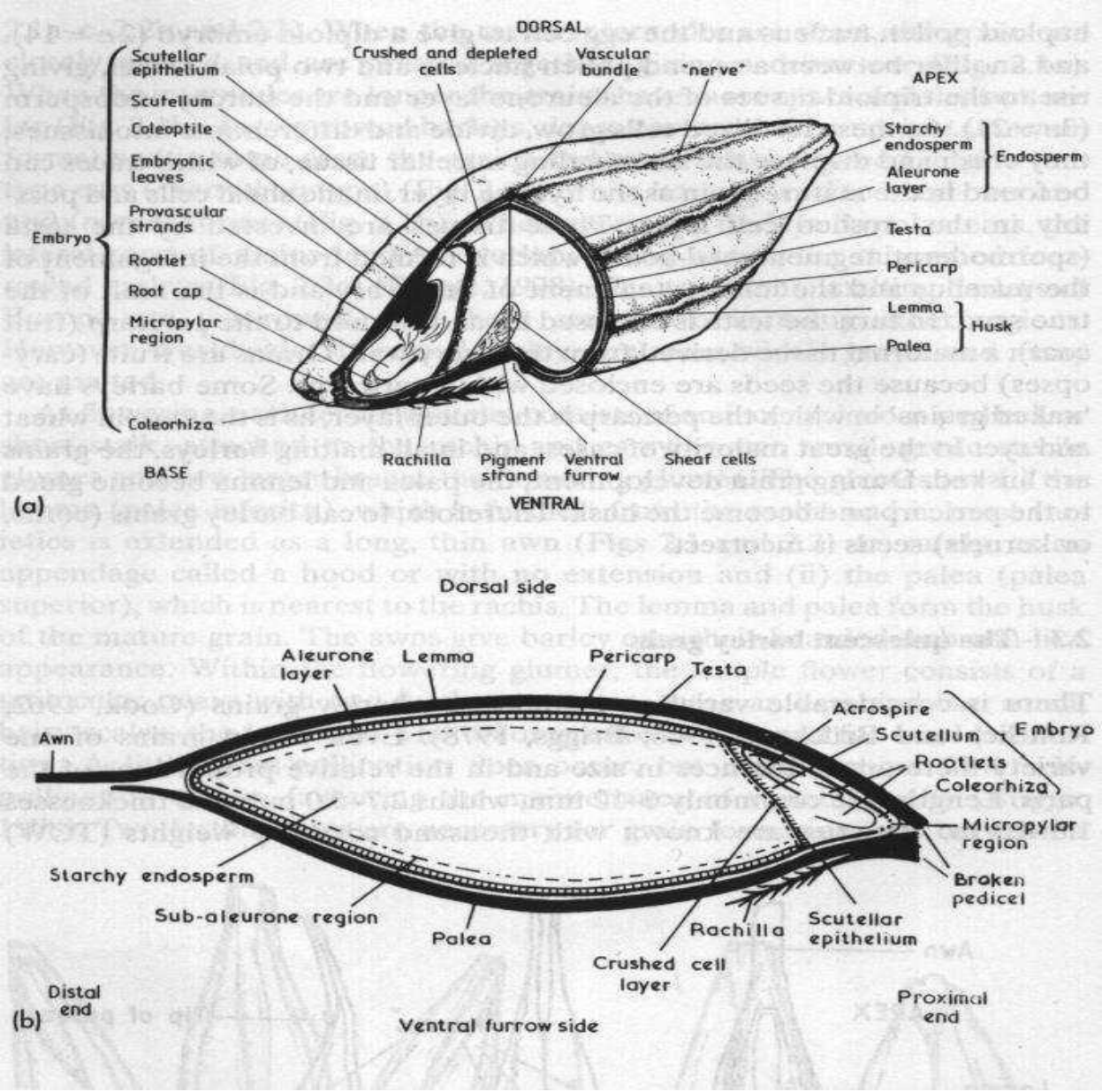

Figure 2.4: Transverse sections on the dorsal sides of a barley grain. (a) A protein-poor (low nitrogen) grain; (b) a protein-rich (high nitrogen) barley grain. (Briggs, 1998, Page 42).

Whole barley grain consists of about 65% to 68% starch, 10% to 17% protein, 4% to 9% β-glucan, 2% to 3% free lipids, and 1.5% to 2.5% minerals (Czuchajowska, Klamczynski, Paszczynska, and Baik, 1998; Izydorczyk, Rossnagel, Labossiere, MacGregor, and Storsley,

2000 ; Quinde, Ullrich, and Baik, 2004). The amylase content of barley starch varies from 0% to 5% in waxy, 20% to 30% in normal, and up to 45% in high-amylose barley (Bhatty and Rossnagel 1997). Barley endosperm protein is rich in prolamin storage proteins (hordeins) and has moderate nutritional quality (Newman and McGuire 1985). High-lysine barley mutants, which contain 2% to 3% greater lysine than normal lysine types could provide high-quality, protein-enriched barley grains for the human diet (high lysine content of 5% to 6% compared to 3% as normal ones), but however after the malting and brewing process not the same degree of nutrition is retained as the proteins are denatured and other nutrients chemically converted from their natural state (Ullrich and Eslick 1978). Among many, proteins are barley components that are essential for the quality of malt and beer. High-protein contents decrease available carbohydrates resulting in a negative influence on the brewing process (Frost, Leeds, Dore, Maderios, Brading, and Dornhurst, 1999; Fox, Onley-Watson, and Osman, 2002). Proteolysis (protease hydrolysis producing amino acids and peptides from hordeins) during malting and mashing is necessary for yeast metabolism (Moll, 1979). Finally, soluble proteins are important in beer head retention and stability.

The kernel has different tissues, with each serving its own purpose. Fincher (1989) justifies that the husk and the pericarp serve to protect interior constituents of the grain from mechanical damage. The husk and pericarp can limit respiration because they provide barriers to gaseous exchange between the interior living tissues and the exterior. This mechanical protection of the grain offered by the pericarp and the husk safeguard the grain from physical damage caused by moving machinery. This is of great importance during processes such as conveyance and turning during the malting process. The testa which is found underneath the pericarp consists of two

cuticularized layers between which polyphenolic proanthocyanidins (colour pigments) usually occur (Briggs *et al*., 2004).

The testa also limits the inward diffusion of solutes which permeate the husk and the pericarp and also prevent the outward diffusion of amino acids, sugars and other essential soluble compounds in the grain (Briggs, 1998). Briggs (1998) also notes that though microbes may be present in this region they can never penetrate the testa therefore it serves to separate the exterior from the interior regions of the grain.

The embryo is another important functional region of the malting barley grain. The embryo is situated within the testa, at the base of the grain, and towards the dorsal side (Brown and Morris, 1890). According to (Briggs *et al*., 2004) the embryonic axis consists of the coleoptile (the maltster's acrospire) and the root sheath (coleorhiza). This appears at the end of the grain, at the onset of germination, as the ‘chit’ and the embryo is the one that grows into a plant by utilizing the reserves in the starchy endosperm. A thin layer called the scutellum shields the embryo from the starchy endosperm.

The starchy endosperm is a dead tissue made up of thin-walled cells packed with starch granules embedded in a protein matrix. The cell walls are mainly β-glucans, with some pentosans and a few holocellulose (Briggs, 2004). Most of the grain's reserves are contained in this tissue, even though others are present in the embryo and in the aleurone layer. Briggs, (2004) further describes that the outer region of the starchy endosperm (the sub-aleurone layer) is relatively richer in protein (including α-amylase) and small starch granules but poor in large starch

granules. The aleurone layer which is about three cells thick surrounds the starchy endosperm. The cells have thick cell walls, contain reserves of lipids, protein, sucrose and possibly fructosans. The cells are also alive but do not multiply during germination and contain a full range of functional organelles but do not contain any starch (Briggs, 2004).

2.2.2 Quality Requirements for Malting Barley

The malt quality of a given barley variety is determined by its genetic background and the physical conditions during growth, harvest and storage (Kunze, 1999). The malting industry requires malt with high extract yield, high levels of enzyme activity and good modification to manufacture beer of excellent quality. The basic raw material for the production of beer is the malting barley whose quality is of paramount importance. Therefore malting barley must be able to germinate vigorously and be post-harvest mature to meet these requirements, (Francakova, Liskova, Bojnaska and Marecek, 2012). Before processing each batch of malting barley is assessed to yield a representative sample in order to verify its suitability for malting. Briggs et al (2004) notes that the frequently assessed quality parameters in malting barley are moisture content , nitrogen content, screenings profiling, germinability and viability.

2.2.3 Storage of Malting Barley

As in other cereals in general, barley is amiable to storage for relatively long periods of time and the storage conditions determine grain quality (Woods et al. 1994; White et al. 1999). Storage time is increased if the grain is stored under ideal conditions which are low temperature and moisture. Since barley is living and produces heat by respiration, the faster the rate of respiration, the warmer the barley gets thus increasing its moisture content and temperature (Kunze, 1999). Such conditions therefore promote the proliferation of microorganisms such as bacteria and

fungi. Most of the insects will also find their ideal conditions for growth hence the need to control both temperature and moisture.

At Kwekwe Maltings barley is stored in eight silos made of reinforced concrete with a capacity of 3500 tonnes each. Such silos have a low conductivity, are fire proof and have low maintenance costs (Kunze, 1999). Aeration and recirculation of the stored malting barley in order to ensure even distribution of moisture and also prevent the building up of heat is important. At Kwekwe Maltings the silos lack mechanical stirrers and to compensate for this, grain is circulated from one silo into another empty silo or simply reticulated by moving it out of the silo and reloading it back into the same silo. To accomplish the recommended temperature control at Kwekwe Maltings the barley is cooled by a grain cooler called the Granifigor, which is connected from the bottom of the silos and blows cold air thus cooling the lowest layer of the barley. The air becomes warmed to the temperature of the grain and flows upwards through the silo to the top where it escapes through air vents. As a result any failure to control both the temperature and moisture during storage causes proliferation of microorganisms and insect infestation which directly causes a negative impact on the germination performance of the grain during malting.

2.3 Overview of the malting process

2.3.1 Preparation of malting grain

Prior to the barley conversion operations, the barley delivered to Kwekwe Maltings is pre-cleaned before storage and further undergoes cleaning and grading before processing. This is achieved through barley screening (cleaning) and grading. The cleaning of barley is achieved by passing it over vibrating sieves with air jets and magnets. The material that is removed includes twigs, leaves straw, stone, metal pieces (nuts, bolts, etc.) and dust. The grading system separates

the barley according to the differences in size. Kunze (1999) notes that different sized grains malt at different rates, leading to non-homogenous batches of poor quality malt. For instance small grains will hydrate and respire vigorously than larger grains. The smaller grains will malt more rapidly and modify greatly resulting in malt containing portions under-modified and over-modified grains. The by-products produced from these processes (thin corns chaff and off chaff) are bagged and sold as stock feeds.

2.3.2 Steeping

Belitz, Grosch, and Schieberle (2009) introduced steeping as soaking the grain in water to initiate hydration and thus inducing germination. According to (Belitz *et al*., 2009) steeping raises the barley moisture content from post-harvest moisture of around 12% to a moisture high as 45% and this process generally takes around 36 to 48 hours. The steep liquor should be of drinking water quality, without taints as they will be carried over to the final product affecting quality and stability. The steep-water temperature should be controlled because at elevated temperatures water uptake is faster but microbial growth is accelerated and the grain may be damaged or killed (Kunze, 1999). The rate of water uptake is affected by the steeping time (water uptake is high at first and slows down with time), steeping temperature (the warmer the steep liquor the faster the uptake due to increased kinetic energy of the water molecules) and kernel size (the smaller the kernel the less the distance for the water to move hence the faster the hydration). Steeping also cleans the grain by removing dust, micro flora and inhibitors (Kunze, 1999). Steeping also removes all floating materials, provide sufficient oxygen to corn respiration and remove produced carbon dioxide (Kunze, 1999).

