KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

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COMPARATIVE EVALUATION OF MALTING PROPERTIES AND PROCESSING CONDITIONS ON ENZYMATIC ACTIVITY OF TEN RICE VARIETIES

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

BY

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DECLARATION

I hereby declare that this submission is my own work towards the MSc. and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

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DEDICATION

This work is dedicated to the Almighty God, theNienufamily, my husband and little daughter Antoinette AbaaSamani.

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ABSTRACT

Diastatic power or activity gives a measure of the saccharifying power of enzymes in prepared malt. There are about 80,000 varieties of rice that exist and new varieties are being developed through continuous plant breeding research. Among these new varieties are some whose malts possess beneficial qualities such as good diastatic activity for the sugar, confectionery, pharmaceutical and brewing industries. Hence, the need to explore these varieties as a cheap source of glucose.Themalting parameters of the ten rice varieties namely; *Wita 7, Digang, Bourke'189, ITA 320, Jasmine'85, China, ITA 324, Viwornor, China and Sikamo* were studied. Different conditions (pH, temperature, substrate conc. and time) were also examined to assess their effects on enzyme activity in ten varieties of malted rice. The effect of pH (2-9), temperature (10-60^{o}C), substrate concentration (0.5-3.0w/v) and time (5-15mins) on the enzyme activity was studied using correlation whiles the optimum conditions for all Rice Malt Extract was determined using principal component analysis and response surface plots. The out-of-steep -moisture content ranged between 30.07% (*Digang*) and 35.26% (*Bourke'189*). With the exception of *Sikamo* (steeping loss of 2.41%), the other varieties showed steeping losses of greater than 3% after grain steeping. Germination energy of >80 % was obtained for each variety and this signified their viability.The findings show that the different varieties had good malting properties. Among all the conditions varied, temperature had the most significant effect on enzyme activity. An increase in temperature increased enzyme activity of the varieties and vice versa. The optimum conditions determined for diastatic activity of the rice malts werepH of 5.5, substrate concentration of 3.0%w/v, temperature of 60ºC and time of 15minutes.*Viwornor*,*China* and *Wita 7* varietieswere found to produce the highestdiastatic power among the ten rice varieties.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Rice (*Oryza sativa L.*) is classified as the most important cereal being the staple for over 50% of the world population (FAO, 2004). Rice contains about 78% of carbohydrates, 7% protein with low levels of fats of about 0.4 to 0.8 (Laureys, 1999). Even though rice has a relatively low protein content, brown rice (*caryopsis*) ranks higher than wheat in available carbohydrates, digestible energy (kilojoules [kJ] per 100 grams), and net protein utilization. Rice is low in sodium, fat and cholesterol which have been implicated in hypertension conditions. It is also free from allergens making it suitable for the formulation of baby foods (James and McCaskill, 1983). Rice is utilized mostly at the household level, where it is consumed as boiled, fried or ground rice with stew or soup. Compared to wheat and maize, rice can support more people per unit land (Chang, 1987). Rice starch can also serve as a substitute for glucose in oral rehydration solution for infants suffering from diarrhea (Juliano, 1985).

Barley has been the major grain used in malting by the brewing industries; however in recent years, malting of cereals other than hulled barley has been used (Suhasini and Malleshi, 1995; Dewar *et al*., 1997; Hammond and Ayernor, 2001). This has been due to economic considerations and local availabilities. Cereals such as maize, sorghum, rice and millet are now being malted for use as sources of enzymes in sugar and brewing industries in Ghana (Hammond and Ayernor, 2000). A study by Ayernor*et al*., (2002) shows that rice malt is rich in many enzymes including alpha and beta amylases,

amyloglucosidase and dextrinases and that based on their diastatic activities, rice has been found to have the highest diastatic activity among cereals such as; maize, sorghum and millet.

In the food industry, malted rice is also gaining importance in the making of infant foods, breakfast cereals, beer, fermented products, as well as rice wine which is a major alcoholic beverage in some parts of East Asia(Cambridge World History of Foods, n.d.). Guinness Ghana, a multinational company in Ghana employs malted sorghum in the production of a non- alcoholic beverage called Malta Guinness (Owusu-Mensah, 2009). The use ofamylase extracted from malted cereal for the conversion of starch to sugar syrups is a common technique in modern biotechnology and this is a common substitute for common sugar in many industries (Bello-Perez *et al*., 2002, Mitchell, 2004). They are used as aids in the preparation of pectin from apple pomace and removes starches in jellies and fruit juices to increase sparkling(Deman, 1999). In the baking industry, amylases are used in the making of bread to break down starch into simple sugars. Of all the staple crops, rice consumption has risen tremendously, due to changing consumer preferences and population increase (Akande, n.d.).It has been estimated that the annual rice production has to increase from 586 million metric tonnes in 2001 to meet the projected world demand of about 756 million metric tonnes by 2030 (FAO, 2002) and therefore the need for new varieties of rice to be developed through continuous plants breeding research. Among these new varieties are some whose malts possess beneficial qualities for the sugar and brewing industries and therefore there is the need to explore them

1.2 Problem statement

The traditional grain for brewing conventional beer has been barley. The barley malt and malt extracts are used in the production of beer, whisky, biscuits, baby food, tonics, health foods and pet foods. In 2009, the total global area and production of barley stood at 54 million ha and 150 million tonnes respectively, of which 70 per cent being contributed by the European Union, Russia and Ukraine. European Union is the largest exporter of barley, with a share of 24 per cent of the total global export of approximately 17 million tonnes in 2009-10. Other major exporters of barley include Australia, Russia and Canada. In 2000, total barley production was at 1.43 million tonnes, which dropped to the level of 1.2 million tonnes (2004-05) and then touched 1.5 million tonnes in 2009-10. With the increasing urbanization and changing food habits, the demand for beer has shown significant increase and therefore is the main influencing factor in the market (Anonymous, 2010) this has made barley very expensive. However in Africa, the cultivation of barley has been met with little success. As such, huge amount of foreign exchange are spent annually in importing barley malt. Industrialists have therefore resorted to importation of microbial amylases for starch hydrolysis. Present realities show that local resources such as maize, rice, sorghum, acha and millet (Egwim, 2006) can be used effectively for malt and enzyme production and therefore there is the need to explore locally grown cereals especially rice for the possibility of alternative source of substrate for the brewery and the other industries. Furthermore, over 10,000 varieties of rice exist and more new varieties of rice are developed though continuous plants breeding research. A great number of these rice varieties and lines have been introduced through varietal improvement and genetic resource conservation, evaluation and

utilization programmes at various national and international institutions. Among these new varieties are some whose malts possess beneficial qualities for our sugar and brewing industry such as good diastatic power. It is therefore proposed in this work that diastatic activity can be greatly enhanced by using the optimum pH, temperature, substrate concentration and time. This study will further diversify the use of rice adding more value to our local food stuff thereby creating a ready market for farm produce reducing postharvest losses.

1.3 Main Objective

The objective of this research was to study the diastase activity in the different varieties of malted rice.

1.4 Specific Objectives

1. To determine the malting properties of ten varieties of rice.
2. To study the effect of pH, temperature, time, and substrate concentration on enzyme activities of these rice varieties.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Malting of cereals

Malting forms an important process in the production of cereal-based beverages in which amylases and proteases embedded in the cereal grain are activated for the purpose of hydrolysis of starch and protein into sugars and amino acids respectively (Okafor, 1987). The primary objective of malting is to promote the development of hydrolytic enzymes which are not present in the ungerminated grains (Dewar *et al*., 1997, Ayernor and Ocloo, 2007). According to Dewar *et al*., (1997) seeds during malting have been found to undergo various changes of modification such as increase in the quantities of alpha and beta-amylases in the grain and the partial degradation (by residual hydrolytic enzymes) of reserve substances such as cell wall, gums, protein, starch in the starchy endosperm. The most important characteristic of good malt are high enzyme levels to degrade starch and obtain high extract yield (Subramanian *et al*., 1995).

Barley has been the major grain used in malting by the brewing industries; however due to economic considerations and local availabilities cereals other than hulled barley has been used (Suhasini and Malleshi, 1995; Dewar *et al*., 1997; Hammond and Ayernor, 2001). In Southern Africa, approximately 200,000 tonnes per annum of malted sorghum are used in the production of traditional (opaque) sorghum beer (Dewar *et al*., 1993) whiles in several other African countries, sorghum and millets are malted and used for the preparation of a popular beverage called ‘pito’ in Ghana and Nigeria, ‘chibuku’ in

Zimbabwe, 'impeke' in Burundi, 'dolo' in Mali and Burkina Faso (Chitsika and Mudimbu, 1992). Also in Nigeria, red sorghum has been malted commercially as sources of amylases, whiles rice and maize malts have been successfully used in maltose syrup production in Vietnam (Dewar *et al*., 1997; Cecil, 1995). Malted maize, sorghum, rice and millet have been used as sources of enzymes by some sugar and brewing industries in Ghana (Hammond and Ayernor, 2000).

Germination is an important part of malting and takes place after the right conditions are set. During germination, there are a lot of modifications that take place that could eventually affect the quality of malt. Modification includesthebreaking down of cell walls and the conversion of starch to sugars due to the action of the enzymes. Enzymes released from the aleurone layer and possibly the scutellum also modify the structure of the endosperm. Modified regions of the endosperm are friable, whereas the unmodified endosperm is structurally strong, compact and relatively non-porous (Palmer, 1988; Oh and Briggs, 1989). The initiation of germination involves the production of giberellic acid which acts as a catalyst for the production of enzymes from 'pre-enzymes' present in the aleurone layer. Gibberellicacid plays an important role during the modification process. It is the most important consequence of germination because it activates the synthesis and secretion of hydrolytic enzymes. The gibberellic acid which cannot pass through the scutellum into the endosperm enters the aleurone layer via the scutellumaleurone junction. Enzymes produced in the aleurone layer then diffuse into the endosperm and act as a catalyst for modification (Palmer, 1980). Ogbonna, (2007) observed that enzyme activity was highest during the early stages of germination since

the first 2-3 days coincide with the movement of the gibberellic acid. Physiological activities in the grains increase due to the action of the enzymes leading to the utilization of the food reserves for energy and growth (Ayernor and Ocloo, 2007). Food reserve starch, decreases as germination progresses due to the action of the hydrolytic enzymes such as α- and β- amylases. These enzymes hydrolyze the starch into low molecular weight carbohydrates such as maltose, glucose and dextrins (Zeeman *et al*., 2007). This implies that the longer the malting period, the smaller the residual starch in the endosperm. The sugars and amino acids released during this stage is used to build new cells and tissues for the germinating seed for the growing radical and new plumule (Ayernor and Ocloo, 2007).

