KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

COLLEGE OF AGRICULTURE AND RENEWABLE NATURAL RESOURCES
FACULTY OF AGRICULTURE DEPARTMENT OF HORTICULTURE

EFFECTS OF PRE-GERMINATION TREATMENTS AND STORAGE PERIODS ON GERMINATION, VIGOUR, CHEMICAL AND HEALTH COMPOSITION OF SEEDS OF THREE CASSAVA (*Manihot esculenta* Crantz) CULTIVARS

A THESIS SUBMITTED TO THE SCHOOL OF RESEARCH AND GRADUATE STUDIES, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MASTER OF PHILOSOPHY SEED SCIENCE AND TECHNOLOGY

BY

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DECLARATION

I hereby declare that this submission is the result of my own work and that it has not been submitted either in part or in whole for any other degree elsewhere. Research by other authors has been duly acknowledged.

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DEDICATION

I dedicate this work to my wonderful parents and my lovely husband and greatly to my exceptional godfather Mr. Gabriel P. K. Maison who saw the good to invest in me to achieve this success. You always believed in me and urged me to work hard to achieve everything I want in this life.

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ABSTRACT

Seeds of three cultivars of cassava were collected from the CSIR-Crops Research Institute at Fumesua in the Ashanti Region to determine **the effect of storage periods and seed pregermination treatments on the germination percentage, physicochemical and health properties of seeds from the three varieties of cassava.** The experiment was conducted under laboratory conditions and a 3 x 3 x 7 factorial in Completely Randomized Design (CRD) with three replications was used. The first factor was cultivar at three levels (Ahwengyanka-1, Ahwengyanka-2 and Aworowa-3); the second factor was pre-germination treatments at seven levels (hot water, cold water, mechanical scarification, three concentrations of acid scarification and no treatment as the control); the third factor was storage period at three levels (no storage, three months storage and six months storage). The study revealed that seeds of cassava stored for up to three months produced about 40% germination after undergoing the various pre-germination treatments. Seeds of the Aworowa-3 cultivar stored for six months produced the highest germination percentage of 61.0%. Seeds mechanically scarified with sand paper produced the highest germination percentage of 48.9% whereas seeds with no treatment recorded the least percentage of 34.8%. Seven fungi pathogens were identified on the three cultivars of cassava seeds irrespective of the cultivar and period of storage. The study concluded that Aworowa-3 seeds without storage produced a higher germination percentage than Ahwengyanka-1 and Ahwengyanka-2 seeds without storage but all the seeds of the three varieties had the highest germination percentage when stored for six months.

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CHAPTER ONE

1.0 INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is the most important tropical root crop in many parts of Africa because of its edible roots (FAO, 2009). It is also cultivated for its leaves and tender shoots which are rich sources of proteins, vitamins A, B, C, and other minerals are consumed as vegetable (Fregene *et al.,* 2000; IITA, 2001). In Africa, Ghana ranks third after Nigeria and the Democratic Republic of Congo as the largest cassava producing country (FAO, 2009). Cassava in Ghana is grown across all the agro-ecological zones and contributes 22% to Ghana's Agricultural Gross Domestic Product (AGDP) (Parkes, 2009).

Cassava is vegetatively propagated by mature stem cuttings. This mode of propagation is the most widely used since cassava does not produce large amounts of sexual seeds; however, the wild species are naturally propagated through seeds (Teixeira, 1987). The seedlings that are obtained from cassava seeds are initially smaller than plants that are developed from vegetative cuttings and they require special care to become established. The seeds of cassava are oval in shape and have a cross section that is elliptic or circular. The seeds are approximately 100 mm long and 4 to 6 mm thick (Alves, 2002).

Seeds of cassava can remain viable when stored under ambient conditions for up to 1 year, although germination percentages may decline substantially after 6 months (Rajendran *et al*., 2000) in some cases. Under cold storage conditions (4°C and 70–80% relative humidity) cassava seeds can remain dormant for up to 7 years with no loss of germination thus dormancy is a usual occurrence in cassava seeds. Dormancy in seeds, therefore is

known to inhibit the germination of intact viable seeds under favorable conditions (Bagheri and Asadi, 2003; Robert, 2003; Finch Savage and LeubnerMetzger, 2006). Germination of seeds of cassava can be favored by dry heat and complete darkness (Ellis *et al.,* 1982). Generally, treatments used to break seed dormancy may include mechanical scarification, chemical scarification (especially sulfuric acid), cold-wet, hot water, electrasonic waves and stratification (Isvand *et al.,* 2004; Dehghani, 2005).

Genetically, cassava clones are highly heterozygous and sexual propagation (propagation through seeds) results in a wide diversity of phenotypes, which is of interest to breeders (Ceballos *et al.,* 2004). This discovery has endeared to breeders who have started to use cassava seeds as the starting material in their breeding programmes. However, a major problem with freshly harvested cassava seeds is its characteristic dormancy which is a well documented occurrence in *Euphorbiaceae* genera (Keleny and VanHaaren, 1967; Eakle and Garcia, 1977; Lago *et al.,* 1978; Nassar and Teixeira, 1983). The period of dormancy in cassava seeds could last for a minimum period of 6-9 months under ambient temperatures (Ellis *et al.,* 1982) with adverse consequences on breeding programmes. To overcome this problem so that cassava seed breeding programmes can be undertaken anytime without the time-related dormancy limitation, development of suitable dormancy-breaking techniques are of paramount importance. The general aim of the study therefore was to determine the most suitable pregermination treatment of seeds for high germination of cassava seeds for breeding programmes.

Specifically, the objective was to;

- Determine the effects of storage periods and seed pre-germination treatments on the germination percentage, physicochemical and health properties of seeds from the three varieties of cassava.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 BOTANY AND USES

Cassava (*Manihot esculenta* Crantz) which belongs to the Euphorbiaceae family is among the main starchy plants of Africa (Legg and Fauquet, 2004) and Asia (Hong *et al*., 1993; Saunders *et al*., 2002). It is found over a wide range of edaphic and climatic conditions and can grow on soils with low fertility which is considered marginal for most crops. It can also survive in areas with low annual rainfall and prolonged periods of drought (El-Sharkawy, 2004) and this makes it an important and readily available source of food.

Of the ninety-eight species in the Manihot genus, only *Manihot esculenta* is cultivated and about 20% of the species are native to North America while 80% occur in South America, mainly in the central and northeastern regions in Brazil (Nassar, 2007). Seeds of cassava contain a high amount of oil as the seeds of Ricinus and Jatropha genera of the Euphorbiaceae family (Akbar *et al*., 2009) which are also known for the production of oils that are used in the production of medicines, cosmetics, industrial applications, and also have tremendous potential as feedstock and biodiesel production. In northeastern Brazil, roots and shoots of some wild shrubby cassava species (*M. caerulescens, M. dichotoma, M. glaziovii, and M. pseudoglaziovii*) known as "manic¸obas" or "mandiocas-bravas" are used for animal feed (Allem *et al*., 1999; Teixeira, 1987).

2.2 VARIETIES

Cultivars of cassava may vary with regard to the flesh colour, yield potential (Howeler, 2007), pest and disease resistance levels, time of planting to harvesting, cooking quality and taste, seed size and texture of seed coat. While cassava occurs in different varieties, breeders are still working hard to improve on the already existing varieties. Varieties are selected by farmers to suit their preferences such as the "Ahwengyanka" (English - takes care of orphans) variety which yields heavily and can be used to feed a large number of people. The different ethnic groups have given numerous names to cassava varieties according to their uses and other existing factors. But this nomenclature has brought about some form of confusion in the exact numbers and identity of cassava varieties under cultivation (Okai, 2001; Baafi and Sarfo-Kantanka, 2008). Some named local varieties available in Ghana are "Aworowa" (name of a town), "Ahwengyanka" (to take care of orphans), "Nkaakom" (name of a town), "Bankyehemaa" (Queen of cassava), "Bankye kokoo" (red cassava) among others.

For generations, most farmers have kept and cultivated ancient cassava varieties although there are improved varieties as a result of accessibility to improved technology (Okai, 2001; Manu-Aduening *et al*., 2005) and this has led to slower adoption rates since some of these farmers and other end-users claim the improved varieties do not satisfy their unique preferences and requirements (Nweke *et al.,* 1994; Benesi, 2005; Manu-Aduening *et al.,* 2005).

2.3 PLANTING MATERIAL

In cassava production as in the production of other crops, quality planting materials are required for good crop establishment. Farmers usually select and distribute cassava planting materials (Kizito *et al.*, 2005; Manu-Aduening *et al.*, 2005) and they have with time selected genotypes that best fit their needs. However, cassava production is somehow constrained by lack of quality planting materials and most of these farmers still recycle poor quality planting stocks which reduce the quality of crops they produce (Okai, 2001) since those genotypes suits them best.