If the oxygen is not supplied intra-molecular respiration will occur (other compounds will replace oxygen) which may lead to death of the grains resulting in a dead steep. To avoid such effects the grain is aerated to certain intervals of the steeping regime. To prevent it packing tightly and wedging in the steep it may be loosened and mixed by blowing air into the base of the steeping vessel (Briggs, 2004). This also adds oxygen to the steep liquor. The oxygen is rapidly taken up, both by the grain and by the microbes that multiply on the grain and in the liquor.

Steeping is followed by the germination stage and through the process activity called steep casting the hydrated grain is transferred from the steep tank to the germination vessel. At Kwekwe Maltings during casting an important plant hormone, gibberrelic acid (GA) is added to the grain. The effects of GA include breaking dormancy, shortening germination time, increasing extract yield by about 1%, increasing extract fermentability and friability and an improvement of malt consistency. GA initiates synthesis of alpha amylase, limit dextrinase, alpha glucosidase, beta glucanase, beta glucan solubilase, pentosanases and proteinases and it has no effect on beta amylase and peptidase (Kunze, 1999).

2.3.3 Germination

Germination is a process in which physical modification of endosperm is undertaken to increase the bioactive compounds (Madhujith and Shahidi, 2007). When the grains reach the desired moisture content (which is around 42-45% at casting) and germination commences, they are allowed to germinate in germination vessels (Belitz *et al*., 2008). Kunze (1999) notes that during germination a new plant develops by utilising energy, other molecular products of respiration and other metabolic processes.

In the germination vessel the grain is kept moist by aerating with cool air saturated with 100% humidity. Aerating also cools the grain and removes the carbon dioxide produced. It is important

that the green malt is kept moist throughout germination because if it dehydrates, modification will cease, hydrolytic enzymes will not be able to progress through the endosperm and thus modification stops (Briggs and Hugh 1985). Turners in the vessel are used to turn the germination bed regularly effecting aeration and cooling. This also prevents matting of the rootlets which tend to entangle as they elongate. Germination lasts 3 – 5 days at temperatures around 14 - 18°C and through applying fine sprays and turning the grain bed the moisture is raised up to 46% during germination (Belitz *et al*., 2009). Respiration increases throughout the process and by the end, temperatures as high as 23°C are normal. The grain grows, producing a tuft of rootlets (culms) at the base of the grain. The extent of acrospires' growth, expressed as a proportion of the length of the grain is used as an approximate guide to the advance of the malting process. Variations in acrospire lengths indicate heterogeneity in growth. The living tissues respire and carbon dioxide and water are generated resulting in a loss of dry matter.

The energy liberated supports growth and is liberated as heat (Briggs *et al*., 2004). Germination is chiefly characterized by enzyme activation and synthesis (Kunze, 1999). Some of these enzymes catalyse the physical modification of the starchy endosperm. During initial stages of germination these hydrolases are released from the scutellum (Briggs, 1998). However, after a short lag the embryo releases gibberellin hormones (gibberellic acid).These diffuse along the grain triggering the formation of some enzymes in the aleurone layer and the release of these and other enzymes into the starchy endosperm. Here they join the enzymes from the embryo in catalysing modification. As germination progresses the starchy endosperm softens and becomes more easily 'rubbed out' between finger and thumb. The stages of physical modification are the progressive degradation of the cell walls of the starchy endosperm, which involves the breakdown of the troublesome glucans and pentosans, followed by the partial degradation of the

protein within the cells and the partial or locally complete breakdown of some of the starch granules, the small granules being attacked preferentially (Briggs, 1998). The main enzymes synthesised and activated during this phase are starch degrading amylases, protein degrading proteolytic enzymes, cytolytic enzymes (glucanases and cytase), and phosphatases (Kunze, 1999). During brewing starch hydrolysis is carried out by the malt enzymes *α*-amylase, *β*-amylase, limit dextrinase, and *α*-glucosidase (Manners, 1985). Limit dextrinase is responsible for hydrolyzing the (1→6)-*α*- glucosidic branch points in low molecular weight (LMW) branched dextrins formed by the action of *α*- and *β*-amylase on starch components (Manners *et al*., 1970). Starch granules can be encapsulated by a rigid protein matrix or by cell walls (Weurding *et al*., 2001). *α*-amylase can solubilize both amorphous and crystalline regions (Lauro *et al*., 1993) of starch granules attacking the (*α*- 4) linkages of starch producing oligosaccharides. *β*-amylase also attacks (*α*-4)-linkages from the non-reducing ends of amylose and amylopectin molecules (Bamforth and Quain, 1989; Lewis and Young 1995). A range of fermentable sugars is produced from the action of these enzymes on starch during the mashing process. These include glucose, sucrose, fructose, and mainly maltose and also some low molecular weight dextrins (Slack and Wainwright, 1980; Lauro *et al*., 1993).

2.3.4 Kilning

Despite highlighting the biochemical changes occurring during this stage of malting it's vital to note that kilning is the most important stage in malting, in terms of operating efficiency. About 80% of the total energy used in the malting plant is at this stage (Kunze, 1999). After the green malt has modified to the extent specified by the brewer, it then becomes necessary to terminate the enzyme activities occurring within the endosperm. As according to Kunze (1999) the main objectives of kilning are discussed below:

Lowering the moisture content

To make the malt storable the moisture content should be decreased from over 40% to less than 5% and this is achieved by passing large amounts of hot air through the green malt. Enzymes which are later used in the brew-house during mashing are easily destroyed in wet heat compared to dry heat so to protect them malt is first pre-dried at low temperatures before subjecting it to higher temperatures.

Termination of germination and modification

As a result of the removal of water, germination is stopped and consequently the rootlets cease to grow any more. A major portion of the kernels are destroyed by the effect of heat and so the malt no longer respires. Modification thus stops as respiratory metabolic changes cease resulting in no further breakdown processes and thus malt becomes a durable good.

Formation of colour and flavour compounds

At temperatures as high as 80^0C attainable during kilning low molecular weight breakdown products react to form a number of colouring and strong flavour compounds. These reactions are complex and collectively referred to as maillard reactions.

Enzyme Inactivation

Enzymes are associated with high molecular weight proteins and as a result of the heating during kilning, the structures of the proteins are altered and become denatured. Denaturation however is dependent on the structure of the carrier proteins and hence affect enzymes to different extents.

For instances in the early phases of kilning enzymatic activity of amylases increase and later decrease as kilning progress. In particular by the end of kilning α-amylase activity is about 15% more than in green malt whilst β-amylase activity is 40% less than in green malt. In the case of

the more sensitive enzymes such as glucanases the loss of enzyme activity is even greater up to 40% for endo β-glucanases and 70% for exo β-glucanases.

2.4 Process flow at Kwekwe Maltings

The barley grains at Kwekwe Malting undergo a series of steps during processing. These steps include quality control (QC) inspections, barley screening, and barley cleaning. A process flow chart of the events that occur from barley intake to malt dispatch is shown below:

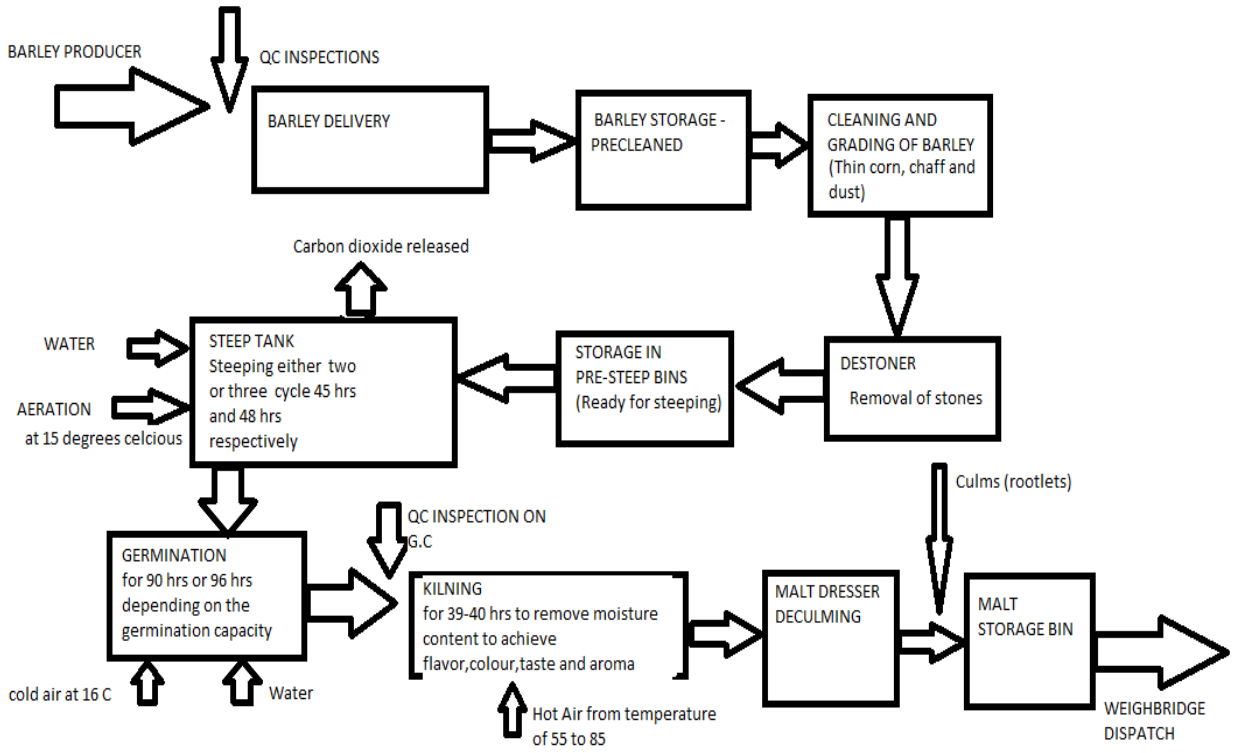

Figure 2.5: Kwekwe Maltings Process Flow diagram prepared by the researcher

2.5 The germination vigour concept (Perry, 1984)

Seed vigour is an important quality parameter which is essential to determine performance of a seed lot during storage and processing. Perry (1984) defines seed vigour as the sum properties of the seed which determines the level of activity and performance of the seed during germination or seedling emergency. Several factors like genetic constitution, environment and nutrition of the mother plant, maturity at harvest, seed weight and size, mechanical integrity, deterioration, ageing and microbes are known to influence seed vigour (Perry, 1984). The principle of seed vigour or growth tests is based on the reality that vigorous seeds grow faster than seeds of poor vigour.

CHAPTER 3: MATERIALS AND METHODS

3.0 Introduction

3.1 Sampling Technique

The research study involved the "*Hope*" variety of barley that was harvested from the 2011 (four years into storage), 2012 (three years into storage), 2013 (two years into storage), 2014 (one year into storage) and 2015 (two months into storage) winter growing season. Samples of 1kg were collected and stored in perforated sample bags under room temperature at Kwekwe Maltings. The simple stratified sampling technique was employed in this study with the five growing seasons (years into storage) considered as separate strata and randomly creating average samples from each.

3.2 Experimental Procedures

The experimental procedures employed in this research study were adopted from the Kwekwe Maltings Laboratory Methods Manual.

3.2.1 Determination of the germinative energy (GE) of malting barley

The Germinative energy (GE) is the proportion of grain percentage that will germinate under the conditions of a specified test. Satisfactory malting barley must be viable and also non-dormant. Despite not being dead, dormant barley will not germinate. The GE shows the preparedness of the grain to germinate under specified periods and conditions. This test is done to predict how the grain will perform during processing. The steeping germination test outlined below was used.