2.2 Malting of rice grains

Rice is a member of the grass family *Poaceae*(Gramineae) and belongs to the genus *Oryza*.The genus *Oryza* is classified to the tribe *Oryzeae*. However, there are 12 genera within the Oryzeae tribe (Vaughan, 1994). It has been consumed by humans for at least 5,000 years. It is one of the most consumed cereals worldwide.The cooked texture of rice can be grouped as glutinous and non-glutinousrice, and sub-grouped as low amylose rice (12–20%), medium amylose rice (20–25%) and high amylose rice (>25%). Since rice is a gluten free cereal containing high quality protein, this is of interest for brewing. Rice seeds however can be modified by germination to improve its functionality. Malting has been identified as a traditional processing technology that could possibly be used to improve the nutritional quality of the protein (Wang and Fields, 1978).

The main reserve compound in rice is starch (Ayernor and Ocloo, 2007) which is stored mainly in the endosperm where it is hydrolyzed during germination to provide soluble sugars to the germination seedling (Sun and Henson, 1991).

Table 2.1 depicts the proximate content of the ten varieties of rice. These varieties contain between 72-79% of carbohydrates, low levels of fats of about 0.5 to 1.0 (Bam *et al*., 2010). Protein content however is important in the generation of diastatic power because of its role in providing amino nitrogen for enzyme synthesis (Gibson and Solah, 1995). It has been observed to be correlated with diastatic power. It has dual effects on malt quality.

Table 2.1. Proximate composition of selected rice varieties grown in Ghana

variety	Moisture Content %	Ash%	Crude protein%	Crude fiber%	Fat%	Carbohydrate %
Wita 7	10.92	0.63	12.52	0.17	0.99	74.74
Digang	9.32	0.82	11.03	0.26	0.51	10.11
Jasmine'85	8.48	0.61	11.00	0.10	0.69	79.12
Sikamo	11.30	0.61	14.70	0.10	1.0	72.29
Bourke'189	10.58	0.43	10.13	0.24	1.0	77.62
ITA 320	10.05	0.42	11.24	0.11	0.50	78.62
ITA 324	9.11	0.42	11.24	0.11	0.50	78.62
Marshal	10.25	0.64	14.88	0.15	1.0	73.08
Viwornor	-	-	-	-	-	-
China	-	-	-	-	-	-

Source; Bam *et al*., 2010

2.3 Factors affecting the malting of cereal grains

2.3.1 Steeping

Steeping which refers to the process of soaking the grains in water forms the most critical stage of any malting process (Briggs *et al*., 1981, French and McRuer, 1990). This is due to the fact that it initiates the modification of the endosperm structure at an increasing rate producing malt of the desired quality (French and McRuer, 1990). The uptake of water by the grain during steeping brings about a rapid increase in the respiratory rate of the grain. Briefly after the absorption of the water by the seed, the enzymes become active which break down the storage materials into simple sugars (Bewley and Black 1985).

Dry mature seeds, in most cases, exhibit very low metabolic rates and needs to be hydrated to stimulate metabolism (Gallardo *et al*., 2001). The water uptake marks the first stage in germination. The dry seed typically has a water content of less than 10% and swells due to the absorption of water in the process. This hydrates the dry components of the seed thereby activating the metabolic system necessary for germination to take place. Owusu-Mensah (2009) studied the uptake rate of water in Jasmine'85 rice grains and found that increase in the steeping period corresponded to an increase in its water uptake rate. The author reported a very rapid uptake of water during the first twelve hours which became fairly constant until the 48 hours. Salisbury and Ross (1992) observed a high osmotic gradient generated by the sugars present mainly in the embryo.

Generally, there is poor modification of the grain when there is no adequate moisture (Coles *et al*., 1991). Steeping increases the quality of the malt produced at least in terms

of brewing quality characteristics such as diastatic power (amylase activity) and free amino nitrogen (free amino acids and short peptides) (Taylor and Dewar, 2001).

2.3.2 Air resting

Germination also requires some amount of oxygen and the term "air resting" is used during malting. Air resting refers to the process where soaked grains are exposed to atmospheric oxygen. Usually grains are first steeped for a brief period and then the steep liquid is drained off to allow for air resting (Wijngaard*et al*., 2005). This allows the grains to undergo aerobic respiration (Saupe, 2008). Respiration supplies most of the energy that drives the grain's metabolism as well as growth of the shoots and roots (Briggs, 1998). The grains are then re-steeped after the duration is complete. Re-steeping plays a very important role because during air-resting the interior surfaces of the grain are hydrated at the expense of the peripheries, and therefore re-steeping helps to reestablish the surface film of the grain again (Briggs, 1998). Oxygen absorbed during air- resting is used by the mitochondria to produce ATP. Thus the availability of oxygen influences the final quality of the malt. A study by Owusu-Mensah (2009) suggests that the rate of water uptake, germination energy and the diastatic activity of the rice malt increased with increasing air rest periods. This study established that the optimum steeping period for rice malt of Jasmine'85 is 48 hours with a 6-hour air-rest. This condition has beenfound to enhance the production of diastase in rice malt for the efficient conversion of starch into fermentable sugars.

2.3.3 Kilning

After the germination, the activities of these enzymes are terminated or halted by drying (kilning) the young plant (green malt) so the endosperms are not completely depleted through respiration of the embryo and its growth. Hence, malt is the modified cereal grain resulting from induced germination under controlled conditions of moisture, aeration and temperature (O'Rourke**,** 2004). Karababa*et al*., (1993) reported that low-temperature kilning schedules produces sorghum malts with greater diastatic power and α-amylase activity. OwusuMensah, (2009) in his studies gave the maximum kilning temperature to be between 40- 45 ^{o}C.Okrah (2008) also reported kilning temperature at 50^{o}Cin an oven.

2.3.4 Malting loss

Malting loss is the material lost, as per cent dry weight in converting the grain into malt. It is incurred as a result of dry matter loss, mainly due to the growth and respiration of the embryo. It is an index of the extent of modification. Malting loss could also be attributed to the chemical changes that occurred during the malting process. The partial degradation of the high molecular weight materials in the starchy endosperm of paddy rice also accounts for the change resulting in decrease yield and increase in malting loss (Ayernor and Ocloo, 2007).

Suhasini and Malleshi (1995) reported malting losses as due to metabolic activity and separation of vegetative growth (root and shoot). In addition, leaching of minerals and other grain constituents during steeping and germination might also account for the malting losses (Ayernor and Ocloo, 2007). Malting loss increases with increase in

duration of germination (Ogundiwin and Ilori, 1991). According to Ayernor and Ocloo (2007), malting loss was highest in the 9- day malt (59.75%).

2.4 Conditions that affect diastatic power of enzymes

George-Kraemer *et al*., (2001) found enzyme activity to be a good predictor of diastatic power which is required in brewing processes and an important characteristic for estimating the quality of malt for beer production (Evans *et al*, 1995). The diastatic power gives an assessment of the saccharifying power of the malt prepared. Originally, it was considered as a relative measure of starch converting power of malt (Ayernor and Ocloo, 2007).Diastatic power has been studied in some cereals.Four different sorghum were malted at room temperature and their diastaticpowerranged between 13°L to 18°L (Owuamaand Adeyemo, 2009). The diastatic power of sprouting acha, maize, rice and sorghum respectively were $16.6 \pm 0.02x10^{-2}$, 14.4 ± 0.06 X 10^{-2}, 11.0 ± 0.04 X10^{-2} and 14.9 ± 0.09 X 10^{-2}mg glucose per minute respectively (Egwim and Oloyede, 2006).There are some conditions that affect diastatic power of enzymes during hydrolysis of starch. These include substrate concentration, pH, temperature and time.

2.4.1 Effect of substrate concentration on enzyme activity

The effect of substrateconcentrationofstarch (0.5%w/v-3.5%w/v) on alpha -amylase activity of Nigerian cereals; maize, acha, rice and sorghum was investigated byEgwimandOloyede, (2006).Enzyme activity increased with an increase in substrate concentration. This is because maximum speed of an enzymatic reaction is reached when the substrate concentration is increased until a constant rate of product

formation.As substrate concentration [S] increases, more of the free enzyme is converted into the substrate-enzyme bound ES form. At the maximum speed (Vmax) of the enzyme, all the enzyme active sites are bound to substrate and the amount of ES complex is the same as the total amount of enzyme. However, at low substrate concentrations, the rate is proportional to the substrate concentration and this shows that there is a linear relation between [S] and the rate. But at higher substrate concentration, the velocity no longer increases with increasing substrate concentration. When the maximum rate is attained, there will be no increase in rate despite increases in the substrate concentration. This at the end results in a hyperbolic curve for the interaction due to the formation of the intermediate E-S complex. In Michaelis- Menten kinetics, the rate of the enzymatic reaction is proportional to the concentration of the ES complex which implies that if the substrate concentration is increased at a fixed level of enzyme concentration, the rate of reaction will be increased as more E-S complex is formed (Copeland, 2000; Nsiah, 2005).The highest enzyme activity was therefore found at substrate concentration of 3.5%w/v for maize, rice and acha (EgwimandOloyede, 2006).

2.4.2 Effect of temperature on enzyme activity

Enzymes work at a range of temperature specific to the organism. When there is an increase in temperature, the reaction rates virtually increases, due to the increase in the kinetic energy of the reactant molecules which allows an increased proportion of the reactants to be able to attain the activation energy to make effective collisions. The effect of varied temperature condition on the amylase activity of the crude extract of rice was studied by Afuikwa*etal*., (2009). The result showed thatthe optimum temperatureof

crude extract of rice is 60°C.Three types of locally available beans such as; green mung beans, black mung beans and soya beans and three types of common cereals such as paddy, glutinous rice, and maize were malted and their amylase activity studied at 30°C, 60°C, 70°C, 80°C, and 90°C.Enzyme activity was low at lower temperature and increased as the temperature increased. Their activity was highest at 60°C (Khin*et al.*, n.d.).The optimum temperature for a-amylase activity was 60°C for maize and rice while that of acha and sorghum was 70°C (Egwim and Oloyede, 2006). Further increase in temperatures led to an abrupt decrease in reaction rates (Copeland, 2000,Schweiger*et. al*., 2007). This is because enzymes are protein in nature and they denature at higher temperatures, resulting from the breakdown of the weak ionic and hydrogen bonding that stabilizes the three dimensional structure of the active site (Daniel*et al*., 2010). Some amino acids are arranged in a three dimensional conformation at the active site. These are present in the tertiary or quaternary structures of the protein which are held by weak forces. Consequently, these forces holding the overall structure of the enzyme in addition to that of the appropriate juxtaposition of amino acids at the active site will be disrupted if the temperature is raised too high. This reduces or completely stops the catalytic activity of the enzyme. Therefore higher temperatures affect the rate of breakdown of the ES complex or the substrate enzyme affinity (Cornish 2004; Nsiah, 2005).

