CHARACTERIZATION OF STARCH ACCUMULATION IN STORAGE ROOT OF MARAMA (*TYLOSEMA ESCULENTUM)*

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ABSTRACT

Starch is of substantial industrial significance for food and non-food uses. Plant roots and tubers such as potato and cassava are rich in starch and they are among the sources of starch for food consumption and industrial use. With climatic changes it is important to find crops that can be used as food and will still be able to survive the arid conditions. Marama is a plant that grows in the arid Namibian conditions, that bears a storage root that it is underutilized and has a potential to serve as an alternative source of starch. The main aim of this study was to determine the most suitable time for harvesting by evaluating the quality of marama root and its starch at the different harvest times. Effects of harvesting time on the proximate analysis of the marama roots as well as the thermal properties, size and physicochemical properties of the starch were investigated. Total starch of the marama roots (dry basis) increased with harvesting time, it ranged from 25.9 to 60.1% while the amylose content on starch basis decreased with harvesting time, ranging from 21.4-50.7%. Starch content was determined by enzymatic hydrolysation of starch to glucose and quantified colorimetrically by the Glucose oxidase-peroxidase reaction. Whereas amylose content was determined by the precipitation of amylopectin with lecin Concanavalin-A protein, amylose was then enzymatically hydrolysed to glucose and quantified using Glucose oxidase-peroxidase. Marama root starch granules were spherical, oval and lenticular in shape, the size of the granules increased with harvesting time and the mean granule (diameter) size ranged from 8.6 – 15.1µm. The youngest (2 month old) marama root had the highest crude protein content (33.6%). Crude protein content decreased from 33.6% down to 2.7% at the 12 months harvest time. The thermal properties of the freeze dried marama roots (2-12 month root samples) showed that it has an endothermic peak of 73.4-93.0 ˚C. This was higher

than the gelatinization temperature of marama starch reported in literature suggesting that the other components of the marama root (proteins, ash, sugars and fibre) can affect the thermal properties. Literature reports that proteins, sugars and fibre delay the gelatinization of the starch. The T_p decreased with harvesting time, while the enthalpy change increased with harvesting time. The thermal properties of marama starch were affected by two factors : 1) the decrease in amylose content of the starch and therefore the increase in amylopectin content; hence an increase proportion of crystalline components (more ordered) the starch and therefore the increase in crystallinity of the starch crystallinity of the starch, which was evident in the increase in the ΔH as crops matured 2) the decrease in other components present in the root flour samples, hence a decrease in the interactions with the starch, the interactions may possibly have been responsible for the delay in endothermic peak, which was evident in the decrease in the T_o, T_p and T_c temperatures as the crop matured. It is therefore safe to conclude that time has an effect on the agronomic and physicochemical properties of the marama storage roots and its starch. The 2 months after planting is the optimal harvesting time for a good nutritional content of marama root, 2 month roots are rich in protein, fibre and ash and are less fibrous. Whereas the optimal harvest time for a good starch is dependent on the intended use of the starch. If a high amylose starch is preferred then the optimal harvesting time would be 2 months, while if normal starch is preferred then the 8 and 12 months present the optimal harvest time. However, the optimal harvesting time for better extractability is at 12 months after planting because of the higher total starch content. It is then recommended that future research focuses on the isolation and application of the marama root starch from roots harvested at different times in the food or non-food industries. This study also recommends that marama should be planted early in summer and harvested after 2 months when it is domesticated for a root vegetable.

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GOPOD – Glucose Oxidase Peroxidase

Con A – Concanavalin A

FAA – Formalin Acetic Acid

DMSO – Dimethyl Sulfoxide

DPX – Di (n-butyl) Phthalate in Xylene

PAS – Periodic acid-Schiff

EDTA - Ethylenediaminetetraacetic acid

NO_X – Nitrogen oxides

AOAC –Association of Agricultural Chemists

DSC – Differential Scanning Calorimetry

Analysis of variance – ANOVA

SE – Standard error

T_o – Onset temperature

T_p – Peak temperature

T_c – Conclusion temperature

ΔH – Enthalpy change

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DEDICATIONS

This thesis is dedicated to my mother, Hendrina Angala. Thank you for being a constant pillar of strength, and for the support and motivation. Thank you for the unconditional love and sacrifices, words are not enough to express my deep appreciation.

DECLARATIONS

I, Maria Hambeleleni Hamunyela, declare hereby that this study is a true reflection of my own research, and that this work or part thereof has not been submitted for a degree in any other institution of higher education.

…….. Date……

Maria Hambeleleni Hamunyela

CHAPTER 1: INTRODUCTION

Starch is the most common carbon reserve stored in plants; it is of a great significance for both food and non-food industrial uses (Geigenberger 2003). In 2012, 75 million tonnes of starch was produced for worldwide industrial applications (Waterschoot et al. 2015) and about 54 % of the starch produced globally is utilized for food applications (Omojola 2013). In the food industry, the main trend in starch applications remains in syrup production and formulation of ready meals, instant food and various sauces (Bertolini 2010). The industrial application of starch is determined by the morphology and physicochemical characteristics of the starch, these characteristics are unique to the biological origin of the starch (Gebre-Mariam et al. 1996). Starch functionality depends on molecular average weight of amylose and amylopectin, as well as their molecular organization within the granule. "The choice of the right starch within food applications must take into consideration such aspects such as food process technology, functional, sensorial, and rheological properties, and co-ingredients" (Bertolini 2010, p.8).

Starch is a major source of energy in the human diet. It accounts for approximately 50 % of the calorie intake in the developed countries and 90% of the calorie intake in the developing countries (Xu et al. 2017). The current sources of starch are a restricted range of crops, the most important being maize, potato, wheat, and tapioca with smaller amounts from rice, sorghum, sweet potato, arrowroot, sago, and mung beans (Wang et al. 1998). Maize is the major source of starch contributing 73%, followed by cassava

(14%), wheat (8%), potato (4%) and others (1%) (Figure 1). The main crops in Namibia are pearl millet, white maize, sorghum and wheat (Kolberg 1996). However, there is no commercialized starch from these crops in Namibia and they are underutilized. The marama bean plant is a storage root bearing plant that is indigenous to the Kalahari sandy region, and could prove to be a starch alternative. Plant roots such as cassava and tubers such as potato are rich in starch and they are among the sources of starch for consumption or industrial use (Shewry 2003). According to Huang et al. (2006) roots and tubers contain 70–80% water, 16–24% starch and trace amounts of protein and lipids. The dry matter of roots and tubers mainly consists of starch, which accounts for approximately 70% of the total solids thus making it the major component (Huang 2009). Due to their high starch content, root and tuber crops are the important staple foods and are also used as ingredients in processed foods across the world (Huang 2009).

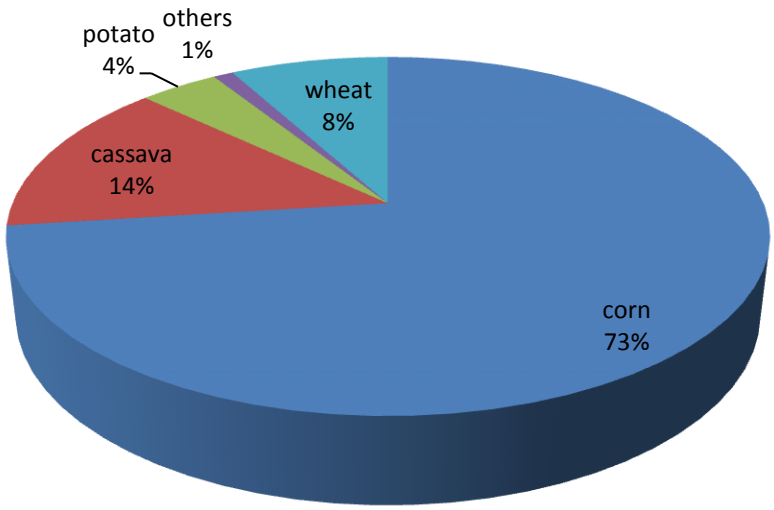

Figure 1: Starch production according to botanic sources (Ropers and Elvers as cited in Bertolini, 2010).

Tylosema esculentum is an underutilized drought tolerant legume, native to the arid and semi-arid regions of southern Africa. It produces protein and oil rich seeds and a storage root used as food (Travlos et al. 2008). In Namibia it grows wild mainly in Omaheke and Otjizondjupa regions, while it grows in the Limpopo, Gauteng and Northern Cape provinces of South Africa (Jackson et al. 2010). Both the seeds and the large storage root are used for consumption by the locals (Powell 1987). Due to the high nutrient value of the seeds and storage root, rich in protein, oil and starch, it is a potential crop for arid areas where few conventional crops can survive. The starch accumulation and physicochemical properties of the marama root have not been studied extensively; there is only one paper by Adeboye and Emmambux (2017) on the marama root starch

characteristics. Thus, research and product development is needed to exploit marama root starch.

1.1. Statement of the problem

T. esculentum is an indigenous plant that grows naturally in the Namibian poor soil and dry conditions. With climatic changes it is important to find climate smart crops that can be used as food and will still be able to survive the arid conditions. Furthermore there is no documentation of starch production from the Namibian plants at a commercial level and the starch that is utilised in the food industry or for other purposes (e.g. laboratory use) is imported. Additionally, there is no published work on when and where starch accumulates in the marama root. Moreover, the utilization of marama roots is low and the marama storage root can serve as an alternative source of starch thus diversifying the sources of starch. Therefore, research to improve utilization of marama storage root starch in the food industry is needed.

1.2. Objectives of the study

The objective was to study the physicochemical and starch accumulation changes of marama roots during growth with the aim of determining the suitable time for harvesting for better nutritional and starch extractability. The specific objectives of this study were to:

i) To determine the size (Length, Diameter, and Mass) and moisture content of the marama roots as they develop over time of 12 months.

ii) To determine the starch accumulation, starch average granule size and shape in the marama roots as they develop over time of 12 months.

iii) To determine the total starch content and amylose/amylopectin content of marama roots as they develop over time of 12 months.

iv) To determine the thermal properties of marama root as the marama roots develop over time of 12 months.

A study on marama root starch accumulation and characterization of the starch will bridge the gap in knowledge and lead to the value addition to the marama root and hence contribute to food security in arid regions. The proposed research is of socio economic significance because it will provide an insight into when to harvest and the potential application of the marama root starch. These findings will facilitate domestication and commercialization of marama. The commercial application of the marama root starch could create a demand for the marama crops, and people can grow this crop and sell to make an income. Characterization of the marama root starch and starch accumulation is therefore very important in establishing its probable applications.

CHAPTER 2: LITERATURE REVIEW

2.1. *Tylosema esculentum* (Marama)

T. esculentum (marama) is an underutilized edible indigenous perennial plant that grows wild under dry conditions in Namibia. *T. esculentum* belongs to the Tylosema genus, which belongs to the Caesalpinioidea subfamily of the Fabaceae family. It is native to the Kalahari desert, the arid and semi-arid areas in Botswana, Namibia and the northern parts of South Africa (Hartley et al. 2002). In Namibia it is distributed in areas in the Omaheke and Otjozondjupa regions (Figure 2).

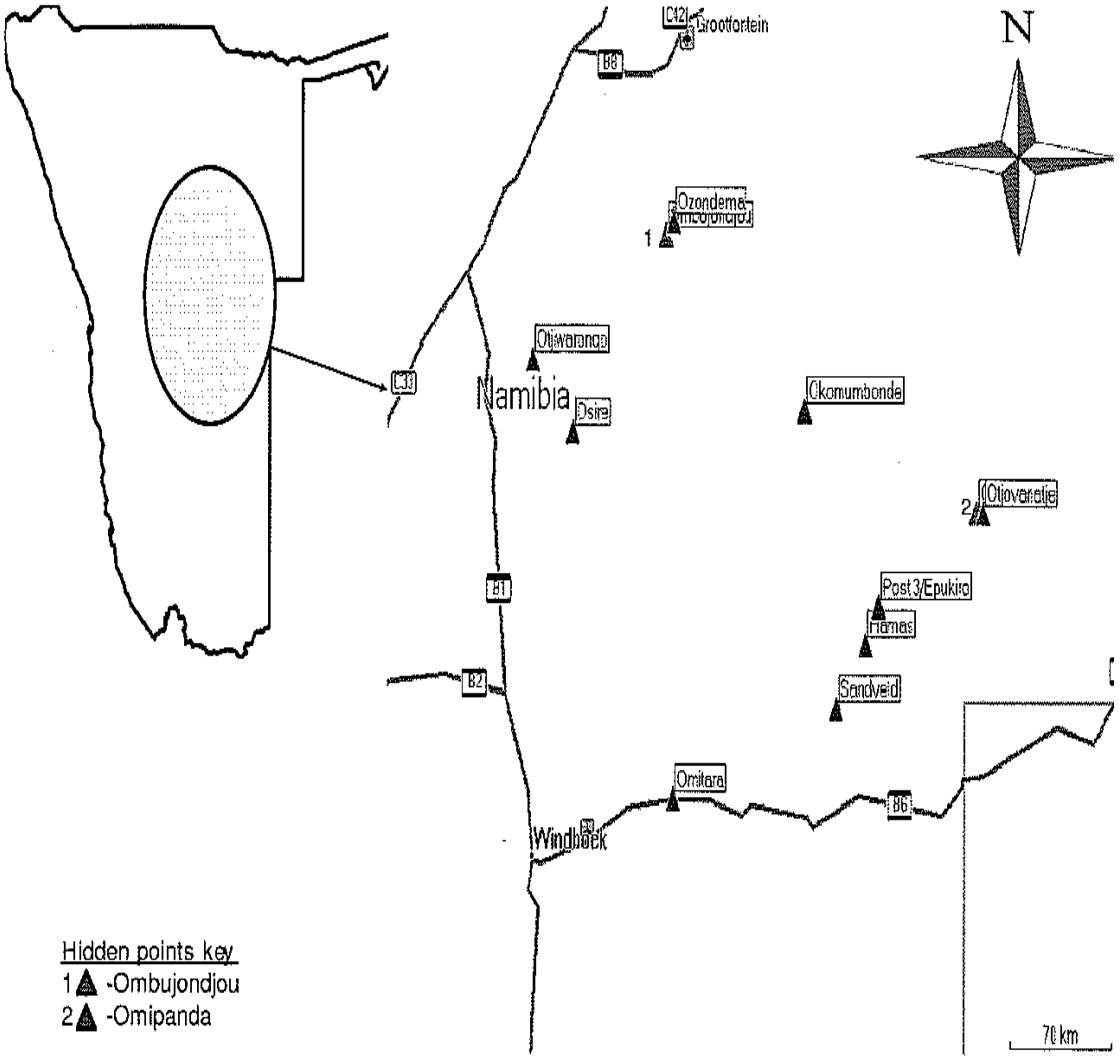

Figure 2: Geographical distribution of *Tylosema esculuntum* in Namibia (Nepolo et al. 2009)

Marama is used for consumption, as feed and medicine by the natives of these areas. It is also known as gemsbok bean, moramaboontjie, elandboontjie, braaiboonjie, marumana, tsi, tsin, gami, and ombanui (Jackson et al. 2010). Marama is a long lived plant that generates from a large, edible, underground storage root which is used to store water.

The above ground vegetation consists of numerous prostate vines which can go up to 6 m in length (Bower et al. 1988)). The plant has bi-lobed leaves that are glaucous green and leathery, and yellow flowers that produce round and oblong shaped pods which contain brown shiny seeds (Keith & Renew 1975). Each of the pods contain one to two seeds encased in a hard coat (Jackson et al. 2010).

The seeds are an excellent source of good quality protein and compares well with other protein foods including soya beans (Bower et al. 1988). Although the marama seed protein content is similar to soya bean, the protein composition of the marama seed is very different from that of the soya bean (Amonsou et al. 2012). The seed contains 29–38% protein content, 32–42% lipids, 19–27% dietary fiber and 2.5–3.7% ash (Holse et al. 2010). The immature seed has a high content of proteins (21%) and low lipid content (1.5%), but the contents increased as the seed matured. The mature seed contains 32% protein content , 40% lipid content and no starch (Mosele et al. 2011). The beans have a high total dietary fibre content that varies between 18.7 and 26.8%. The lipid, protein, total dietary fiber and minerals content on average makes up more than 97% of the beans. The beans have a great potential both as a nutritive food on its own and as a protein-rich food supplement in food products (Holse et al. 2010). The mature seeds are roasted and eaten as a snack by the native Ovaherero people who dominate the Otjozondjupa and Omaheke regions of Namibia where *T. esculentum* are found (Nepolo et al. 2009). The immature green seeds are also boiled and eaten as a vegetable and are comparable to green peas (Keith & Renew 1975).