Although, for agricultural and commercial purposes or livelihood, cassava cultivation is exclusively performed by means of vegetative propagation by using "seed-cuttings" or stem cuttings, majority of accessors such as breeders maintain active the sexual propagation system (Vieira *et al.*, 2008) to raise seedlings to aid in their selection programs.

Cassava stakes planted in moist soils under favorable conditions produce sprouts and adventitious roots within a week but with seed propagation, plant establishment is considerably slower and the plant itself is smaller and weaker than that produced from the stakes. However, seedlings produce healthy plants and some farmers select from these healthy and vigorous plants and add them to their varieties (Onwueme, 1978; IITA, 1990; Osiru *et al.*, 1996; Pujol *et al*., 2002; 2007) to improve the quality of the crop.

Due to variability in fertility among cultivars, an average of one seed can be found in each fruit (capsule) instead of a maximum of three from the trilocular ovary and these seeds when planted, germinate within 7 to16 days (Jennings and Iglesias, 2002).

2.4 SEED HEALTH

The presence or absence of disease-causing and non disease-causing organisms such as fungi, nematodes, bacteria, viruses and insects on seeds in a particular seed lot is referred to as seed health (Mathur and Kongsdal, 2001; Mew and Gonzales, 2002). In the set up of a healthy field, healthy seeds are required to produce good yield and quality seeds, because seed-borne pathogens such as, fungi, cause poor germination and vigour, poor crop establishment and crop stands as well as non-healthy plants (Wiese, 1984). The health condition of seeds at the time of storage or planting plays a major role in conserving and producing seed quality and quality seeds respectively. One of the factors known to reduce seed viability in storage seeds are fungi and these storage fungi are particularly insidious because they have the tendency to invade seeds that are stored at moisture contents considered safe by farmers and seed producers (Syed *et al.*, 2013). However, contamination of seeds cannot be avoided completely since temperature and humidity are not controlled and the means to establish proper drying and storage facilities may be expensive and or inadequate. Therefore, in order to promote healthy seeds, contamination, must be reduced.

In Nigeria, maize farmers were able to reduce aflatoxin contamination by 80 percent and also in the United States of America, a safe and natural method was developed to prevent aflatoxin formation in maize which has drastically reduced contamination levels in maize and cotton seed successfully thus enhancing the health quality of the seeds (The Organic Farmer, 2010).

Loss in quality and yield can be attributed to microorganism infestation especially disease causing organisms. Neergaard (1977) reported that two-thirds of total losses of maize caused by diseases are due to seed-borne diseases, of which Diplodia and Fusarium account for about 25% and Drechslera species for almost 20 % of the total loss. The proximate and nutrient composition of seeds and other crops are also affected by the presence of microorganisms on those seeds and crops (Onifade and Agboola, 2003). Therefore seeds have to be tested to ensure the absence of pathogens before propagating.

2.5 STORAGE

In Euphorbiaceae, storage behavior of seeds is generally orthodox. According to the Food and Agriculture Organisation, 40 % of grain harvest is spoiled due to unsafe storage practices in Africa. The ability of seeds to store for a specific length of time without appreciable loss in viability is primarily dependent on the nature of the seed, the moisture content before storage, storage material used and the temperature and relative humidity of the storage environment. Seeds must be dried to a required and acceptable moisture content below 8% (Daun, 1995) because improperly dried seeds lose their viability rapidly which leads to decrease in seed quality. Also, storage conditions must be favorable to meet the quality demands of seeds (Suttle, 2007).

2.5.1 Storage and Germination

Cassava seeds can remain viable when stored under ambient conditions for up to one year after which viability may decline (Rajendran *et al.*, 2000). High germination rates are

usually obtained after cassava seeds have undergone a period of storage for at least six months after which its inherent dormancy would have been released.

Seeds of cassava can survive for up to seven years with no loss of germination when stored under cool temperature conditions (4°C and 70–80% relative humidity) because the length of storage has a tendency of inducing germination in dormant seeds (Cheema, 2010) such as cassava seeds.

2.5.2 Storage of Planting Material

Proper storage of cassava planting material (mature stem cuttings or seeds) must be done to conserve or improve their quality. However, the length of storage must be considered since stakes (mature stem cuttings) have a tendency to dehydrate during long periods of storage. On the contrary, long-term storage of cassava seeds have the tendency of enhancing the germination ability of the seeds since dormancy in cassava seeds take up to 3 to 6 months to be released although 12 months storage in ambient temperature may lead to loss of germination, long-term storage in cold temperatures preserve the viability of the seeds (Jennings and Iglesias, 2002; Garcia *et al.*, 2006).

2.5.3 Storage Effects on Health Quality of Seeds

Storage may decrease or increase the susceptibility of stored materials to microorganisms and or diseases due to the proper or improper regulation of factors such as temperature, relative humidity, light, moisture, and etcetera. When the storage environment and facilities are favorable for the proliferation of microorganisms and or insects, attack of

these organisms on seeds becomes rampant leading to the gradual susceptibility of seeds to the organisms (Aliyu and Kutama, 2007). However, it is important to study the presence of fungi on stored seeds to determine their possibility of causing either physical or biochemical damage to crops under favorable conditions (Ingale and Shrivastava, 2011). This can help in creating measures to either control or avoid these fungi.

2.5.4 Storage and Dormancy

Storage can induce germination in dormant seeds as seen in newly harvested seeds which exhibit physiological dormancy and require 3 to 6 months of storage at ambient temperature before germination can occur (Jennings and Iglesias, 2002). The conditions of the storage environment contribute immensely to the length of dormancy in seeds and seeds stored at temperatures below 3°C or above 30°C are able to sprout prematurely (Law and Suttle, 2004). Furthermore, fluctuating storage temperatures have been known to shorten dormancy more than constant high temperatures (Eremeev *et al.,* 2008) therefore, it is important to keep the temperature of the storage environment as consistent as possible.

2.6 DORMANCY

Baskin and Baskin (2004) defined dormancy as the lack of the capacity for a seed "to germinate in a specified period of time under any combination of normal physical environmental factors such as temperature, light or darkness among others that are otherwise favorable for its germination". Therefore seeds that fail to germinate in such conditions are known as dormant seeds. Seeds obtained from tuber crops naturally express

dormancy (Burton, 1989) due to their physiological state which prevent sprouting even when they are placed in ideal germination conditions (Reust, 2002; Sonnewald and Sonnewald, 2014).

In order for germination to occur in dormant seeds, some seeds have to undergo morphological changes, while in others; part of the seed must undergo physiological changes. Although, dormancy may be a problem, in the temperate areas, dormancy in seeds of potato plays a significant role in keeping them from sprouting in the fall, thereby reducing chances of the species being killed by unfavourable winter conditions (Essah and Honeycutt, 2004). But dormancy may vary from seed to seed especially in the size of the seed (Vreugdenhill, 2004).

The reduction in the period of dormancy and acceleration of physiological development of the seeds can occur when salient conditions of the storage environment (Krugman and Jenkinson., 1974) such as temperature and light (Knowles and Knowles, 2006; Aksenova *et al.,* 2013) are enhanced.

2.7 PRE-GERMINATION TREATMENTS

Several methods have been used to induce germination in dormant seeds. Some of these methods are mechanical scarification, chemical scarification, soaking seeds in hot water, soaking seeds in cold water, dry heat application, fire, mulch, use of other chemicals as well as cold and warm stratification (Emery, 1987). A complex sequence of events takes place for dormancy to be broken, such as cell division and elongation, chemical and biochemical reactions (Teper-Bamnolker *et al.,* 2010), leading to emergence and

germination of seeds. Mechanical scarification of dormant seeds, chemical scarification with concentrated sulfuric acid, soaking seeds in boiling water or a combination of these treatments could result in high germination percentages (Bonner, 2000; Pasiecznik *et al.*, 2001; Shiferaw *et al*., 2004).

2.7.1 Mechanical Scarification

Mechanical scarification which is known to be one of the best methods used to induce germination in seeds with hard seed coat dormancy. It is done by rubbing seeds between two sandpaper pieces (Schmidt, 2000) or by using a pin, knife or file to scrape the seed coat partially or entirely depending on the thickness of the seed coat. Similarly, seeds could be mixed with coarse sand or gravels and shaken vigorously in a container. This must be done carefully in order not to injure the embryo. Nartey (1978) recommended that for high germination results, seeds should be scraped near the micropyle.

2.7.2 Acid Scarification

Acid scarification is the method of soaking dormant seeds in different concentrations of acid to break dormancy and it is similar to mechanical scarification (Sadeghi *et al.,* 2009). This is because the aim is to scratch, scrape or entirely remove the seed coat to make it permeable to water leading to easy and fast imbibition of water to increase germination (Sadeghi *et al.,* 2009). Acid scarification is also known as chemical scarification. Different concentrations of acid have been used to break seed coat dormancy but the concentrated sulfuric acid treatment has been widely used on several species with hard

seed coats to improve their germination potential (Tigabu and Oden, 2001). Care must be taken during the use of this method because prolonged immersion of seeds in sulfuric acid (H_2SO_4) could injure the seeds by destroying some vital parts of the embryo (Aliero, 2004) which could cause the death of seeds leading to no emergence and germination.