2.4.3 Effect of pH on enzyme activity

pH is a measure of the concentration of hydrogen ions in a solution. Most enzymes function efficiently over a narrow pH range (Copeland, 2000). The activity of an enzyme is however affected by the changes in pH either above or below its range. This is due to the fact that changes in pH affects the state of ionization of the component amino acid residues and leads to the breaking of the ionic bonds that hold the tertiary structure of the enzyme in place. This causes the enzyme to lose its functional shape, particularly the shape of the active site, such that the substrate will no longer be able to bind resulting in decrease in activity. However when the proper ionic form of the amino acid residues are created, there will be the proper maintenance of the active site conformation as well as the proper binding of the substrate which will enhance activity. The effect of varied pH on the amylase activity of rice was studied by Afuikwa*etal*., (2009) which showed that the amylase activity initially increased with increase in pHuntil it reached its optimum at about pH 6.0. Beyond this pH value, the activity declined progressively. Local Saudi Arabian wheat (*Triticumaestivum*) variety was malted and the pH optimum for α-amylase activity was determined using buffers ranging in their pH values from 2.0-9.0. From the results it was revealed that the enzyme exhibited high activity at pH 5.0. Increasing or decreasing the pH resulted in decrease in the activity of the enzyme in the pH range (Saleh*et al*., 2009). The optimal pH for the activity ofalpha-amylase from sprouting maize, rice, acha, and sorghum were exhibited at 6.5, 5.5, 6.5 and 5.8 respectively(EgwimandOloyede, 2006).When extremes of pH are used, there is the inactivation of the protein structure due to denaturation (Cornish, 2004 and Nsiah,2005).

2.4.4 Effect of time on enzyme activity

During an enzyme catalyzed reaction, the loss of substrate increases as the time increases, these results in the increase in the formation of products. In the initial stages when the time is minimal, substrate loss and product appearance change rapidly over with time, however as time increases these rates diminish reaching zero when all the substrate has been converted to product by the enzyme (Copeland, 2000).Different incubation periods were used to test the optimum incubation period for fourdifferent sweet sorghum varieties. From the results, it was observed that as time of incubation increased reducing sugars produced also increased. Therefore maximum activity took place at the highest incubationperiod of 24hours (Mesta, 2005**)**.

2.5 Conclusion on Literature

There has been little work done on the diastatic activity of new rice varieties in Ghana though among these new varieties, there are some whose malts possess beneficial qualities for the sugar and brewing industries. Therefore there is the need to explore these varieties especially as a cheap source of glucose for the confectionery and pharmaceutical industries (Egwim, 2004). This study will investigate the effect of pH, temperature, starch concentration and time on the enzyme activity as well as the activity of the crude diastase extract on cassava starch.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Collection and preparation of rice grains

Ten different viable varieties with eight crossed varieties of rice namely: *Jasmine '85, Sikamo, Bouake 189, ITA 320, ITA 324, Marshall, China, Viwornor* and two viable local varieties: *Wita 7* and*Digang* were obtained from the Council for Scientific and Industrial Research (CSIR), Crop Research Institute (CRI) Fumesua, Kumasi, Ghana. The rice grains had been harvested and stored for exactly one year.Samples of each variety of rice grains were screened to remove broken grains and foreign materials such as stones, dusts and plant parts.

3.1.2 Chemicals

All the chemicals used for analysis were analytical grade purchased from Sigma – Aldrich.

3.2 METHODS

3.2.1 Steeping of rice grains

Three replicates, 1.5 g each of the different varieties of rice grains were soaked separately each in 50-100ml of 5% sodium metabisulphite for 20 seconds respectively to eliminate microorganisms which may retard germination. Thereafter, the grains were

washed with water several times to remove residual sodium metabisulphite. The replicateswere then steeped each in 200 ml of distilled water for 48 hours at room temperature of 28 ±1 ^{o}C. The steep water was changed every twelve hours to prevent the growth of microorganisms.

3.2.2 Malting and kilning of rice grains

The soaked grains were placed on pieces of jute sacks in Petri dishes and incubated at 28 ±1^{o}C. The jute sack pieces were moistened at regular intervals of 12 hours with 5 ml of distilled water to avoid drying and to maintain adequate moisture content. Germination was allowed to proceed and stopped on the 8th day by freezing. During kilning, the malted rice grains were dried with warm air in a forced air conventional oven at 40^{o}C for 5 hours. The dried malted grains together with their plumule and radicle were then ground in Bruder blender (BR-333, Korea), sieved, packed and labeled in cellophane bags pending further analysis.

3.2.3 Preparation of rice malt extract (RME)

Accurately weighed mass of 0.75 g of each of the ground rice malt was put into centrifuge tubes and 4ml of sodium phosphate buffer (pH 8) was added into each whilst mixing. The mixture was then allowed to stand for 30 minutes with intermittent stirring after which it was centrifuged (MSE Mistral 3000E, UK) for 10minutes at 2000 x g. The supernatants were filtered through wet filter paper (1001110) into measuring cylinders and the volumes were recorded. The final liquid was diluted by a factor of 50 using 0.1M sodium phosphate buffer (pH 5.5) and used as RME in all the experimental runs.

3.2.4 Determination of Out-of-Steep Moisture Content (SMC)

The soaked grains were also analyzed for SMC according to the Association of Official Analytical Chemist approved method (AOAC, 1990). In this method,two grams each of the pre-soaked rice grain samples was weighed using a laboratory electronic balance (Ohaus, model; AS260D, USA) into a previously dried and weighed glass crucible. The glass crucibles with the samples were placed in a forced air oven (Genlab, model; N53EF, England) for five (5) hours at a temperature of 105^{o}C. The glass crucibles together with its content were weighed again after cooling in a dessicator. The differences in weights were used for the calculation of the SMCs mean values as shown in appendix A.

3.2.5 Determination of steeping losses

Steeping losses of the soaked grains were determined according to the protocol given by Hammond and Ayernor (2001). The determination was carried out by accurately measuring the dry weight of the grains before and after soaking at 105^{o}C for 5hours. Two grams of each variety of the rice grains were dried before and after soaking in 200ml distilled water for 48 hours. The differences in the dry weights were divided by the initial dry weight, and multiplied by 100 to give the percentage of weight.

3.2.6 Determination of grain viability

The grain viability tests were run in order to perform related tests such as germination energy, rate of emergence and mean germination time (MGT). Three replicates of 1.5g of each variety of rice grains were soaked for 48 hours in distilled water. The soaked grains were placed in Petri dishes lined with Whatman's (1001/110) filter paper. Germination was carried out in the dark at a temperature of 28 $\pm 1^{o}$C for eight days during which about 5ml of distilled water was sprinkled onto the grains daily to maintain adequate moisture content. Counts of germinated grains were taken daily and the germination energy was calculated as the ratio of germinated grains to the total grains.

The rate of emergence and the mean germination time used were also calculated for the first 24 and 48 hours. From the counts, the mean germination time (MGT) was calculated using the formula given by Bam *et al*., (2006) and the rate of emergence was deduced as the inverse of the MGT.

$$\text{MGT (days)} = \frac{\sum nidi}{n}$$

Where; ni = number of seed germinated on day, di = day during germination period, n = total number of grains germinated during the experimental period.

3.2.7 Determination of shoots and root length

The shoot and root lengths of ten seedlings per replicate of each of the variety of rice grains were measured with a measuring rule. The average lengths were calculated as the mean of three shoots or roots (Appendix A).

3.2.8 Determination of malting loss

Malting loss after each germination period was determined by taking the weight of 50 grains before malting, after malting and after drying. Malting losses were calculated using the difference in dry weights divided by the initial dry weight and expressed in percentages.

3.2.9 Experimental design

The diastatic activity conditions were optimized using response surface methodology. The optimization for the diastatic activity focused on four important parameters: substrate concentration (0.5 – 3%w/v), temperature of incubation (10 – 60^{o}C), pH of buffer (2- 9) and time of incubation (5 – 15min). The composite design was chosen to evaluate these four parameters. The combination of the process variables provided 25 experiments(table 3.1) as the experimental design by using the design expert.

Table 3.1; Process variables used in the central composite design for the diastatic power study of ten varieties of rice

Run	A:pH	B:starch slur% w/v	C:Temp ^{o}C	D:Time/mins
1	2.0	3.0	35	5.0
2	2.0	3.0	60	10.0
3	2.0	0.5	60	5.0
4	2.0	3.0	60	5.0
5	2.0	3.0	60	10.0
6	2.0	3.0	10	15.0
7	2.0	0.5	60	15.0
8	2.0	3.0	10	5.0
9	2.0	3.0	10	15.0
10	2.0	0.5	60	15.0
11	2.0	1.75	60	5.0
12	2.0	0.5	10	5.0
13	5.5	1.75	35	10.0
14	5.5	1.75	35	10.0
15	5.5	3.00	60	15.0
16	5.5	0.5	10	15.0
17	5.5	3.0	10	5.0
18	5.5	3.0	60	15.0
19	9.0	3.0	10	15.0
20	9.0	0.5	35	15.0
21	9.0	0.5	10	5.0
22	9.0	3.0	60	5.0
23	9.0	1.75	60	15.0
24	9.0	0.5	60	5.0
25	9.0	3.0	10	5.0

Five percent (5%) of the cassava starch of moisture 10.2 % was gelatinized and different concentrations (0.5, 1.75 and 3.0% w/v) hydrolyzed using 0.05 mL appropriately diluted RMEs prepared from the ten different varieties of rice. The hydrolysis was carried out in a thermo-regulated bath (Grant, model; SBB14, UK) according to the preparations shown in table 3.1. The reaction was terminated by adding 2mL of 0.1M NaOH after which 1 mL of freshly prepared 3, 5-dinitrosalicylic acid solution was added and the

mixtures heated at 80^{o}C for 5minutes and the absorbance read from the spectrophotometer at 480nm. Diastatic activity of each variety was calculated for each set of parameters.

3.2.10 Statistical analysis

Malting parameters

Data obtained from the malting parameters were subjected to one-way ANOVA to determine significant differences between treatment means at $p<0.05$ using Microsoft excel (2007). Correlation was then used to study the effect of the varied conditions; pH, temperature, substrate concentration and time on enzyme activity.The variety with the highest amylase activity was then selected from the data.

Selection of optimum conditions

Using the experimental design (table 2) from the design expert, the response data were subjected to the statistical tool Simca-p (2007) using the Principal Component Analysis model. The statistical tool was used to select the optimum temperature, pH, substrate concentration and time of amylase activity for the ten different varieties of rice. The optimum conditions were then verified by response surface plots for a selected variety.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 MALTING PARAMETERS

4.1.1 Out-of-Steep Moisture Content and steeping losses of the rice grains

Table 4.1 shows theout-of-steep moisture content for the ten rice varieties. An out-of-steep moisture content (OSM) of 30.07% was observed for *Wita7* as the lowest whiles *Bourke' 189*had the highest OSM of 35.26%.