The storage root, is juicy and sweet and is comparable to a sugar beet (Vietmeyer 1986). The roots are eaten as a vegetable, they are usually harvested when they are still fairly small (less than 1kg) as older roots (more than two years) become fibrous and astringent. The younger roots are boiled or roasted before consumption while the older fibrous roots are used as a source of drinking water (Keith & Renew 1975). The storage root is equally important and highly sought after by the natives because it is available even during the dry season unlike the seeds (Bousquet 1982).

In addition, the marama plant loses its leaves and the vines die during winter , however the plant is maintained by the perennial storage root (Hartley et al. 2002). Despite this commendable nutritional status and the ability to survive aridity, marama is not yet commercially cultivated or utilised due to the lack of knowledge about the properties of marama root starch. Marama root starch, like potato and cassava, may be applied in food systems to improve nutrition and/or functionality. However, this will require a fundamental understanding of when to harvest, the starch composition and structure as well as its functionality. As a drought-tolerant legume, marama root has great potential as an alternative source of starch in semi-arid environments.

2.2. Starch

Starch is the main carbohydrate reserve in plants and it is an important part of our diets (Geigenberger 2003). Starch is increasingly being seen as useful raw material to include in foodstuffs (Wang et al. 1998). Starch occurs in plants as semi-crystalline granular structures and the granules from different botanical sources have different characteristics, such as, shapes, sizes and morphology (Jane 2006). Starch granules are composed mainly of two homopolymers with different structures: amylose, is composed of units of D-glucose linked through α-D-(1-4) linkages and amylopectin, the branching polymer of starch, composed of α-D-(1-4)-linked glucose segments containing glucose units in α-D-(1-6) branches (Bertolini 2010). The starch granules consist of amorphous regions predominated by amylose molecules (single helical structures). The starch granule also consists of crystalline regions that are dominated by radially arranged amylopectin molecules (may form double helices) (Figure 3). The degree of the crystalline regions is determined by the branch length of the amylopectin (Martin & Smith 1995).

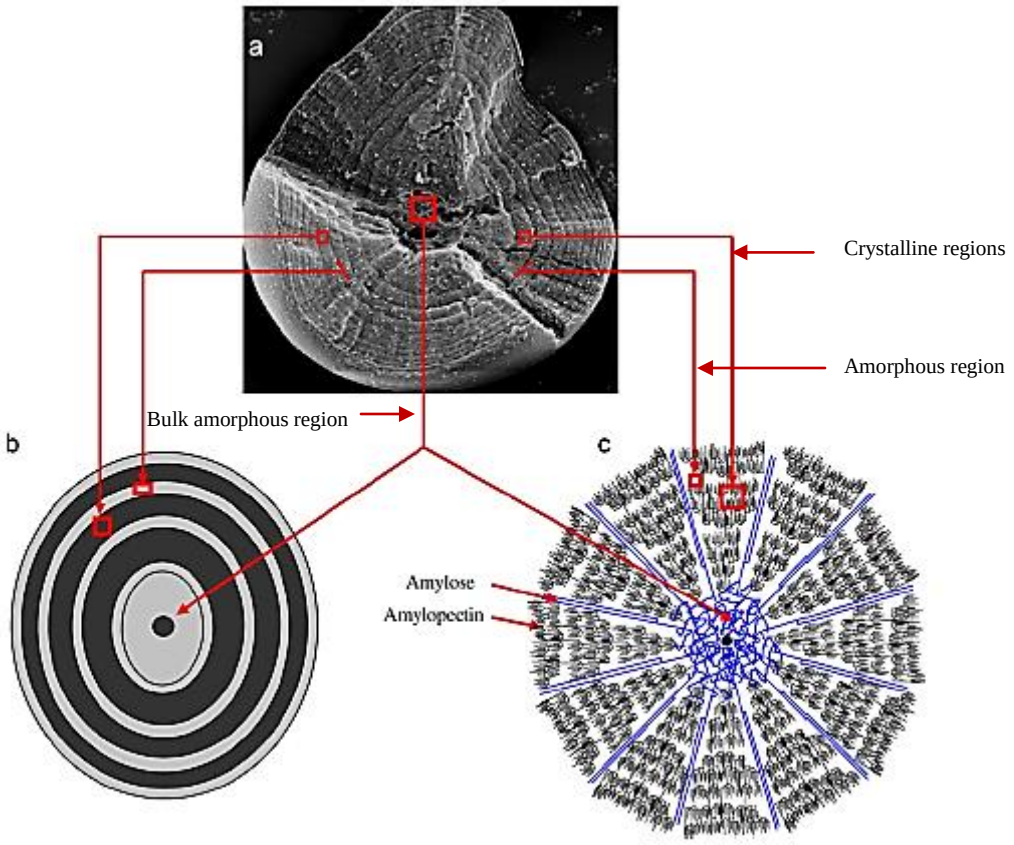

Figure 3: Starch granule organization showing the amorphous regions predominated by amylose and crystalline regions dominated by amylopectin a) SEM image of a pea starch granule b) Growth ring structure c) Chain distribution (adopted from Wang et al. 2012).

Amylopectin has a molecular weight which ranges from 10^7 to 10^8 while amylose has a molecular weight ranging from 5 x 10^5 to 10^6 (Kossmann & Lloyd 2000). The amylose to amylopectin ratio is unique to the botanical source of the starch. Generally, starch contains 20–35% amylose, however waxy starches contain less than 15% amylose and high amylose starches contain more than 40% (Tester et al. 2004). The proportion of amylose does not only depends on the biological origin, but also on the plant organ and

the developmental stage of the organ (Martin & Smith 1995). The amylose to amylopectin ratio is a major factor that controls all the physiochemical properties of starch due to its effect on gelatinization and other functional properties (Wani et al. 2012). In addition, amylopectin is responsible for the crystalline structure of starch granules (Jane 2006). Furthermore, swelling is predominantly a property of amylopectin (Tester & Morrison 1990). Therefore the measurement of amylose content is an important quality parameter for starch. The amylose content of the starch can be quantified by the precipitation of amylopectin with lecin Concanavalin-A (con A) protein followed by an estimation of glucan content using Glucose oxidase-peroxidase following the hydrolysis with amyloglucosidase and α- amylase. A method described by Yun and Matheson (1990), but has been modified by Gibson et al 1997 to develop the amylose/amylopectin Megazyme kit. Con A reacts with the terminal α-D-Glucose groups of amylopectin, therefore differentiating amylopectin from amylose, which has a minimal and insufficient proportion of terminal end groups (Zobel & Stephen 2006). The advantages of this modified Con A procedure for amylose determination are; its applicable to flour samples without the need for prior starch isolation, its suitable for multiple sample analysis and it also allows for the simultaneous estimation of total starch and a calibration curve is not required (Gibson et al. 1997).

Another method of determining amylose content has been reported in literature. The amylose is determined by the colorimetric measurement of the iodine binding capacity of the amylose. Amylose and iodine bind to form a complex that results in a blue color (Chrastil 1987). However this method has been reported to be inconsistent and

inaccurate because amylopectin-iodine complexes also form which absorb a similar wavelength as the amylose-iodine complex and this leads to an overestimation of amylose (Gibson et al. 1997). Therefore, the Con A precipitation of amylopectin method was used in this study.

2.3. Starch accumulation

In higher plants, starch is synthesized in plastids; it is synthesized in the chloroplast of the leaves during the day by fixing carbon through photosynthesis and is mobilized at night. It is also synthesized transiently in other organs, such as meristems and root cap cells. Its major site of accumulation is in storage organs, such as seeds, fruits, tubers, and storage roots. In plant storage organs starch is manufactured within amyloplasts (Martin & Smith 1995). A study by Rouse-Miller et al. (2013) on the storage root of cassava reported that starch accumulation was observed earliest in the first formed cells of the secondary tissue and radiated outward with tuber development. According to Buléon et al. (1998) the starch is stored for a long term in the parenchyma of tubers (Figure 4). Parechyma cell division leads to the increase in bulk of tubers or roots and hence increases in size. Starch is deposited in the form of partially crystalline granules whose morphology, chemical composition and super molecular structure are characteristics of plant origin (Figure 5).

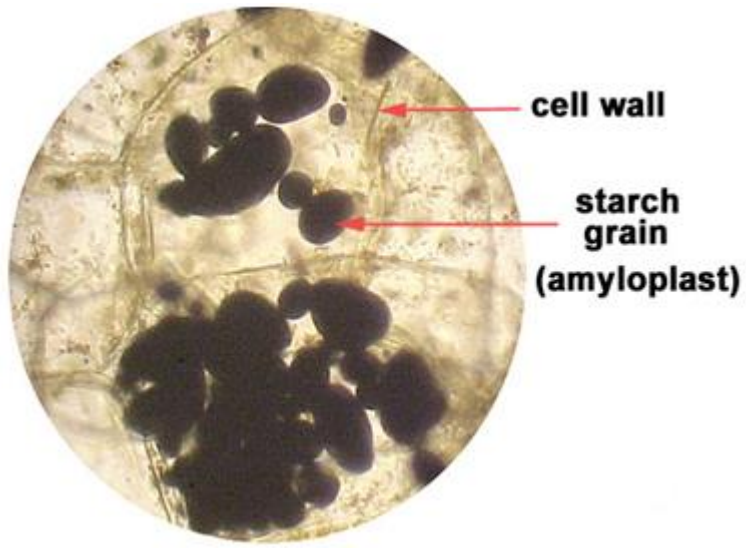

Figure 4: A micrograph showing clusters of starch within potato tuber parechyma cells. the starch granules were stained black with Lugols solution (Armstrong 2001).

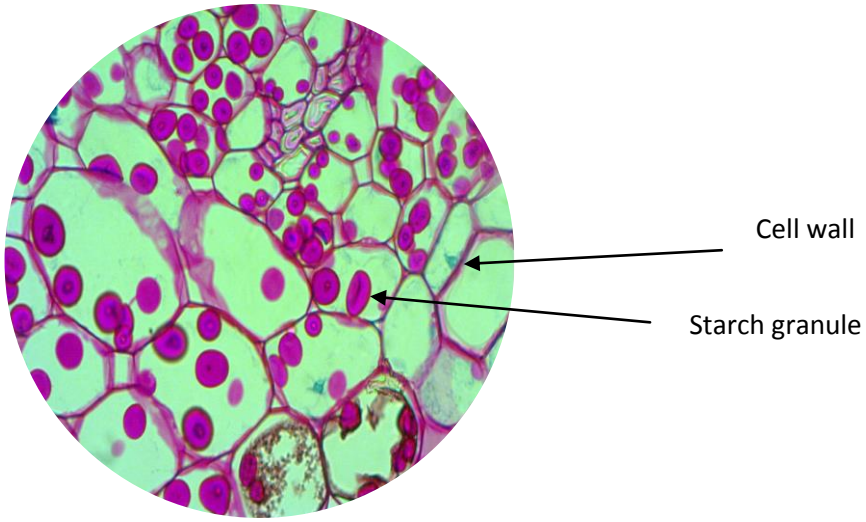

Figure 5: A micrograph showing starch granules within marama root parechyma cells. The cell walls and starch granules were stained a bright fuschia colour with Periodic acid-Schiff.

A study by Turesson et al. (2010) on the characterization of starch accumulation in the tubers of *Cyperus esculuntus* reported that, a high sugar load correlated with the onset of starch accumulation at the beginning of tuber development. It further reported that starch accumulates slowly at the beginning of tuber development until day 7 after tuber initiation and eventually starch content increases rapidly by more than 600% over a period of 10 days. Starch accumulates more efficiently in the central part of the tuber than in the cortex, most likely due to the vicinity of several vascular bundles translocating sucrose to the growing tubers (Turesson et al. 2010). There is currently no literature on starch accumulation in the marama roots.

Sucrose is the first precursor for the starch biosynthesis in the roots and tubers (Figure 6). In the cell cytosol, the sucrose derived from photosynthesis is converted into uridine diphosphate glucose (UDP-glucose) and fructose by the sucrose synthase enzyme. The UDP-glucose is then converted into glucose-1-phosphate (G-1-P) by the UDP-glucose pyrophosphorylase enzyme; this is done in the presence of pyrophosphate. The G-1-P is converted into glucose- 6-phosphate (G-6-P) by the cytoplasmic phosphoglucomutase. The G-6-P is then translocated into the amyloplast. In the amyloplast, the G-6-P is converted to G-1-P by phosphoglucomutase. Thereafter, the G-1-P within the amyloplast is converted into ADP-glucose by the ADP-glucose pyrophosphorylase. The ADP-glucose is finally converted into both amylopectin and amylose. The amylopectin is synthesized by the starch synthase and starch branching enzyme, while the amylose is synthesized by the granule bound starch synthase (Tester et al. 2004).

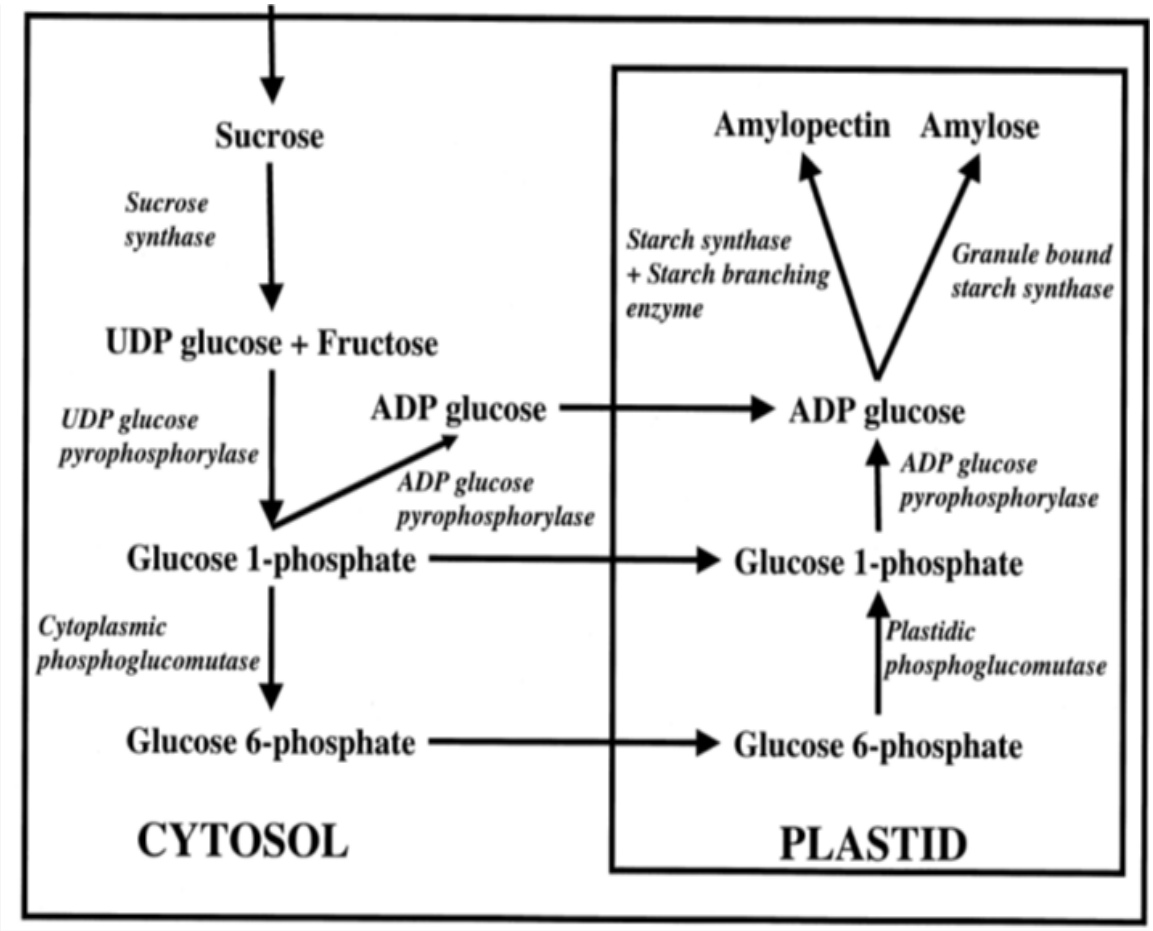

Figure 6: Pathway showing the breakdown of sucrose and starch synthesis in storage organs (Kossmann & Lloyd 2000).

In general, starch structure and functional properties alter according to the stage of development of the plant and the botanical source. There is a trend between amylose content and developmental age (Sriroth et al. 1999). As storage tissues mature, starch content, percentage amylose and average starch granule size increase (Preiss 2009). In most cereals the amylose content is lower at early stages of grain development (Inouchi

et al. 1984). Similarly, this has been observed in potatoes whereby the amylose content and viscosity significantly increased with crop maturity (Ezekiel & Rana 2009). However, a study by Sriroth et al. (1999) further reported that the amylose content of cassava decreased in the older roots. The study suggested that starches extracted from the older roots will have better pasting and swelling properties, which may be due to the amylose size or the greater proportion of amylopectin observed in the older roots.