2.7.3 Hot Water Soaking

Pre-treatment of seeds with hot water is one of the effective treatments used to enhance germination (Basbag *et al.,* 2010) in dormant seeds; the water softens the seed coat and the heat helps in the opening of the pores of the seed coat making it more permeable to water. Regulation of temperatures and length of soaking must be controlled to achieve increasing germination (Pallavi *et al*., 2010).

2.7.4 Cold Water Soaking

Soaking seeds in cold water can also be termed as priming. A successful utilization of priming which led to the release of dormancy was recorded in priming sunflower seeds in cold water for 12 to 24 hours (Maiti *et al*., 2006). In cold water soaking, the germination process is first activated, and, there is also the washing away of retardants and any other compounds that may inhibit germination and finally, imbibition of the water weakens the seed coat and hard structures, thereby removing physical dormancy (Marchetti, 2012).

2.8 GERMINATION

The growth and viability of seeds is indicated by germination (Barua *et al.,* 2009). Germination of seeds usually occurs under favorable conditions (optimum temperature, light and moisture), although some seeds may fail to germinate under such conditions due to dormancy, low seed vigour and death of seed. Several other factors including complete darkness, dry heat as well as the moisture content of seeds are critical factors which trigger cell elongation and initiate radicle emergence which is generally known as germination (Willenborg *et al.,* 2005). The seeds of cassava can germinate within 7 to 30 days after planting but due to their inherent dormancy, they may require pretreatment before planting.

Temperature plays a major role in germination. High and low temperatures (alternating temperatures) have positive effects on germination of seeds (Ellis *et al.,* 1982) depending on the type of seed and its temperature requirement but Pujol *et al.* (2002) expressed uncertainties as to whether fluctuation between high and low temperatures triggers germination in seeds regardless of their type or temperature requirement or the simple exposure of seeds to high temperatures improves germination. However, it is clear from some experiments conducted that higher constant temperatures are also required for high and quick germination (Ellis and Roberts, 1979; Ellis *et al.,* 1982). Therefore the temperature during planting of seeds should be considered to ensure germination.

Germination tests under a wide range of constant and alternating temperature regimes have clearly indicated that certain minimum conditions must be provided before cassava

seeds can germinate; the maximum temperature during part of the day must exceed 30°C and the mean temperature must be 24°C or more (Ellis *et al.,* 1982).

2.9 VIGOUR

According to ISTA (2007), seed vigour is the sum of those properties of the seed that determine the potential level of activity and performance of the seed or seed lot during germination and seedling emergence. The vigour of seeds can also be defined as the set of characteristics that determine the activity and behaviour of the seed lots of commercially acceptable seed germination in different environmental conditions. Seed vigour is a good determinant of the length of period seeds can be stored (ISTA, 2007). A decline in seed vigour leads to loss of seed viability and thus low germination rates, therefore seed vigour must be given utmost attention in seed production (Caddick, 2007). Low vigour seeds usually show stunted growth and abnormalities in the developing shoot and root system and subsequently crop establishment is affected (Caddick, 2007).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 EXPERIMENTAL SITE

The experiment was conducted at the CSIR-Crops Research Institute (CRI), Fumesua, near Kumasi from June, 2015 to January, 2016. Specific tests and analyses were carried out in laboratories at the CRI, Faculty of Agriculture, KNUST, Kumasi and University of Ghana, Legon.

3.2 SOURCE OF CASSAVA SEEDS

Cassava seeds were obtained from cassava trial fields at the CSIR-Crops Research Institute (CRI), at Fumesua, near Kumasi. The seeds were harvested from growing cassava plants. The cultivars collected were Ahwengyanka-1, Ahwengyanka-2 and Aworowa-3. The seeds were cleaned, dried and put in paper bags. Seeds were stored under ambient temperature conditions in the cassava seed storage room for periods of three and six months. The storage environment had a temperature range of 25ºC – 30ºC. Daily monitoring and recording of temperature and relative humidity were done for the entire storage period.

3.3 EXPERIMENTAL DESIGN

A 3x3x7 factorial arrangement in Completely Randomized Design was used. The experiment was replicated three times. The first factor was variety at three levels (Ahwengyanka-1, Ahwengyanka-2 and Aworowa-3). The second factor was storage period at three levels (0 month, 3 months and 6 months). The third factor was pregermination treatment at seven levels (Hot water soaking (HW), Cold water soaking (CW), Acid scarification for 5 minutes (AS5), Acid scarification for 10 minutes (AS10), Acid scarification for 30 minutes (AS30), Mechanical scarification (MS), and No treatment as Control). The details of the pre-germination treatments (PGT) were as follows:

Hot water bath (HW): Thirty seeds of cassava were placed in conical flasks containing 200ml of water and put into a water bath at a temperature of 100^{o}C for 30mins. The seeds were allowed to cool after 30 minutes and they were placed into Petri dishes containing wetted paper towels for the germination test.

Cold water (CW): Thirty seeds were soaked in tap water for 24 hours after which they were placed on the paper towels in the Petri dishes and observed for occurrence of germination.

Acid scarification (AS): Thirty seeds were soaked in 50 mls of concentrated sulfuric acid for periods of (i) 5 minutes, (ii) 10 minutes and (iii) 30 minutes. After each period, the seeds were thoroughly washed with tap water to remove any acid residue and a litmus paper was used to test for the presence or absence of acid. The seeds were placed in the Petri dishes when the absence of acid was confirmed.

Mechanical scarification (MS): The seed coat of thirty seeds was scraped with sand paper to remove some part of the seed coat for easy imbibition of water. The seeds were placed on the paper towels in the Petri dishes and germination was observed.

3.4 DATA COLLECTED

3.4.1 1000 Seed Weight

Four replicates of hundred seeds from the pure seed fraction was selected at random and weighed on an analytical balance and the weight was recorded. The mean weight was multiplied by 10 and the weight of 1000 seeds was determined as recommended by the International Seed Testing Association (ISTA) (2007).

3.4.2 Seed Purity Determination

A working sample was taken from the submitted sample by thorough mixing and repeated halving to 100g instead of the required weight of 500g due to limited quantity of seeds. The working sample was then separated into three components; pure seed, other crop seed and inert matter through visual assessment. The various components were weighed and the percentage by weight of each of the components was calculated using the ISTA (2007). This was done for all three experiments at the various storage periods.

3.4.3 Temperature and Relative Humidity of Storage Room

The temperature and relative humidity readings of the storage room (ambient) were taken at specific times during the day. An Acurite manufactured indoor digital temperature and humidity monitor (00325) was used in taking the various readings.

3.4.4 Moisture Content

The moisture content of the cassava seeds was determined by using the low constant temperature oven method (Association of Official Analytical Chemists (AOAC), 2007). An empty moisture dish was thoroughly washed, cleaned and dried for 4 hours at 105 °C and cooled in a desiccator for 30 minutes. The weight of the empty dish was recorded and about 3 grams of milled cassava seeds from each variety was weighed and transferred into a previously weighed empty dish and placed in an oven and maintained at a temperature of 105 °C for 5 hours. The samples were taken out of the oven and placed in a desiccator to cool for about 30 minutes at the end of the prescribed period.

After cooling, the dish and the sample were reweighed and the weights were recorded. The moisture content was calculated from the loss in weight (AOAC, 2007).

Calculation of moisture content

$$\%Moisture = \frac{(weight\ of\ wet\ sample - weight\ of\ dry\ sample)}{weight\ of\ wet\ sample}\ x\ 100$$

3.4.5 Crude Fat Content

For fat determination, 3grams of the milled sample was weighed into a paper thimble, carefully folded, labeled and placed into a thimble holder. The weight of an empty 500ml round bottom flask was recorded and 150 ml of petroleum ether was poured into the flasks and they were assembled on a semi-continuous soxhlet extractor and refluxed for 16 hours at a condensation rate of 6-8 drops per second at 100^{o}C. After the prescribed period, the hexane was recovered after removing the paper thimble from the thimble holder and the flask was heated for 30 min in an oven at 103^{o}C to remove the residual hexane. The flasks were later placed in a desiccator to cool for 30 minutes after which the flask was re-weighed and the weight recorded (AOAC, 2007).

The increase in weight was calculated as percentage of crude fat as shown below:

$$\%Fat = \frac{(weight\ (flask+fat) - weight\ (empty\ flask)}{weight\ of\ sample} x\ 100$$

3.4.6 Protein Content

The protein content of the samples was determined using the macro Kjeldahl method; digestion, neutralization and distillation and titration.