Table 4.1: Out-of-Steep Moisture Content of the different rice grains

Variety	OSM%
Wita 7	30.07± 0.02
Digang	31.57±0.01
Jasmine'85	34.98±0.01
Sikamo	31.85±0.01
ITA 320	34.86±0.02
ITA 324	31.42±0.004
Bouake'189	35.26±0.003
Marshal	33.12±0.01
Viwornor	33.16±0.01
China	33.84±0.01±

An increase in OSM increases germination such that the right modification of the endosperm structure will progress at an increasing rate producing malt of the desired

quality. An out-of-steep moisture content above 30% is good enough for modification (Smart *et al*., 1993). Statistically, there were significant differences between OSMof the different varieties ($p=3.17x10^{-18}$). This result is comparable to thatobtained by Owusu-Mensah (2009). Ogu*et al*., (2006) also had an out-of-steep moisture ranging between 33-35% for different varieties of sorghum. An out of steep moisture content of 43–46% was alsoobserved for the barley grain. The reason for the differences can be attributed to the fact that each grain differed in shape, size, nitrogenous content and initial moisture content (Brookes *et al*., 1976). Apart from the grain property, the differences in the kind of cereal, cultivar as well as the temperature used also had an influence on the out-of-steep moisture content (Ulaiwan*et al*., 2009**).**

Sikamo had the lowest steeping loss of mean value 2.41% with the others ranging between 3-7%. *Bourke'189* however had the highest mean value (table 4.2). Steeping losses for barley are reported to fall within the range of 0.5- 1.5% of the initial dry weight of the grains (Briggs, 1998).Statistically, there were significant differences between the steeping losses of the different varieties ($p=3.17x10^{-18}$). The results obtained in the study were also higher than that reported by Owusu-Mensah (2009). This was due to the fact that testinic acid, mineral salt such as phosphates, amino acids, simple sugars and phenolic acids which are also dissolved substances might have leached out of the grain into the steep water when the grains were steeped (Briggs, 1998).

Table 4.2; Steeping losses of the ten rice varieties

Variety	Mean values%
Wita 7	2.97±0.007
Digang	7.30±0.007
Jasmine'85	5.44±0.01
Sikamo	2.41±0.008
ITA 320	3.91±0.006
ITA 324	3.70±0.005
Bouake'189	7.60±0.01
Marshal	5.34±0.005
Viwornor	5.23±0.004
China	3.44±0.004

Bam *et al*., (2006) reported that phosphate is among the essential constituents of metabolic enzymes that catalyses germination processes and also influences early seedling growth. Materials that leached out also support the growth of microbes that compete with the grain for oxygen. The microbes produce acidic metabolites while consuming oxygen which certainly interfere with germination and modification of the malt thereby hampering its final quality (Briggs, 1998).

4.1.2 Germination energy of the rice grains

The germination energy for the different varieties from day 1 to day 8 is shown in figure 4.1. Germination of grains was observed on the 1st day of malting after 48 hours of steeping. Most of the varieties had their maximum seed germination on day 4. *Bourke' 189* and *Marshal* however obtained the highest number of ungerminated grains.

*Viwornor*had the highest energy of 98.2% with *Marshal* having the lowest energy of 85.8%.

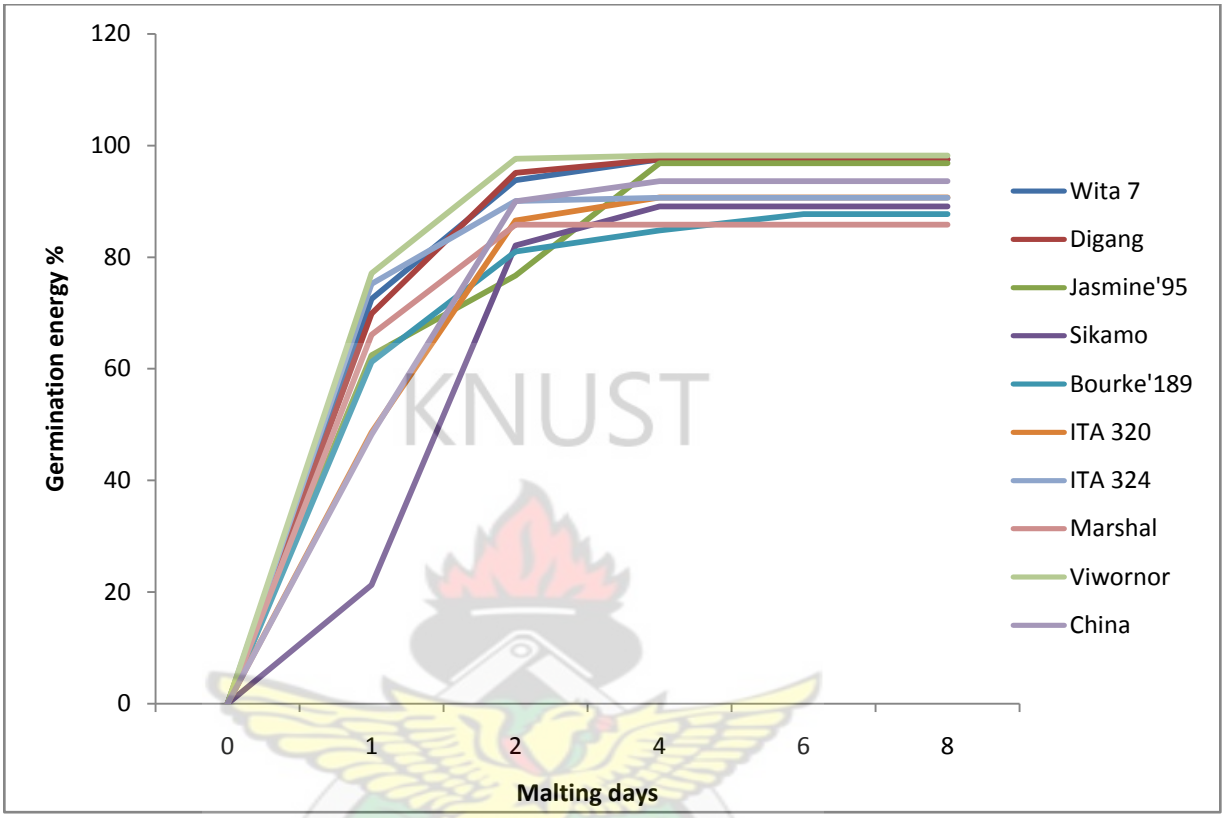

Fig.4.1; Germination energy of the ten varieties of rice

At the end of the germination period, most of the grains had their germination energy above 85.0%, thus these grains had the potential of producing good quality malt (Agbale*et al*., 2007).

Votong and Vonone (1976) have shown that cereal germination of up to 80% is recommended as viable. Statistical analysis showed significant differences between the germination energies of the different varieties ($p=7.12x10^{-23}$). Egwim and Oloyede (2004) also obtained similar results; 96% for maize, 98% for Acha, 98% for rice and

97% for sorghum whiles Eneje*et al*., (2004) obtained 95% for white maize and 97% for yellow maize.Owuama and Adeyemo (2008) also obtained 91- 97% for the different varieties of sorghum. These differences in germination energies could be probably due to the differences in storage conditions and germination temperature (Novellie, 1962).

4.1.3 Mean Germination Time (MGT) and Rate of Emergence (Rm)

The MGT ranged between 1.155 and 1.604 among the rice grains. *Sikamo* had the highest value of 1.604 whiles the other grains were within the range (A-B)(Figure 4.2). Mean germination time (MGT) directly expresses the rapidity of the germination (Munesh*et al*., 2007) and the time it takes to get 50% emergence (Bam *et al*., 2006). It also represents the vigor levels present in grains.

Rate of emergence (Rm) is calculated as the inverse of MGT. Rm ranged between 0.623 for *Sikamo* and 0.866 for ITA 324. Statistically, the variations in the MGT and the Rm of the different varieties of rice grains were significant ($p=4.54x10^{-5}$)due to the differences in vigor levels present in the grains.

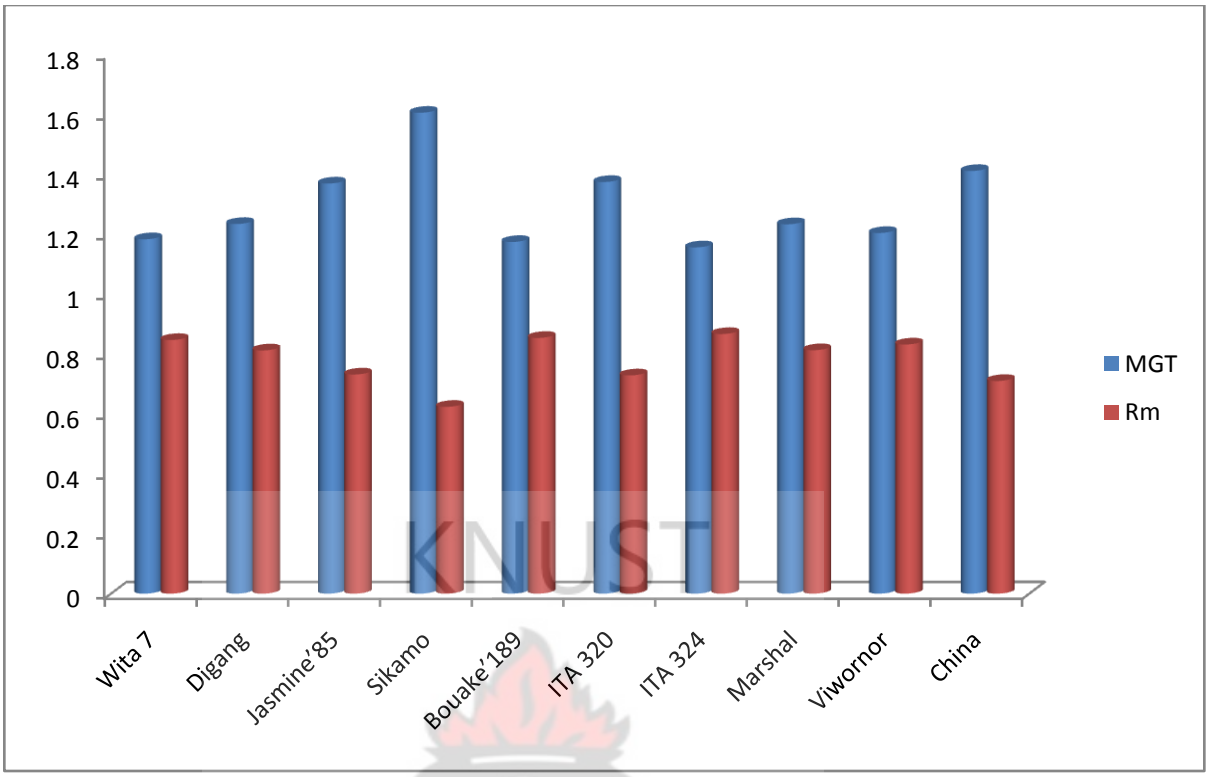

Fig. 4.2 Mean germination time (MGT) and Rate of emergence (Rm) of ten varieties of rice

4.1.4 Shoot and root length development of rice grains

The development of the shoot and root are shown as the germination advances in Figure 4.3 and 4.4. Growth development of the various rice grains could be observed after rice grains were steeped for 48 hours. The figures show the development of the shoot and root as the germination advances. Growth development of the various rice grains could be observed after rice grains were steeped for 48 hours. Whilst in steep, the grains started to chit. Between the 1st and the 2nd day of the malting period, one rootlet and a cotyledon erupted from the embryo and appeared outside the grain for most grains. The shoots and roots developed slowly as the germination time increased.