2.4. Tuber and root starches

Roots and tubers crops such as potato, cassava and yams are plants that are grown for edible purposes. Because of their high starch content, root and tuber crops are the important staple foods and are also used as ingredients in fabricated foods across the world. Root and tuber starches have unique physicochemical properties mostly because of their amylose and amylopectin ratio (Huang, 2009). According to Billiaderis (as cited in Pérez-Pacheco et al. 2014), the ratio of these two components is important given the functional properties they provide; amylose is responsible for the formation and stability of the gels while amylopectin provides viscosity. The tuber and root starches have an amylose content in the range of 1-38% and a lipid content less than 1% (Hoover 2001). In addition the granular size of root and tuber starches ranges from 1-100µm in size, with most granules oval, however spherical, round, polygonal and irregular shaped granules are also found (Hoover 2001). *Solanum tuberosum* (potato) has been reported to have 15-110 µm starch granules with spherical and oval shape, 0.19 % lipids and an amylose content of 25.4%. *Dioscorea alata* has 6-100 µm starch granules with round-

oval shape, 0.03 lipids and an amylose content ranging from 22.8-30 %. Cassava root starch has been reported to have 5-40 µm starch granules that are round and an amylose content in the range of 18.6-23.6% (Hoover 2001). Starch from roots and tubers shows some particular rheological and physical properties, such as clear gel, high viscosity, and lower retrogradation, which are required in the formulation of specific products (Bertolini 2010). The purity, the amylose, amylopectin content, of starches and the shape and size of the granule affect the pasting and gelling properties. Furthermore, harvesting dates influence both the starch molecular structure and pasting and gelatinization properties (Zobel & Stephen 2006). Age root had an effect on the granule structure and hydration properties of cassava root starch (Sriroth et al. 1999). Potato starch was also influenced by harvest time; mean granule size, phosphorus content and peak viscosity increased with harvest time while amylose content decreased with harvest time (Noda et al. 2004). Effect of harvest time was also reported for sweet potato starch, the mean granule size increased with harvest time (Noda et al. 1992) Bridging the gap in knowledge about the accumulation and properties of marama roots starches according to the stage of development might provide insight for industrial application of non-official starches such as *T. esculentum* starch and the best time for harvesting.

2.5. Marama root starch

Although extensive research has been done on the marama bean seeds there has been very little research done on the root. Marama root has been reported to have a starch yield of 9% (Nepolo 2014) and 8.1% (Adeboye & Emmambux 2017), which are low in comparison to those reported for other starch sources such as potato tubers and cassava root. According to Abera and Rakshit (2003) cassava has a starch yield ranging from 25-27.8%. Whereas, potatoes has been reported to have a starch yield of 32% (Hoover & Hadziyev 1981). However, the marama plant grows in arid and dry conditions as opposed to the commercial competitors such as maize, that are grown under favourable costly conditions. In countries such as Namibia, that have poor soil quality and are facing severe dry spells and a water shortage, marama root production may be more suitable. African countries do not have an advantage when it comes to corn production, this is because of high production costs due to high requirements of fertilizers and pesticides and also due to severe droughts (Omojola 2013).

According to Adeboye &Emmambux, (2017) marama root starch granules are spherical, lenticular and oval in shape, with mainly large granules and small granules in between the larger granules (Figure 7). It was further reported that the marama root starch granules shape were similar with potato tuber granules but different from those of cassava and maize. The marama starch granules ranged from 5–38μm and had a mean diameter size of 15.15 μm, lower than the potato starch sample but higher than that of the cassava (Adeboye & Emmambux 2017).

Adeboye &Emmambux, (2017) further reported that, the gelatinization temperature of the marama root starch ranged from 67.5–79.0°C, close to the commercial cassava and maize starches, while the enthalpy change for the marama root starch was 4.7 J/g, a value lower than that reported for the commercial cassava, maize and potato starches (10.32, 9.33 and 9.70 J/g respectively) (Adeboye & Emmambux 2017).

In addition, marama root starch paste has a peak viscosity which ranges from 5350–5475mPas which is double the viscosity reported for cassava and maize. The gel firmness of marama root starch was reported to be higher than that of cassava starch but lower than that of potato and maize starches. The amylose content of the marama root is 192 g/kg of starch which is in the same range as the content reported for cassava (196g/kg). Furthermore, marama root has an A type molecular structure, the A type molecular structures are densely packed (Adeboye & Emmambux 2017).

Adeboye and Emmambux (2017) therefore concluded that marama root starch is similar to cassava starch, both in crystallinity and amylopectin content. A study by Sriroth et al (1999) suggests that time has an effect on the structural and functional properties of cassava root starch. Presumably, time will have an influence on the structural and functional properties of the marama root starch as well.

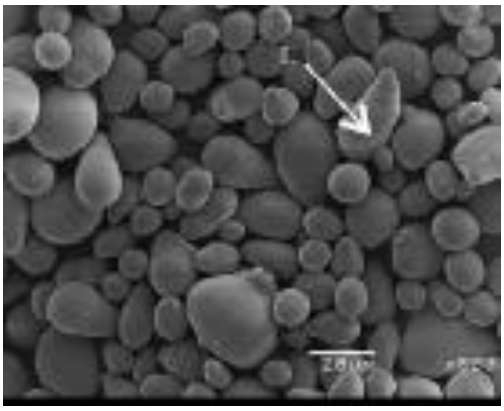

Figure 7: Scanning electron micrograph of isolated starch granules from marama root. The starch granules of the marama root are lenticular, spherical and oval. (Adeboye & Emmambux, 2017).

2.6. Starch uses

According to Singh et al. (2010), starch is a global multi-billion dollar business used in several industries such as the food and non-food industry. Starch has been widely studied because of its availability and a combination of other factors such as price, abundance, easy degradability and extensive use in food products (Pérez-Pachecoa et al. 2014, p.920). Starch is mainly included in the diet as a source of food that is high in calories; it is also used in the manufacturing of food, as it improves the properties of foods such as gelling and pasting. Starch pastes and gels provide consistency and texture for sauces, soups, dressings and spreads. Starch gels also provide structures to bread, cakes, and pudding. In addition, starch gels provide textures for desirable properties, such as a crispy coating for fried foods (Wang et al. 1998). Most of the starch used in the

processing of food is hydrolysed to produce glucose, fructose, maltose, and syrups for the production of drinks and confectionery.

There is a new trend of starch application in the formulation of health and nutritional food products such as, frozen, chilled, gluten free and low fat foods. Starch is used as a main ingredient in the formulation of gluten free food products, such as gluten free cakes and pasta. (Bertolini 2010). Starch is also used as a co-ingredient to replace fat in the production of low-fat food, such as dairy products (Lillford & Morrison 1997). Starch is used in low fat products to improve texture, colour, palatability and stability of the food, thus serving as a fat replacer. A fat replacer is produced by acid hydrolysis of maize starch and the shearing of the insoluble starch product in water to produce a firm deformable crème (Harris & Day 1993).

The largest fraction of starch produced is mainly used in the food industry. However, there is also a significant use of starch in the non-food industries. In the non-food industries, starch is used for production of textiles, paper, non-biodegradable plastics, ethanol and bio-fuels industry. Starch is also used for sewage and water treatment, and in the pharmaceuticals and cosmetics industries (Lillford & Morrison 1997). The paper industry is the second largest consumer of starch, with an estimated 10 million tons of starch used per year (Bertollini 2010).

Table 1 Starch uses (Lillford & Morrison 1997)

Food uses		**Industrial uses**	
Sauces	Thickening	Paper and board	Sizing
Soups	Gelling	Textiles	Coating
Dressings	Stabilising	Plastics	Texturizing
Baked goods	Sweetening	Rubber	Viscosity control
Dairy products	Bulking	Oil	Flocculation
Meat products	Texturizing	Pharmaceuticals	Ion exchange
Drinks	Fat replacement	Cosmetics	Adhesive
Ice cream		Alcohol	Dusting
		Adhesives	Fuel
		Sewage& water treatment	

2.7. Starch thermal properties

The heating of starch in the presence of excess water results in the progressive uncoiling of the double helices of the crystalline structures in the starch granules (Tester 1997). Starch swells when it is in water, the starch granules increases with temperature and this leads to a transfer of water from the sample to water associated with amylose and amylopectin. Amylopectin is responsible for the swelling of the starch granules, while the amylose severely restricts the extent of swelling of the starch granules, the swelling results in the disruption of the starch granule (Hermansson & Svegmark 1996). Hence waxy starch swells to a greater extent than normal amylose starch (Tester & Morrison 1990). The insoluble starch granules are disrupted when starch temperature reaches 60-

70˚C which results in loss of molecular organization and crystallinity (Bertolini 2010). The disorder in the amylopectin structure is responsible for the major changes observed during gelatinization (Zobel & Stephen 2006). Amylopectin chains are stripped from the crystalline region as a result of the swelling of the amorphous regions by absorbed water (Donovan 1979).

Gelatinization is measured using the differential scanning calorimetry (DSC). The gelatinization temperatures measured using the DSC are, the onset temperature (T_o), the peak temperature (T_p) and the conclusion temperature (T_c). The enthalpy changes (ΔH) are also measured during this process. The gelatinization temperature is a reflection the crystallinity of the starch (Tester & Morrison 1990). The gelatinization temperatures therefore provides a measure of the energy required for disruption of the starch structure (Zobel & Stephen 2006).

There are two endothermic transitions observed which are dependent on the moisture content (Donovan 1979). According to a review by Wang et al. (1998), melting occurs in the presence of small amounts of water (less than 30 %) and gelatinization occurs in excess water (1:3, starch: water). A high temperature endotherm that is wide is observed during melting, which is strongly dependent on the water content. Whereas, a high temperature is not observed during gelatinization, only the 66 ˚C endotherm is observed. In excess water, all the crystallites are pulled apart by the swelling of the granules. During gelatinization there is a near-solubilization of the starch, this however does not happen during melting (Donovan 1979). During gelatinization there are sharp changes in

absorption of heat, which is called the enthalpy changes (ΔH) (Wang et al. 1998). Gelatinization seems to be a suitable parameter of the cooking process. The study of starch gelatinization is therefore essential for understanding starch structure and supporting its applications (Bertolini 2010).

There is currently no literature on the starch accumulation and characteristics of the marama root. There is also only one paper on the properties of starch from marama roots by Adeboye and Emmambux (2017) However, the effects of harvesting time on the starch properties have not been studied. Therefore, bridging the gap in knowledge about the accumulation and properties of marama root starch according to the stage of development of the root might provide an insight for industrial application of non-official starches such as *T. esculentum* starch and the best time for harvesting.

CHAPTER 3: MATERIALS AND METHODS

3.1. Plant materials

Marama plants were grown in a greenhouse on the University of Namibia NEUDAM campus, which is located 30 km outside Windhoek on the way to the Hosea Kutako Airport. Marama seeds collected from an experimental field in Omitara which is located in the Omaheke region were supplied by the supervisor. The marama seeds were planted in 20L plant pots in a greenhouse; 144 seeds were planted, 1 seed placed in each pot. The seeds were planted in September and grown for 12 months before the final harvesting day. Figure 8 shows the seeds and marama plants growing in plant pots inside a green house. Roots were randomly selected at different stages of development for analysis. The roots were harvested in November, January, May and finally in September. The different analyses were done at 2, 4, 8 and 12 months after planting based on the harvesting times. Some of the analyses were done on the fresh root while some were on the freeze dried root, the roots were freeze dried and ground into flour for proximate, total starch content, amylose content and thermal properties analysis.

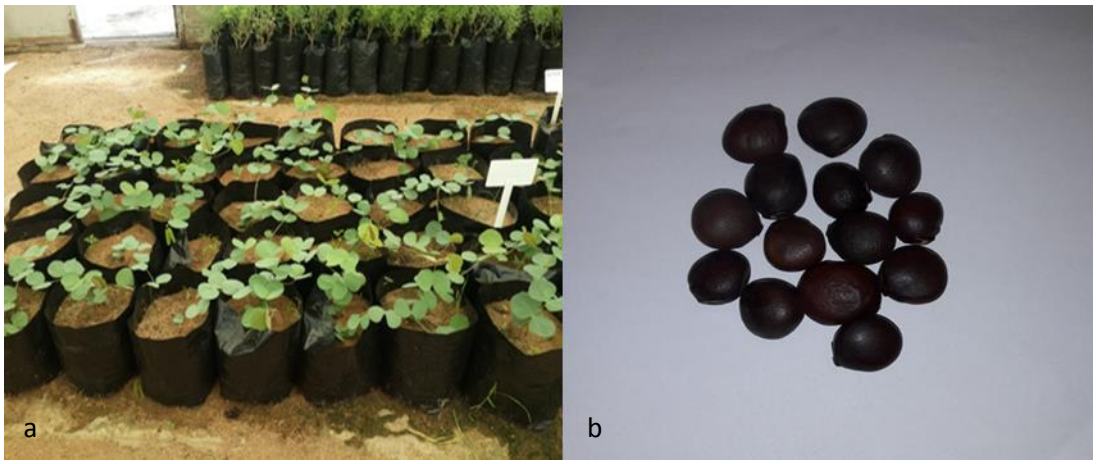

Figure 8 Shows marama: a) marama plant 3 months after planting (left), b) marama seeds (beans).

3.2. Fresh root Analysis

3.2.1. Size determination

The fresh mass (using a weighing balance) and diameter (using a vernier calliper) of roots, this was measured at 2, 4, 8 and 12 months after planting. The diameter was measured in the middle section of the marama root.

3.2.2. Root Microstructure

A protocol was devised using fixing and staining procedures described by Ruzin (1990). The storage root (2cm slices) was fixed in formalin-acetic acid (FAA) then dehydrated in an ethanol series, wax infiltrated and embedded. Cross sections of 10 μm thickness were prepared and mounted on slides before staining with Periodic acid-Schiff (PAS), and counter staining with amido black 10B. PAS stains starch a bright fuchsia and amido

black stains protein cell walls a deep blue colour. Slides were viewed using a Zeiss Axio Imager 2 microscope (Oberkochen, Germany) and digital images taken using an Axiocam ERC5S camera. This was done to determine starch accumulation, granule size and shape of the starch.

3.2.2.1. Fixing of marama root

Sections of marama root (2 cm in size) were immediately fixed in FAA after harvesting. The FAA fixative was prepared by adding 50% Absolute ethanol, 5% Glacial acetic acid and 10% Formalin to 35 % distilled water. The root sections were then submerged in the FAA fixative for 2-3 weeks before processing.

3.2.2.2. Histoprocessing

Smaller sections were cut from the fixed root and fitted into Sacura embedding cassettes, the sections where washed in 50% Ethanol before loading into a Thermo Scientific Excelsior ES histoprocessor (Thermo Fisher Scientific, United States of America) . The sections were first dehydrated in 50% ethanol for an hour, dehydrated in 70% ethanol for an hour, dehydrated in 90% ethanol for an hour, dehydrated in 95% for an hour, dehydrated in 100% ethanol for an hour and finally dehydrated in 100% for another hour. The sections were then cleared in 2 series of xylene, 1 hour for each series. After clearing the sections were wax infiltrated, first for 2 hours and finally for 3 hours. The dehydration, clearing and wax infiltration was all done using a Thermo Scientific Excelsior ES histoprocessor.

3.2.2.3. Embedding and Sectioning

The sections were then removed from the processor and embedded in wax, oriented ensuring that all structures in cross section were included. The blocks were then left to solidify on ice. The embedding was done using a Thermo Scientific Histo Star work station. Cross sections of 10 μm thickness were sectioned using a Thermo Scientific Finesse 325 rotary microtome. Sections were placed in a water bath at 40°C and picked up with a slide, using the frosted end of the slide. The slides were placed in an oven at 37°C to dry.

3.2.2.4. Staining

The slides were dewaxed by leaving them in xylene for 3 minutes two times. Thereafter, the slides were rehydrated by first placing them in 100% ethanol for 2 minutes, placed in 96% ethanol for 2 minutes, placed in 70% ethanol for 2 minutes and lastly placed in distilled water for 2 minutes.

PAS was used to stain the slides and amido black was used as a counter stain. PAS stains the carbohydrates a bright fuschia whilst amido black stains the cell wall proteins blue. The slides were placed in 0.5% periodic acid for 10-20 minutes, after that they were rinsed in 3 changes of distilled water. The slides were then stained in Schiff's reagent for 15 minutes at room temperature. Thereafter, the slides were washed gently under tap water for 5 minutes. Subsequently, the slides were bleached in 2% sodium bisulfite for 1 minute and then washed gently in running water. To counter stain, the slides were placed in 7% acetic acid for 2 minutes before they were stained in 1%

aqueous amido black 10B diluted in 7% acetic acid for 2 minutes. Afterwards, the slides were rinsed in 7% acetic acid before they were washed gently in running tap water. Subsequently, slides were dehydrated in 96% ethanol for 2 minutes two times, placed in 100% ethanol for 2 minutes before finally clearing in xylene for 3 minutes two times.