3.4.6.1 Digestion

The first step in analysis of protein content is digestion; 2 grams of the sample was weighed into a digestion flask and mixed with 25 ml of concentrated H_2SO_4, selenium

catalyst and few anti-bumping agents. The contents of the flask were digested by heating in a fume chamber to obtain a colorless solution.

3.4.6.2 Neutralization and Distillation

After the digestion, the digestion flask was allowed to cool and the solution transferred into a 100 ml volumetric flask and the volume will be increased up to the 100 ml mark with distilled water. The distillation apparatus was flashed out with water and 10 ml of digested sample transferred into the distillation apparatus. Neutralization of the solution was done with 18 ml NaOH and boiled under distilled water in a steam generator. Circulation was carried out for about 10 min. A conical flask was filled with 25 mL of 2% boric acid and 3 drops of mixed indicator (methylene blue and methylene red) added. The conical flask and its content were placed under the condenser for 10 minutes.

3.4.6.3 Titration

The ammonium borate formed in the conical flask was titrated with 0.1M HCl solution for the Nitrogen content estimation. Titre values of the replicate samples were recorded and the percentage Nitrogen was calculated as shown below. A blank sample was run at the same time the sample was analyzed.

Calculation of crude protein content

$$\%Nitrogen = \frac{(S_t - S_b)x\ NA\ x\ 100\ x\ 0.1\ x\ 0.014\ x\ 100}{Sample\ weight\ x\ 10}$$

S_t = Titre of sample; S_b = Titre of blank; NA = Normality of acid; % Protein = % N x F;

N= Nitrogen ; * F = Factor (5.71)

*Conversion factor (F) was provisionally adapted from the conversion factor of the Kjeldahl Nitrogen to soybean protein since soybean is the oil seed protein well studied.

3.4.7 Seed Health

The blotter method was used in testing for the health of the seeds (Marthur and Kongsdal, 2001). Instead of four hundred seeds, fifty seeds were randomly taken from the pure seed sample of each variety and carefully dipped into 5% ethanol. Three blotter papers were soaked in tap water and placed at the bottom of the Petri dish. Ten (10) seeds were plated in each of the five Petri dishes and incubation was done at 20 + 2°C near ultraviolet (NUV) light and darkness at alternating cycles for 7 days. After the required period, the seeds were examined first under a stereo binocular microscope and then a compound microscope was used for more detailed identification.

Evaluation of seeds was done on the basis of visible vegetative growth fruiting bodies and characteristic symptoms on seedlings (Mathur and Kongsdal, 2001). The results were expressed as percentage of the number of seeds affected, number of organisms in the weight of sample examined (Mathur and Kongsdal, 2001).

3.4.8 Germination Percentage

The method used for the germination analysis was the top of paper method; paper towels were cut into disc shapes and placed in Petri dishes after thorough wetting. Three disc-

shaped wetted paper towels were placed in each Petri dish. Thirty (30) seeds of each variety after subjection to the various seed treatments; mechanical scarification, acid scarification, hot water, cold water and no treatment were carefully placed on the paper towels in the Petri dishes (ten seeds per plate resulting in three replicates) and labeled.

First count was recorded on day 7 after plating after which subsequent counts were observed every other day. After sixty (60) days the last count was done as well as the evaluation which led to the grouping of seeds into normal, abnormal and dead seeds (ISTA, 2007).

Calculation of germination percentage:

$$\%Germination = \frac{number\ of\ germinated\ seeds}{number\ of\ total\ seeds\ planted}\ x\ 100$$

3.4.9 Determination of Seed Vigour

Seed vigour was determined using the conductivity test. Four replicates of 50 seeds of each of the three varieties were selected at random and tested for their electrical conductivity. Selected seeds were placed in Erlenmeyer flasks containing 75 ml ultra pure deionized water which was equilibrated to and maintained at 25 °C for 24 hours.

After the requisite period, the flasks were swirled for 10-15 seconds and the seeds were taken out of water with a clean forcep.

An electrical conductivity dip cell was inserted into the leach water until a stabilized reading was obtained and recorded (ISTA, 2007). Electrical conductivity was calculated using the formula below:

$$Conductivity\ (\mu S/cm^{-1}g^{-1}) = \frac{conductivity\ reading - background\ reading}{weight\ of\ replicate}$$

3.4.10 Mineral Composition Determination

One gram of the milled sample was weighed into a clean pyrex conical flask, 4ml of concentrated sulfuric acid (H_2SO_4) was added and the flask was swirled carefully to ensure complete wetting of the sample. The flask and its content were heated on an electric hot plate at medium heating in a fume hood after which it was removed and cooled. 10 drops of hydrogen peroxide (H_2O_2) was added; 3-4 drops at a time to avoid vigorous reaction of the contents. The flask was swirled keeping the contents at the bottom and reheated at medium heat. The flask and its contents were cooled and 6 drops of H_2O_2 was carefully added and reheated, this was repeated until there was a change in color from black to dark brown. The set up was heated at high heat and cooling was done continuously as 6 drops of H_2O_2 was added repeatedly. Finally, perioxide was added and placed on high heat hot plate for 10-15 minutes after the solution remained colorless from cooling. The content was cooled and transferred quantitatively into a 50ml volumetric flask using distilled water. This solution was used in determining the N, P, K, Ca and Mg (Okalebo, 1985; Anderson and Ingram, 1993).

3.4.10.1 Method of Determination of Calcium (Ca) and Magnesium (Mg)

Ethylene diamine tetra acetic acid **(**EDTA) titration is used in calcium and magnesium determination and it involves the addition of several reagents such as; Buffer solution which was prepared by dissolving 60 g of ammonium chloride in 200 ml of distilled water. 570 ml of concentrated ammonium hydroxide was added and diluted to 1000 ml in a volumetric flask (Motsa and Roy, 2008; Anderson and Ingram, 1993; Okalebo *et al.*, 1993).

3.4.10.2 Determination of Calcium

Five milliliters (5.0 ml) of sample solution was transferred into a 100 ml Erlenmeyer flask. 10 ml of 10 % Potassium hydroxide (KOH) solution was added followed by 1 ml of 30 % TEA, five drops of 2 % Potassium cyanide (KCN) and one drop of Erichrome Black T (EBT) indicator solution. The mixture was shaken to ensure homogeneity. The mixture was titrated with 0.02 N EDTA solution from a red to blue end point (Motsa and Roy, 2008; Anderson and Ingram, 1993; Okalebo *et al.*, 1993).

Calcium in mg = Titre value of EDTA x 0.40

$$\%Calcium = \frac{mg\ Ca}{sample\ weight}\ x\ 100$$

3.4.10.3 Determination of Magnesium

5.0 ml sample solution was emptied into a 100 ml Erlenmeyer flask 5 ml of ammonium chloride – ammonium hydroxide buffer solution was added followed by 1 ml 30% Triethanol amine (TEA). Three drops of 10 % KCN and a few drops of EBT indicator solution. The mixture was shaken to ensure homogeneity. The mixture was titrated with 0.02 N EDTA solution from a red to blue endpoint (Motsa and Roy, 2008; Anderson and Ingram, 1993; Okalebo *et al.*, 1993).

Magnesium in mg = Titre value of EDTA x 0.24

$$\%Mg = \frac{mg\ Magnesium}{Sample\ wt} x\ 100$$

3.4.10.4 Method of Determination of Phosphorus (P)

A vanadomolybdate reagent was prepared by dissolving 22.5 g of ammonium molybdate in 400 ml of distilled water and 1.25 g of ammonium vanadate in 300 ml of boiling distilled water. The vanadate solution was added to the molybdate solution and cooled to room temperature. 250 ml of analytical grade HNO_3 was added to the solution mixture and diluted to 1 litre with deionized water. The standard phosphate solution was also prepared by dissolving 0.2195 g of analytical grade KH_2PO_4 in 1000 ml distilled water. This solution contains 50 µg P/ml. A standard curve was prepared by pipetting 1, 2, 3, 4, 5 and 10 ml of standard solution (50 µg P/ml) in 50 ml volumetric flasks. 10 ml of vanadomolybdate reagent was added to each flask and the volume made up to 50 ml. This gave a P content of the flasks as 1, 2, 3, 4, 5, and 10 µg P/ml. These concentrations were measured on the Jenway 6051 colorimeter to give absorbance measurements at a

wavelength of 430 nm. A plot of absorbance against concentration was used to prepare the calibration curve.

Five (5) ml of the sample solution from the digest was put into a 50 ml volumetric flask. 10 ml of vanadomolybdate reagent was added and volume made up to 50 ml with deionized water. The sample was allowed to stand for 30 minutes undisturbed for colour development. A stable yellow colour was developed. The sample was read on the colorimeter at 430 nm. The observed absorbance was used to determine the P content from the standard curve (Motsa and Roy, 2008; Anderson and Ingram, 1993; Okalebo *et al.*, 1993).