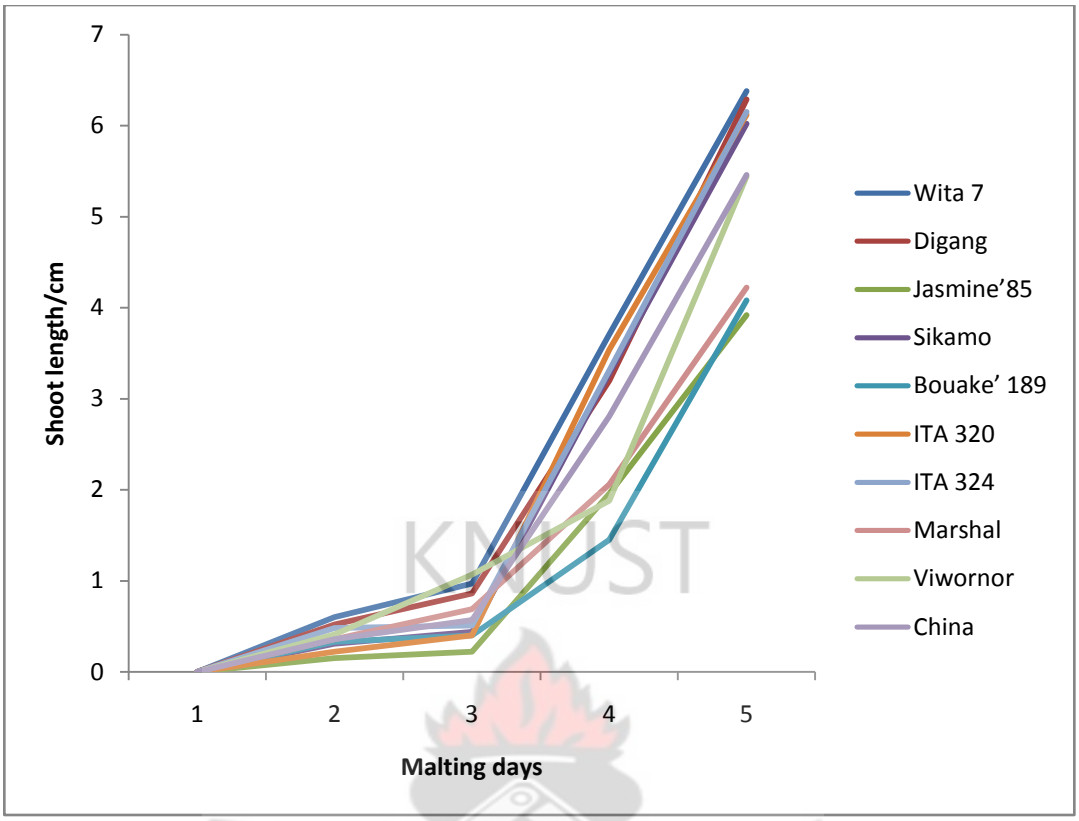

Fig.4.3 The effect of malting days on the shoot length of the ten varieties of rice

However, there was rapid growth between the 3rd and the 8th day. The shortest shoot length for all the varieties were observed on the 2nd day of the malting period with the longest at the end of the malting period, day 8. Shoot and root length thereby increased with germination time (Hammond and Ayernor, 2001).

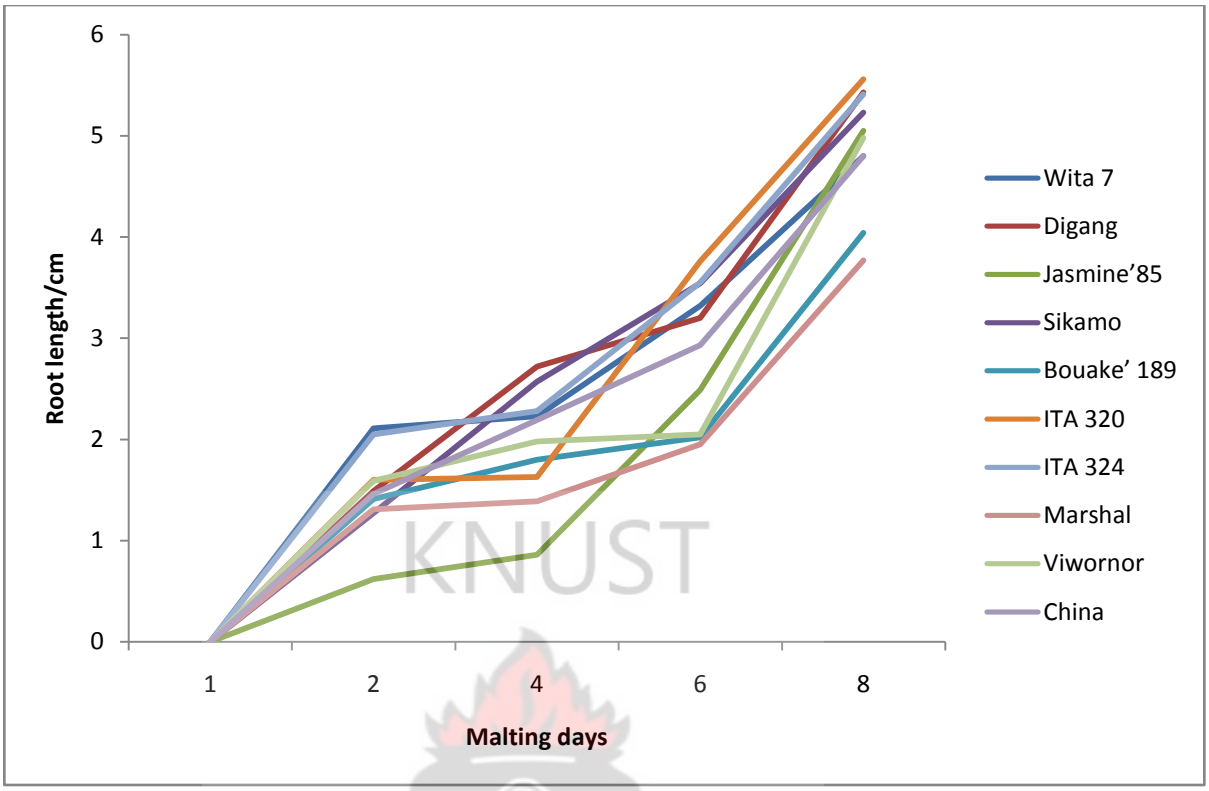

Fig.4.4 The effect of malting days on the root length of the ten varieties of rice

The longest shoot length of 6.38 cm was observed for *Wita 7*. The other varieties had their shoot length ranging from 4–6 cm at the end of the malting period. There were significant differences between the shoot ($p = 1.79x10^{-25}$) and root length of the varieties ($p = 1.32x10^{-22}$) due to the fact that the varieties were different from each other and therefore differed in germination. This is similar to results obtained in earlier studies by Pelembe*et al*., (2002).

4.1.5 Malting loss of rice malt

The malting losses of the different varieties of rice are depicted in Figure 4.5. *Jasmine'85*had the lowest of 25.9% with *Digang* recording the highest among all the

varieties of about 55.0%. The lowest average loss obtained was 0.8% on day zero due to the chitting of the grains whilst in steep.

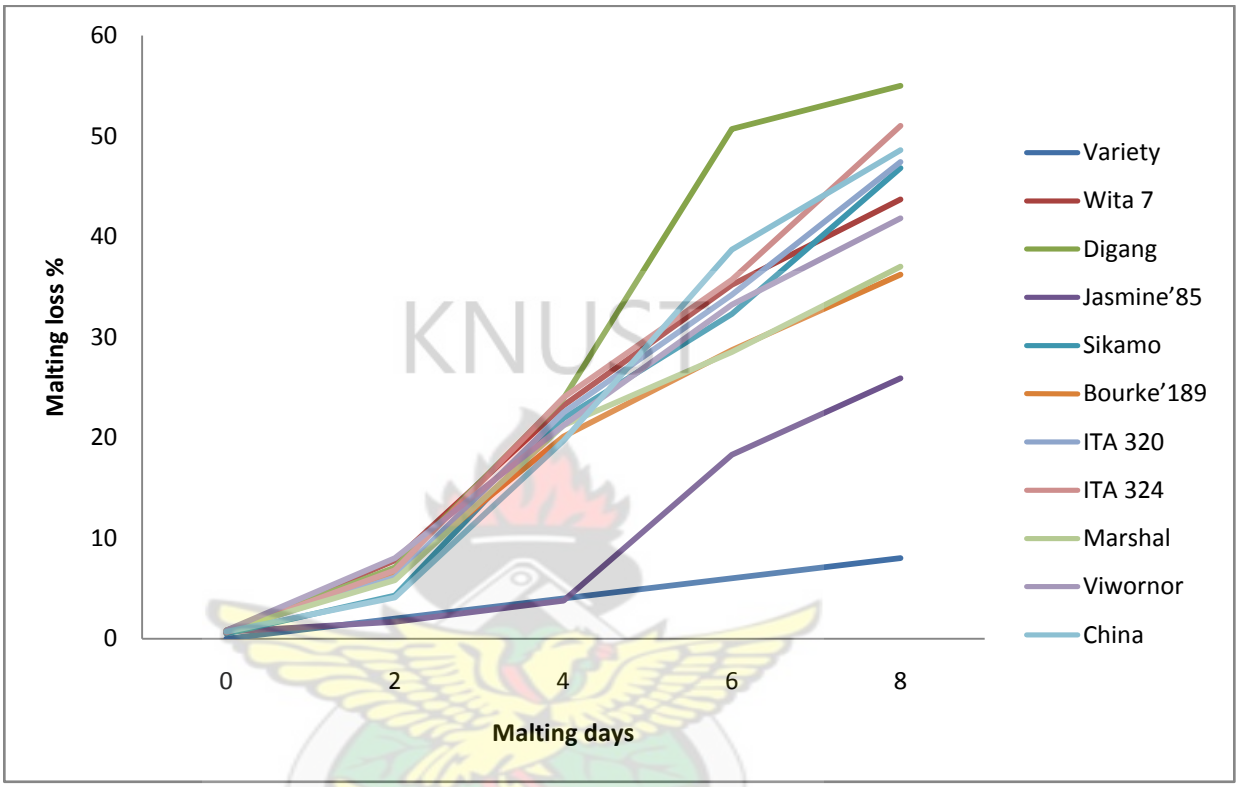

Fig 4.5. Malting losses of ten varieties of rice

The highest malting loss obtained for the different rice varieties was on the 8th day of germination. This could be as a result of dry matter loss, mainly due to the growth and respiration of the embryo. Other factors include leaching of compounds during steeping from the grain, respiration of the grain and the fermentative processes that are involved during malting (Smart *et al*., 1993; Wijgnaard*et al*., 2005). Therefore, the longer the duration of malting the smaller the residual starch (Ayernor and Ocloo, 2007). Malting losses are an outcome of respiratory metabolism and therefore has an effect on the final

malt quality (Dewar *et al*., 1997). The results were similar to that reported by Ayernor and Ocloo (2007); a malting loss of 59.75% was obtained at the end of the germination for paddy rice whiles Awoyinka and Adebawo (2008) obtained a different trend for malting Nigerian amylolytic maize cultivars, barley, red and white sorghum. Palmer *et al.*, (1989) also reported malting losses of 15-20% in sorghum, compared to 7% in barley. Another trend was observed by Owuama and Adeyemo (2008). They obtained a malting loss of 7.1 and 10.6 for two different varieties of sorghum. The differences in malting loss among the different varieties however were statistically significant ($p=6.0x10^{-21}$). These differences in malting loss were due to the differences in variety, cultivar, different malting conditions such as steepingandgerminationperiod and temperatures.