One hundred kernels of barley were randomly selected from the primary samples. Filter papers (Whatman number one) were placed in the bottom half of each Petri dish (ninety millimetres diameter). Four cm^3 of distilled water were then added onto the papers and the 100 kernels were

then evenly added on the filter papers. The Petri dishes were then closed with the other half (lids) which were labelled. The kernels were then germinated in an incubator at 18^0C –21^0C for seventy two hours. Germinated kernels were then removed at twenty four hours. The total number of kernels that had germinated at seventy two hours was recorded as a percentage (x %). Percentage of germinated kernels at twenty four and fourty eight hour intervals were noted for the determination of the germination Index (indicator of the rate of seed germination) as required by the formulae given below:

$$\text{GE (\%)} = \frac{NUMBER\ OF\ GERMINATED\ KERNELS}{TOTAL\ NUMBER\ OF\ KERNELS} \times 100$$

Calculation of the Germination Index (GI):

$$\text{GI} = \frac{10 \times (x+y+z)}{(x+2y+3z)}$$

Where:

x is the number of germinated kernels at 24 hours.

y is the number of germinated kernels at 48 hours.

z is the number of germinated kernels at 72hours.

3.2.2 Determination of Water Sensitivity

Water sensitive kernels are those which show a decreased ability to germinate in the presence of water exceeding the minimum amount required to promote germination .The extent of water sensitivity of kernels is evaluated by performing germination tests in Petri dishes with 4cm^3 and 8cm^3 of water. The difference in percentage germination in the tests gives a measure of water sensitivity. A difference of > 20% indicates that the kernels are water sensitive.

Method

A random selection of one hundred barley kernels from the primary samples was done. Whatman number 1 filter papers were placed in the bottom half of each Petri dish (ninety millimetres diameter). Eight cm^3 of distilled water was pipetted and added onto the papers and the one hundred kernels were then evenly distributed on the filter papers. The Petri dishes were then closed with their labeled lids and the kernels were allowed to germinate in an incubator at 18^0C – 21^0C for 72 hours. Germinated kernels were then removed at twenty four hour intervals. Recording of The total number of grains which had germinated at seventy two hours was recorded as a percentage (x %).

The water sensitivity was then calculated using the formulae below:

$$\%\ \text{germinated in 4 cm}^3 - \text{germinated in 8 cm}^3\ \text{test}$$

References: BIRF – Studies in Barley and Malt – JIB 1955, page 25

3.2.3 Determination of the germination capacity of barley

The grain's potential to germinate (viability) if the minimum requirements of germination are provided is determined by this test (determination of germination capacity in barley). The main objective of determining germinative capacity (GC) is to measure the percentage of living corn in barley sample. This measure shows the degree of dormancy. In the event that dormancy has already been broken the GC helps to determine the grain is still alive or dead. The 2, 3, 5 triphenyl tetrazolium chloride (TTC) acts as an indicator of viable dormant seeds

Method

One hundred barley kernels were selected at random from each sample. The kernels were then soaked in tap water for about 5 minutes to soften them. The kernels were longitudinally dissected and one half of each kernel was discarded. The retained halves of each kernel were then placed in labelled test tubes in order of their harvest years. 2, 3, 5-triphenyl-tetrazolium chloride (TTC) solution was added in each test tube and then these were placed in a water bath set at 40^0C for thirty minutes to allow for the reaction to take place. The excess TTC solution was then poured off, the half kernels emptied onto labelled white tiles and forceps and classified as follows:

i. Kernels in which the embryos did not stain red are either dead or will not modify during the process of malting.
ii. Corns in which the embryos stained red are live, viable and will germinate during malting (x).

3.2.4 Moisture content determination in barley kernels

A rapid moisture meter (Brand Name: Pfeuffer, Model Number: He Lite), manufactured in 2010 was used. The Pfeuffer He Lite grain moisture meter determines the actual internal moisture content of the sample by grinding and suitably compressing it.

Method

Barley kernels were randomly selected from each sample. Each sample was then put into the grinding chamber of the moisture meter. The samples were grinded and homogenized on the rough grinding surface of the moisture meter instrument's measuring cell. A reading of the moisture content of each sample was displayed digitally on the display unit and was recorded.

MALTING AND MALT ANALYSIS

Each sample (2011, 2012, 2013, 2014 and 2015) was then micro-malted at KKM and the full malt analysis of each sample was done at Kwekwe Maltings laboratory.

3.2.5 Determination of friability (physical parameter)

Friability gives an indication of how malt is modified. A friabilimeter is used to determine friability and it gives the amount of energy needed to mill the malt, modified malts require less energy. Some of the malt grains are not milled in a friabilimeter, and these are wholly unmodified grains (WUG) and their mass is determined using a balance. WUG should not exceed three %. Other corns are only partly milled and these are termed partially unmodified grains (PUG) and they should not exceed two %. Homogeneity gives a measure of how evenly modified the malt is. It is calculated as below:

$$\text{Homogeneity} = 100 - (\text{PUG} + \text{WUG}).$$

3.2.6 Determination of diastatic power in malt

Diastatic power is the measure of how much starch converting enzymes any given malt contains.

Method

20g of each sample were weighed using an analytical balance and milled. Metal beakers each with a stirrer were weighed using a digital scale. To each weight, five hundred and twenty grams were added and recorded on the tin using a mighty marker. The milled twenty grams of each sample were then added to each metal beaker. Two hundred millilitres of water were measured using a measuring cylinder and then added to each metal beaker with contents. These were then placed in a water bath for seventy minutes. During the first thirty minutes of the mashing process, one hundred millilitres of warm water were added into each beaker. After the mashing,

the beakers were removed and cooled to 20^0C in a water bath. Topping up to the weight labelled on each tin with distilled water was done using a digital scale. The mash was then filtered using Whatman number one filter papers and funnels into labelled two hundred and fifty millilitres measuring cylinders. The filtrate of each sample was collected in labelled measuring cylinders and the first two hundred millilitres of each filtrate were discarded. The next fifty millilitres were collected for the analysis. Five millilitres acetate buffer (pH 4.3) was pipetted using a pipette into labelled two hundred millilitres volumetric flasks. One hundred millilitres of a starch solution was then be added into each volumetric flask. A stopper was used to close each flask and these were placed in a water bath set at 20^0C for twenty minutes. Five millilitres filtrate from the labelled measuring cylinders was added to respective volumetric flasks and each volumetric flask was upended twice to mix the contents. The timer was immediately started. The flasks were removed from the water bath after thirty minutes and 4mls of sodium hydroxide (NaOH) was added. Distilled water was used to top up to the two hundred millilitres mark of the flasks. Fifty millilitres of the solution in each volumetric flask was pipetted into labelled two hundred and fifty millilitres conical flasks. Twenty five millilitres of Iodine solution was added to each conical flask. Three millilites of sodium hydroxide was also added into each conical flask. The conical flasks were stored in a cupboard for fifteen minutes after which 4.5mls of sulphuric acid (H_2SO_4) was added. The unreacted Iodine was titrated against sodium thiosulphate until the solution turned colourless. The results were recorded on a worksheet. To calculate the diastatic power (DP), the formula below was used:

$$\text{Diastatic Power} = (\text{Blank} - \text{Test Titration}) \times \text{Factor},$$

Where the value for the blank is known and the factor value is 34.2. The DP value of each sample was reported to the nearest whole number.

In the tests to determine colour, total extracts, filtration speeds and total soluble nitrogen, the labelled metal beakers, each with a stirrer were weighed using a digital scale. Four hundred and fifty grams was added to the each weight. The overall weight was recorded on each metal beaker. The different malt samples (2011, 2012, 2013, 2014 and 2015) were milled using a small scale milling machine. Fifty grams of each sample was added into its respective beaker. These beakers were then placed in a mash bath which runs for seventy minutes. During the first thirty minutes, two hundred millilitres of tap water was added. After mashing, the beakers were placed in a water bath and cooled to 20^0C.

3.2.7 Determination of filtration speed

In the determination of filtration speed, each mash was filtered using Whatman number one filter paper and funnels into labelled two hundred and fifty millilitres beakers. These were re-filtered and the first one hundred millilitres of each filtrate was discarded. The timer was started and the time taken in minutes until the mash was all filtered was recorded. This filtrate was then used to analyse for colour and extracts.

3.2.8 Determination of total extracts

In the determination of extracts tests, the empty specific gravity (SG) bottle was weighed using an analytical balance and its mass was recorded. The SG bottle with distilled water was also weighed using an analytical balance and its mass was recorded. Again, the mass of the empty SG bottle with each liquid (filtrate) was weighed using an analytical balance and recorded. All weights were weighed will the liquid in the SG bottle at 20^0C.

Calculation

To get the Mass Of distilled Water (MOW), weight of SG bottle was subtracted from weight of SG bottle + distilled water. To get the mass of liquid (MOL), subtract the weight of the empty SG bottle from the weight of the SG bottle + the liquid (filtrate). To get the specific gravity, the following formula was used:

$$\text{SPECIFIC GRAVITY} = \frac{MASS\ OF\ LIQUID(MOL)}{MASS\ OF\ DISTILLED\ WATER(MOW)}$$

The SG values and the percentage moistures were used to get the percentage of extracts from the extract tables.

3.2.9 Determination of total soluble nitrogen

In the determination of total soluble nitrogen content in the malt, each sample was milled using a small scale miller. 1,4grams of each sample was weighed using an analytical balance. These were added into labelled two hundred and fifty millilitres conical flasks. Ten millilitres of hydrogen peroxide (H_2O_2) were measured using a measuring cylinder and added to each flask. Thirty millilitres of concentrated sulphuric acid (H_2SO_4) were also added in each flask. In each flask, two Kjeldahl tablets were added. These act as catalysts. The mixtures were digested in a fume hood until the solution turned green. The flasks were then cooled. Two hundred millilitres of water were measured using a measuring cylinder and added in each flask. Pieces of granulated zinc were also added in each flask. One hundred millilitres of caustic soda measured using a measuring cylinder was also added in each flask. One drop of methyl blue indicator was added in separate labelled two hundred and fifty millilitres conical flasks. Thirty millilitres of boric acid measured using a measuring cylinder was also added in each conical flask. The nitrogen is absorbed by the boric acid in a process of distillation. The process of distillation was terminated

when the conical flasks' contents reached the two hundred and fifty millilitres mark. These contents were then titrated against ammonia (NH_3) until the solution turned colourless. The results were recorded.

3.2.10 Determination of Free Amino Nitrogen

In this test, two millilitres of the extract filtrate (from each sample) obtained from the mashing process described in the determination of DP in malt was pipetted using a pipette into labelled one hundred millilitres volumetric flasks. Distilled water was used to top up to the mark of each volumetric flask. Two millilitres of each diluted sample was then pipetted into separate labelled test tubes. One millilitre of Ninhydrin colour reagent and two millilitres of the diluted glycine standard stock solution were added into each test tube which were then stopped using glass balls to avoid loss by evaporation. The tests tubes were then heated in a boiling water bath for exactly sixteen minutes and then cooled in a water bath at 20^0C for twenty minutes. Eighty five millilitres of dilution solution were then added into each test tube and stopped with plastic stoppers. These were then mixed thoroughly by inverting the test tubes several times. Using a spectrophotometer, the absorbance of the sample, blank and glycine standard was read at wavelength five hundred and seventy nanometres.