The % P was calculated as:

$$P(g) in\ 100g\ sample\ (\%P) = \frac{C\ x\ df\ x\ 100}{1\ 000\ 000} = \frac{C\ x\ 1000\ x\ 100}{1\ 000\ 000} = \frac{C}{10}$$

C = concentration of P (µg /ml) as read from the standard curve; *df*

= dilution factor, which is 100 *10 = 1000, as calculated below:

1 g of sample made to 100 ml (100 times);

5 ml of sample made to 50 ml (10 times)

1 000 000 = factor for converting µg to g

3.4.10.5 Method of Determination of Potassium (K)

1.908 g and 2.542 g of analytical grade KCl and NaCl respectively previously dried in an oven for 4 hours at 105^{o}C were each dissolved in 200 ml of deionised water. The two solutions were mixed together and volume made up to 1000 ml. This gave a combined standard of 1000 ppm. For K, a calibration curve (standard curve) of 200, 400, 600 and

800 ppm was prepared. All the absorbance reading was taken using the flame photometer. The sample solution from the digest was read on the flame photometer. From the standard curve, the concentration of K and Na were calculated using the particular absorbance observed for the sample (Motsa and Roy, 2008; Anderson and Ingram, 1993; Okalebo *et al.,* 1993).

Calculation:

K (g) in 1.0 g of plant sample = C x df

$$K(\mu g) in\ 100 g\ sample\ (\%K) = \frac{C\ x\ df\ x\ 100}{1\ 000\ 000} = \frac{C\ x\ 100\ x\ 100}{1\ 000\ 000} = \frac{C}{10}$$

C = concentration of K (g / ml) as read from the standard curve

df = dilution factor, which is 100 x1 = 100, calculated as : 1.0 g

of sample made up to 100 ml (100 times)

1000 = factor for converting g to g.

3.4.10.6 Method of Determination of Nitrogen

Determination of Nitrogen was based on the calculation used under the method of determination of protein in 3.4.6.1-3.

3.5 ANALYSIS OF DATA

Data collected were subjected to Analysis of Variance (ANOVA) using STATISTIX version 10. Data on germination were square root transformed before analysis. Tukeys Honest Significant Difference (HSD) was used for the separation of treatment means. The probability of declaring significant differences between treatments was set at P=0.01.

CHAPTER FOUR

4.0 RESULTS 4.1 AMBIENT TEMPERATURE AND RELATIVE HUMIDITY OF STORAGE

ENVIRONMENT

The storage temperature ranged from 26ºC to 32ºC whereas the relative humidity ranged from 84% to 91%. The minimum temperature was recorded in September, 2015 while the maximum was recorded in November, 2015. The minimum relative humidity was recorded in July, 2015 while the maximum was recorded in August, 2015 (Table 4.1).

Table 4.1: Minimum and maximum temperature and relative humidity of storage environment

Month	Minimum Temperature (ºC)	Minimum Relative humidity (%)	Minimum temperature (ºC)	Maximum relative humidity (%)
June	29	88	24	88
July	27	84	23	89
August	28	86	22	91
September	26	89	21	87
October	30	86	24	88
November	32	84	25	86
December	30	85	24	86

4.2 EFFECTS OF CULTIVAR AND STORAGE PERIOD ON PERCENTAGE GERMINATION (%) OF CASSAVA SEEDS.

Significant variety x storage period interactions ($P \leq 0.05$) were observed in the percent germination of cassava seeds (Table 4.2). Seeds of all three cultivars stored for six months produced significantly the highest percent germination, while seeds of Ahwengyanka-1 and Ahwengyanka-2 without storage produced the least germination percentages.

Among the cultivars, Aworowa-3 seeds produced significantly higher germination percentages than Ahwengyanka-1 and Ahwengyanka-2 which produced the least yet similar germination percentages.

Among the storage periods, seeds stored for six months produced significantly the highest germination percentage, 1.94 times greater than the least obtained from seeds without storage (Table 4.2).

Table 4.2: Effects of cultivar and storage period on the germination percentage of cassava seeds

Storage	Germination Percentage (%)			
	Cultivars			
	Ahwengyanka-1	Ahwengyanka-2	Aworowa-3	Mean
No storage	28.1	27.6	36.2	30.6
Three months	38.6	38.6	41.4	39.5
Six months	58.6	58.6	60.9	59.4
Mean	41.8	41.6	46.2	
HSD (0.01): Cultivar =2.54; Storage =2.54; Cultivar x Storage = 5.40				

4.2.1 Effects of Cultivar and Pre-Germination Treatments on the Percent Germination of Cassava Seeds

Significant cultivar x pre-germination treatments interactions were observed for percent germination (Table 4.3). Aworowa-3 seeds treated by mechanical scarification with sand paper produced significantly the highest percent germination although similar to Aworowa-3 seeds treated with hot water and Aworowa-3 seeds soaked in cold water. The least percent germination was recorded for Ahwengyanka-2 seeds without any pregermination treatment.

Among the cultivars, Aworowa-3 seeds produced significantly higher percent germination than Ahwengyanka-1 and Ahwengyanka-2 which produced the least yet similar germination percentages.

Among the pre-germination treatments, seeds treated with mechanical scarification with sand paper (MS) produced significantly the highest percent germination, 40.4% greater than the least obtained from seeds without treatment any pre-germination treatments (Control) (Table 4.3). The other pre-germination treatment methods also produced germination percentages which were 28.7% (Hot and cold water) and 23.4% (acid scarifications 5, 10 and 30 mins) greater than the control.

Table 4.3: Effects of cultivar and pre-germination treatments on percent germination of cassava seeds

Pre-germination treatments	Germination Percentage (%)			
	Cultivars			Mean
	Ahwengyanka-1	Ahwengyanka-2	Aworowa-3	
Mechanical scarification	45.6	44.4	56.7	48.9
Control	30.0	30.0	44.4	34.8
Cold water	43.3	43.3	47.8	44.8
Hot water	43.3	43.3	47.8	44.8
Acid scarification-5mins	43.3	43.3	42.2	42.9

Acid scarification-10mins	43.3	43.3	42.2	42.9
Acid scarification -30mins	43.3	43.3	42.2	42.9
Mean	41.8	41.6	46.2	
HSD (0.01): Cultivar =2.54; PGT =4.59; Cultivar x PGT = 9.18				

4.3 EFFECT OF STORAGE PERIODS ON THE VIGOUR OF CASSAVA SEEDS

Significant differences were obtained in the storage periods for the electrical conductivity of the seeds. Seeds without storage produced significantly the highest electrical conductivity value (1.28 S/cm-^{1}g-^{1}) whereas the least conductivity value was obtained from seeds stored for six months (1.04 S/cm-^{1}g-^{1}) (Table 4.4).

Table 4.4: Effect of storage period on electrical conductivity (vigour) of cassava seeds.

Storage period	Electrical conductivity (S/cm-^{1}g-^{1})
No storage	1.28
Three months	1.13
Six months	1.04
HSD (0.01)	0.092

4.4 EFFECTS OF CULTIVARS, STORAGE PERIODS AND PREGERMINATION TREATMENTS ON THE PURITY OF CASSAVA SEEDS

There were no significant main as well as interactive effects of treatments for cassava seed purity. Percent seed purity ranged from 99.5 % to 99.9 %.

4.5 EFFECTS OF STORAGE PERIODS ON THE 1000 SEED WEIGHT OF SEEDS OF CASSAVA

Seeds stored for six and three months were significantly heavier than those without storage. Seeds without storage had the least 1000 seed weight, 9.56 % and 7.78 % less in weight than those stored for six months and three months, respectively (Table 4.5). Table 4.5: Effect of storage period on 1000 seed weight of cassava seeds

Storage period	1000 seed weight (g)
No storage	110.44
Three months	118.22
Six months	120.00
HSD (0.01)	7.253

4.6 EFFECTS OF CULTIVARS ON MOISTURE CONTENT OF CASSAVA SEEDS

There were significant differences in the cultivars for the moisture content of the seeds. The highest moisture content of 7.3 % was recorded by Ahwengyanka-2 seeds,

significantly greater than the moisture contents of seeds of Aworowa-3 and Ahwengyanka-1. The least moisture content was recorded by Aworowa-3 seeds though similar to seeds of Ahwengyanka-1 (Table 4.6).

Table 4.6: Effect of cultivar on moisture content of cassava seeds

Cultivar	Moisture content/ %
Ahwengyanka-1	6.6
Ahwengyanka-2	7.3
Aworowa-3	6.4
HSD (0.05)	0.38

4.7 EFFECTS OF STORAGE PERIODS ON THE MOISTURE CONTENT OF CASSAVA SEEDS

There were significant differences in the moisture content of the cassava seeds stored for different periods. Seeds stored for six months recorded the highest moisture content (7.4 %), which was significantly higher than seeds stored for three months (6.9 %) and seeds without storage (5.9 %). The least moisture content was obtained from seeds without storage (Table 4.7).