The slides were then immediately mounted using DPX mounting media and cover slips were used to cover while ensuring that no air bubbles were formed. The slides were allowed to stand for 2 days to dry before observing under a Carl Zeiss light microscope.

3.3. Physicochemical properties

3.3.1. Moisture analysis of marama fresh marama root and flour

Moisture content analysis of the marama root was carried out using the oven drying temperature (105°C) using the AOAC 925.45 B Method. About 4g of sample was weighed into a porcelain crucible and the final weight recorded. The crucibles were then placed in an oven at 105 °C for 24 hours. After heating in the oven the crucibles were removed from the oven and placed in a dessicator to cool. The cooled crucibles were then measured and weight recorded. The weight was then calculated as moisture loss divided by initial sample weight and multiplied by a 100. The moisture content was reported on wet basis.

Moisture % = moisture loss/sample weight *100

3.3.2. Ash content

The ash content analysis was determined using the AOAC 942.05 method. A sample of 2 g freeze dried and milled marama root was weighed into a porcelain crucible and placed in a muffle furnace at 600°C. The sample was incinerated for 5 hours until the sample turned to a light grey colour. The crucibles were then cooled in a dessicator that contains blue silica gel. This was done in triplicates. The crucibles were then weighed immediately and ash content was determined by subtracting the weight of sample after incinerating from the weight of sample before incinerating. The ash content was converted to dry weight basis to be able to compare different roots as they have different moisture content.

% Ash = weight of ash/weight of sample * 100

Dry weight basis = (wet basis*100)/ (100- moisture content)

3.3.3. Crude protein

Crude protein was determined using the Dumas combustion method which is an AOAC 990.03 method. The nitrogen content is determined by total combustion of milled freeze dried marama root at 950˚C in the presence of oxygen, nitrogen is converted to NO_x. Approximately 100 mg of milled freeze dried marama root was weighed into a tin foil cup, the cup was folded and molded into a ball and the samples were loaded into the auto sampler of the Leco CHN 628 series. EDTA powder was used as the calibration standard and an empty cup was used as a blank. A protein factor of 6.25 was used to determine the protein for this analysis. This analysis was done in triplicates. The crude

protein content was converted to dry weight basis to be able to compare different roots as they have different moisture content.

3.3.4. Crude fibre

Crude fibre analysis was done using the AOAC 962.09 method. About 1 gram of ground freeze dried marama root was weighed into a filter crucible. The crucible was placed in the hot extraction unit of the Velp Scientifica raw fiber analyzer ensuring that all crucibles are snugly fitted. The valves were closed and cooling water system was turned on. Approximately 150 ml of preheated 0.128 M sulphuric acid was added to the tube making use of a funnel and three drops of n-octanol was added to minimize foaming. The heating section was covered, heating element turned on fully and contents of the tube boiled and allowed to cool for 30 minutes. Afterwards the heating element was turned off and the water suction pump and vacuum turned on. The sample was rinsed 3 times with about 30 ml hot distilled water each time, while ensuring that all the sample material has been washed of the condenser tube.

About 150 ml of preheated 0.313 M sodium hydroxide was added. About 3 drops of n-octanol was added to the tube and contents were boiled and allowed to cool for 30 minutes. The heating element was turned off and contents were filtered by vacuum. The contents of tube were once again rinsed three times with hot distilled water. Thereafter, the tube was rinsed with 20 ml acetone to remove traces of water. The crucible was removed and placed in an oven at 105 °C to dry overnight. Afterwards, the crucible was cooled in a desiccator for about 30 minutes and weighed. After weighing the crucibles

were placed in a muffle furnace at 500 °C for about 4 hours. The crucible was then removed and cooled in a dessicator for 30 minutes and weighed. The crude fibre content was calculated using the formula below. This was done in triplicates. The crude fibre was converted to dry weight basis to be able to compare different roots as they have different moisture content.

Crude fibre (%) = W1-W2/m x100

Where:

W1 = Mass of residue in crucible after drying, in grams

W2 = Mass of residue in crucible after ashing, in grams

m = original sample mass, in grams

3.3.5. Total starch content

The Megazyme total starch assay kit (Megazyme International Bray, Ireland) was used to determine the percentage composition of total starch (TS) as described by McCleary et al. (1994) and reported on dry basis. A mass of 100mg of freeze dried marama root flour was weighed accurately into a glass test tube. This was done in duplicates for maize starch (positive control) and each sample. The test tubes were tapped to ensure that the entire sample dropped to the bottom of the test tube. A volume of 0.2 ml 80% ethanol was added to wet the sample and aid dispersion of the sample. This was then mixed thoroughly using a vortex mixer. Immediately 3 ml of thermostable α-amylase was added, samples were vortex and incubated in a boiling water bath for 6 minutes. The

contents of the tubes were mixed vigorously using a vortex after every 2 minutes ensuring that all the lumps have been completely homogenized.

The test tubes were placed in a water bath at 50°C, a volume of 0.1 ml amyloglucosidase was added and test tubes were mixed using a vortex and incubated in a 50°C water bath for 30 minutes. Entire contents of the test tubes were transferred to 15 ml centrifuge tubes. A water bottle was then used to rinse the tubes contents thoroughly and volume was adjusted to 10 ml with distilled water. The tubes were then centrifuged for 10 minutes at 1800 × g. Duplicate aliquots 0.1 ml was transferred to the bottom of glass test tubes for each sample. A volume of 0.1 ml of D-Glucose standard was added to 2 test tubes (positive control), and 0.1 ml of distilled water was added to 2 other glass test tubes (reagent blank which is the negative control).

A volume of 3 ml GOPOD reagent was added to each test tube and test tubes were incubated in a water bath at 50 °C for 20 minutes. The absorbance for each sample, D-glucose was then read at 510nm against the reagent blank using an Mrc Spectro UV- 11 spectrophotometer.

3.3.6. Amylose/amylopectin ratio

Megazyme amylose/amylopectin assay kit was used to determine the percentage composition of amylose and amylopectin as described by Gibson et al. (1997). Freeze dried and milled marama root flour sample of 20mg was accurately weighed into a 10 ml glass test tube. This was done in duplicates for each sample. A volume of 1 mL DMSO was added to the tube while gently mixing at low speed on a vortex, the tube was capped

and placed in a boiling water bath for approximately 1 minute and vigorously stirred to ensure that no gelatinous lumps form. The tube was then further incubated in boiling water bath for 15 minutes with intermittent mixing at high speed using a vortex. The tube was then removed from the water bath and left to stand for 5 minutes to cool to room temperature.

A volume of 2 mL 95% ethanol was added to the tube while continuously stirring with a vortex, further 4 mL was added and test tube was inverted to mix and allowed to stand for 15 minutes. The tube was centrifuged for 5 minutes at 2000 × g, and supernatant was discarded and tube was drained on tissue paper for 10 minutes, ensuring that all ethanol has been drained. A volume of 2 mL DMSO was added to the tube containing the starch pellet and tube was incubated for 15 minutes with occasional mixing to ensure that no gelatinous lumps form. Immediately after incubation 4 mL Con A solvent was added and contents of the tube were mixed thoroughly and transferred to a 25 mL volumetric flask by repeated action with Con A solvent. Volume was adjusted to 25 mL with Con A solvent; making the Solution A.

A 1 mL aliquot of solution A was transferred to a 2 mL eppendorf tube and 0.5 mL Con A solution A was added. Contents were then mixed gently by repeated inversion while avoiding frothing. The tube was then allowed to stand at room temperature for 1 hour and thereafter it was centrifuged at 14000 g for 10 minutes. After centrifuging, 1 mL of the supernatant was transferred to a 15 mL centrifuge tube and 3 mL of 100mM sodium acetate buffer pH 4.5 was added to reduce the pH to approximately 5 pH. The contents

were mixed and incubated in a boiling water bath for 5 minutes to denature the Con A. The tube was then placed in a 40 °C water bath and incubated for 30 minutes.

A volume of 0.1 mL enzyme mixture of amyloglucosidase and α- amylase was added and the tube was centrifuged at 2000 × g for 5 minutes. A volume of 1.0 mL supernatant was transferred to a tube; 1.0 mL of distilled water was added to another tube and 1.0 mL of D-glucose to another. The distilled water serves as the reagent blank and the D-Glucose is the positive control. A volume of 4 mL GOPOD reagent was added to all tubes and they were all incubated at 40 °C. The absorbance for each sample, D-glucose was then read at 510nm against the reagent blank using an Mrc Spectro UV- 11 spectrophotometer.

The total starch was determined by adding 4 mL of 100mM sodium acetate buffer pH 4.5 to 0.50 mL and 0.1 mL of amyloglucosidase and α-amylase solution. The contents of the tube were then incubated at 40 °C in a water bath for 10 minutes. An aliquot of 1.0 mL was transferred to test tubes, in duplicates and 4 ml of GOPOD reagent was added and contents mixed well. The test tubes were incubated at 40 °C in a water bath for 20 minutes. The incubation was done concurrently with the samples, reagent blank and D-Glucose. The absorbance then read at 510nm using an Mrc Spectro UV- 11 spectrophotometer.

3.3.7. Determination of total soluble solids

Marama root flour was mixed with distilled water (10% slurry), filtered and the total soluble solids were measured using a digital refractometer. The total soluble solids were expressed as a brix percentage. The refractometer was calibrated with distilled water before taking the measurements. The total soluble solids was converted to dry weight basis to be able to compare different roots as they have different moisture content.

3.3.8. Determination of thermal properties

Thermal properties of ground freeze dried marama root were determined using a method described by Wokadala et al. (2012). Thermal properties were analyzed using a high pressure differential scanning calorimetry (DSC) system with STARe software (HPDSC-827, Mettler Toledo, Greifensee, Switzerland). A mass of 10 mg (dry weight basis) of freeze dried marama root flour was dissolved in 30 mg distilled water and allowed to equilibrate for at least 2 hours at room temperature. Scanning was done from 40 to 125 °C at a rate of 10°/ min. Indium (Tp = 156.61 °C, 28.45 J/g) was used as a standard to calibrate DSC and an empty pan as a blank reference.

3.4. Statistical analysis

Statistical analysis was conducted using the SPSS 21 statistical package (Chicago, IL, USA).The data was subjected to a one way analysis of variance (ANOVA) at a 5% significance level at p= 0.05. A p-value $\leq$ 0.05 was considered significant and the null hypothesis was therefore rejected. The Duncan's multiple range tests was used to further compare the means to determine which of the means is significantly different. Data was

presented as means ± standard deviation. The independent variable in this study is time, while the dependent variables are the root and starch characteristics. A randomized block design was used in this study.

CHAPTER 4: RESULTS

4.1. Marama fresh root size

Generally each plant contained one storage root; the marama plants grown in this study did not produce any pods and hence no seeds. In addition, the average fresh mass of the marama storage roots ranged from 14.6 g to 420 g (Figure 9). The root harvested early (at 2 months) weighed about 14.6 g while the root harvested at 8 months weighed 420 g and the late harvested root (12 months) weighed 326 g. Marama root mass significantly differed with age ($P<0.05$). However, the Duncan test revealed that the mass of the root harvested 2 months after planting and the root that was harvested 4 months after planting was not significantly different.

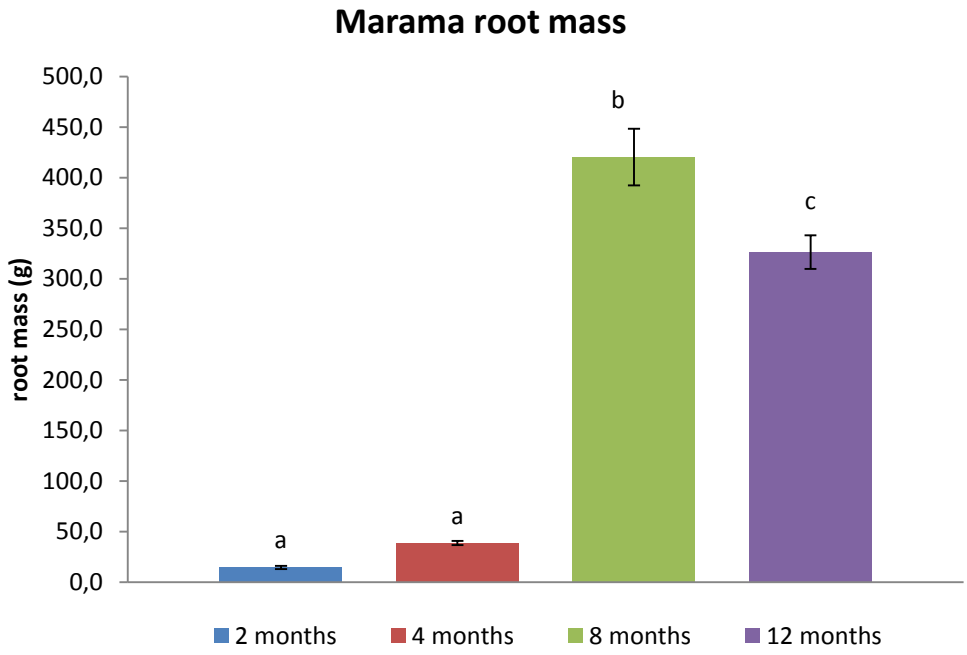

Figure 9: The fresh mass of marama roots at different age. The bars with different letter are significantly different at $P \leq 0.05$. Experiment replicated 5 times, and data are presented as means with standard error bars. Standard error bars are an indication of variability within the samples.

The average diameter for the marama storage roots ranged from 1.4 cm to 6.5 cm (Figure 10). The roots harvested at 2, 4, 8 and 12 months had the average diameter of 1.4, 2.7, 6.5 and 5.7, respectively. The One way ANOVA test revealed the diameter of the marama storage roots differed significantly with time ($P<0.0005$). Although the 4 month roots appeared to be shorter in length than 2 months roots, the diameter increased with time, and therefore the 4 months roots were larger than the 2 months roots.

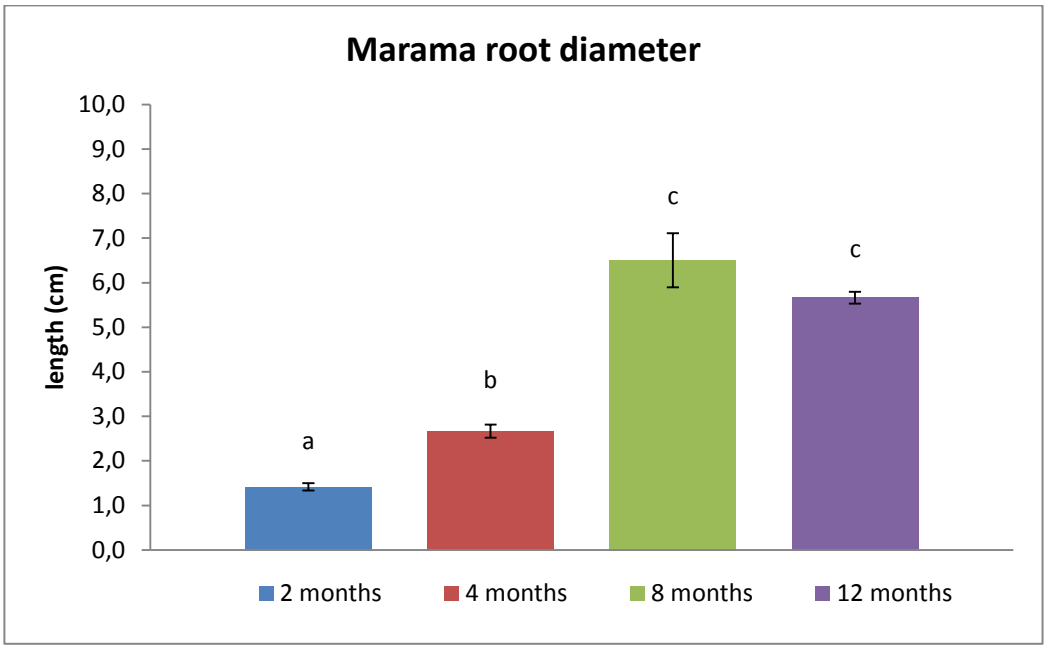

Figure 10: The storage root diameter of the marama roots at different age. The bars with different letter are significantly different at P≤0.05.Experiment replicated 5 times and data was presented as means with standard error bars. Standard error bars are an indication of variability within the samples.

The older roots appeared to be more fibrous then the younger roots. Whereby, the youngest root (2 months roots) appeared to consist mostly of water. The average moisture content of the fresh marama storage root ranged from 80.8 to 91% (Figure 11). The youngest marama root contains a moisture content of 91% while the oldest root has a root content of 80.8%. While the roots harvested at 4 months and 8 months had an average moisture content of 89.4% and 86.6%, respectively. The One way ANOVA test revealed that the p value was less than 0.0001 and therefore the null hypothesis was rejected. Therefore, the moisture content of the marama roots significantly differed as

root development progressed. However the Duncan test revealed that moisture contents of the 2 months roots and that of the 4 months roots were not significantly different.