Calculation:

$$\text{FAN (mg/dm}^3) = \frac{NET\ ABSORBANCE\ OF\ SAMPLE}{NET\ ABSORBANCE\ OF\ GLYCINE\ STANDARD} \times 2 \times DILUTION$$

3.3 DATA ANALYSIS

Analysis of variance (ONE WAY ANOVA) was used to compare the parameters measured before and after malting amongst the years into storage (2011-2015), (SPSS version 21).

CHAPTER 4

4.0 RESULTS

4.1 General results of the parameters measured before and after malting

A total number of 12 parameters were measured, before malting and after malting. Parameters measured prior to malting are: moisture content, germination capacity, germination energy and water sensitivity. The results showed a general increase as years into storage decreased, that is, 2011- 2015 (Table 4.1). The lowest moisture content was recorded in 2011 and the highest in 2015 (Table 4.1). Most of the parameters were below spec (acceptable limits set by KKM) with exceptions of 2015 sample having a percentage higher than the spec in GE (Table 4.1). The highest GE was recorded in 2014 while the lowest was recorded in 2011 with 75%. GC also increased with decrease in storage time. 2013 and 2014 samples showed water sensitivity greater than 20% (spec) with 40% and 45% respectively.

Table 4.1 Summary of the results of parameters measured before and after malting

PARAMETER	SPEC	2011	2012	2013	2014	2015
Moisture content (%)	≤13	7	9	10	10	14
Germination Capacity (%)	≥97	78	87	95	97	79
Germination Energy (%)	≥98	75	83	94	99	89
Water Sensitivity (%)	>20	30	40	20	20	13
Diastatic Power (WK)	≥260	213	256	274	280	298
Extracts (%)	≥80	80.2	80.8	81.8	82	82.3
Total Soluble Nitrogen (%)	≤1.0	0.69	0.72	0.75	0.79	0.81
Free Amino Nitrogen (mg/L)	155-190	161	169	177	186	191
Wholly Unmodified Grains (%)	≤2.0	2.4	2.2	1.4	0.6	0.4
Partially Unmodified Grains (%)	≤5.0	8.2	6.4	5	4.4	3.8
Friability (%)	≥78	79	60	45	35	81
Filtration Speed (minutes)	≤50	125	110	45	47	95

The parameters that were measured after malting are diastatic power (DP), extracts, total soluble nitrogen (TSN), free amino nitrogen (FAN), wholly unmodified grains (WUGS), partially unmodified grains (PUGS), friability and filtration speed. The DPs, TSN, FAN, and extracts were increasing with decrease into storage years, that is, from 2011-2015 while PUGS and WUGS decreased with increase in years (decrease into storage time) (Table 4.1). Friability generally showed a decrease with decrease into storage years, with a sudden increase from the downfall in 2015 (81%) (Table 4.1). Also in filtration speed, an increase in 2015 (95mins) was recorded after a downfall pattern in other years (Table 4.1).

4.2 Results of the parameters measured before malting

Moisture content generally increased with decrease into storage time that is, from 2011- 2015. The lowest moisture content was recorded in 2011 with 7% while the highest was recorded in 2015 with 14% (Table 4.1). There were significant differences among years that were observed in moisture content levels (Figure 4.1; $p = 0.016$, Appendix 18).

Significant differences among years in GC (Figure 4.1; $p = 0.000$, Appendix 18) were observed. From the results in Table 4.1, an increase in GC as years into storage decreased was observed. However there is a sudden decline in 2015 (79%) from the 97% recorded in 2014. In other years, the GC was below spec, that is, less than 97% except in 2014 were a GC of 97% was observed (Table 4.1).

The results show an increase with decrease into storage years, with the highest GC being recorded in 2014 (99%) and the lowest in 2011 (75%). GE trends showed significant differences among years (Figure 4.1; $p = 0.000$, Appendix 18). 2011, 2012, 2013 and 2015 samples were

below spec because they recorded percentages less than 98%. 2014 sample had a GE of 99% and was in spec (Table 4.1).

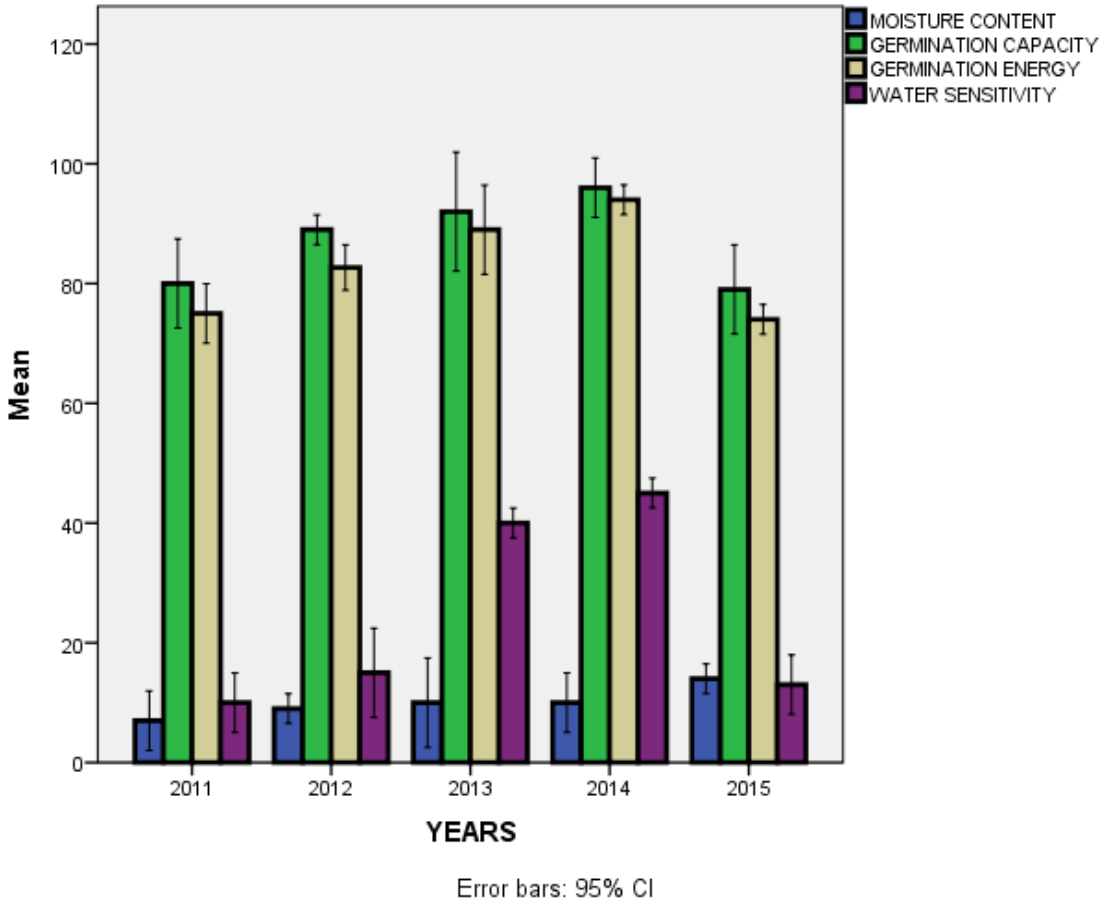

Figure: 4.1: Trends of parameters measured before malting

The results displayed significant differences among years in water sensitivity (Figure 4.1; p = 0.000, Appendix 18). A decrease as years into storage decreased was observed from the results in Table 4.1 in water sensitivity. 2011 and 2012 samples recorded a percentage greater than the spec which is 20% (Table 4.1). 2013- 2015 had percentages less than the spec (Table 4.1).

4.3 Results of the parameters measured after malting

The results showed a general increase in DP as years into storage decreased (Figure 4.2), with significant differences being observed among years (Figure 4.2; p = 0.000, Appendix 15). The 2013 sample recorded the lowest DP of 213KW units while the 2015 recorded the highest DP of 298KW units (Table 4.1). The 2011 and 2012 samples recorded DPs that are below the spec which is 260WK units while the 2013, 2014 and 2015 DPs were above spec (Table 4.1).

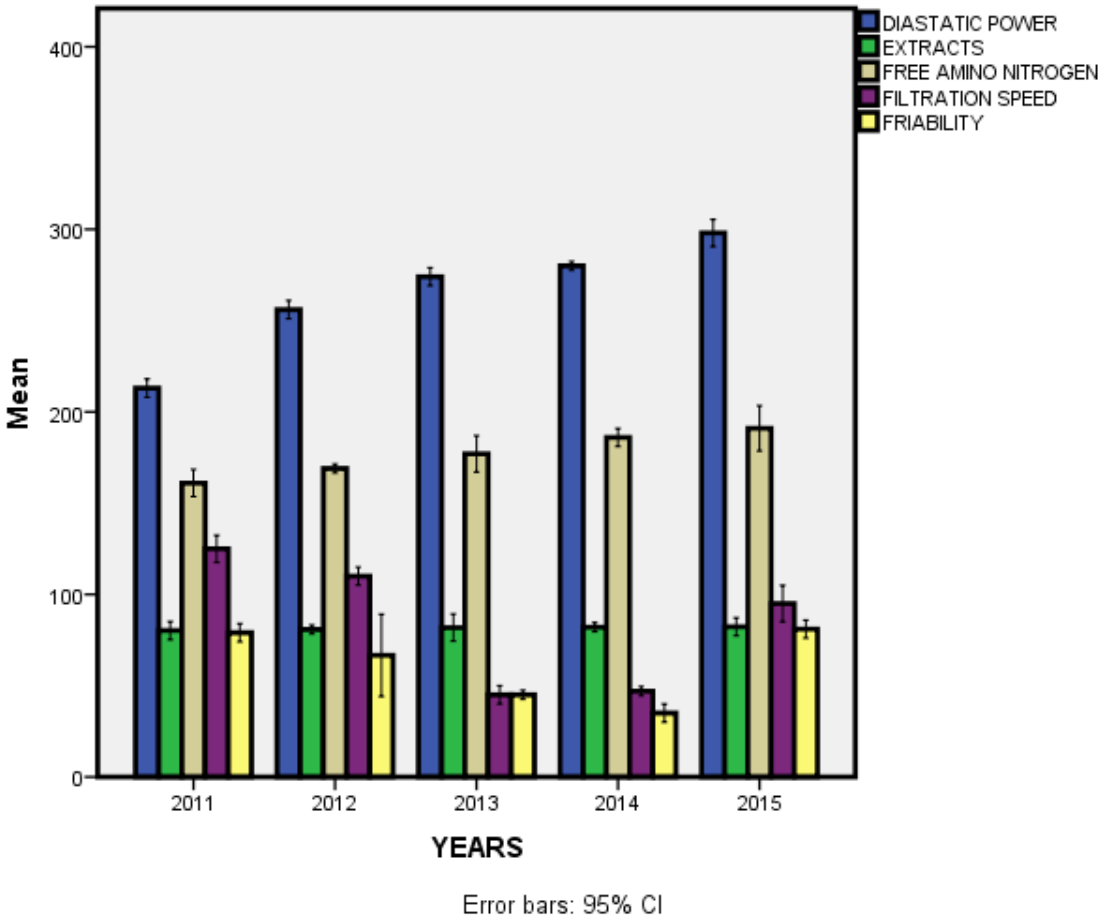

Fig: 4.2: Trends of the parameters measured after malting

From the results obtained there were no significant differences among years (Figure 4.2; p = 0.660, Appendix 15) in percentage extract. Percentage extract increased with a decrease into

storage years (Figure 4.2). The highest percentage extract (82.3%) was recorded in the 2015 sample while the lowest was recorded in the 2011 sample with a percentage of 80.2 (Table 4.1). The spec is ≥80% and all the samples (2011, 2012, 2013, 2014 and 2015) were above the specification (Table 4.1).