Table 4.7: Effect of storage period on moisture content of cassava seeds

Storage	**Moisture content (%)**
No storage	5.9
Three months	6.9
Six months	7.4
HSD (0.05)	0.38

4.8 EFFECT OF CULTIVARS AND STORAGE PERIODS ON PERCENT OIL CONTENT OF CASSAVA SEEDS

There were significant cultivar x storage period interactions for percent oil content of cassava seeds (Table 4.8). Aworowa-3 seeds stored for three months produced significantly the highest percent oil content (28.6%) which was similar to the percent oil content of seeds stored for six months (28.5%). The least percent oil content was recorded in Ahwengyanka-2 seeds without storage (23.8%).

Among the storage periods, seeds stored for three months produced the highest percent oil content (26.7%) while seeds without storage recorded the least (25.1%).

Among the cultivars, Aworowa-3 seeds produced significantly the highest percent oil content (27.8%) while the least was produced by Ahwengyanka-2 seeds (24.7%) (Table 4.8).

Table 4.8: Interactive effects of storage period and cultivar on percent oil content of cassava seeds

Storage	Percent oil content (%)			Mean
	Cultivars			
	Ahwenyanka-1	Ahwenyanka-2	Aworowa-3	
No storage	24.9	23.8	26.5	25.1
Three months	26.2	25.2	28.6	26.7
Six months	24.9	25.0	28.5	26.1
Mean	25.4	24.7	27.8	
HSD (0.01): Cultivar = 0.52; Storage = 0.52; Cultivar x Storage = 1.23				

4.9 EFFECT OF CULTIVARS ON PERCENT PROTEIN OF CASSAVA SEEDS

There were no significant differences among the cultivars for percent protein content. Protein content ranged between 16.18% and 17.07%.

4.10 EFFECT OF STORAGE PERIOD ON THE PERCENT PROTEIN CONTENT OF CASSAVA SEEDS

There were significant differences in storage periods for the percent protein content of the cassava seeds. Seeds without storage contained the highest protein content (17.6%), significantly greater than seeds stored for three and six months. The least percent protein content was recorded in seeds stored for six months (15.04%), about 1.2 times lower than seeds without storage (Table 4.9).

Table 4.9: Effect of storage period on percent protein content of cassava seeds

Storage period	Protein content (%)
No storage	17.6
Three months	16.8
Six months	15.0
HSD (0.01)	1.03

4.11 INTERACTIVE EFFECTS OF CULTIVAR AND STORAGE PERIOD ON PERCENT MAGNESIUM CONTENT OF CASSAVA SEEDS

There were significant storage period x cultivar interactions for the percent magnesium content of cassava seeds. Aworowa-3 seeds stored for six months contained the highest percent magnesium, 2.2 times greater than the least contained in Ahwengyanka-1 seeds stored for six months (Table 4.10).

Among the storage periods, seeds stored for three months recorded the highest percent magnesium content (1.04%), significantly greater than seeds stored for six months and those without storage. The least percent magnesium content was found in seeds stored for six months (0.79%).

Among the cultivars, Aworowa-3 seeds produced the highest magnesium content whereas Ahwengyanka 1 and Ahwengyanka 2 seeds recorded the least, the difference being 1.4 times (Table 4.10).

Table 4.10: Effects of cultivar and storage on the magnesium content (%) of cassava seeds

	Percent magnesium content (%)			
	Cultivars			
Storage Periods	Ahwengyanka-1	Ahwengyanka-2	Aworowa-3	Mean

No storage	0.93	0.93	1.05	0.97
Three months	0.96	0.98	1.17	1.04
Six months	0.56	0.59	1.23	0.79
Mean	0.82	0.83	1.15	
HSD (0.01): Variety =0.06; Storage Periods =0.06; Variety x Storage Periods = 0.14				

4.12 INTERACTIVE EFFECT OF CULTIVAR AND STORAGE ON THE PHOSPHORUS CONTENT OF CASSAVA SEEDS

Significant cultivar x storage periods interactions were observed for percent phosphorus in cassava seeds.

The highest phosphorus content was observed in Aworowa-3 seeds stored for three months which was significantly greater than the other treatment combinations except seeds of Aworowa-3 and Ahwengyanka-2 stored for six months each. Ahwengyanka-1 seeds without storage had the least phosphorus content. The phosphorus content of Aworowa-3 seeds stored for three months was 1.33 times higher than seeds of Ahwengyanka-1 without storage (Table 4.11).

Table 4.11: Effects of cultivar and storage period on the percent phosphorus content of cassava seeds

Storage Periods	Percent phosphorus content (%)			
	Cultivars			
	Ahwengyanka-1	Ahwengyanka-2	Aworowa-3	Mean
No storage	0.87	0.95	0.89	0.90
Three months	0.89	0.95	1.16	1.00
Six months	0.95	1.01	1.12	1.03
Mean	0.90	0.97	1.06	
HSD (0.01): Variety =0.09; Storage Periods =0.09; Variety x Storage Periods = 0.21				

4.13 EFFECT OF CULTIVAR AND STORAGE PERIOD ON PERCENT POTASSIUM CONTENT OF CASSAVA SEEDS

The interaction between the cultivars and storage periods produced significant differences in the percent potassium content. The highest potassium content was recorded in Aworowa-3 seeds without storage while the least was recorded in Ahwengyanka-2 seeds

stored for three months which was 1.54 times less in content than the Aworowa-3 seeds without storage (Table 4.12).

Across the varieties, seeds that were not stored at all produced the highest (0.57%) mean potassium content while seeds stored for six months recorded the least (0.48%).

Across the storage periods, the least mean potassium content was obtained in the Ahwengyanka-2 seeds while the highest was obtained in the Ahwengyanka-1 and Aworowa-3 seeds (Table 4.12).

Table 4.12: Effects of cultivar and storage period on percent potassium content of cassava seeds

	Percent potassium content (%)	
Storage Periods	Cultivars	Mean

	Ahwengyanka-1	Ahwengyanka-2	Aworowa-3	
No storage	0.55	0.53	0.63	0.57
Three months	0.60	0.41	0.50	0.50
Six months	0.46	0.48	0.50	0.48
Mean	0.54	0.47	0.54	
HSD (0.05): Variety =0.02; Storage Periods =0.02; Variety x Storage Periods = 0.05				

4.14 VARIETY AND STORAGE PERIOD EFFECTS ON OCCURRENCE OF FUNGAL PATHOGENS ON SEEDS OF THREE CASSAVA VARIETIES.

A total of seven fungal pathogen species were identified on the seeds of the three cassava cultivars. The pathogens included *Aspergillus flavus, Aspergillus niger, Bipolaris oryzae, Curvularia lunata, Fusarium moniliforme, Penicillium spp., and Rhizopus*. Of the seven pathogens, *Aspergillus flavus* recorded the highest pathogen occurrence on the seeds, followed by *Penicillium sp* and *Curvularia lunata* as the least.

For cultivar, the highest occurrence of fungal pathogens was observed on seeds of Ahwenyanka-1 whiles the lowest occurrence was recorded on seeds of Aworowa-3. In terms of storage periods, the seeds without storage recorded the highest number of fungal occurrence; whiles the seeds stored for six months recorded the least occurrence (Table 4.13 – Table 4.15).

Table 4.13: Frequency of occurrence of pathogens

Pathogens	Frequency of occurrence
Aspergillus flavus	53.33
Pennicillium spp.	29.67
Aspergillus niger	28.00
Rhizopus spp.	13.67
Bipolaris oryzae	8.67
Fusarium monilliforme	7.00
Cuvalaria lunata	6.67

Table 4.14: Frequency of occurrence of pathogens on three cultivars of cassava

Pathogens	Cultivars		
	Ahwengyanka 1	Ahwengyanka 2	Aworowa 3
Aspergillus flavus	4.67	2.56	1.78
Aspergillus niger	2.11	1.44	1.89
Pennicillium spp.	2.44	2.56	2.11
Bipolaris oryzae	1.22	1.44	1.00
Fusarium monilliforme	1.00	1.00	1.11
Cuvalaria lunata	1.00	1.00	1.11
Rhizopus spp.	1.67	1.78	1.89

Table 4.15: Frequency of occurrence of pathogens during storage

Pathogens	Storage period		
	0 month	3 months	6 months
Aspergillus flavus	1.56	2.78	4.67
Aspergillus niger	2.56	1.33	1.56
Pennicillium spp.	3.22	1.56	2.33
Bipolaris oryzae	1.67	1.00	1.00
Fusarium monilliforme	1.11	1.00	1.00
Cuvalaria lunata	1.11	1.00	1.00
Rhizopus spp.	1.00	1.00	3.33

4.15 CORRELATION ANALYSIS

Correlation relationships among the seed quality parameters throughout the storage periods were conducted but there were no significant effects recorded.