4.3 Variation in pH, substrate concentration, temperature, and time on amylase activity

The effect of varying these parameters such as pH, substrate concentration, temperature and time on amylase activity was done using correlation.Thedata from the experiment (table 3.1) wereanalysed with simca-p graphical software for general purpose multivariate data analysis. This software was used to obtain the optimum pH, temperature, substrate concentration and time for all rice varieties.

Table 4.3; Correlation of pH, substrate concentration, temperature and time on diastatic activity of the ten rice varieties

	pH		sub conc		temp		time	
Variety	corr.	p	corr.	p	corr.	p	corr.	p
Wita_7	0.24	0.24	0.19	0.36	0.71	0	-0.09	0.66
Digang	0.34	0.1	0.29	0.17	0.57	0	-0.16	0.44
Sikamo	0.26	0.22	0.16	0.46	0.72	0	0.02	0.94
Jasmine	0.26	0.21	0.14	0.5	0.67	0	-0.18	0.39
Bourke	0.3	0.15	0.22	0.3	0.66	0	-0.14	0.52
ITA_320	0.39	0.05	0.22	0.28	0.57	0	-0.15	0.48
ITA_324	0.26	0.21	0.17	0.42	0.68	0	-0.17	0.43
Marshal	0.29	0.16	0.18	0.39	0.66	0	-0.17	0.43
Viwornor	0.39	0.06	0.2	0.34	0.46	0.02	-0.15	0.48
China	0.3	0.15	0.16	0.46	0.7	0	-0.12	0.55

corr. = correlation; p = significance

4.3.1 Effect of pH variation on diastatic power of varieties

Generally, the diastatic power increased with increasingpH of the varieties with a positive correlation however the correlation for all the varieties was weak as shown on table 4.3 except for ITA 320 which had a strong positive correlation between diastatic power and pH ($r= 0.389$, $p<0.005$). Enzyme activity increases over some pH range to an optimum pH range. Beyond this range, results in the decline in activity of the enzyme which may be due to the improper ionic form of the enzyme and the inactivation of the protein structure due to denaturation especially when extremes of pH are used (Nsiah, 2005).

4.3.2 Effect of substrate concentration variation

Increase in substrate concentration had an increase in diastatic power of the varieties however these were not strongly correlated as shown on Table4.3. Generally, diastatic power increases with increase in starch concentration. This is probably because as substrate concentration increases more of the active sites of the enzyme are being occupied which results in more reactions until enzyme saturation occurs.

4.3.3 Effect of temperature variation

Temperature had an effect on the diastatic power of the various varieties of rice. An increase in temperature had an increase in diastatic power. The correlation between the diastatic power of the varieties and temperature was strongly correlated as shown on Table 4.3. Generally, increase in temperature increases diastatic power to an extent.This is probably due to the fact that an increase in temperature provides more kinetic energy to the molecules involved which increases the numbers of collisions between enzyme and substrate resulting in an increase in the rate. However beyond a certain temperature, the activity of the crude extract will decrease sharply. This may probably be accounted for by denaturation of the enzyme protein at higher temperature. This leads to loss of activity (Schweiger*et al*., 2007) as a result of the breaking of the bonds holding the structure resulting in the loss in shape of the active site.

4.3.4 Effect of time variation

Increase in time produced a decrease in the diastatic power of the varieties with the exception of *Sikamo* which was positively correlated; however the correlation was weak as shown in Table 4.3 below.

4.4 Optimum conditions for diastatic activity of rice varieties

The results from the principal component analysis to select optimum conditions for diastatic activity are shown graphically in Fig 4.6. A pH range of 2.0, 5.5, and 9.0 was chosen for the study. Though there were variations in pH among the varieties, on the average pH of 5.5 (the longest among the three bars) which is represented by the middle green bars was the optimum pH chosen for all varieties of rice.

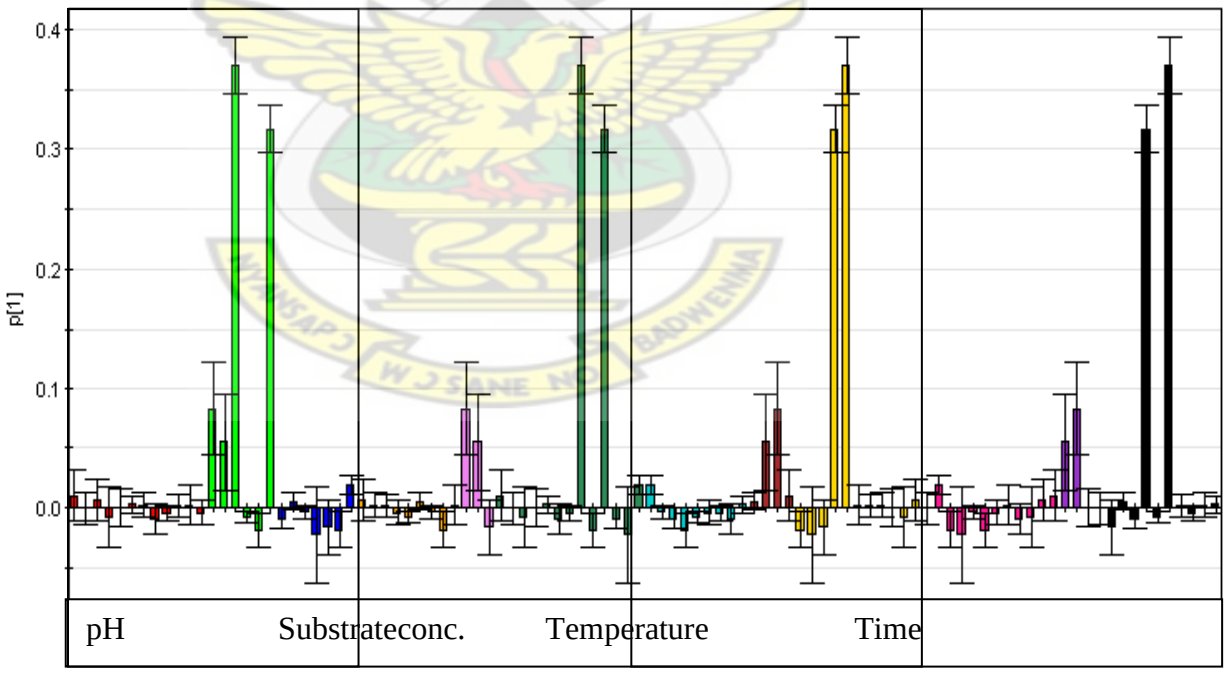

Fig. 4.6The variation of pH, substrate concentration, temperature and time

Egwim and Oloyede (2004) andAfuikwa*et al*., (2008) obtained a similar trend of optimum pH of 6.0. This conforms to that used in literature for starch hydrolysis using amylase from rice malt.

A range of 0.5%w/v, 1.75%w/v and 3.0%w/v was chosen for the substrate concentration. The results indicated that on the average a starch concentration of 3.0w/v% (the longest among the three bars) was the optimum for all varieties of rice.

Optimization was carried out by incubating the samples at temperatures of 10, 35 and 60°C.The longest group of yellow bars represents activity at 60°C.This represents the optimum temperature obtained for all the varieties of rice. The trend obtained above is similar to that obtained by Afuikwa*et al*., (2008), they obtained the optimum temperature of 60^{o}C for amylase. Egwim and Oloyede (2004) also obtained the same trend of 60^{o}C optimum temperature for paddy rice malt.The last black bars represent the optimum time of 15mins of rice varieties.

The results obtained also showed that*Viwornor* variety had the highest diastatic activity, closely followed by *China and Wita 7*.

4.5Validation of optimum conditions on *Wita 7*

The diastatic activity of *Wita 7* was studied under conditions of pH, temperature, starch concentration and time. The effect of these conditions on the diastatic activity is shown in the response surface plots in figures 4.7 and 4.8. The results obtained were found to validate the optimum conditions determined by principal component analysis.

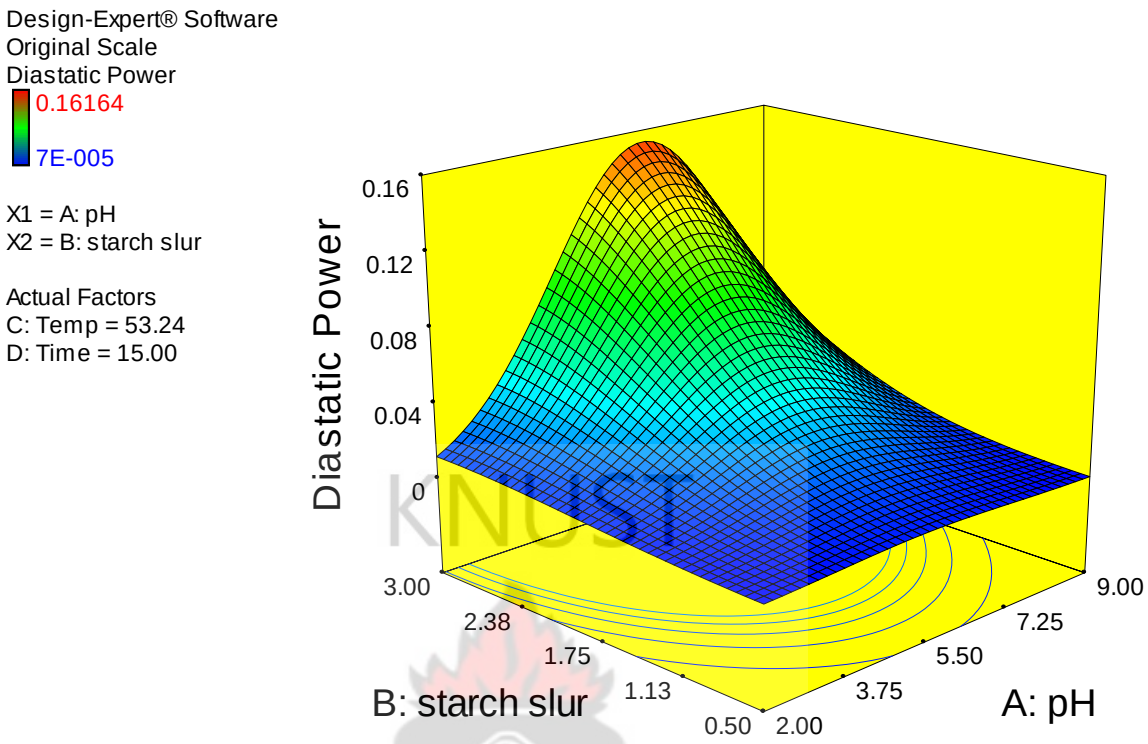

Fig. 4.7Effect of pH and starch slurry on diastatic power

The effect of varying both pH and starch slurry on the diastatic power of *Wita 7* is depicted in figure 4.7. The diastatic power increased as the starch slurry increased from 0.5% to 3.0% and with a corresponding increase in pH to 6.3. The enzyme was found to show optimum activity under slightly acidic conditions.