Free amino nitrogen (FAN) significantly differed among years (Figure 4.2; $p = 0.000$, Appendix 15). FANs increased as years into storage decreased. The lowest FAN (161mg/L) was recorded in 2011 while the highest (191mg/L) was recorded in 2015 (Table 4.1). All the samples were in range as the spec is 155- 190mg/L with the 2015 sample being an exception with a FAN value of 191mg/L.

In filtration speed, significant differences among years were observed (Figure 4.2; $p = 0.000$, Appendix 15). There was a general decrease in filtration speed as years into storage decreased. However there was an increase in 2015 with a filtration speed of 95minutes. The highest speed was recorded in 2011 (125mins) while the lowest was recorded in 2013 (45mins), (Table 4.1).

There were significant differences among years in friability (Figure 4.2; $p = 0.000$, Appendix 15). The highest percentage friability was recorded in 2015 with a percentage of 81 while the lowest (35%) was recorded in 2014. 2012, 2013 and 2014 samples were below spec which is ≥78% (Table 4.1). 2011 and 2015 sample had a percentage higher than the spec. The results generally showed a decrease as years into storage also decreased.

In percentage total soluble nitrogen (TSN), there were no significant differences among years (Figure 4.3; $p = 0.841$, Appendix 15). There was a general increase in TSN as the years into storage decreased. The lowest percentage was recorded in 2011 with 0.69% and the highest in

2015 with 0.81% (Table 4.1). All the samples were in range because the spec is ≤1.0% (Table 4.1).

Significant differences among years in partially unmodified grains (PUGS) were observed (Figure 4.3; p = 0.001, Appendix 15). PUGS decreased as years into storage decreased (Table 4.1). The lowest PUGS were recorded in 2015 (3.8%) and the highest in 2011 with 8.2% (Table 4.1). 2011 and 2012 samples the PUGS recorded were higher than the spec (Table 4.1).

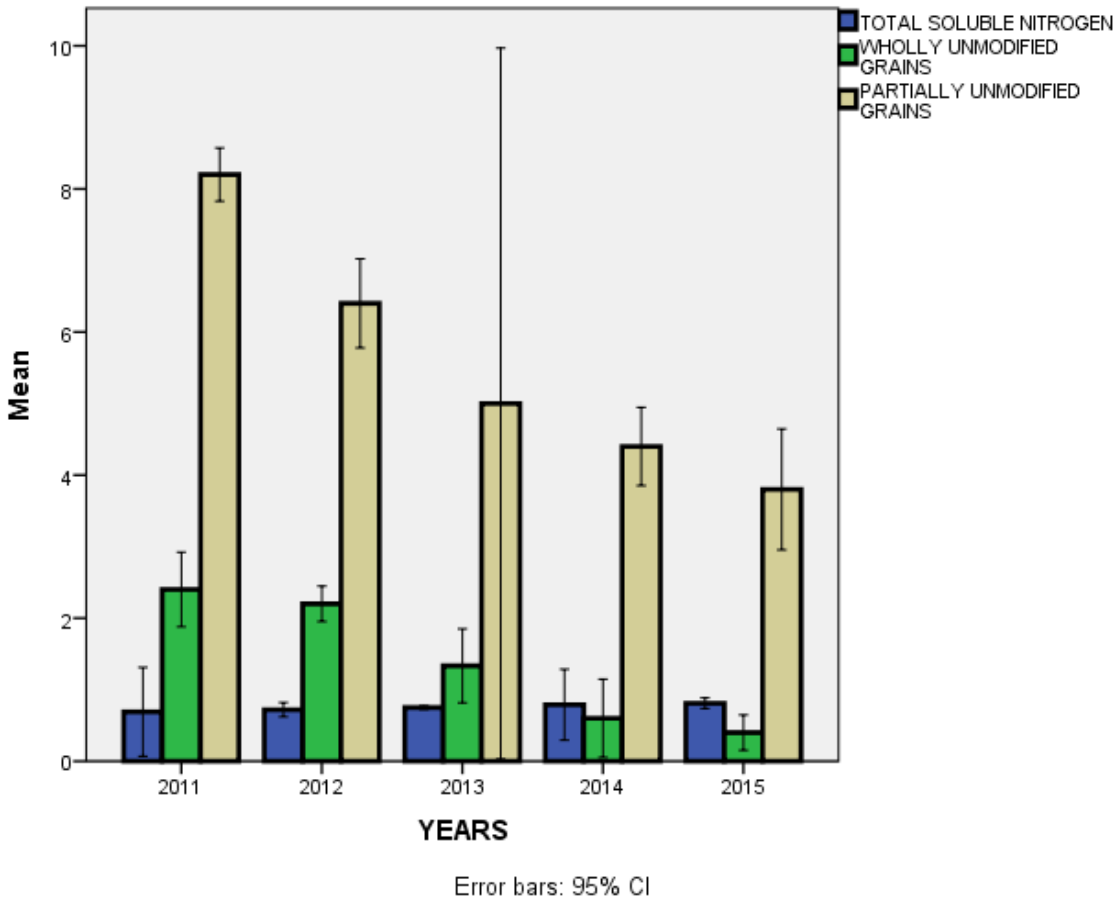

Fig: 4.3: Trends of results measured after malting

There were significant differences among years (Figure 4.3; p = 0.000, Appendix 15). In wholly unmodified grains (WUGS). Generally WUGS were decreasing as years into storage were decreasing with the highest percentage being recorded in 2011 (2.4%) (Table 4.1). The lowest WUGS percentage was recorded in 2015 with 0.4% (Table 4.1). 2011 and 2012 samples were out of spec having percentages of 2.4 and 2.2 respectively (Table 4.1).

4.4 Correlations of parameters measured before malting

Germination capacity (GC) had strong positive correlation with germination energy (GE) and water sensitivity (WS) with r values of 0.889 and 0.862 respectively (Appendix 20) showing that there is a strong positive linear relationship. However there is a weakly negative correlation between germination capacity (GC) and moisture content (MC) with r value of -0.230 (Appendix 20). A weak negative relationship therefore exists.

There is a strong positive correlation between germination energy (GE) with germination capacity (GC) and water sensitivity (WS) with r values of 0.889 and 0.924 respectively (Appendix 20).There is a strong positive linear relationship. There is a weakly negative correlation between germination energy (GE) and moisture content (MC) however exists (r value = -0.408, Appendix 20) showing the existence of a weak negative relationship.

Water sensitivity (WS) shows a strong positive correlation with germination capacity (GC) and germination energy (GE), (r values = 0.862 and 0.924 respectively, Appendix 20). There is therefore a strong linear relationship. However a weakly negative correlation is observed between water sensitivity (WS) and moisture content (MC), (r value = -0.369, Appendix 20). A weak negative relationship exists between the parameters.

Weakly negative correlations exist between moisture content (MC) with germination capacity, germination energy (GE) and water sensitivity (WS), (r values = -0.230, -0.408 and -0.369 respectively, Appendix 20). This shows that there is a weak negative linear relationship between the parameters.

4.5 Correlations of parameters measured after malting

There are weakly positive correlations between diastatic power (DP) with total extracts, total soluble nitrogen (TSN) and free amino nitrogen (FAN), (r values = 0.113, 0.023 and 0.041 respectively, Appendix 21). This shows that weak positive linear relationships exist between these parameters.

Weakly positive correlations exists between total extracts with diastatic power (DP) and total soluble nitrogen (TSN), (r values = 0.113 and 0.390 respectively, Appendix 21) showing weak positive linear relationships. Weakly negative correlation is however observed between total extracts and free amino nitrogen (FAN), (r value = -0.375, Appendix 21). A weak negative linear relationship therefore exists between the parameters.

There are weak positive correlations between total soluble nitrogen (TSN) with diastatic power (DP) and total extracts (r values = 0.230 and 0.039 respectively, Appendix 21). There is therefore a weak positive linear relationship. However a weakly negative linear relationship exists between total soluble nitrogen and free amino nitrogen (FAN) which is shown by the weak negative correlation (r value = -0.031, Appendix 21).

A weak positive correlation was observed between diastatic power (DP) and free amino nitrogen (FAN), (r value = 0.041, Appendix 21). This shows the existence of a weak positive linear relationship between the two parameters. Weak negative correlations were observed between

diastatic power (DP) with total extracts and total soluble nitrogen (TSN), (r values = -0.375 and -0.031 respectively, Appendix 21). This shows that a weak negative correlation exists between these parameters.

Weak positive linear relationships exist between wholly unmodified grains (WUGs) with partially unmodified grains (PUGs), friability and filtration speed (FS). This was shown by the observed weak positive correlation (r values = 0.302, 0.426 and 0.458 respectively, Appendix 22).

There were weak positive correlations between partially unmodified grains (PUGs) with wholly unmodified grains (WUGs), friability and filtration speed (FS), (r values = 0.302, 0.357 and 0.382 respectively, Appendix 22). Therefore weak positive linear relationships exist between these parameters.

There are weak positive linear relationships that exist between friability with wholly unmodified grains (WUGs) and partially unmodified grains (PUGs). This was shown by the weak positive correlations (r values = 0.426 and 0.357 respectively). A strong positive correlation was observed between friability and filtration speed (r value = 0.926, Appendix 22) showing a strong positive linear relationship between these two parameters.

Weak positive correlations were observed between filtration speed with WUGs and PUGs (r value = 0.458 and 0.382 respectively, Appendix 22). Weak positive linear relationships therefore exist between these parameters. A strong positive linear relationship exists between filtration speed and friability which was shown by a strong positive correlation (r value = 0.926, Appendix 22).

CHAPTER 5

5.0 DISCUSSION

In this research a commercially grown Zimbabwean bred malting barley variety Hope was assessed, with the aim of comparing the changes in germination vigour, malt quality and general storage stability. Results obtained showed that germinability and malt quality improved with storage of less than or equal to two years. This means that storage periods of more than two years will yield undesirable quality results in malting barley variety "*Hope*". Results for parameters were undesirable for 2011 and 2012 samples probably because these grains were now too old resulting in death. This might have also been caused by damage of the grain during cleaning (barley recycling). Break down of grains during cleaning leads to the production of malt which is not homogenous as these grains tend to take up and lose water at different rates. Malt which is not homogenous is not desirable. Damage of the pericarp and husk during cleaning deprives the grain from mechanical protection which safeguards the grain from any physical damage. Desirable quality trends were observed from samples of 2013 and 2014 which was evident from the results obtained. The 2015 sample which was two months into storage also showed undesirable quality trends. This was probably due to dormancy. Studies done by Woonton, Jacobsen, Sherkat and Stuart (2005) for the Intentional brewing institute on the storage stability of Australian malting barley varieties showed that storage at room temperature positively influenced the germination characteristics of all samples, with concomitant improvements in hydrolytic enzyme production during malting and in a number of malt quality parameters. This research commenced with the 2011- 2014 samples having minimal or no dormancy, while the 2015 sample was still dormant as signified by the germination capacity and energy scores (Table 4.2).

5.1 Germinability Trends (before micro-malting)

5.1.1 Germination Capacity

For germination capacity, only the 2014 sample retained the desired score of GCs which ≥ 97% (Fig 4.1).The germination capacity score gives an indication of whether the barley grain would germinate or not germinate when exposed to the conditions necessary for germination. In this case almost all samples demonstrated undesirable germination capacity which is the disability to germinate with the exception of the 2014 sample (Table 4.1). Briggs (1998) denotes that the failure of grains to germinate (low viability) is because they are dormant or dead. Therefore the low GC of 79% recorded for the 2015 sample explains that this study commenced before the dormancy had been broken, despite that the grains were still alive. For 2011- 2013 samples, the low GCs recorded might be due to the fact that the grains were dead at the time this study was conducted. The grains might have been exposed to harmful toxic chemicals, insect or fungal attack and damaging physical conditions during storage thus rendering them dead and not viable. The 2014 sample retained a desirable germination capacity score (Table 4.1), showing that it was neither too old to be dead nor dormant.