CHAPTER FIVE

5.0 DISCUSSION

5.1 PURITY AND WEIGHT OF CASSAVA SEEDS UNDER VARYING STORAGE PERIODS IN AMBIENT CONDITIONS

The percent purity obtained from the three varieties of cassava seeds used in this study was high and this indicated that, after harvesting, the seeds were properly cleaned before storage. Seed lots with high purity rates mostly produce good quality crops but seed quality is reduced when seed lots have a significantly lower purity rate or a high level of contamination (van-Gastel *et al.,* 1996). In the present study, cassava seeds stored for six months produced the highest 1000 seed weight. Interestingly, increasing seed weight was observed to be directly proportional to the lengthening of the storage period. This observation could be attributed to the high relative humidity recorded during the time of storage which resulted in the imbibition of moisture by the seeds during the period of storage. The 1000 seed weight obtained for the three cultivars of cassava seeds used in this study agrees with the findings of Roger (1965) that on the average, the 1000 seed weight of cassava seeds was 100g.

5.2 GERMINATION DYNAMICS OF SEEDS OF THREE CASSAVA CULTIVARS

The pre-germination treatment afforded seeds of a cultivar may have an effect on their germination response (van-Gastel *et al.,* 1996). This finding was evident in the present

study where Aworowa-3 seeds recorded profound differences in germination percentage depending on the pre-germination treatment. When Aworowa-3 seeds were mechanically scarified with sand paper they produced a germination percentage of 56.7% as against when similar Aworowa-3 seeds were acid-treated (42.2%). The seeds that were treated by mechanical scarification using sand paper produced the highest germination rate while the seeds with no treatment produced the least. This observation agrees with Pujol *et al.* (2002) who reported that pre-treatment of seeds by mechanical scarification had significant effects on the probability of water imbibition leading to increase in germination by seeds. Mechanical scarification of dormant seeds has also been found to be an efficient method in increasing the rate of germination over time in *Acacia* spp. seeds (Mohammed, 1981), seeds of *Adesmia* spp. (Tedesco *et al.,* 2001) and cassava seeds (Pujol *et al.,* 2002). Cassava seeds exhibit the exogenous type of dormancy due to the hardness of the seed coat and mechanical scarification is one of the best methods used in breaking physical or exogenous dormancy in cassava seeds. Moreover, Hartman and Kester (1983) indicated that mechanical scarification was an effective method used in breaking physical, mechanical and chemical dormancy in seeds since the impermeability of hard seed coat to water and gases had been found to be part of the many causes of poor germination in seeds (Ibrahim, 2004).

In the present study, seeds treated with sulfuric acid recorded lower germination rates but this contradicts an earlier study by Gupta *et al.* (2001), who reported that treating *Peltophorum pterocarpum* seeds with sulfuric acid yielded no germination. He explained that, the acid acted mainly on the micropylar and hilar regions of seeds and higher acid concentrations and long soaking periods not only damaged the soft tissues, but it created

cracks on the testa, which allowed the acid to gain access into the embryonic tissue causing the death of the seed. In the present study, the rate of germination of seeds treated with sulfuric acid was about 42 %, an indication that sulfuric acid had a positive effect on the germination of cassava seeds, an observation also found by Lefèvre (1989). Both short (5 minutes) and long (30 minutes) periods of immersion recorded germination. In other studies, the dormancy in *Cassia fistula* seeds was released when the seeds were soaked in concentrated sulfuric acid for 15 minutes (Karaboon *et al.*, 2005). Similarly, Zodape (1991) obtained significantly higher germination rate of *Peltophorum pterocarpum* seeds treated by soaking in sulfuric acid for 5 – 75 minutes, washed and soaked in distilled water for 8-24 hours. These results however contradict those of Aliero (2004) and Adjata *et al.* (2013) who indicated that prolonged immersion of seeds in sulfuric acid (H_2SO_4) injured the seeds and also destroyed some vital parts of the embryo leading to low or no germination. The occurrence of germination in the seeds pretreated with hot water but not stored could be due to the ability of the hot water to cause rupture of the hard seed coat which allows water imbibition leading to seed germination and further rapid emergence (Travlos *et al.*, 2007; Lebutswe *et al.*, 2003). In the present study, the germination percentages recorded for cassava seeds were higher than the10-40% previously reported (Ekandem, 1970).

5.3 CULTIVAR AND STORAGE EFFECTS ON MOISTURE, MINERAL, OIL AND PROTEIN CONTENTS OF SEEDS OF CASSAVA

The quality of seeds is adversely affected by its moisture content which usually increases during harvesting, processing and storage and therefore needs to be kept at low levels at all times (van-Gastel *et al.,* 1996) if the quality of the seeds needs to be protected. High moisture content in seeds is known to initiate fungal development during seed storage and also causes rapid seed deterioration (van-Gastel *et al.,* 1996). In the present study, the range of moisture content of the cassava seeds falls within the safe moisture limit for long term storage thereby implying that the seeds were properly dried before storage. Storage of oil seeds for a long period of time is possible only when the moisture content of the seed is less than 10% or when seeds are dried to 8% (Daun, 1995).

Several factors are involved in the germination process in seeds; some of which are the presence of calcium, magnesium, nitrogen in appreciable amounts to prevent the inhibition of germination (Eze *et al.,* 2013). In the present study, the amounts of calcium and nitrogen in the seeds were found to decrease with increasing storage period though no significant differences were observed. This observation is similar to the findings of Fagbohun *et al.* (2011) who reported a decrease in Na, Ca, Mg, Fe, Mn and Cd in soyabean seeds stored for a period of 5 months. Conversely, the content of magnesium increased with increasing storage in the Aworowa-3 variety. This agrees with the findings of Amusa *et al.* (2003), who reported an increase in the mineral constituents of African star, including magnesium, after nine days of storage.

The content of potassium was observed to decline with increasing storage and this agrees with the report of Fagbohun *et al.* (2011).

Aworowa-3 seeds recorded an oil content of 27.8% which was significantly higher than the oil content of the other two varieties. This notwithstanding, the range of values obtained in the present study (24.7%– 27.8 %) is in agreement with the range (24.527.3%) reported for five cassava seed varieties by Tanjararux *et al.* (1985). In terms of storage, the reduction in the oil content at six months of storage could be explained by the occurrence of auto-oxidation of lipids which causes an increase in free fatty acids (Reuzeau and Cavalie, 1995; Balašević-Tubić *et al*., 2005; Kauser *et al.,* 2009). Similarly, Šimić *et al.* (2007) observed a significant decrease in seed oil content of soyabean and sunflower as storage progressed and concluded that the difference in seed oil content was affected by storage longevity. Oil seeds such as cassava are usually short-lived due to their high oil content, chemical composition and processes that take place during storage which lead to loss of germination and viability if proper care and handling are not given to the seeds (Balašević-Tubić *et al*., 2007).

The percent protein content of the three cassava varieties is similar to that of Tanjararux *et al.* (1985) who reported a protein content range of 15.1 to 19.5% for seeds of five varieties of cassava. These ranges of protein content, exceed that of soya bean (12.5 %) and castor oil seed (13.2 %) (Nzelu, 2006; Achi, 2005).

5.4 CULTIVAR AND STORAGE EFFECTS ON VIGOUR AND GERMINATION OF SEEDS OF CASSAVA VARIETIES

Among all three varieties, Aworowa-3 had the highest mean germination percentage. This could be attributed to several factors such as varietal differences, the oil and protein contents, weight of seed, moisture content, among others. The Aworowa-3 seeds stored for six months produced the highest germination. It proved to be the best of the varieties used in this study since germination even occurred without storage whereas the remaining two varieties failed to germinate. From the present study, percentage vigour increased as storage increased implying that vigour of cassava seeds increases over time; this could be due to the chemical processes that take place in the seeds during storage such as the release of dormancy which occurs as storage increases. The increase rate of vigour could ascertain the reason why germination rates increased with increasing storage. However, even though the seeds without storage had a significantly high leachate rate which indicates low percentage vigour (ISTA, 2007), they still produced an appreciable rate of germination (about 40%). The pattern of vigour of cassava seeds does not agree with the findings of Pratt *et al*. (2009) that vigour declines in storage as a result of deterioration of seed quality which precedes loss in standard germination and this could be attributed to the inherent dormancy exhibited by seeds of

cassava.

The increasing rate of germination with increasing storage confirms reports of Girase *et al.* (2002) that the length of storage caused the release of dormancy in seeds of *Peltophorum pterocarpum*. Martins *et al.* (2009) also observed that an increase in the rate of germination occurred with an increasing storage period, especially in *Manihot glaziovii*

and *Manihot pseudoglaziovii* seeds. Similarly, Mezzalira *et al*. (2013) reported that there was an increase in the rate of germination of stored seeds of different plant species and explained that the room temperature adequately attenuated the intensity of dormancy and caused acceleration in the germination and emergence of seedlings.