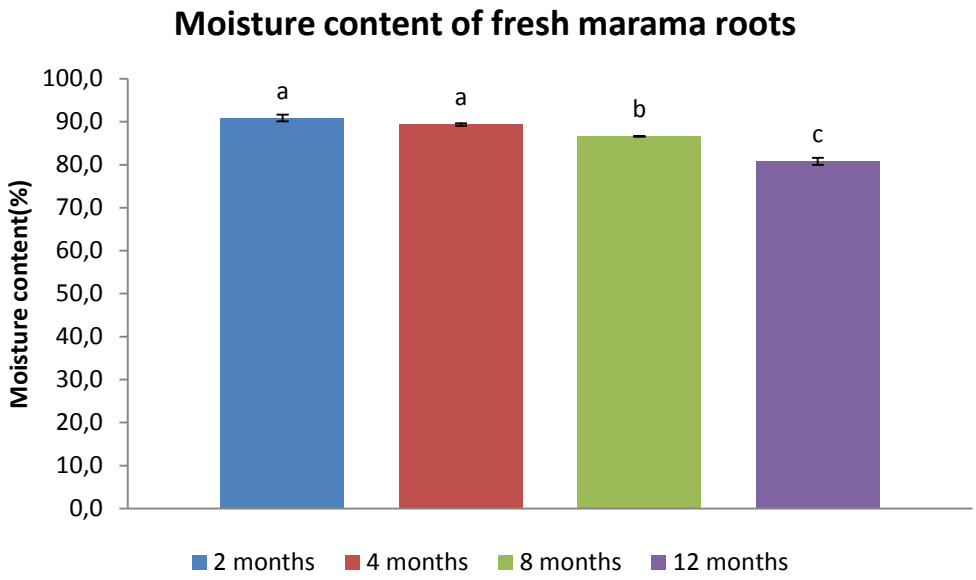

Figure 11: The moisture content of the marama roots at different age. The bars with different letter are significantly different at P≤0.05.This was done in triplicates and data was presented as means with standard error bars. Standard error bars are an indication of variability within the samples. Standard error bars are an indication of variability within the samples.

After freeze drying of the marama roots, the 4 months roots, 8 months roots and 12 months roots were dried into white coloured products. However, discolouring was observed in the 2 months roots after moisture loss, the dried root product had a bright orange- brown colour (Figure 12). In addition, The 12 month fresh root appeared shrivelled with a darker shade peel then the rest of the samples (Figure 13).

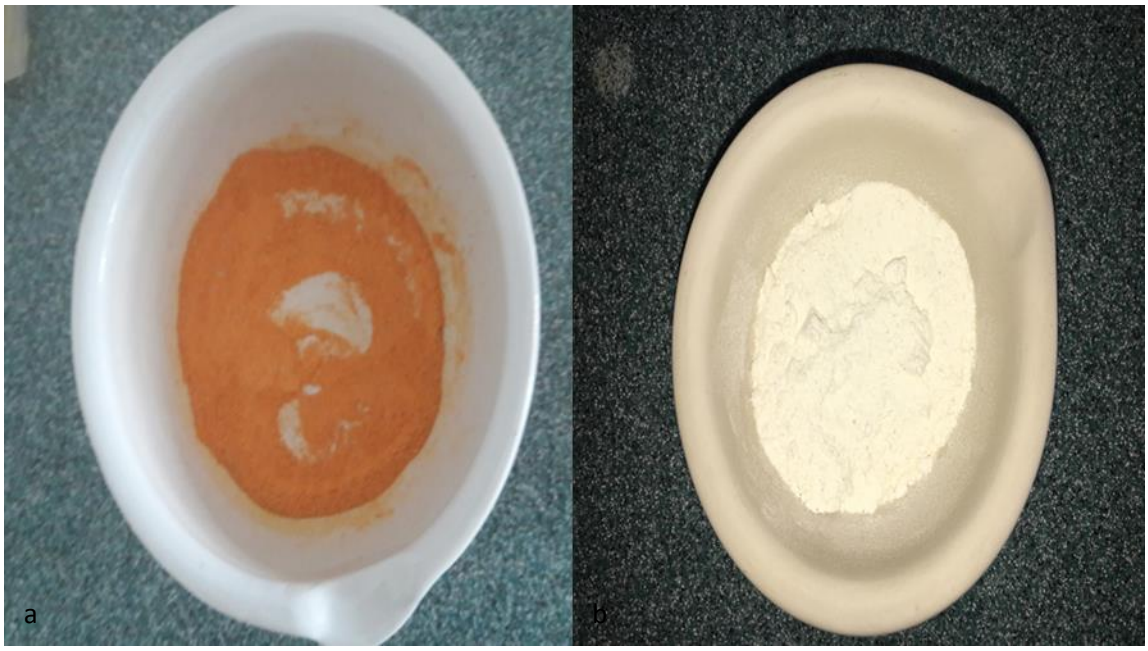

Figure 12: Ground marama storage root flour: a) flour of ground marama roots harvested after 2 months, showing an orange colour after moisture loss (left), b) flour of ground marama roots harvested after 8 months (right), no discolouring was observed.

Figure 13: Different marama roots harvested at different times: a) marama roots harvested 2 months after planting (top left), b) marama roots harvested 4 months after planting (top right), c) marama roots harvested 8 months after planting (bottom left), d) marama roots harvested 12 months after planting (bottom right), appears shrivelled and darker. All the pictures were taken with a vernier calliper opened up to 1 cm (10mm).

4.2. Root microstructure

Figures 14-17 show cross sections of marama storage roots harvested at different times. The sections were stained with PAS and counterstained with Amido black. All the roots were characterised by parenchyma cells which contained the starch granules. Starch accumulation was observed in all the different aged marama roots and it was reflected by the purple or magenta colour. The PAS stained the cell walls and the starch granules a purple to magenta colour while the Amido black was used as a counter stain. The amido black stained the cell wall proteins a blue colour, the counter stain also stained the starch granule surface proteins a blue colour. The 2 month root has more cells that contain no starch granules as compared to the other root samples.

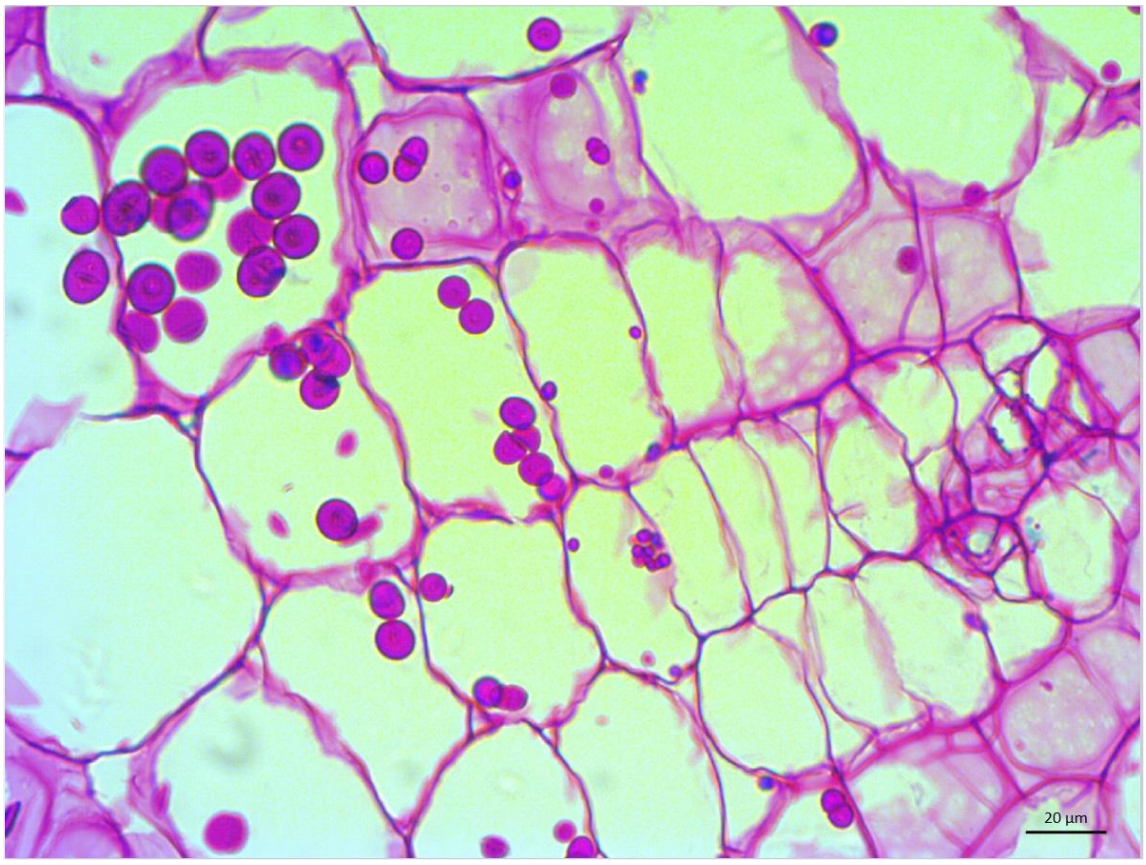

Figure 14: A micrograph of a 2 month old marama root, the starch granules are stained magenta with Periodic schiffs acid and counter stained with amido black. Periodic schiffs acid stains carbohydrates a purple or magenta color; while the amido black stains cell wall proteins and the granule-bound proteins a blue color. The scale bar represents 20 µm, respectively.

The starch granule morphology of the marama root was determined from the prepared cross sections. All the marama storage root samples had similar starch granule morphology; however the granule average size was different. The starch granules were

spherical, oval and lenticular in shape with a few irregular shaped granules observed. The average starch granule size increased as the roots developed further (as shown in Table 2). The youngest roots (2 months roots) have an average granule size of 8.6 µm, while the oldest roots (12 months roots) had the largest average granule size of 15.1 µm. The 4 months roots and 8months roots had the average granule size of 9.3 µm and 11.9 µm, respectively. The One way ANOVA test revealed that the p value was 0.002 and the null hypothesis was therefore rejected. Thus the average granule size of the root samples harvested at different times was significantly different. However the Post hoc (Duncan) test revealed that the average granule size for 2 months roots and 4 months roots were not significantly different, and neither were the average granule size for 4 months roots and 8 months roots.

The granule size range for the roots harvested at different times was also determined from the observed cross sections of the marama storage roots (as shown in Table 2). The granule size for the 2 months roots ranged from 1.2-14.2 µm, the 4 months roots ranged from 2.6-17.3 µm, while 8 months roots ranged from 2.9-21.4 µm and 12 months roots ranged from 3.8-27.1 µm. The size distribution of the marama roots harvested at different times displayed were quiet variable (Table 2). With more than 60% of the granules presenting size between 1.0 and 10.0 µm, while more than 30% of granules presenting size between 10.1 and 20.0 µm and no granules were >20 µm for the 2 months roots and 4 months roots. In addition, the size distribution for the 8 month root was, over 50% of the 10.1-20.0 µm granule size, almost 40% for the 1.0-10.0 µm granule size and about 2% of the>20 µm. Finally, the size distribution for the 12 months

roots was over 70% of the 10.1-20.0 µm granule size, 13 % of the >20 µm and about 12% for the 1.0-10.0 µm granule size.

Table 2 Starch granular structure and granule size

Sample	Average granule size (µm)	Granule range (µm)	Size distribution		Granule shape
			Size(µm)	%	
2 month roots	8.6^{a} ±0,91	1.2-14.2	1.0-10.0	63.7	spherical, oval, lenticular
			10.1-20.0	36.3	
			>20.0	0	
4 month roots	9.3^{ab} ±1,02	2.6-17.3	1.0-10.0	80.2	spherical, oval, lenticular
			10.1-20.0	19.9	
			>20.0	0	
8 month roots	11.9^{b} ±0,99	2.9-21.4	1.0-10.0	39.2	spherical, oval, lenticular
			10.1-20.0	58.3	
			>20.0	2.5	
12 month roots	15.1^{c} ±1,03	3.8-27.1	1.0-10.0	12.1	spherical, oval, lenticular
			10.1-20.0	74.9	
			>20.0	13.0	

Values followed by a different superscript letter in the same column are significantly different.

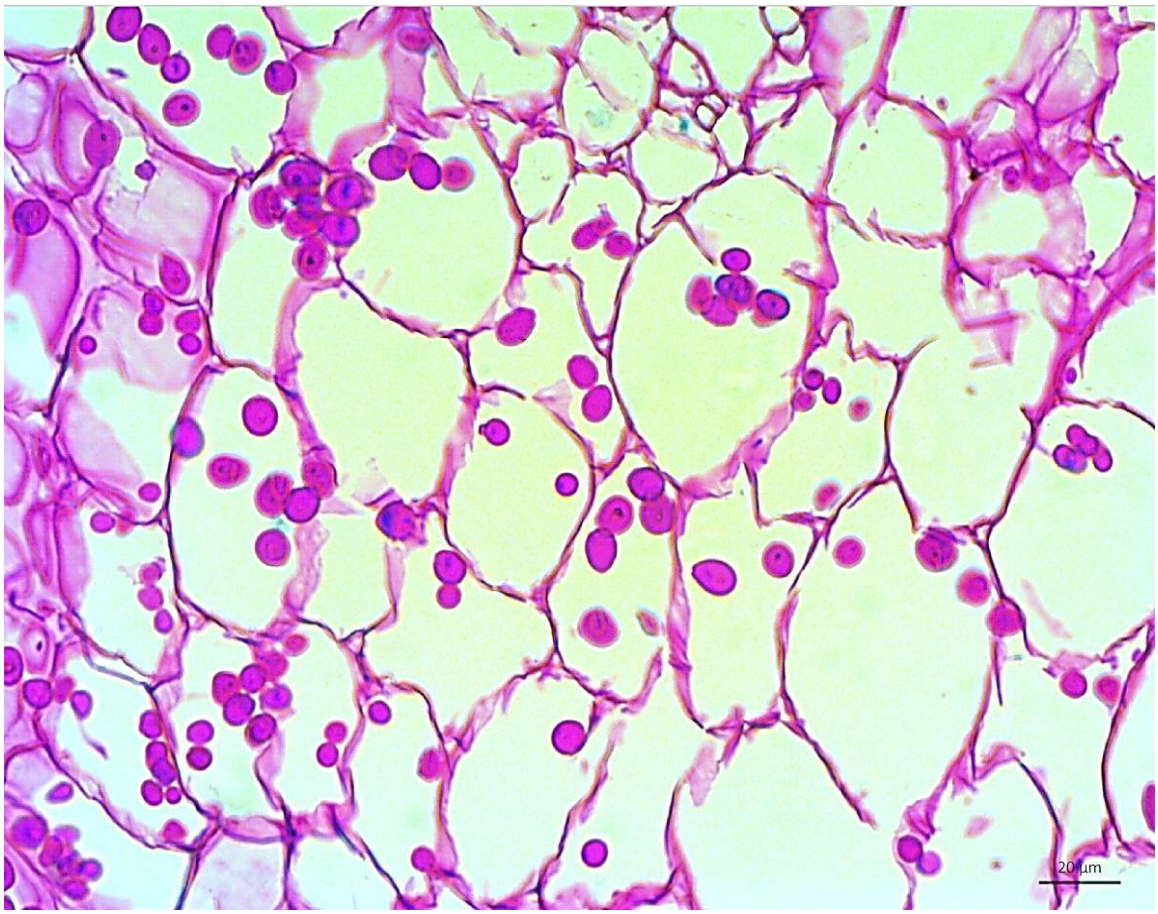

Figure 15: A micrograph of a 4 month old marama root, the starch granules are stained magenta with Periodic schiffs acid. Periodic schiffs acid stains carbohydrates a purple or magenta color; while the amido black stains cell wall proteins and granule-proteins a blue color. Cell walls are not intact, probably due to poor fixation of the root. The scale bar represents 20 µm, respectively.