5.1.2 Germination Energy and Water Sensitivity

A measure of the extent of germination provided by the 4ml GE test supply the conditions necessary for germination and thus is a test for dormancy and malting ability. GE indices of scores less than 98% (below the acceptable limits set by Kwekwe Maltings) were observed for 2011, 2012, 2013 and 2015 samples, with results from Table 4.1 clearly showing that these samples have their extent of germination increasing with decrease in storage time. An exception is seen with the 2015 sample that showed a sudden downfall from the trend with a GE of 89% owing to the fact that these grains were still dormant at the time this study was carried out.

Dormancy that persists after harvest is highly undesirable because it prevents malting of newly received barley (Jacobsen *et al*, 2002). Physiological differences and storage conditions affect germination ability and recovery from dormancy. Many other possible factors may be influencing the changes in germination, enzyme production and malt quality with barley storage. Changes in the rate and extent of water uptake, the quantity of endogenous hormones and the aleurone response to hormones may all be associated with the observed changes during storage of barley (Woonton *et al*., 2005). This serves to then explain why significant differences were observed in the germination trends for all samples. Since the 8 ml GE test makes use of double the amount of water, it therefore gives a measure of water sensitivity of the grain. Results from Table 4.1 show a trend that barley becomes water sensitive with increase in storage time. As the grain ripens the optimum amount of water for germination apparently declines from 4 ml to about 3 ml/dish. In the GE (4 ml) test, which provides the optimum amount of water for the germination of water sensitive grains, the grain attains a moisture content of around 35%, and the surface film of water is absorbed during the test (Briggs 1998). If water sensitive grains are steeped (hydrated by immersion to about 45% moisture) and then drained and set to germinate under malting conditions, germination is ragged and slow, or fails, and the moisture film only slowly dissipates because the grain is virtually saturated with moisture (Briggs 1998). The mechanisms responsible for water sensitivity are unknown, though microorganisms present in grain among other factors are known to contribute (Kelly and Briggs, 1992). Although not included in the methodology and objectives of this study, thus not quantified, mould taints were observed from some samples in the Petri dishes during germination energy tests and this could be another probable explanation as to how the grains became water sensitive with time. Other studies have suggested that the pericarp is the main controller of water sensitivity and dormancy,

as removal or damage to this tissue has shown to decrease both dormancy and water sensitivity without affecting the microbial load (Harvey and Rossnagel, 1983, Jansson; Kirsop and Pollock, 1959). Therefore the differences in the water sensitivity behaviour of the grains might be due to the structural differences in the properties of their pericarp.

Results of the GE scores (Table 4.1) showed that the grains' malting abilities increased as years into storage decreased. Figure 4.1 clearly shows that the GE was improving as storage time decreased implying that the germination vigour improves with less storage times of grains. The GE of the 2015 sample was however lower (89%) probably because the grains were still dormant.

5.1.3 Moisture Content

One of the parameters used in comparing the storage stability of the barley variety was moisture content. From the study the variety had its moisture content decreasing from 14% in 2015 (2 months into storage) to 7% in 2011 (4 years into storage), (Table 4.1). This was because the grains naturally continued to dry out which results in a change in the mass of the grains and obviously shrinkage of the kernels.

In addition to germination tests other several quality parameters of malting barley had their trends determined and analysed so as to assess the storage stability of the variety "*Hope*". Hoseney (1994) explains the major cause of functional changes during postharvest storage of cereals when he states that as long as grain retains its viability, grain respires and is thus "alive". Hoseney (1994) further elaborates that grains stored under reasonable conditions (avoiding high temperatures and moisture augmentation) will slowly lose weight because of its respiration.

5.2 Malt Quality Trends (After micro-malting)

Parameters that were analysed after micro-malting are DP, total extracts, friability, total soluble nitrogen, filtration speed and free amino nitrogen.

5.2.1 Diastatic Power (DP) Trends

DP shows the strength of starch- reducing enzymes in malt. It is an indication of how well a given malt sample will respond to mashing. Higher DP malts have more protein and thus more enzymes to convert other materials (Noonan, 1997). The DP of all malt samples increased slightly with decrease of years into storage (Fig. 4.2). It has been established that β amylase originating in the starchy endosperm is the major enzyme contributing to malt DP and that, during germination, proteases synthesised by the aleurone release β amylase from the protein complex rendering it active. Therefore, the observed increase in malt DP is presumably due to increased protease synthesis during malting, resulting in increased starchy endosperm protein hydrolysis and greater release of β amylase (not measured). Increased synthesis of other starch hydrolases during malting, such as α amylase could also be partly responsible for the increase in diastatic power (Woonton *et al*., 2005).

5.2.2 Total Soluble Nitrogen (TSN) Trends

An increase with decrease in storage time was observed for total soluble nitrogen (Fig. 4.3). This trend gives a good indication of proteolysis during germination. The ability of the grain to hydrolyse protein during malting increased, presumably due to an increase in protease synthesis during germination (Woonton *et al*., 2005). All samples recorded total soluble nitrogen less than 1% which is desirable (Table 4.1). Storage length therefore has no effect on TSN concentrations in malt.

5.2.3 Total malt Extract Trends

The total malt extract values of all samples increased with a decrease in barley storage time (Table 4.1). It is well established that extractable substances from malt and hence extract values are influenced by the extent of endosperm cell wall and protein modification during malting. The increased synthesis of hydrolases (α amylase, β glucanase and presumable proteases) during malting of stored barley and would have resulted in greater endosperm cell wall (predominately β glucan) and protein modification during germination (Woonton *et al*., 2005), thus leading to the observed increases (Fig. 4.2).

5.2.4 Free Amino Nitrogen (FAN) Trends

The malt's free amino nitrogen (FAN) values increased progressively with decrease in barley storage time. Due to the increase in hydrolytic enzyme activities and protein modification, storage of barley most probably also led to an increased level of fermentable sugars and or FAN in malt (Fig. 4. 2), (Woonton *et al*., 2005). FAN deficiencies in wort can lead to poor yeast nutrition and health. Other studies have shown that excess FAN levels in finished beer can significantly reduce product flavor stability. These effects are amplified in all-malt beer production. High finished beer FAN levels can result in decreased flavor and biological stability in the package. As all-malt brands continue to grow geographically, the amount of time from brewery to consumer increases. High FAN levels in finished beer mean that over time product stability is threatened. In contrast lower FAN levels in finished beer actually contribute positively to product stability. The usual concentration of soluble free amino nitrogen (FAN) in wort is required to be above 160 mg/l, lower levels can lead to a defective fermentation (O'Rourke, 2002).

5.2.5 Friability Trends (WUGs and PUGs)

The friability meter is a device whose role is to physically disintegrate the grain of malt and to separate its friable constituents from the hard constituents. The more friable malt is, the better it will be disintegrated or malted. The friabilimeter allows simplifying the analysis of malt while giving the degree of accessibility to enzymes (Kumar *et al*., 2013). Some of the malt grains are not milled in a friability meter, and these are wholly unmodified grains (WUG) and their mass is determined using a balance. WUG should not exceed 3%. Other corns are only partly milled and these are termed partially unmodified grains (PUG) and they should not exceed 2%. Homogeneity gives a measure of how evenly modified the malt is. There is accumulating evidence relating brew house performance of malt to either the β-glucan content of the malt or to aspects of wort viscosity. Malts are occasionally found with adequate standard analyses yet produce worts with higher than average viscosities (Bathgate, 1983). It has been demonstrated that, in many cases, wort viscosity is strongly influenced by small proportions (<5%) of water sensitive grains which fail to germinate properly, as well as by the overall degree of modification. Such grains are more troublesome in this respect than dead grains which fail to germinate at all (Bathgate, 1983). The results from the study show that 2011 and 2015 samples had friabilities above 78% which is the spec (Table 4.1). 2012, 2013 and 2014 samples recorded friabilities below the spec probably because of damages encountered during cleaning and recycling stages in storage.

5.2.6 Filtration Speed Trends

Filtration speeds decreased as years into storage decreased (Table 4.1). The 2015 sample however recorded a higher speed than the 2014 sample. Filtration speed is influenced by the levels of β-glucans and heteroxylans (Stone, 2006) and their modifications on malting and

mashing. Higher amounts of these hot water soluble high molecular weight materials would result in viscous mash that lowers the speed of filtration, which is manifested by longer periods of filtration time (Stone, 2006) as observed for the 2011, 2012 and 2015 samples. Longer filtration time (greater than 50 min) delays the brewing time by lowering the speed with which the fermentable extract is obtained and thus lagging beer production (Stone, 2006). In this study, only 2013 and 2014 samples showed desirable filtration speeds of less than 50 minutes (Table 4.1).

5.3 CORRELATIONS BETWEEN YEARS AND PARAMETERS MEASURED

A strong positive linear relationship exists between germination energy (GE), germination capacity (GC) and water sensitivity (WS), (Appendix 20). This shows that these parameters strongly depend each other. Water sensitive grains show slow or no germination in water exceeding the minimum required amounts thus strongly and directly affecting germination energy (GE) and germination capacity (GC), (Briggs, 1998). Grains with a low GC will probably show a low GE and be water sensitive. A weak negative linear relationship exists between moisture content (MC) with germination capacity (GC), germination energy (GE) and water sensitivity (WS). This means that initial amount of water in a grain has a negative effect on germination capacity (GC), germination energy (GE) and water sensitivity (WS). A high MC results in low GC, GE and WS. A low MC results in high GC, GE and WS. In this case however, the effect is weak which is evidenced by the weak negative correlations (Appendix 20).

Strong positive linear relationships exist between friability and filtration speed. This is shown by a strong positive correlation (Appendix 22). Non friable grains are not perfectly modified leading to a coarsely wort. This coarsely wort will then form a mat in the filter bed that will reduce

filtration speed. This therefore explains the positive linear relationship, as friability increases, filtration speed also increases and the vice versa is also true. Weak positive and negative linear relationships exist between parameters that were measured after malting. This means that each parameter either negatively or positively affect other parameters. The effects are however weak in this study showing that there is not much effect.

5.4 CONCLUSIONS

The germination of malting barley variety "*Hope*" can be improved by storage through the loss of dormancy and water sensitivity. At the same time, germination may be lost, especially with extended storage periods at elevated temperatures. The effect of storage temperatures on barley dormancy have been previously reported (Briggs *et al*., 1994; Woods and McCallum, 2000). From the findings of this study it can be concluded that malting barley variety "*Hope*" is best malted at storage periods of less than or equal to two years. After two years, the quality of "*Hope*" starts to deteriorate leading to undesirable malt quality. Water sensitive grains must not be used for malting as this strong affects germination energy and germination capacity.

5.5 RECOMMENDATIONS

Three options for managing barley dormancy to provide opportunities to malt and export barley earlier can be recommended. These involve the use of agricultural chemicals to break dormancy before or after storage. Another option is the use of dry heat, since it avoids difficulties such as chemical residues and market sensitivities to chemical use. Finally, by understanding and carefully manipulating the storage process, postharvest dormancy breakdown can be accelerated without compromising barley quality. "*Hope*" should not be malted after storages of more than 2 years as it showed undesirable maltability trends evident from the results in this study. Also, Kwekwe Maltings must take in quantities of barley that will be maltable within periods of not

more than two years. This is because after storage periods of more than two years, the germination vigour and hence malt quality starts to decline. This was also evidenced by the results obtained in this study. For verification of these results the researcher proposes that this study be repeated using the same variety and methodology employed. To improve the quality of wort, gibberelic acid must be added at the first stages of germination. Gibberellins from the embryo of germinating grains are necessary for the synthesis of α-amylase by the cells of the aleurone layer, which, in turn is necessary for the hydrolysis of starch within the endosperm. In the brewing industry, the production of beer relies on this hydrolytic breakdown of starch in barley grains to yield fermentable sugars, principally maltose, which are subjected to fermentation by yeast. During fermentation glycolytic enzymes from yeast break down the sugars, resulting in ethanol. Increase of these gibberellins through addition of giberrelic acid will therefore be an added advantage to brewer companies.