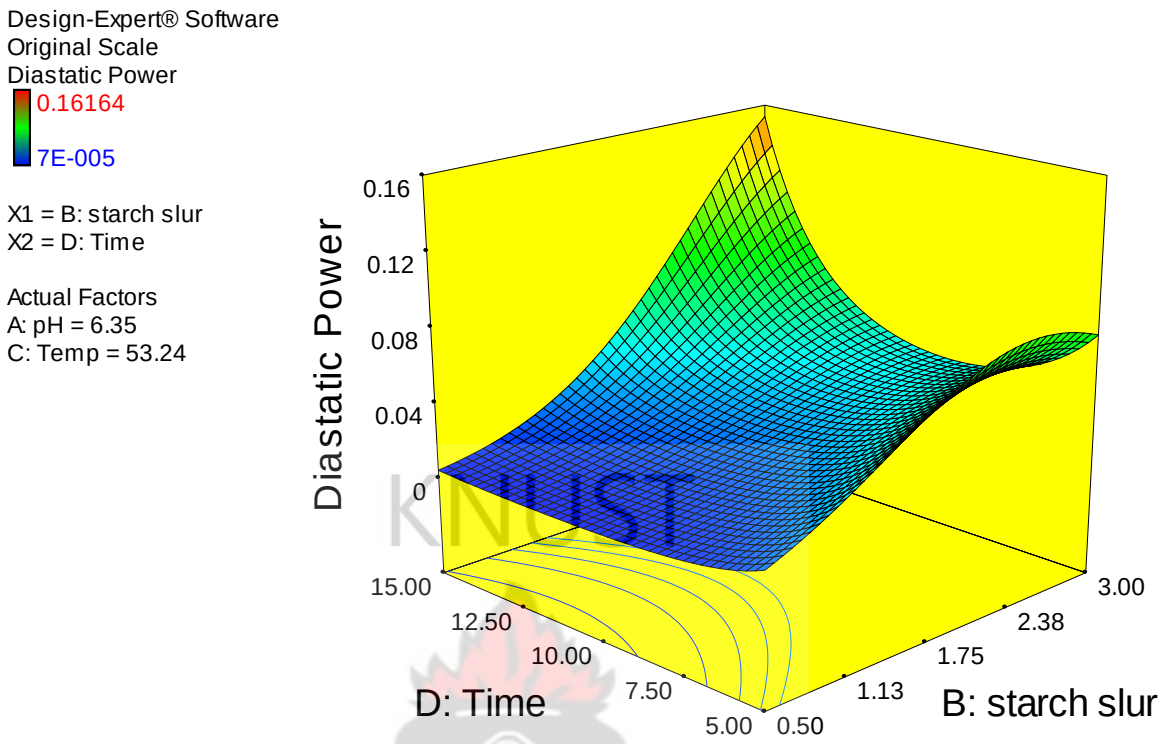

Fig. 4.8 Effect of time and starch slurry on diastatic power of *Wita 7*.

The effect of varying both starch concentration and time on the diastatic power is shown in figure 4.8. The highest diastatic power was obtained at time of 15 mins and starch concentration of 3.0 %w/v. Thus optimized conditions were at starch concentration of 3.0w/v, pH of 6.3, temperature of 53.,24^{o}C, and time of 15mins with the highest diastatic power obtained at 1.41µmol/min. This trend is not similar to that obtained by Afuikwa*et al*., (2008), they obtained the optimum pH for diastase in general to be 6.0 with optimum temperature of 60^{o}C. Egwim and Oloyede (2004) also obtained an optimum pH of 5.5, and an optimum temperature of 60^{o}C for paddy rice malt. This slight difference may be due to difference in variety of rice.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

At the end of this study, all the varieties had good malting properties. Out-of-steep moisture of above 30% was obtained for the varieties which is good enough for modification to take place. Most of the grains had germination energy of above 85.0% at the end of the germination process thus these grains had the potential of producing good malt.

This study showed the importance ofpH, temperature, substrate concentration and time of incubation during enzymatic hydrolysis of these varieties. This was indicated by the effect of these conditions on the diastatic power of enzyme during hydrolysis. However, among these conditions, variation in temperature had the most significant effect on the diastatic activity of the varieties. An increase in temperature had an increase in diastatic power of the varieties. The optimum conditions determined for maximum diastatic activity in all the rice varieties werepH of 5.5, temperature of 60^{o}C, substrate concentration of 3.0%w/v and time of 15minutes.These optimum conditions were validated by response data on *Wita 7* which exhibited the highest diastatic activity at pH of 6.3, 60^{o}C, 3.0%w/v substrate concentration and 15min incubation time. *Viwornor*, a local variety exhibited the highest diastatic power due to itssaccharifying power and enzyme activity on the cassava starch along with China and *Wita 7*.

5.2 Recommendation

Malted rice could be a potential ingredient in the breweries, pharmaceutical and sugar industries. It could be used as a perfect substitute for common sugar in the beverage and pharmaceutical industries. This will be a cheap form of glucose syrups for these industries due to easy access, since these cereals are locally available.

From the study, the recommendation below can be made:

1. Further studies should be done on *Viwornor* for feasibility of the above parameters on industrial scale production for glucose sugar production in Ghana.

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APPENDIX

APPENDIX A

variety	**wita 7**	**Digang**	**Sikamo**	**Jasmine'85**	**Bourke'189**
Number of values	25	25	25	25	25
Minimum	7.0 e-005	0.00015	0.00011	0.00023	0.00011
Maximum	0.1616	0.1151	0.1555	0.1017	0.1335

variety	**ITA 320**	**ITA 324**	**Marshal**	**Viwornor**	**China**
Number of values	25	25	25	25	25
Minimum	0.00011	0.0003	0.00026	0.00015	0.00011
Maximum	0.1019	0.06704	0.1410	0.1689	0.1613

Loss of moisture content = Initial weight – Final weight

$$\text{Moisture content (\%)} = \frac{\text{loss of moisture}}{\text{Initial dry weight}} \text{ x } 100$$

$$\text{Steeping losses} = \frac{\text{Initial dry} - \text{ Final dry weight}}{\text{Initial dry weight}} \text{ x } 100$$

Table A1: Steeping losses for the 10 varieties of rice

Variety	Mean values%
Wita 7	2.97
Digang	7.30
Jasmine'85	5.44
Sikamo	2.41
ITA 320	3.91
ITA 324	3.70
Bouake'189	7.60
Marshal	5.34
Viwornor	5.23
China	3.44

Table A2: Out- of- Steep Moisture Content of the different rice grains

Variety	OSM %
Wita 7	30.07
Digang	31.57
Jasmine'85	34.98
Sikamo	31.85
ITA 320	34.86
ITA 324	31.42
Bouake'189	35.26
Marshal	33.12
Viwornor	33.16
China	33.84

Table A3: Germination energy of the 10 rice varieties

Variety	Germination energy (%) of rice malt					
	0	1	2	4	6	8
Wita 7	0	72.5	93.8	97.5	97.5	97.5
Digang	0	69.9	95.1	97.5	97.5	97.5
Jasmine' 85	0	62.4	76.7	96.8	96.8	96.8
Sikamo	0	21.3	82.1	89.1	89.1	89.1
Bouake'189	0	61.2	81.0	84.8	87.7	87.7

ITA 320	0	48.6	86.6	90.7	90.7	90.7
ITA 324	0	75.2	90.0	90.6	90.6	90.6
Marshal	0	66.1	85.8	85.8	85.8	85.8
Viwornor	0	77.1	97.6	98.2	98.2	98.2
China	0	48.2	90.0	93.6	93.6	93.6

Table A4: MGT and Rm

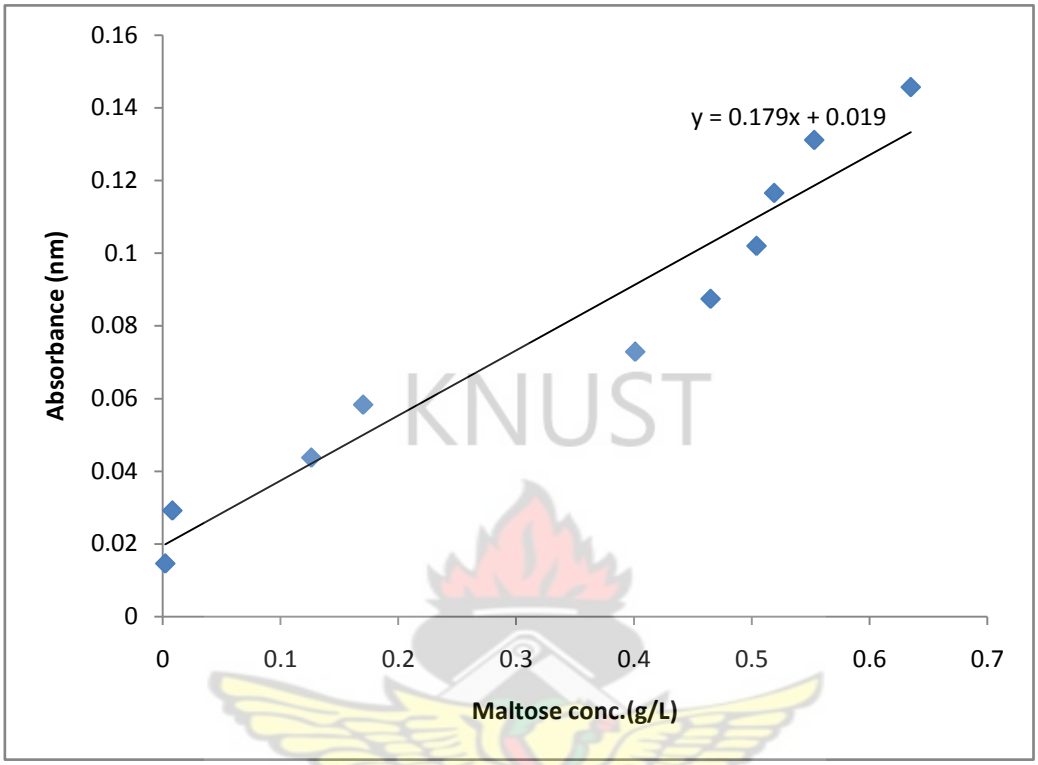
Variety	Mean MGT	Mean Rm
Wita 7	1.182	0.8460
Digang	1.233	0.8112
Jasmine'85	1.368	0.731
Sikamo	1.604	0.623
Bouake'189	1.173	0.853
ITA 320	1.373	0.728
ITA 324	1.155	0.866
Marshal	1.232	0.812
Viwornor	1.203	0.831
China	1.4097	0.7094