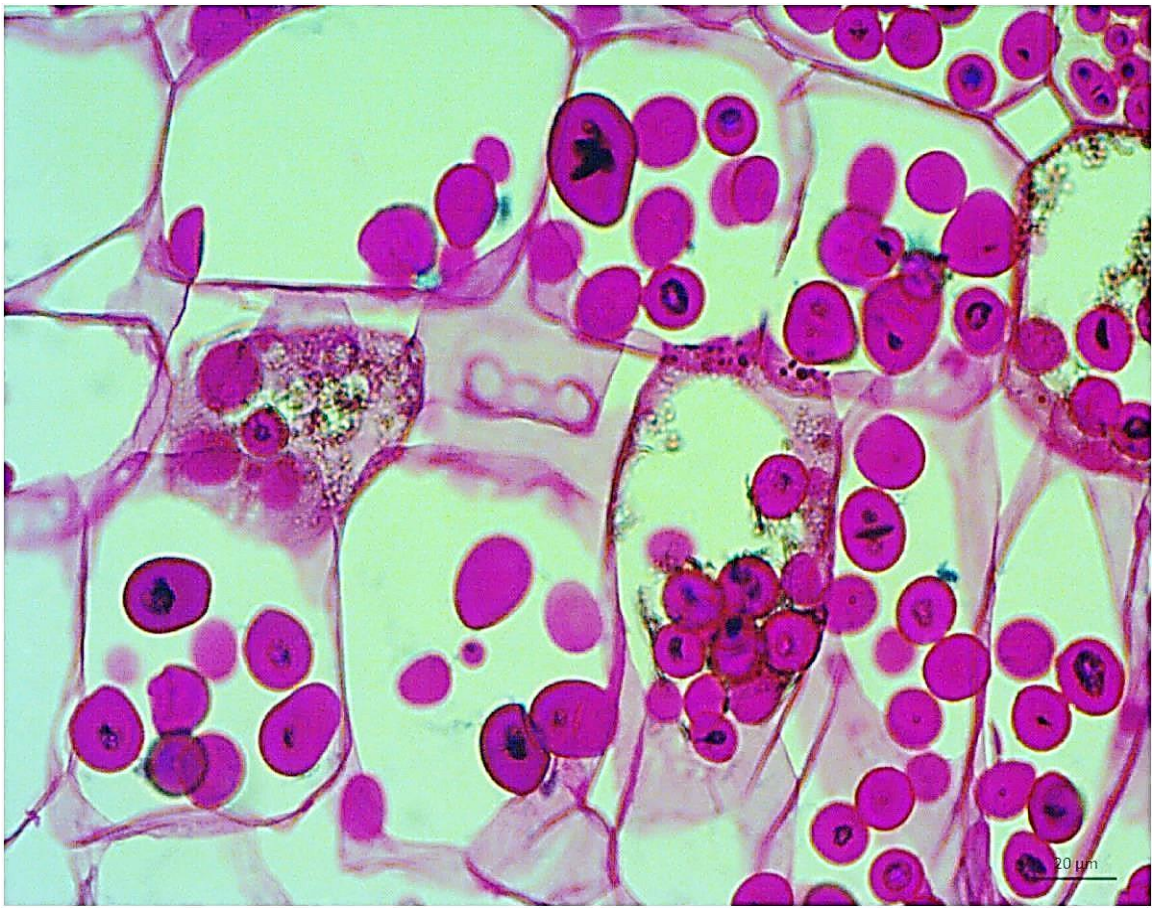

Figure 16: A micrograph of a 8 months marama root, the starch granules are stained magenta with Periodic schiffs acid. Periodic schiffs acid stains carbohydrates a purple or magenta colour; while the amido black stains cell wall proteins and granule-proteins a blue to black colour. The scale bar represents 20 µm, respectively.

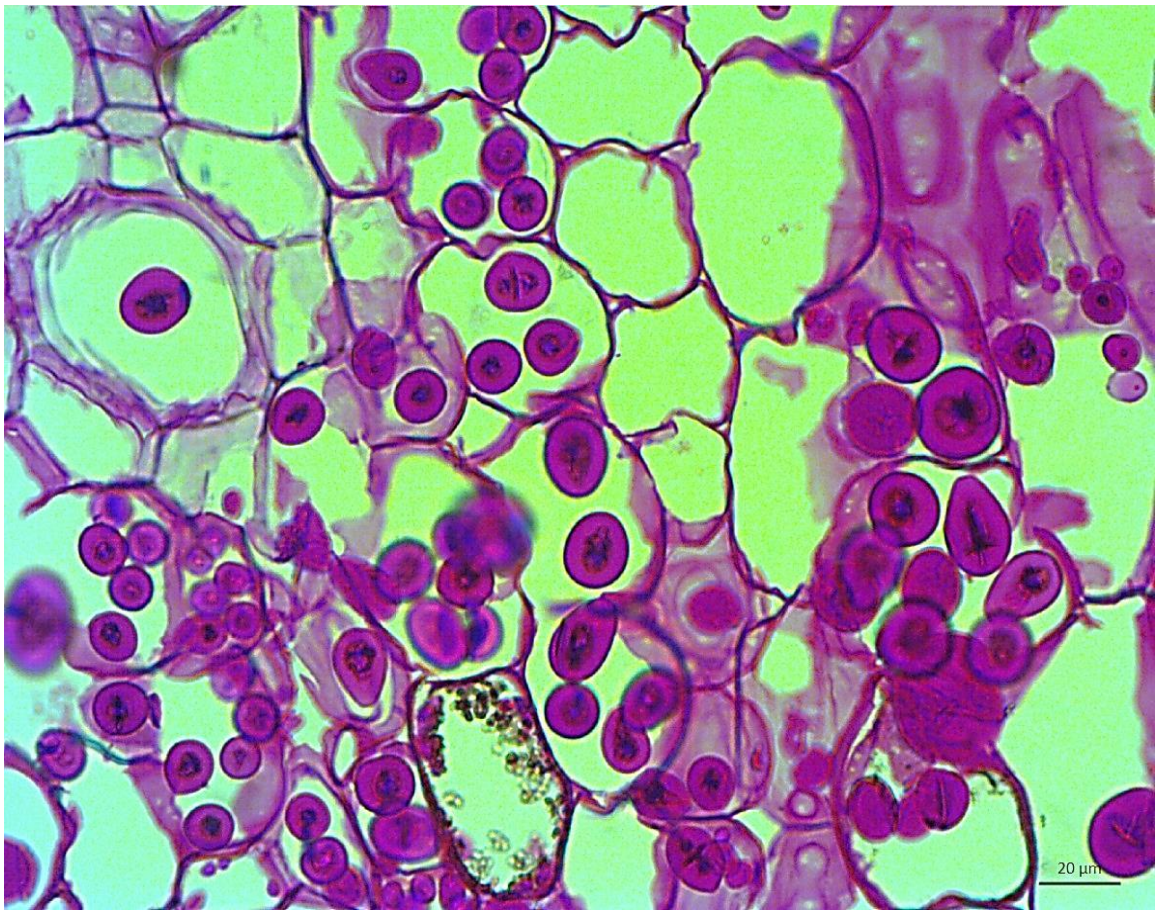

Figure 17: A micrograph of a 12 months marama root, the starch granules are stained magenta with Periodic schiffs acid. Periodic schiffs acid stains carbohydrates a purple or magenta colour; while the amido black stains cell wall proteins and granule-proteins a blue colour. The bars represent 20 µm, respectively.

4.3. Physicochemical properties

4.3.1. Proximate analysis of the root (freeze dried)

The moisture content of the ground marama root (Table 3) ranged between 4.3% and 10.8%. The 12 months flour and 2 months flour had the lowest (4.3%) and highest (10.8%) average values of moisture content, respectively. The average values of the moisture content (ground root) for the 2 month roots, 4 month roots, 8 month root and 12 month roots were 10.8%, 6.7%, 6.4%, 4.3%, respectively. The One way ANOVA test revealed that p-value ≤ 0.05 and therefore the moisture content of marama storage root flour were significantly different ($P<0.05$).

Table 3 Proximate analysis results of marama root flour after freeze drying

Sample	Moisture (%)	Ash (%)	Crude protein (%)	Total soluble solids (%)	Crude fibre (%)
2 months root	10.8^{a} ±0.31	6.3^{a} ±0.12	33.6^{a} ±0.06	6.4^{a} ±0.0	7.2^{a}±0.26
4 months root	6.7^{b} ±0.30	5.9^{a} ±0.34	14.0^{b} ±0.06	$5,8^{b}$ ±0.06	6.7^{b}±0.17
8 months root	6.4^{b} ±0.30	4.3^{b} ±0.25	3.3^{c} ±0.06	$3,7^{c}$ ±0.11	6.8^{b}±0.18
12 months root	4.3^{c} ±0.96	3.1^{c} ±0.06	2.7^{d} ±0.05	$2,3^{d}$ ±0.10	5.6^{c}±0.13

The data presented as means of 3 marama roots samples with standard deviation.

Values followed by a different superscript letter in the same column are significantly different at $P\leq0.05$. Ash, crude protein, total soluble solids and crude fibre are reported on dry basis.

The ash content of the ground marama storage root flour also decreased with time, the values ranged between 3.0% and 5.6% (shown in table 3). The average values of the ash content for the 2 month roots, 4 month roots, 8 month root and 12 month roots were 6.3%, 5.9%, 4.3% and 3.1%, respectively. The lowest ash content value was in 12 month root flour (3.1%) and the highest was in 2 months root flour (6.3%). The one way ANOVA test revealed that the average values for ash content where significantly different (p value < 0.0005).

The same trend was observed for the total soluble solids (Table 3), the total soluble solids average values decreased with time. Among the 4 samples, the average values for the total soluble solids ranged between 2.2% and 5.7%. The average values of the total soluble solids for the 2 month roots, 4 month roots, 8 month roots and 12 month roots were, 5.7%, 5.4%, 3.5%, and 2.2%, respectively. The average was lowest in 12 months root flour (2.2%) and the highest in 5.7%), respectively. The One way ANOVA test revealed that the variation in the average values for total soluble solids of the marama roots harvested at different times was significant (p value < 0.0005).

The crude protein content determined using the Dumas method was in the range of 2.7% to 33.6%. The average protein content values for the 2 month roots, 4 months roots, 8 month roots and 12 month roots samples were 33.6%, 14.0%, 3.3%and 2.7%, respectively. The trend (decreasing crude protein content with time) was similar to all the other proximate average values. The lowest average value of the crude protein was in 12 months root flour (2.7%) and the highest in 2 months root flour (33.6%),

respectively. The One way ANOVA test also revealed that the variation in the average values of the crude protein content was significant (p value<0.0005).

Finally, the crude fibre content was in the range of 6.5% to 5.4%. The average crude fibre values for the 2 month roots, 4 months roots, 8 month roots and 12 month roots samples were 7.2%, 6.7%, 6.8% and 5.6%, respectively. The lowest average value of the crude fibre was in 12 months root flour (5.6%) and the highest in 2 months root flour (7.2%), respectively. The One way ANOVA test also revealed that the variation in the average values of the crude fibre content was significant (p value<0.0005).

4.3.2. Total starch content

The total starch content of the ground marama storage roots (Figure 18) ranged between 25.9% and 60.1% on dry basis. The 2 months root and 12 months root had the lowest (25.9%) and highest (60.1%) average values of total starch content (dry basis), respectively. The average values of the total starch content (dry basis) for the 2, 4, 8 and 12 month roots were 25.9%, 26.5%, 49.0%, 60.1%, respectively. The One way Anova revealed that the variation in the average values of the total starch content (dry basis) of the marama roots harvested at different times was significant (p value<0.0005), therefore the total starch content increased significantly with age of the roots.

In addition, the total starch content for the marama root was also determined on fresh basis. The total starch content (fresh basis) of the marama root (Figure 19) ranged between 2.3 and 11.5%. The 2 months root and 12 months root had the lowest (2.3%)

and highest (11.5%) average values of total starch content (fresh basis), respectively. The average values of the total starch content (fresh basis) for the 2, 4, 8 and 12 month roots were 2.3%, 2.8 %, 6.6%, 11.5%, respectively. The one way ANOVA test revealed that the total starch content (fresh basis) values for the different marama storage root samples differed significantly (p value< 0.0005).

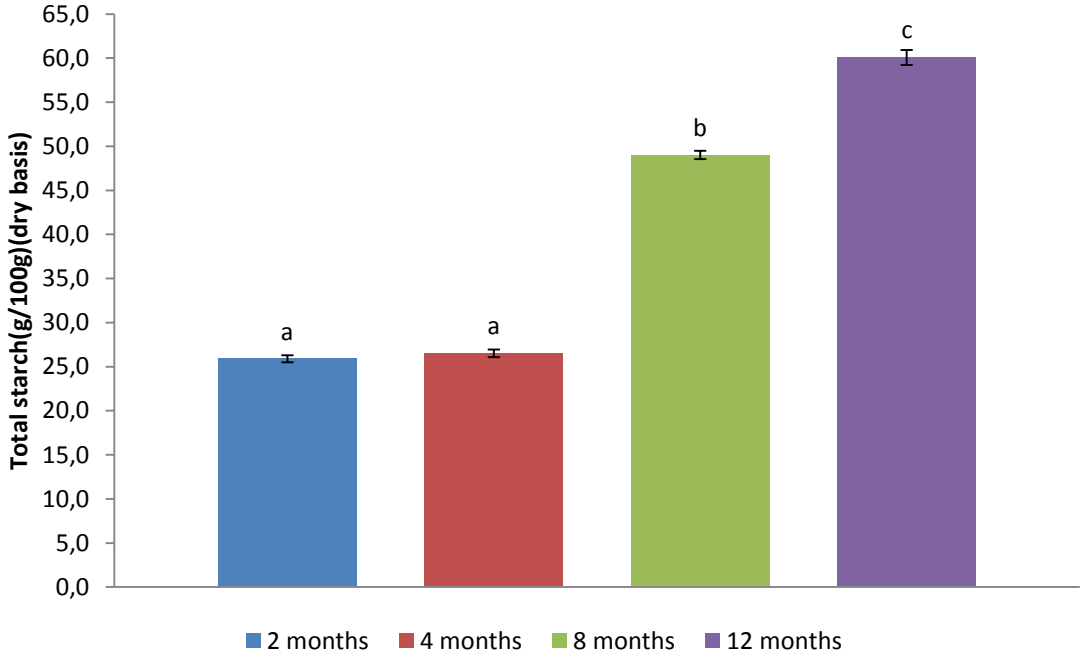

Figure 18: Total starch content (dry basis) of marama storage roots harvested at different times. The bars with different letter are significantly different at P≤0.05. This was done in triplicates and data was presented as means with standard error bars. Standard error bars are an indication of variability within the samples.

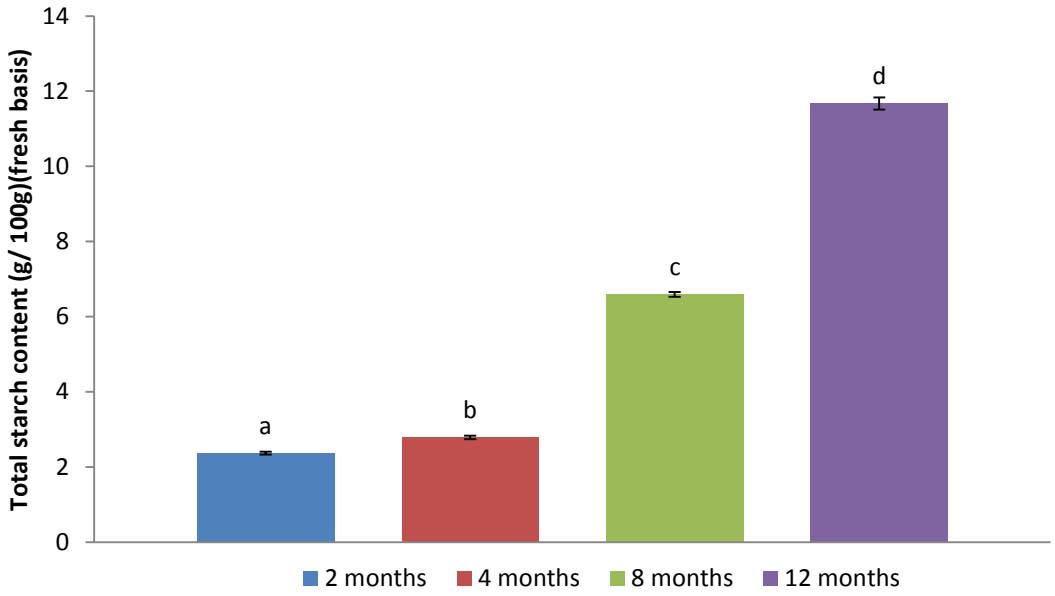

Figure 19: Total starch content (fresh basis) of marama storage roots harvested at different times. The bars with different letter are significantly different at P≤0.05. This was done in triplicates and data was presented as means with standard error bars. Standard error bars are an indication of variability within the samples.

4.3.3. Amylose content

The amylose content of the marama storage root starch determined by the precipitation of amylopectin was in the range of 21.4% to 50.7 % on starch basis (Figure 20). The lowest average value for the amylose content was in 12 months root flour (21.4%) while the highest was in 2 months root flour (50.7%). The average values of the amylose content for the 2, 4, 8 and 12 month roots were 50.7%, 40.6%, 25.3%, 21.4%, respectively. The one way ANOVA test revealed that the variation in the average values of the amylose content of the different marama storage roots samples was significant (p

value< 0.0005). Therefore the amylose content significantly decreased with the maturity of the storage root. The starches of the earlier harvested 2 months root flour and 4 months root flour marama roots were high amylose starches. The amylose content of the older roots starches was in the range of the normal amylose content for native root and tuber starches.