REFERENCE LIST

Aastrup, S., Riis, P., & Hansen, J.R. (1989) *Proceedings of the European Brewery Convention Congress, Zurich* 1989, 171-178.

Abu-Ghannam, N. and Gallaghar, E. (2010) *Barley for brewing: Characteristic changes during malting, brewing and applications of its by-products*, Ireland, Teagasc Press

Bamforth, C.W, & Quain, D.E. (1989*). Enzymes in brewing and distilling*. UK: Aberdeen Univ. Press

Bamforth, C.W., & Barclay A.H.P. (1993). Malting technology and the uses of malt. *Barley: Chemistry and Technology. American Association of Cereal Chemists Inc. St. Paul*, 297–354.

Bathgate, G. N. (1983). The relationship between malt 'friability' and wort viscosity. Jnl Institute Brewing, 89: 416–419. doi: 10.1002/j.2050-0416.1983.tb04217.x

Belitz, H.G, Grosch, W, & Schiebrle, P. (2009*). Food Chemistry.* Berlin: Springer.

Bhatty, R.S, & Rossnagel, B.G. (1997). Zero amylose lines of hull-less barley. *Cereal Chem,* **74**:190–1.

Bingham, M.K. (1990). Field detection and implications of mercury in natural gas. *SPE Prod Engg*, **5**:120–4.

Briggs, D.E. (1995). *Aspects of Dormancy*. Pauls Malt, London, 31–35.

Briggs, D. E. (1998). *Malts and Malting*. London: Blckie Academic & Professional. .

Briggs, D.E, Boulton, C.A., Brookes, P.A., & Stevens, .R. (2004). *Brewing Science and practice*. New York: Woodhead publishing limited.

Briggs, D. E., Hugh, S.J .(1985), *Malting and Brewing Science Volume 1(* 2nd edition). London: Chapman and Hall Publishing House.

Czuchajowska, Z., Klamczynski, A., Paszczynska, B., & Baik, B.K., (1998). Structure and

functionality of barley starches. *Cereal Chem*, **75**:747–54.

Enari, T.M., & Sopanen, T. (1968). Mobilisation of endospermal reserves during the germination of barley. *Centenary review. J Inst of Brew*, 92, 25–31.

Faivre, J., & Bonithon-Kopp, C. (1999). Diets, fibers and colon cancer. *Adv Exp Med Biol,* **72**:199–206

Fox, G.P., Onley-Watson, K., & Osman, A. (2002). Multiple linear regression calibrations for barley and malt protein based on the spectra of hordein. *J Inst Brew*, **108**:155–9.

Frančáková, H., Líšková, M., Bojňanská, T., & Mareček, J. (2012): Germination index as an Indicator of malting potential. *Czech J. Food Sci*ence, **30**: 377–384.

Frost, G., Leeds, A.A., Dore, C.J., Maderios, S., Brading, S., & Dornhurst, A. (1999). Glycaemic index as a determinant of serum, cholesterol concentration. *Lancet,* **353**:1045–8.

Gallagher, D.D., Hassel, C.A., Lee, K.J., & Gallagher, C.M. (1993). Viscosity and fermentability as attributes of dietary fibre responsible for hypocholestrolemic effects in hamsters. *J Nut,***123**:244–52.

Gavin, H. (2008).*Understanding Research Methods and Statistics* .London: SAGE Publications

Gupta, M., Abu-Ghannam, N., & Gallaghar, E. (2010). Barley for Brewing: Characteristic Changes during malting, brewing and applications of its by-products. *Comprehensive Reviews In Food Science And Food Safety,* **9**:318-325

Hart, C. (2005). *Doing your Masters Dissertation*. London: SAGE Publications

Harvey, B.L., & Rossnagel, B.G. (1983). Sprouting resistance in barley. *Proceedings of the 3rd International Symposium on Pre- harvest Sprouting in Cereals, Epping Bowker: Winnipeg, Canada*, 239–243.

Henry, R.J. (1988). Changes in *β*-glucan and other carbohydrate components of barley during

malting. *J Sci Food Agric*, **42**:333–41

Hoseney, R.C. (1994). *Principles of Cereal Science and Technology* (2nd Edition). USA, American Association of Cereal Chemistry

Izydorczyk, M.S., Storsley, J., Labossiere, D., MacGregor, A.W., & Rossnagel, B.G. (2000). Variation in total and soluble *β*-glucan content in hulless barley: effects of thermal, physical, and enzymic treatments. *J Agric Food Chem,* **48**:982–9.

Jacobsen, J.V., Pearce, D.W., Poole, A.T., Pharis, R.P. and Mander, L.N. (2002) Abscisic acid, phaseic acid and gibberellin contents associated with dormancy and germination of barley. Physiologica Plantarum, 115, 428–441.

Jansson, C., Kirsop, B.H., & Pollock, J.R.A. (1959). Studies in barley and malt: XIII. Watersensitivity as an intrinsic property of certain barleys. *Journal Institute of Brewing*, 65, 165–168.

Keenan, J.M., Coulson, M., Shamliyan, T., Knutson, N., Kolberg, L., & Curry, L. (2007). The effects of concentrated barley *β*-glucan on blood lipids in a population of hyper cholesterolaemic men and women. *Br J Nutr,* **97**:1162–8.

Kelly, L. & Briggs, D.E. (1993).Relationship between duration of steeping, grain microbes, grain maturity and the response of de-embryonated grains to gibberellic acid. *Journal Institute of Brewing*, 99, 57–62

Kumar, D., Kumar, V., Verma, R.P.S., Kharub, A.S., and Sharma, I. (2013) *Quality parameter requirements and standards for malt barley* – A review, Dictorate of Wheat Research, Karnal – 132001, India, Agri. Reviews, **34** (4): 313 – 317.

Kunze, W. (1999) .*Technology: Brewing and Malting*. Berlin: VLB Berlin.

Larsenet, J. (1994). Targets for tailored breeding. *Symposium Malting Technology, Andernach, European Brewing Convention. Monograph. Fachverlag Hans Carl, Nürnberg*, 152–164.

Lauro, M., Suortti, T., Autio, K., Linko, P., & Poutanen, K. (1993). Accessibility of barley starch granules to *α*-amylase during different phases of gelatinisation. *J Cereal Sci*, **17**(2):125–36.

Lewis, M.J, & Young, T.W. (1995). *Mashing biochemistry*. London,UK: Chapman & Hall.

Li, J., Kaneko, T., Qin, L.Q., Wang, J., & Wang, Y. (2003). Effects of barley intake on glucose tolerance, lipid metabolism, and bowel function in women. *Nutr,* **19**:926–9.

Lu, M.Q., O'Brien, L., & Stuart, I.M. (2000). Barley malting quality and yield interrelationships and the effect on yield distribution of selection for malting barley quality in early generations. *Australian J Agric Research,* **51**: 247–258.

Madhujith, T., & Shahidi, F. (2007). Antioxidative and antiproliferative properties of selected barley cultivars and their potential for inhibition of low-density lipoprotein (LDL) cholesterol oxidation. *J Agric Food Chem,* **55**:5018–24.

Manners, D.J., Marshall, J.J, & Yellowlees, D. (1970). The specificity of cereal limit dextrinase. *Biochem J,* **116**:539–41

Manners, D.J. (1985). Some aspects of the metabolism of starch. *Cereal Foods World*, **30**:722–7.

Moll, M., (1979). *Water in malting and brewing.* London: Academic Press.

Munck, L., & Moller, B. (2004). A new germinative classification model of barley for prediction of malt quality amplified by a near infrared transmission spectroscopy calibration for vigour "on-line" both implemented by multivariate data analysis. *J Inst Brew*, **110**: 2–17.

Naidu, S. (2008). *International Encyclopedia of Agricultural Science and Technology. Volume 4*. New Delhi: Krishan Mittal

Newman, R.K., McGuire, C.F., & Newman, C.W. (1990). Composition and muffin-baking

characteristics of flours from four barley cultivars. *C F W,* **35**:563–6.

Noonan, G. (1997) "Understanding Malt Analysis Interpretion" , Brewers' Market Guide, Brewing Techniques Magazine.

O'Rourke, T. (2002) The Brewer International. www.Igb.org.uk. Volume 2. Issue 9.

Peltonen, J., Rita, H., Aikasalo, R., Home, S. (1994). Hordein and malting quality in northern barleys. *Hereditas,* **120**:231–9.

Psota, V., Vejrazka, K., Famera, O., & Hrcka, M. (2007). Relationship between grain hardness and malting quality of barley (Hordeum vulgare L.). *J Inst Brew,* **113**:80–86.

Quinde, Z., Ullrich, S.E., & Baik, B.K. (2004). Genotypic variation in color and discoloration potential of barley-based food products. *Cereal Chem,* **81**:752–8.

Riss, P., & Bang-Olsen, K. (1991). Germination profile – A new term in malting barley analysis. *Proceedings 23rd European Brewery Convention. Lisabon,* 100–108.

Simpson, B. and M. Ogorzaly. (2001) Economic Botany: Plants in our world. McGraw-Hill, New York.

Slack, P.T., & Wainwright, T. (1980). Amylolysis of large starch granules from barleys in relation to their gelatinisation temperatures. *J Inst Brew,* **86**(2):74–7.

Stewart, D.C., Freeman, G., and Evans (2000). Development and assessment of a small-scale wort filtration test for the prediction of beer filtration efficiency. *J Inst Brew,* 106:361–6.

Stone, B. A (2006). Cell walls of cereal grains. Cereals Food World. **51**:62-65

Swanston, J.S., & Taylor K. (1990): The effects of different steeping regimes on water uptake, germination rate, milling energy and hot water extract. *J Inst Brew,* **96**:3–6.

Ullrich, S.E., & Eslick, R.F. (1978). Lysine and protein characterization of spontaneous shrunken endosperm mutants of barley. *Crop Sci,* **18**:809–12.

White, N.G.G., Hulasare, R.B. & Jayas, D.S. (1999). Effect of storage conditions on quality loss of hull-less and hulled oats and barley. *Canadian Journal of Plant Science*, 79, 475–482.

William. (2005).Your research project (3rdedition).London: SAGE Publications

Woods, J.L., Favier, J.F., & Briggs, D.E. (1994). Predicting the germinative energy of dormant malting barley during storage. *Journal of the Institute of Brewing*, 100, 257–269.

Woonton, B., Jacobsen, J., Sherkat, F. and Stuart, M. (2002) Effect of post-harvest storage period on barley germination and malt quality. In: Proceedings of the 10th Australian Barley Technical Symposium, Canberra, ACT, 16–20 September 2001. Australian Barley Technical Symposium Incorporated.

Woonton, B., Jacobsen, J.V., Sherkat F., & Stuart I.M. (2005). Effect of post-harvest storage period on barley germination and malt quality. *J Inst brew and distilling*, **111**(1), 33–41.