Table A5: Malting loss of ten varieties of rice

	Malting days (%)				
Variety	0	2	4	6	8
Wita 7	0.60	7.80	23.20	35.20	43.70
Digang	0.70	7.10	23.90	50.70	55.00
Jasmine'85	0.70	1.70	3.80	18.30	25.90
Sikamo	0.50	4.30	21.90	32.30	46.80
Bourke'189	0.8	6.50	20.10	28.70	36.20
ITA 320	0.70	6.10	22.50	34.20	47.40
ITA 324	0.60	6.80	24.00	35.70	51.00
Marshal	0.80	5.80	21.20	28.50	37.00
Viwornor	0.80	8.00	21.20	33.20	41.80
China	0.70	4.10	19.70	38.70	48.60

APPENDIX B

Standard curve for maltose standard



APPENDIX C

C1: Analysis of variance for steeping losses and out of steep moisture content of the ten varieties,

Anova: Single Factor

SUMMARY

Groups	Count	Sum	Average	Variance
Column 1	10	330.13	33.013	3.075579
Column 2	10	47.34	4.734	3.093916

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	3998.509	1	3998.509	1296.219	3.17E-18	4.413873

Within Groups	55.52545	18	3.084747			
Total	4054.035	19				

C2; Analysis of variance for germination energy of the ten varieties of rice

Anova: Two-Factor Without Replication

SUMMARY	Count	Sum	Average	Variance
Row 1	11	0	0	0
Row 2	11	603.5	54.86364	577.9805
Row 3	11	880.7	80.06364	710.5665
Row 4	11	928.6	84.41818	733.8556
Row 5	11	933.5	84.86364	702.9325
Row 6	11	935.5	85.04545	671.7507
Column 1	6	21	3.5	9.5
Column 2	6	458.8	76.46667	1498.107
Column 3	6	457.5	76.25	1512.879
Column 4	6	429.5	71.58333	1428.466
Column 5	6	370.7	61.78333	1621.49
Column 6	6	402.4	67.06667	1178.487
Column 7	6	407.3	67.88333	1378.422
Column 8	6	437	72.83333	1310.391
Column 9	6	409.3	68.21667	1178.938
Column 10	6	469.3	78.21667	1538.562
Column 11	6	419	69.83333	1489.191

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	63008.18	5	12601.64	81.68063	7.51E-23	2.400409
Columns	26256.89	10	2625.689	17.01905	7.12E-13	2.026143
Error	7713.969	50	154.2794			
Total	96979.04	65				

C3; Analysis of variance for Mean germination time and Rate of emergence of the ten varieties

Anova: Two-Factor Without Replication

SUMMARY	Count	Sum	Average	Variance
Row 1	2	2.028	1.014	0.056448
Row 2	2	2.0442	1.0221	0.088958
Row 3	2	2.099	1.0495	0.202885
Row 4	2	2.227	1.1135	0.481181
Row 5	2	2.026	1.013	0.0512
Row 6	2	2.101	1.0505	0.208013
Row 7	2	2.021	1.0105	0.041761
Row 8	2	2.044	1.022	0.0882
Row 9	2	2.034	1.017	0.069192
Row 10	2	2.1191	1.05955	0.24521
Column 1	10	12.9327	1.29327	0.020394
Column 2	10	7.8106	0.78106	0.006267

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	0.018706	9	0.002078	0.084549	0.999457	3.178893
Columns	1.311795	1	1.311795	53.36111	4.54E-05	5.117355
Error	0.22125	9	0.024583			
Total	1.551752	19				

C4; Analysis of variance for shoot length of the ten varieties

Anova: Single Factor

SUMMARY

Groups	Count	Sum	Average	Variance
Column 1	10	0	0	0
Column 2	10	3.74	0.374	0.01836

Column 3	10	6.13	0.613	0.076757
Column 4	10	27.2	2.72	0.653822
Column 5	10	54.08	5.408	0.949729

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	205.4386	4	51.35966	151.1763	1.79E-25	2.578739
Within Groups	15.28801	45	0.339734			
Total	220.7267	49				

C5; Analysis of variance for root length of the ten varieties of rice

Anova: Single Factor

SUMMARY

Groups	Count	Sum	Average	Variance
Column 1	10	0	0	0
Column 2	10	14.91	1.491	0.173899
Column 3	10	19.65	1.965	0.316383
Column 4	10	28.81	2.881	0.48921
Column 5	10	49.07	4.907	0.350046
Column 6	0	0	#DIV/0!	#DIV/0!

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	131.7761	5	26.35522	96.91167	1.32E-22	2.42704
Within Groups	11.96584	44	0.271951			
Total	143.7419	49				

C6; Analysis of variance for malting loss of the ten varieties of rice

Anova: Single Factor

SUMMARY

Groups	Count	Sum	Average	Variance
Column 1	10	6.9	0.69	0.009889
Column 2	10	58.2	5.82	3.784
Column 3	10	201.5	20.15	35.16722
Column 4	10	335.5	33.55	67.965
Column 5	10	433.4	43.34	72.06489

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	12998.09	4	3249.522	90.77332	6E-21	2.578739
Within Groups	1610.919	45	35.7982			
Total	14609.01	49				

APPENDIX D

CORRELATION ANALYSIS

D1: Summary of descriptive statistics of pH, temperature, substrate concentration and time of the varieties of rice

Parameter	Number of values	Min. VALUE	Max. VALUE	Mean	Standard Deviation	STD. ERROR	LOWER95% C.I.	UPPER95% C.I
pH	25	2.00	9.00	4.800	1.031	0.6062	3.549	2.471
Temp.	25	10	60	38.00	23.18	4.637	28.43	47.57
Sub. Conc.	25	0.500	3.000	2.000	1.141	0.2282	1.529	2.471
Time	25	5.000	15.00	9.800	4.673	0.935	7.871	11.73

D2: Summary of descriptive statistics of the varieties of rice

variety	**wita 7**	**Digang**	**Sikamo**	**Jasmine'85**	**Bourke'189**
Number of values	25	25	25	25	25
Minimum	7.0 e-005	0.00015	0.00011	0.00023	0.00011
Maximum	0.1616	0.1151	0.1555	0.1017	0.1335
Mean	0.01587	0.01370	0.01722	0.01213	0.01455
Std. Deviation	0.04057	0.02925	0.03868	0.02694	0.007130
Std. Error	0.008115	0.005850	0.007736	0.005389	0.007130
Lower 95% CI	-0.000880	0.001625	0.001255	0.001013	-0.0001622
Upper 95% CI	0.03262	0.02577	0.03319	0.02326	0.02927

D3: Summary of descriptive statistics of the varieties of rice

variety	**ITA 320**	**ITA 324**	**Marshal**	**Viwornor**	**China**
Number of values	25	25	25	25	25
Minimum	0.00011	0.0003	0.00026	0.00015	0.00011
Maximum	0.1019	0.06704	0.2410	0.1689	0.1613
Mean	0.01418	0.01019	0.02343	0.01943	0.01758
Std. Deviation	0.005492	0.003844	0.01198	0.008887	0.008790
Std. Error	0.005492	0.003844	0.01198	0.008887	0.008790
Lower 95% CI	0.002846	0.002259	-0.001294	0.001086	-0.000559
Upper 95% CI	0.02551	0.01813	0.04815	0.03777	0.03572

D4: Summary of descriptive statistics of pH of the varieties of rice

Spearman's pH	Wita_7	Digang	Sikamo	Jasmine	Bourke	ITA_320	ITA_324	Marshal	Viwornor	China
Correlation coefficient	0.243	0.342	0.257	0.259	0.297	0.389	0.262	0.289	0.388	0.300
Sig.(2-tailed)	0.243	0.095	0.215	0.211	0.150	0.054	0.205	0.161	0.055	0.145

D5: Summary of descriptive statistics of substrate concentration of the varieties of rice

Spearman's substrate concentration	Wita_7	Digang	Sikamo	Jasmine	Bourke	ITA_320	ITA_324	Marshal	Viwornor	China
Correlation coefficient	.191	.286	.155	.143	.216	.224	.170	.180	.198	.156
Sig.(2-tailed)	.362	.165	.460	.497	.299	.281	.416	.388	.343	.457

D6: Summary of descriptive statistics of temperature of the varieties of rice

Spearman's temperature	Wita_7	Digang	Sikamo	Jasmine	Bourke	ITA_320	ITA_324	Marshal	Viworor	China
Correlation coefficient	0.711	0.573	0.720	0.670	0.658	0.572	0.679	0.662	0.461	0.700
Sig.(2-tailed)	0.000	0.003	0.000	0.000	0.000	0.003	0.000	0.000	0.020	0.000

D7: Summary of descriptive statistics of time of the varieties of rice

Spearman's time	Wita_7	Digang	Sikamo	Jasmine	Bourke	ITA_320	ITA_324	Marshal	Viworor	China
Correlation coefficient	-.093	-.163	.016	-.181	-.136	-.149	-.167	-.167	-.148	-.124
Sig.(2-tailed)	.658	.436	.938	.386	.517	.479	.425	.426	.481	.554

D8: Enzyme activity of varieties

variety	wita 7	Digang	Sikamo	Jasmine'85	Bourke'189
Number of values	25	25	25	25	25
Minimum	7.0 e-005	0.00015	0.00011	0.00023	0.00011
Maximum	0.1616	0.1151	0.1555	0.1017	0.1335

D8: Enzyme activity of varieties

variety	ITA 320	ITA 324	Marshal	Viwornor	China
Number of values	25	25	25	25	25
Minimum	0.00011	0.0003	0.00026	0.00015	0.00011
Maximum	0.1019	0.06704	0.1410	0.1689	0.1613

D9: Constraints used in experimental design

		Lower	Upper	Lower	Upper	
Name	Goal	Limit	Limit	Weight	Weight	Importance
starch slur	is in range	0.5	3	1	1	3
Temp	is in range	10	60	1	1	3
Time	is in range	5	15	1	1	5
Diastatic Power	maximize	0.00011	0.13352	1	1	5
Solutions						
Number	pH	starch slur	Temp	Time	Diastatic Power	Desirability
1	8.299998	2.999998	10	5.000002	0.072526	0.542806

Plate 1; Rice grains in steep.

Plate 2; 2nd day of malting

Plate 3; 4th day of malting

Plate 6; Culture before addition of DNSA

Plate 5; Enzymes, starch, buffer after DNSA is added.

Plate 7; culture after incubation