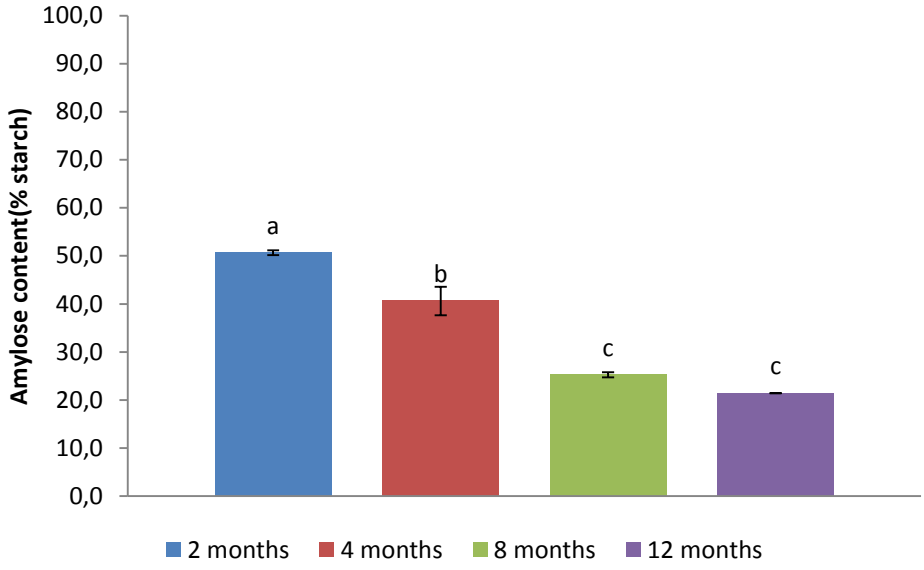

Figure 20: The amylose content of marama storage roots harvested at different times. The bars with different letter are significantly different at P≤0.05. Experiment was done in triplicates and data presented as means with standard error bars. Standard error bars are an indication of variability within the samples.

4.3.4. Thermal properties

The thermal properties of the marama roots were determined by the DSC. The three samples (4, 8 and 12 month roots) yielded an endothermic peak while the 2 months roots sample yielded no visible peak between the temperature range 30-120˚C (Figure 20). The DSC thermogram peak in terms of onset temperatures (T_o), peak temperature (T_p) and conclusion temperature (T_c) for the samples are shown in Table 5. The T_o, T_p and T_c for the 4 months were 77.4˚C, 84.9˚C and 93.0˚C, respectively. In addition, T_o, T_p and T_c for the 8 months were 73.4˚C, 81.2˚C and 89.6˚C, respectively. Finally, T_o, T_p and T_c for the 12 months root samples were 74.18˚C, 79.1˚C and 84.6˚C, respectively. The enthalpy temperature (ΔH) for the 4, 8 and 12 months root samples were 2.2, 8.2 and 12.3 J/g, respectively and these were significantly different ($P<0.05$).

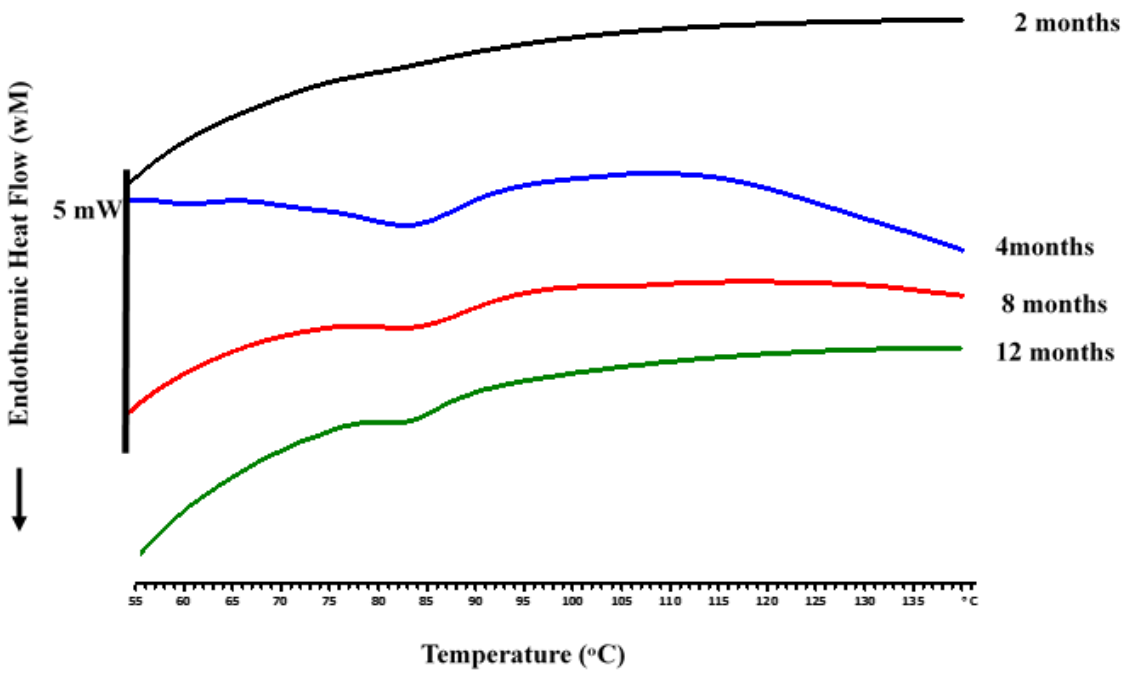

Figure 21: DSC curves of marama root harvested at different months

Table 4 Thermal properties of marama root starch harvested at different months

Sample	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
2 months	ND	ND	ND	ND
4 months	77.4^{a}±0.11	85.0^{a}±0.10	93.0^{a}±0.50	2.2^{a}±0.12
8 months	73.4^{c}±0.27	81.2^{b}±0.00	89.6^{b}±0.50	8.2^{b}±0.40
12 months	74.2^{b}±0.15	79.1^{c}±0.10	84.6^{c}±0.2	12.3^{c}±0.70

The data presented as means of 3 marama roots samples with standard deviation.

Values followed by a different superscript letter in the same column are significantly different.ND is not detected

CHAPTER 5: DISCUSSION

5.1. Marama fresh root size and moisture

This study is the first study to investigate the relationship between time and the characteristics of the marama storage roots and its starch. It is also one of the very few studies on the marama storage roots that are known to the author. Adeboye and Emmambux (2017) and Nepolo (2014) isolated and characterized starch isolated from 6 months old roots.

The fresh mass of the marama root increased with time as expected, however there was a decrease in the mass of the root between the 8 months and 12 months. This study showed that the weight of the marama root ranges from 14.9g to 420 g, the root can attain a weight of up to 420g in 12 months. Bousquet (1982) reported that the storage root can reach a weight of up to 12 kg within a few unspecified years. However, roots can grow larger and a root that weighed 277 kg was found in Botswana (National Academy of Sciences 1996). As expected, the average values for the weight of marama root significantly increased with time up until 8 months after planting. However the 8 month root weighs more than that of 12 month root and this might have been due to the loss of leaves during winter. The marama plants lose their leaves and vines during winter and they sprout back after winter. Consequently the plants could not produce their own food through the process of photosynthesis and thus the 12 month root weighs less than the 8 month root although older. Since 12 month root were harvested right after winter

(first week of September), it is suspected that the plants would then have had to rely on the reserves to survive winter.

The diameter is also significantly different for the marama storage roots harvested at different times. The average values for the diameter are also higher in the 8 month root than in 12 month root, the variability between these 2 samples is however insignificant. The diameter (1.4-6.5 cm) also follow the same trend as that of the mass of the marama storage root and therefore a conclusion is drawn that the size of the marama storage roots increases until winter, when the plants lose their leaves. There are no known studies investigating the marama storage roots harvested at different times and therefore this study is unique. However a study on the agronomic characteristics of marama roots harvested approximately 3 months after sowing was done, the storage roots had a diameter of 2.82 cm, a fresh weight of 25.12 g, a length of 13.5 cm and finally a moisture content of 89.21% (Travlos & Karamanos 2006). The size of the marama roots reported by Travlos and Karamanos (2006) was bigger than that of the 2 month root but smaller than that reported for the 4 month root, which further confirms that time has a positive effect on the root size. A review by Alves (2002) on the agronomic traits of cassava roots reports the weight of the cassava roots to be in the range of 0.7-2.5 kg. The diameter of the cassava roots was in the range 3-15cm while the length of the storage roots was in the range of 15-100 cm. These values reported for the cassava storage root are higher than those found in this study. However, a reference to harvest time was not mentioned and the age of the cassava storage roots is unknown.

The moisture content of the fresh marama root decreased with the age of the root. The moisture content of all the fresh marama storage root samples is higher than 80% with the youngest roots having a moisture content of 90.9%. As expected, the moisture content of the roots decreases with the age, the young roots have higher moisture content than the older roots. Similarly, young cassava roots contain more water than starch in comparison to the older roots that contain more starch and a high fibre content (Sriroth et al. 1999). After freeze drying (moisture loss) the youngest roots (2 month roots) turned an orange-brownish colour that is not observed in the other root samples. The orange colour may be attributed to carotenes and other phenolic compounds that are concentrated after moisture loss. Carotenes are found in plant products, β-carotenes are particularly found in most fruits and vegetables (Zobel & Stephen 2006). Phenolic compounds in sweet potatoes increased by 116-225% after the drying of the sweet potatoes (Yang et al. 2010). Presumably, dehydration of the marama roots also increases the carotenes and phenolic compounds of the earliest harvested roots (2 month roots). The absence of the orange-brownish colour in the other root samples (4, 8 and 12 months) is an indication that the amount of carotenes probably reduce with harvesting time. It is necessary to study the different marama root samples and quantify total phenolics and carotenes in the marama storage roots.

5.2. Root microstructure

As expected, the cell walls of the marama storage roots are stained a purple to magenta colour while the proteins of the cell walls are stained a blue colour. The PAS stains insoluble carbohydrates that contain 1-2 glycol groups (Mosele et al. 2011). The marama starch granules are contained in parenchyma cells where they are synthesised in the amyloplasts. The micrographs of the marama storage roots cross section slides were similar to those that were prepared by Rouse-Miller et al. (2013) from cassava roots. Both micrographs showed purple to magenta stained starch granules contained in parenchyma cells. There is a deformation in the cell walls of the 4 month root cross section slides which happened during the preparation of the slides. However, the starch granules are still intact.

The marama starch granules are stained a blue to black colour on the surface by the amido blue, this indicates the presence of surface proteins on the marama starch granules. Starch granules contain a small amount of granule bound proteins, the granule proteins are found on the surface of the granules and on the interior parts (Pérez & Bertoft 2010). The shapes of the marama starch granules are similar in all the marama root samples and time has no effect on the shape of the starch granules. The marama starch granules are spherical, oval and lenticular in shape. This is in agreement with Adebola and Emmambux (2017) who reported that the shapes of marama starch granules were rounded, oval and lenticular, similar to those of the potatoes although smaller in size. However, very few irregular shaped granules are observed in this study. Marama starch granules are almost similar in shape to the cassava starch granules,

except from the truncated shape of some of the cassava starch granules. Cassava starch granules, were described as round, oval and truncated in shape (Zhu 2015). While the sweet potato starch granules were reported to be polygonal in shape (Peroni et al. 2006).

There are no other studies on the effect of harvest time on the marama starch granule known to the author, and this study is therefore unique. As expected, the average granule size of the marama root starch significantly increased with the time. The earlier harvested roots have a smaller average size as compared to the roots harvested later. The average granule size of the marama root granule is in the range of 8.6 - 15.1 µm, while the granules for all the samples are all in the range of 1.2-27.1 µm. There is no significant variation between the younger roots (2 month roots and 4 month roots) and between 4 month roots and 8 month roots. A similar trend was also observed in potato tubers during growth, the average granule size of potato tubers increased as potato growth time increased until it reached its highest level and then it decreased (Liu et al. 2003). Similarly, the average granule size of two different varieties of sweet potatoes increased with the stage of development (time), the average granule sizes for the 2 varieties were in the range of 8.58-11.0 µm and 8.67-11.9µm (Noda et al. 1992). The sweet potatoes were grown over a period of 6 months as opposed to the 12 months period in this study; however, the range of the average granule size was in the same range as that of the marama storage roots (8.6-11.90 µm) observed in this study between 2 months and 8 months (2 month roots-8 month roots). This study is also in agreement with the observations of Noda et al. (2004) ,which suggests that the average starch

granule size of potatoes also increased with the stage of development. Noda et al. (1992) states that the bigger the storage root gets the larger the granules of the starch become.

The size distribution of the marama root starch granules was as expected. The larger sized starch granules of more than 20 µm increased from 0 % (2 month roots) to 13% (12 month roots) throughout development of the storage root. Similarly, the starch granules in the range of 10.1 - 20.0 µm also increased from 36.3 % (2 month roots) to 74.9% (12 month roots) throughout the development of the marama storage root. In addition the starch granules equal to and less than 10 µm in size decreased from 80.2 % (4 month roots) to 12.1% (12 month roots) during the experiment period. A similar trend was observed for the starch granules of sweet potatoes, the granules of more than 14 µm also increased throughout the development of sweet potatoes (Noda et al. 1992). According to Peroni et al. (2006), the size distribution of cassava roots starch granules was; 8.6% for the granules less than 10 µm, 71.4% for the granules less than 10.0 - 20.0 µm and 20.0% for the granules more than 20.0 µm. This size distribution is similar to the size distribution observed in the 12 month roots. Cassava roots are considered mature from the age of 12 months and are harvested from 12 months onwards (Sriroth et al. 1999). This is probably the reason for the similarity in the size distribution of cassava and the 12 month roots. The size distribution of cassava roots was also affected by the age of the root. The average granule size for the cassava root starch for several varieties was around 15 µm and the granules were in the range of 8-22µm (Sriroth et al. 1999). These values were also similar to values observed in this study for the marama storage roots harvested later in the trial (12 month roots).

5.3. Physicochemical properties

5.3.1. Proximate analysis of the root flour

The physicochemical properties of the marama root with respect to harvesting times has never been studied, and thus makes this study unique as it fills a gap in knowledge. The flour (freeze dried ground marama root) of the earlier harvested roots (2 month roots) has a moisture content of 10.8%, which decreases down to 4.3 % in the flour of the older marama storage roots (12 month roots). The variation might have been due to the different moisture absorption by the flours. The younger roots are higher in protein content and the protein hydrophilic groups might have bonded with water molecules, resulting in higher moisture content of the root flours. Legume plants that are rich in protein have a great amount of hydrophilic groups that are exposed to water (Hermanson as cited in Ayodele & Ade-omowaye 2015). Moisture content values for the 2, 4 and 8 month root flours are in the same range as those reported for sweet potato, taro and yam; however the value for 12 month root flour is lower. The moisture content for sweet potato, taro and yam flours were 7.07%, 8.19% and 10.51%, respectively. There was however no significant variation between the moisture content values of the flours of these root crops and their starches. The moisture contents for the sweet potato, taro and yam starches were 9.96%, 8.99% and 11.16%, respectively (Aprianita et al. 2009). The variation in moisture content values of the different marama root flours in this study may probably be due to different moisture absorption.

Ash content average values of the different ground (2 month roots) marama storage root flours decrease with time. The younger marama roots (12 month roots) have higher ash

content values than the older marama roots. Ash content values were in the range of 3.0–5.6%. Ash content of the starch of marama roots harvested after 6 months was reported to be 3.1% by Adeboye and Emmambux (2017) , a value within the range of the ash content values for the marama root flours in this study. Ash content values reported by (Osundahunsi et al. 2003) for sweet potatoes were in a range of 1.7- 3.1%, with the highest value similar to the ash content value determined for 12 month roots in this study. According to Ravindran et al. (1995) the ash content of varieties of sweet potatoes was in the range of 2.7-4.2 %. A study by Mosele et al. (2011) found that the ash content for the marama seeds was 3.2 %, a value that is also in the same range as the ash content value for the 12 month roots.

Crude protein is very high in the 2 month roots sample (33.6%) and is not comparable to the 4 month roots (14.0%), 8 month roots (3.3%) and 12 month roots (2.7%). Crude protein values for the marama root samples in this study significantly decrease with time. This means, the roots harvested early have a higher crude protein content then those harvested later on in the trial. Mosele et al. (2011) reported that the marama seed (bean) had a high protein content (32%), a value comparable to the value of protein content found in the marama roots harvested earliest (2 month roots with 33.6%) in this trial. Marama storage root has a high protein content, higher than that of the potato, yam and the sugar beet (National Academy of Sciences 1996). Marama roots also have crude protein content higher than that of cassava roots. Cassava roots are relatively low in protein, their crude protein ranges from 0.95 to 6.42 % (Cellabos et al. 2006). Protein content for the sweet potato, taro and yam was 3.15%, 6.28% and 10.46 % respectively

(Aprianita et al. 2009). Protein content for these root crops were lower than that of the 2 month roots and 4 month roots (earliest harvested marama roots), however the protein content value for sweet potatoes (3.15%) was in the same range as that for 8 month roots and 12 month roots (roots harvested latest).