APPENDIX

APPENDIX 1: Results for percentage germination capacity

SAMPLE NUMBER	2011	2012	2013	2014	2015
1	77	90	92	96	76
2	80	88	96	94	82
3	83	89	88	98	79

APPENDIX 2: Results for percentage germination energy

SAMPLE NUMBER	2011	2012	2013	2014	2015
1	77	84	89	93	74
2	73	83	86	94	75
3	75	81	92	95	73

APPENDIX 3: Results for water sensitivity (%)

SAMPLE NUMBER	2011	2012	2013	2014	2015
1	8	18	40	46	13
2	12	15	41	44	11
3	10	12	39	45	15

APPENDIX 4: Results for moisture content (%)

SAMPLE NUMBER	2011	2012	2013	2014	2015
1	9	10	10	12	14
2	5	9	13	10	15
3	7	8	7	8	13

APPENDIX 5: Results for diastatic power (Wk)

SAMPLE NUMBER	2011	2012	2013	2014	2015
1	211	258	274	281	295
2	213	254	276	280	301
3	215	256	272	279	298

APPENDIX 6: Results for total percentage extracts

SAMPLE NUMBER	2011	2012	2013	2014	2015
1	78.2	80.8	84.8	81	84.3
2	80.2	79.8	81.8	83	82.3
3	82.2	81.8	78.8	82	80.3

APPENDIX 7: Results for total soluble nitrogen

SAMPLE NUMBER	2011	2012	2013	2014	2015
1	0.44	0.72	0.75	0.99	0.84
2	0.94	0.76	0.74	0.59	0.81
3	0.69	0.68	0.76	0.79	0.78

APPENDIX 8: Results for Free amino nitrogen

SAMPLE NUMBER	2011	2012	2013	2014	2015
1	164	170	173	186	191
2	161	168	177	184	196
3	158	169	181	188	186

APPENDIX 9: Results for Wholly unmodified grains

SAMPLE NUMBER	2011	2012	2013	2014	2015
1	2.61	2.1	1.1	0.6	0.5
2	2.4	2.3	1.4	0.38	0.4
3	2.19	2.2	1.5	0.82	0.3

APPENDIX 10: Results for Partially unmodified grains

SAMPLE NUMBER	2011	2012	2013	2014	2015
1	8.35	6.65	3	4.62	3.8
2	8.2	6.15	5	4.18	4.14
3	8.05	6.4	8	4.4	3.46

APPENDIX 11: Results for friability

SAMPLE NUMBER	2011	2012	2013	2014	2015
1	81	77	45	37	83
2	77	60	46	33	81
3	79	63	44	35	79

APPENDIX 12: Results for filtration speed

SAMPLE NUMBER	2011	2012	2013	2014	2015
1	128	112	45	48	91
2	125	108	43	47	99
3	122	110	47	46	95

APPENDIX 13: p values for anova of germinability (before micro-malting)

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
GERMINATION CAPACITY	Between Groups	668.400	4	167.100	21.423	.000
	Within Groups	78.000	10	7.800		
	Total	746.400	14			
GERMINATION ENERGY	Between Groups	906.267	4	226.567	65.356	.000
	Within Groups	34.667	10	3.467		
	Total	940.933	14			
WATER SENSITIVITY	Between Groups	3279.600	4	819.900	215.763	.000
	Within Groups	38.000	10	3.800		
	Total	3317.600	14			
MOISTURE CONTENT	Between Groups	78.000	4	19.500	5.132	.016
	Within Groups	38.000	10	3.800		
	Total	116.000	14			

APPENDIX 14: p values for homogeneity of variances of germinability (before micro-malting)

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
GERMINATION CAPACITY	.667	4	10	.630
GERMINATION ENERGY	.838	4	10	.532
WATER SENSITIVITY	.737	4	10	.588
MOISTURE CONTENT	.737	4	10	.588

APPENDIX 15: p values for homogeneity of variances of malt quality results

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
DIASTATIC POWER	.455	4	10	.767
EXTRACTS	.737	4	10	.588
TOTAL SOLUBLE NITROGEN	2.327	4	10	.127
FREE AMINO NITROGEN	.909	4	10	.495
FILTRATION SPEED	.765	4	10	.572
FRIABILITY	6.426	4	10	.008
PARTIALLY UNMODIFIED GRAINS	4.704	4	10	.021
WHOLLY UNMODIFIED GRAINS	.669	4	10	.628

APPENDIX 16: p values for tests of normality of malt quality results

Tests of Normality

	YEARS	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
TOTAL SOLUBLE NTROGEN	2011	.175	3	.	1.000	3	1.000
	2012	.175	3	.	1.000	3	1.000

	2013	.175	3	.	1.000	3	1.000
	2014	.175	3	.	1.000	3	1.000
	2015	.175	3	.	1.000	3	1.000
	2011	.175	3	.	1.000	3	1.000
	2012	.175	3	.	1.000	3	1.000
WHOLLY UNMODIFIED GRAINS	2013	.292	3	.	.923	3	.463
	2014	.175	3	.	1.000	3	1.000
	2015	.175	3	.	1.000	3	1.000
	2011	.175	3	.	1.000	3	1.000
	2012	.175	3	.	1.000	3	1.000
PARTIALLY UNMODIFIED GRAINS	2013	.219	3	.	.987	3	.780
	2014	.175	3	.	1.000	3	1.000
	2015	.175	3	.	1.000	3	1.000

APPENDIX 17: **p values for tests of normality before malting**

Tests of Normality

	YEARS	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
	2011	.175	3	.	1.000	3	1.000
	2012	.175	3	.	1.000	3	1.000
GERMINATION CAPACITY	2013	.175	3	.	1.000	3	1.000
	2014	.175	3	.	1.000	3	1.000
	2015	.175	3	.	1.000	3	1.000
	2011	.175	3	.	1.000	3	1.000
	2012	.253	3	.	.964	3	.637
GERMINATION ENERGY	2013	.175	3	.	1.000	3	1.000
	2014	.175	3	.	1.000	3	1.000
	2015	.175	3	.	1.000	3	1.000
	2011	.175	3	.	1.000	3	1.000
	2012	.175	3	.	1.000	3	1.000
WATER SENSITIVITY	2013	.175	3	.	1.000	3	1.000
	2014	.175	3	.	1.000	3	1.000
	2015	.175	3	.	1.000	3	1.000
	2011	.175	3	.	1.000	3	1.000
	2012	.175	3	.	1.000	3	1.000
MOISTURE CONTENT	2013	.175	3	.	1.000	3	1.000
	2014	.175	3	.	1.000	3	1.000
	2015	.175	3	.	1.000	3	1.000

APPENDIX 18: p values test of normality for malt quality

Tests of Normality

	YEARS	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
	2011	.175	3	.	1.000	3	1.000
	2012	.175	3	.	1.000	3	1.000
DIASTATIC POWER	2013	.175	3	.	1.000	3	1.000
	2014	.175	3	.	1.000	3	1.000
	2015	.175	3	.	1.000	3	1.000
EXTRACTS	2011	.175	3	.	1.000	3	1.000

	2012	.175	3	.	1.000	3	1.000
	2013	.175	3	.	1.000	3	1.000
	2014	.175	3	.	1.000	3	1.000
	2015	.175	3	.	1.000	3	1.000
	2011	.175	3	.	1.000	3	1.000
	2012	.175	3	.	1.000	3	1.000
FREE AMINO NITROGEN	2013	.175	3	.	1.000	3	1.000
	2014	.175	3	.	1.000	3	1.000
	2015	.175	3	.	1.000	3	1.000
	2011	.175	3	.	1.000	3	1.000
	2012	.324	3	.	.878	3	.317
FRIABILITY	2013	.175	3	.	1.000	3	1.000
	2014	.175	3	.	1.000	3	1.000
	2015	.175	3	.	1.000	3	1.000
	2011	.175	3	.	1.000	3	1.000
	2012	.175	3	.	1.000	3	1.000
FILTRATION SPEED	2013	.175	3	.	1.000	3	1.000
	2014	.175	3	.	1.000	3	1.000
	2015	.175	3	.	1.000	3	1.000

APPENDIX 19: p values for anova of the malt quality results

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
DIASTATIC POWER	Between Groups	12530.400	4	3132.600	711.955	.000
	Within Groups	44.000	10	4.400		
	Total	12574.400	14			
EXTRACTS	Between Groups	9.384	4	2.346	.617	.660
	Within Groups	38.000	10	3.800		
	Total	47.384	14			
TOTAL SOLUBLE NITROGEN	Between Groups	.029	4	.007	.345	.841
	Within Groups	.210	10	.021		
	Total	.239	14			
FREE AMINO NITROGEN	Between Groups	1790.400	4	447.600	40.691	.000
	Within Groups	110.000	10	11.000		
	Total	1900.400	14			
FILTRATION SPEED	Between Groups	16101.600	4	4025.400	591.971	.000
	Within Groups	68.000	10	6.800		
	Total	16169.600	14			
FRIABILITY	Between Groups	5062.667	4	1265.667	66.381	.000
	Within Groups	190.667	10	19.067		
	Total	5253.333	14			
PARTIALLY UNMODIFIED GRAINS	Between Groups	36.443	4	9.111	6.921	.001
	Within Groups	13.165	10	1.316		
	Total	49.607	14			
WHOLLY	Between Groups	9.851	4	2.463	79.016	.000

UNMODIFIED GRAINS	Within Groups	.312	10	.031		
	Total	10.162	14			

APPENDIX 20: r values for correlations before malting

Correlations

Control Variables			GERMINATION CAPACITY	GERMINATION ENERGY	WATER SENSITIVITY	MOISTURE CONTENT
YEARS	GERMINATION CAPACITY	Correlation	1.000	.889	.862	-.230
		Significance (2-tailed)	.	.000	.000	.429
		df	0	12	12	12
	GERMINATION ENERGY	Correlation	.889	1.000	.924	-.408
		Significance (2-tailed)	.000	.	.000	.147
		df	12	0	12	12
	WATER SENSITIVITY	Correlation	.862	.924	1.000	-.369
		Significance (2-tailed)	.000	.000	.	.194
		df	12	12	0	12
	MOISTURE CONTENT	Correlation	-.230	-.408	-.369	1.000
		Significance (2-tailed)	.429	.147	.194	.
		df	12	12	12	0

APPENDIX 21: r values for correlations after malting

Correlations

Control Variables			DIASTATIC POWER	TOTAL EXTRACTS	TOTAL SOLUBLE NITROGEN	FREE AMINO NITROGEN
YEARS	DIASTATIC POWER	Correlation	1.000	.113	.023	.041
		Significance (2-tailed)	.	.699	.937	.889
		df	0	12	12	12
	TOTAL EXTRACTS	Correlation	.113	1.000	.039	-.375
		Significance (2-tailed)	.699	.	.895	.187
		df	12	0	12	12
	TOTAL SOLUBLE NITROGEN	Correlation	.023	.039	1.000	-.031
		Significance (2-tailed)	.937	.895	.	.917
		df	12	12	0	12
	FREE AMINO	Correlation	.041	-.375	-.031	1.000

	NITROGEN	Significance (2-tailed)	.889	.187	.917	.
		df	12	12	12	0

APPENDIX 22: r values for correlations after malting

Correlations

Control Variables			WHOLLY UNMODIFIED GRAINS	PARTIALLY UNMODIFIED GRAINS	FRIABILITY	FILTRATION SPEED
YEARS	WHOLLY UNMODIFIED GRAINS	Correlation	1.000	.302	.426	.458
		Significance (2-tailed)	.	.293	.128	.100
		df	0	12	12	12
	PARTIALLY UNMODIFIED GRAINS	Correlation	.302	1.000	.357	.382
		Significance (2-tailed)	.293	.	.211	.178
		df	12	0	12	12
	FRIABILITY	Correlation	.426	.357	1.000	.926
		Significance (2-tailed)	.128	.211	.	.000
		df	12	12	0	12
	FILTRATION SPEED	Correlation	.458	.382	.926	1.000
		Significance (2-tailed)	.100	.178	.000	.
		df	12	12	12	0