Conditions such as the temperature and relative humidity of the storage environment also play major roles in seed quality maintenance or deterioration. High temperature and relative humidity (25°C/75%) in storage environments cause seeds to deteriorate at a faster rate thereby leading to lower seed vigour and germination percentages than storage environments with low temperature and relative humidity (12°C/60%) (Branimir *et al.,* 2006; Al-Yahya, 2001).

5.5 SEED HEALTH OF STORED SEEDS AND THEIR POTENTIAL GERMINATION

The number of pathogens associated with the cassava seeds may lead to reduction in germination and storability of the seeds (Shelar *et al.*, 2008) and not necessarily cause diseases (Agarwal, 1995) since three components (host, pathogen and environment) must come together and interact to cause a disease (Agrios, 2005). The high mineral content of the seeds in the present study could possibly be the cause of the presence of *Rhizopus* spp, *Penicillium* spp, *Aspergillus niger* and *Aspergillus flavus* since minerals are useful for the occurrence of such fungi as exemplified in groundnut seeds (Sullivan, 1984).

The presence of fungi on the seeds might have played a significant role in reducing the percentage of germination of the seeds. Ibrahim (2004) reported a low germination rate

of seeds when he identified the infection of fungi on the seeds. Similarly, Ingale and Shrivastava (2011) reported that the presence of moulds on seeds caused physical and biochemical damages to crops stored under conditions that were likely to promote their growth and these damages could have a tendency of reducing or terminating germination.

Furthermore, since mineral elements needed for germination (Eze *et al.*, 2013) were also responsible for the infection of fungi, the presence of such elements could have affected the proximate and nutrient composition resulting in a low germination rate as observed in the *Cocos nucifera* fruit (Onifade and Agboola, 2003).

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

- A rate of 40% germination was obtained from cassava seeds stored up to three months. This is appreciable since seeds stored up to three months are known to exhibit dormancy which is usually released after a period of time.
- Aworowa-3 seeds without storage produced a higher germination rate than Ahwengyanka-1 and Ahwengyanka-2 seeds without storage but all the seeds of the three varieties had the highest germination percentage when stored for six months.
- Mechanical scarification with sand paper proved to be the best method in breaking dormancy of cassava seeds especially seeds stored up to three months.

6.2 RECOMMENDATIONS

1. Other forms of mechanical scarification could be adopted to determine the response of seeds of cassava cultivars especially seeds with no storage to imbibition of water, emergence and establishment.
2. The use of other pregermination treatments such as growth hormones should be studied for seeds of these cassava cultivars to determine their germination response
3. Enzyme activity test should be conducted to determine the causes of dormancy of these cassava cultivars as well as others which are of importance to breeders and seed users.

4. Other cultivars of cassava seeds should be studied to assess the quality attributes of the seeds under different storage periods and environments.

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APPENDICES

Appendix 1: Analysis of Variance Table for Germination

Source	**DF**	**SS**	**MS**	**F**	**P**
Storage	2	27260.3	13630.2	715.58	0.0000
Variety	2	860.3	430.2	22.58	0.0000
Dormancy	6	2917.5	486.2	25.53	0.0000
Storage*Variety	4	307.9	77.0	4.04	0.0041
Storage*Dormancy	12	295.2	24.6	1.29	0.2313
Variety*Dormancy	12	1473.0	122.8	6.44	0.0000
Storage*Variety*Dormancy	24	1181.0	49.2	2.58	0.0004
Error	126	2400.0	19.0		
Total	188	36695.2			

Grand Mean 43.175 CV 10.11

Appendix 2: Analysis of Variance Table for Transformed Germination

Source	**DF**	**SS**	**MS**	**F**	**P**
Storage	2	2.92613	1.46306	713.34	0.0000
Variety	2	0.10195	0.05097	24.85	0.0000
Dormancy	6	0.36912	0.06152	30.00	0.0000
Storage*Variety	4	0.04105	0.01026	5.00	0.0009
Storage*Dormancy	12	0.05082	0.00424	2.06	0.0238
Variety*Dormancy	12	0.18187	0.01516	7.39	0.0000
Storage*Variety*Dormancy	24	0.15496	0.00646	3.15	0.0000
Error	126	0.25843	0.00205		
Total	188	4.08433			

Grand Mean 0.7137 CV 6.35

Appendix 3: Analysis of Variance Table for vigour

Source	**DF**	**SS**	**MS**	**F**	**P**
Storage	2	0.05927	0.02963	3.05	0.0721
Variety	2	0.03449	0.01724	1.78	0.1975
Storage*Variety	4	0.21158	0.05289	5.45	0.0047
Error	18	0.17467	0.00970		
Total	26	0.48000			

Grand Mean 1.1567 CV 8.52

Appendix 4: Analysis of Variance Table for Purity

Source	**DF**	**SS**	**MS**	**F**	**P**
Storage	2	0.09852	0.04926	0.82	0.4558
Variety	2	0.16519	0.08259	1.38	0.2778
Storage*Variety	4	0.33259	0.08315	1.39	0.2784
Error	18	1.08000	0.06000		
Total	26	1.67630			

Grand Mean 99.770 CV 0.25

Appendix 5: Analysis of Variance Table for 1000 seed weight

Source	**DF**	**SS**	**MS**	**F**	**P**
Storage	2	464.89	232.444	6.40	0.0079

Variety	2	18.67	9.333	0.26	0.7761
Storage*Variety	4	65.78	16.444	0.45	0.7689
Error	18	653.33	36.296		
Total	26	1202.67			

Grand Mean 116.22 CV 5.18

Appendix 6: Analysis of Variance Table for moisture

Source	**DF**	**SS**	**MS**	**F**	**P**
Storage	2	11.1785	5.58926	57.38	0.0000
Variety	2	3.7785	1.88926	19.40	0.0000
Storage*Variety	4	0.7126	0.17815	1.83	0.1672
Error	18	1.7533	0.09741		
Total	26	17.4230			

Grand Mean 6.7630 CV 4.61

Appendix 7: Analysis of Variance Table for Fat

Source	**DF**	**SS**	**MS**	**F**	**P**
Storage	2	11.8656	5.9328	32.12	0.0000
Variety	2	50.7263	25.3631	137.31	0.0000
Storage*Variety	4	3.2896	0.8224	4.45	0.0112
Error	18	3.3249	0.1847		
Total	26	69.2064			

Grand Mean 25.967 CV 1.66

Appendix 8: Analysis of Variance Table for Protein

Source	DF	SS	MS	F	P
Storage	2	31.1679	15.5840	21.09	0.0000
Variety	2	4.4713	2.2357	3.03	0.0737
Storage*Variety	4	4.0259	1.0065	1.36	0.2862
Error	18	13.3016	0.7390		
Total	26	52.9668			

Grand Mean 16.491 CV 5.21

Appendix 9: Analysis of Variance Table for Calcium

Source	DF	SS	MS	F	P
Storage	2	5.70480	2.85240	596.09	0.0000
Variety	2	0.00336	0.00168	0.35	0.7090
Storage*Variety	4	0.03078	0.00769	1.61	0.2155
Error	18	0.08613	0.00479		
Total	26	5.82507			

Grand Mean 1.2011 CV 5.76

Appendix 10: Analysis of Variance Table for Magnesium

Source	DF	SS	MS	F	P
Storage	2	0.28405	0.14203	55.58	0.0000
Variety	2	0.63267	0.31634	123.78	0.0000
Storage*Variety	4	0.32799	0.08200	32.09	0.0000
Error	18	0.04600	0.00256		
Total	26	1.29072			

Grand Mean 0.9326 CV 5.42

Appendix 11: Analysis of Variance Table for Nitrogen

Source	**DF**	**SS**	**MS**	**F**	**P**
Storage	2	0.95027	0.47514	20.95	0.0000
Variety	2	0.13736	0.06868	3.03	0.0735
Storage*Variety	4	0.12273	0.03068	1.35	0.2892
Error	18	0.40820	0.02268		
Total	26	1.61856			

Grand Mean 2.8870 CV 5.22

Appendix 12: Analysis of Variance Table for Phosphorus

Source	**DF**	**SS**	**MS**	**F**	**P**
Storage	2	0.07529	0.03764	6.99	0.0057
Variety	2	0.10949	0.05474	10.17	0.0011
Storage*Variety	4	0.06856	0.01714	3.18	0.0384
Error	18	0.09693	0.00539		
Total	26	0.35027			

Grand Mean 0.9778 CV 7.51

Appendix 13: Analysis of Variance Table for Potassium

Source	**DF**	**SS**	**MS**	**F**	**P**
Storage	2	0.03956	0.01978	72.18	0.0000
Variety	2	0.02792	0.01396	50.93	0.0000
Storage*Variety	4	0.04713	0.01178	42.99	0.0000

Error	18	0.00493	0.00027
Total	26	0.11954	

Grand Mean 0.5185 CV 3.19