Crude fibre content (dry basis) was significantly different in the marama roots harvested at different times. Crude fibre of marama root ranged from 5.6 to 7.2%, higher than reported values for other root and tuber crops such as taro (1.7-2.7%) , sweet potato(1.89 -3.48 %) and yam (0.6-15%) (Aregheore & Perera 2003; Ravindran et al. 1995; Bhandari et al. 2003). In addition, marama root crude fibre was also higher than the values reported for cassava, cassava crude fibre ranges from 1.5- to 3.5% (Charles et al. 2005).

In addition to there being no literature on the proximate analysis of marama storage root, literature on the total soluble solids of roots and tuber crops is also not known to the author and thus this study is unique. Total soluble solids are used together with titratable acidity (the ratio of total soluble solids to titratable acidity) to determine the sweetness of fruits and vegetables, sweetness is a flavour quality of fruits and vegetables (Kader 2008). The total soluble solids for the marama storage roots were analysed by using a refractometer and the values significantly decreased with time, thus the roots harvested earlier in the trial had higher total soluble solids than the roots harvested latest in the trial. The 2 month roots samples had the highest total soluble solids (5.7%), followed by 4 month roots (5.4%), then by 8 month roots (3.5%) and finally 12 month roots with the lowest total soluble solids content (2.2%). The 2 month roots have lower starch and

higher total soluble solids, the total soluble solids decreased with an increase in starch content as the crop matured. Therefore the decrease in total soluble solids is most probably due to the synthesis of starch from the simple sugars. Simple sugars (glucose and fructose) are synthesised into starch in the amyloplast of storage organs; ADPglucose pyrophosphorylase, starch synthase and starch-branching enzyme are involved in the synthesis of starch in the amyloplast (Kossmann & Lloyd 2000). In general, reducing sugars correlate with total soluble solids content and thus total soluble solids analysis is a good estimate of the sugar levels (Georgelis 2002). Therefore the total soluble solids in this study are only for estimating the sugar levels and are not a measure of the sweetness flavour quality. Total soluble solids does not only include sugars, but also includes organic acids ,ascorbic acid, soluble pectins, anthocyanins and other phenolic compounds (Kader 2008). Titratable acidity is therefore recommended to accurately determine the sweetness flavour of the different marama roots samples.

5.3.2. Total starch content

As expected, both the dry weight basis and fresh basis total starch content of the marama root increased significantly with age, the total starch content of roots harvested earlier in the trial had less total starch amount then those harvested later in the trial. The dry basis total starch content slowly increased between the 2^{nd} and 4^{th} months after planting but almost doubled between the 4^{th} and 8^{th} months. The total starch content on dry weight basis varies from 25.9 (2 month roots) to 60.1% (12 month roots). Similarly, there was a variation in the starch content of potato tubers harvested at different times, the highest

starch content was recorded for 2-3 months potato tubers (Liu et al. 2003). The total starch content of cassava roots harvested at different times also varied, the starch content increased with time until it reached its maximum at 14th month (Sriroth et al. 1999).

The total starch content in fresh marama roots was derived from the total starch content of the dried flour. The values for the total starch content on fresh basis of the marama root for all the root samples were lower than 15%. With the lowest being only 2.3% (2 month), and highest being only 11.5% (12 month). This means that in 100 g of fresh 2 month marama root there is only 2.3 g of starch, while in a 12 month marama root there is only 11.5 g of starch. Nepolo (2014) reported that 1 g of fresh marama root contains 87mg of starch. This is in agreement with results in this study that show that the major component of the fresh marama root is water. The marama root therefore contains very low starch content and genetic modifications might be required to increase the starch production of the roots. However, one can safely conclude that the total starch of the marama roots increases with maturity of the root. Therefore, this study suggest that time has a positive impact on the total starch content. Literature shows that the harvest dates had an effect on starch properties (Sriroth et al. 1999).

5.3.3. Amylose content

The amylose content showed a decreasing trend with an increase in time growth from 50.1 to 21.4% with increase in harvesting time. The younger roots (2 month and 4 month roots) contain high amylose starch, while the older roots contain normal amylose starch. A similar trend was observed for sweet potatoes whereby the amylose content of the different variety of sweet potatoes increased with harvest time. The amylose content varied from 19.7 to 20.5% for Koganesengan sweet potato variety and from 21.9 to 23.1% for the Shiroyutaka sweet potato variety (Noda et al. 1992). Similarly, the amylose content of cassava root starch of different cassava varieties was also highest in the roots harvested early but it remained constant after 14 months. The amylose content for the cassava varieties varied from 20.6 to 24.1 for the Rayong 1 cassava variety, from 20.8 to 22.5% for the Rayong 60 variety, from 22.5 to 23.1% for the Rayong 90 variety and finally from 19.6 to 21.4 % for the KU 50 variety (Sriroth et al. 1999). In much the same way, the amylose content for potato starch was also highest in the tubers harvested earlier, it decreased and remained constant during further growth of the tubers. The amylose content of the potato starch varied from 28.3 to 29.5% for Superior starch, from 29.0 to 29.7 % for Shepody starch and from 29.7 to 31.1% for Snowden. However the differences were only significant for the Shepody potato variety (Liu et al. 2003). Noda et al. (2004) also reported that the amylose content of potato starch also decrease with time, the starch of the tubers harvested early in the trial had the highest amylose content compared to the tubers harvested late in the trial. The amylose content ranged from 20.2 to 21.2 %. Although the trend observed in this study is similar to the trends in literature,

the amylose content of the starch in younger roots is higher than any amylose content reported in literature for root and tuber starches. The roots and tuber starches are reported to have an amylose content ranging from 10- 38% (Hoover 2001). Farmers can decide when to harvest depending on the amylose content and thus the functional properties of the starch which in turn is dependent on the intended use of the starch. The desired functional properties of starch can therefore be achieved by controlling growth time without the need for chemical or physical modifications of the starch for specific applications (Liu et al. 2003)

5.3.4. Thermal properties

The endotherm peak for all the marama root flour samples in this study is in the range 73.4-93.0 ˚C, this study is the first to investigate the thermal properties of marama root flour. However, one paper in literature reported the thermal properties of extracted marama root starch. Adeboye & Emmambux (2017) reported that the gelatinization temperature for marama root starch is 67.5 – 79.0 ˚C. As expected time had an effect on the thermal properties of the marama root starch, this may be attributed to the difference in the amylose content of the starch of roots harvested at different times and also the difference in other components of the ground root flour samples. No endothermic peak was yielded by the 2 months root flour sample in the temperature range between 30-120˚C; this was probably due to the high amylose content and high concentration of other components in this root sample (low starch). It is difficult to accurately define the gelatinisation temperature of high amylose starch because of the flat endotherm (Tester

1997). Therefore no endothermic temperatures could be determined for the freeze dried 2 months root flour sample in the specified temperature range due to the high amylose. High amylose starch has high gelatinization temperatures (Jane et al. 1999). In addition, the freeze dried 2 month root sample contains a low starch content, it only contains 25.1% total starch content, of which 50.7% is amylose content, which is very high; it also contains 33.6% protein content, 6.3% ash content, 7.2% fibre content, 6.4% total soluble solids and 10.8% moisture content.

Furthermore, the peak temperatures of the marama roots starch in this study decreased with maturity of the marama roots, this correlated with the decrease in amylose content; which was expected because high amylose starch has high gelatinization temperatures. According to Chen et al. (2017) high amylose maize starch granules exhibits high resistance to gelatinization.

The T_o, T_p and T_c temperatures decreased with crop maturity, this correlated with a decrease in other components of the starch such as, the protein, total soluble solids, ash content and fibre content. The other components decreased with the maturity of the crop, thus the younger roots had higher endotherm temperatures as compared to the older roots. The higher endothermic temperatures could be due to the interactions of the starch with other starch components. The other components may have an effect on the endothermic temperatures of the marama root starch. This is because the study of starch gelatinization in flour samples is a bit more complex due to the interactions that can occur between starch and other components present (Torres et al. 2013). Starch

gelatinization is delayed by the presence of sugars, sugars decrease the water activity and it also interacts with the starch chains (Moreira et al. 2011). The effect of sugars on the gelatinization of potato starch has been reported in literature. Similarly, there was a decrease in peak temperature in this study as the total soluble solids decreased. The T_p for the gelatinization of potato starch increased with increasing sugar content. The T_p for the gelatinization of potato starch shifted to higher temperatures due to the interactions of the sugar with the starch and also the immobilization of the water molecules (Kohyama & Nishinari 1991). Hirashima et al. (2005) also reported that starch gelatinization temperatures are shifted to higher temperatures with an increase in sucrose. Moreover, proteins have an effect on the availability of water needed to interact with the starch and hence causes an increase in gelatinization temperatures (Larrosa et al. 2012). The proteins form complexes with starch on the starch granule surface decreasing amylose leaching, they also have an effect on the water availability (Sumnu et al. 1999). In addition to proteins and sugars, dietary fibre also shifts the starch gelatinization temperatures to a higher range by competing with the starch for water (Srichuwong et al. 2017). Therefore it is expected that there was no endothermic peak observed for the 2 month old roots as at this stage the sample contains high amounts of other components and the lowest amount of total starch.

Furthermore, the enthalpy change increased with the maturity of the marama storage roots; therefore the enthalpy change was lowest in the earliest stage of development of the marama roots (4 months root). This correlated with the decrease of amylose and an increase in amylopectin between 2-12 months, thus the crystallinity of the starch

increases with crop maturity. Amylopectin content is a determining factor for the starch crystallinity and hence the thermal properties (Jane et al. 1999). As the stability of the crystallites increase with crop maturity, the enthalpy change increases. A similar trend was observed in sweet potatoes whereby the enthalpy change was lowest in the sweet potatoes harvested earlier (earlier stage) (Noda et al. 1992). When amylopectin content increases enthalpy change also increases, thus normal starch has a lower enthalpy change then waxy starch. Waxy starch displays a higher enthalpy change which reflects the higher percentage of crystallinity of the amylopectin. Thus swelling is predominantly a characteristic of amylopectin (Tester & Morrison 1990).

Depending on the preferred starch, marama roots can be selected according to the different properties that are due to the different harvesting times. The younger roots can be boiled and consumed as a vegetable. The root also has potential as a source of starch, due to the difference in the properties of the starch at different harvest times. Marama roots could be a new source of food, the root does not take a long time to grow and can be harvested as a cash crop.

CHAPTER 6: CONCLUSIONS

The marama plants are successfully grown at the NEUDAMM campus. However the plants did not produce any pods during the 12 month trial. In addition, the characteristics of the marama roots are all significantly different at different harvest times. It is therefore safe to conclude that time has an effect on the agronomic and physicochemical properties of the marama roots and its starch. The tuber size increased as time progressed, however winter has a negative effect on the size of the tuber due to the loss of leaves during winter. The granule size of the starch from the different marama roots also significantly increase with the time.

Although the total starch content of the marama roots increases with crop maturity (harvesting time), the amylose content decreases with crop maturity and thus the lowest at the latest harvest time in the trial. Moreover, the ash, protein, moisture, and total soluble solids all decrease with crop maturity (harvesting time). Marama roots have high protein content, especially the younger roots (2 month roots) which have a protein content similar to that of the marama seed. The younger roots also have the highest total soluble solids which is an estimate of the sweetness of the root. The older roots are more fibrous although they have higher total starch content. Hence the younger roots are more suitable to be consumed as a vegetable.

Furthermore, marama root flour endotherm are affected by two factors : 1) the decrease in amylose content of the starch and therefore the increase in crystallinity of the starch, which was evident in the increase of the ΔH as crops matured 2) the decrease in other

components present in the root flour samples, hence a decrease in the interactions with the starch, the interactions may possibly have been responsible for the delay in endothermic peak, which was evident in the decrease in the T_o, T_p and T_c temperatures .

In conclusion, the findings from this study might therefore boost the consumption of the younger marama root as a vegetable. Furthermore, marama can be domesticated, one will be able to produce marama roots for different applications or root consumption. The marama plants should be planted during summer and roots should be harvested before winter, due to the effects of winter on the root. This study is the first known to the author that investigated the effect of harvest time on the properties of both the root and its starch.

CHAPTER 7: RECOMMENDATIONS

Marama has been dubbed as the lost crop of Africa, this is due to the fact that although both its seed and root have commendable nutritional qualities and it thrives in poor soils and grows under arid conditions; it is still being underutilized. It is therefore recommended that future research focuses on the isolation and application of the marama root starch from roots harvested at different times in the food or non-food industries. The findings of this study can therefore be used as a guide for future studies. It is also recommended that the carotenoids and total phenolic compounds as well as the digestibility of the different aged marama roots are investigated. In addition, a study focusing on when the plants produce its first pods is also recommended. Finally, younger roots can be harvested for consumption as a vegetable. The planting can be done early summer and the roots can be harvested as a fresh root vegetable at 2 and 4 months so that one can have two seasons.

CHAPTER 8: REFERENCES

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CHAPTER 9: APPENDICES

Appendix 1

Marama root mass results

marama root mass(g)								
sample code	replicate 1	replicate 2	replicate 3	replicate 4	replicate 5	mean	standard deviation	standard error
2 months	11,7	11,8	17,4	19,9	12,4	14,6	3,376743994	1,510125823
4 months	37,35	36,11	41,71	32,91	46,43	38,9	4,704544186	2,103936121
8 months	464,3	364,1	326,6	468,1	479	420,4	62,61723086	28,00327695
12 months	302,5	303,9	397,7	328,1	299,8	326,4	37,07182218	16,57902289

Appendix 2

Marama root mass statistical analysis

Post Hoc Tests

Homogeneous Subsets

root mass

Duncan[a]

		Subset for alpha = 0.05		
time	N	1	2	3
2	5	14,6400		
4	5	38,9020		
12	5		326,4000	
8	5			420,4200
Sig.		0,361	1,000	1,000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

Appendix 3

Marama root diameter results

marama root diameter(cm)								
sample code	replicate 1	replicate 2	replicate 3	replicate 4	replicate 5	mean	standard deviation	standard error
2 months	1,179	1,261	1,553	1,685	1,397	1,4	0,185299757	0,082868571
4 months	2,2	3	2,8	2,34	3	2,7	0,336	0,150263768
8 months	7,586	4,723	5,518	8,465	6,223	6,5	1,35936441	0,607926246
12 months	5,914	5,296	6,086	5,587	5,43	5,7	0,295527731	0,132164019

Appendix 4

Marama root diameter statistical analysis

Post Hoc Tests

root diameter

Duncan[a]

time	N	Subset for alpha = 0.05		
		1	2	3
2	5	1,4150		
4	5		2,6680	
12	5			5,6626
8	5			6,5030
Sig.		1,000	1,000	0,119

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

Appendix 5

Marama root crude fibre statistical analysis

crude fibre

Duncan[a]

Time	N	Subset for alpha = 0.05		
		1	2	3
12	3	5,5903		
4	3		6,6738	
8	3		6,8020	
2	3			7,2421
Sig.		1,000	0,431	1,000

Means for groups in homogeneous subsets are

a. Uses Harmonic Mean Sample Size = 3,000.

Appendix 6

Marama average granule size (diameter) statistical analysis

Post Hoc Tests

Homogeneous Subsets

granule average size

Duncan[a]

time	N	Subset for alpha = 0.05		
		1	2	3
2	4	8,5550		
4	4	9,3400	9,3400	
8	4		11,9050	
12	4			15,0500
Sig.		0,584	0,091	1,000

Means for groups in homogeneous subsets are displayed.

Appendix 7

Mass per sample (Wet basis) used for DSC analysis

Mass per sample used for DSC analysis

sample	2 months	4 months	8 months	12 months
dry basis(mg)	10	10	10	10
moisture %	10,8	6,7	6,4	4,3
wet basis(mg	8,9	9,3	9,4	9,6

Appendix 8

DSC results

	4 months marama root					8 months marama root					12 months marama root			
	To	Tp	Tc	Change		To	Tp	Tc	Change		To	Tp	Tc	Change
replicate 1	77,44	84,99	93,28	2,3		73,53	81,24	89,83	7,94		74,31	79,21	84,67	12,31
replicate 2	77,24	84,99	92,38	2,2		73,03	81,24	89,93	8,7		74,01	79,04	84,62	13
replicate 3	77,44	84,83	93,28	2,06		73,48	81,24	89,06	8,05		74,22	79,04	84,5	11,61
mean	77,37333	84,93667	92,98	2,186667		73,34667	81,24	89,60667	8,23		74,18	79,09667	84,59667	12,30667
standard deviation	0,11547	0,092376	0,519615	0,120554		0,275379	0	0,47606	0,410731		0,153948	0,09815	0,087369	0,695006
standard error	0,05164	0,041312	0,232379	0,053914		0,123153	0	0,212901	0,183685		0,068848	0,043894	0,039073	0,310816