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Alpha Amylase Activity and Sprouting During Short Term Storage of Taro Corms

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ABSTRACT

The objective of this study was to investigate taro [C*olocasia esculenta* (L.) Schott] corm quality as determined by changes in starch morphology and degradation during storage after harvest. Starch is the major nutrient component of taro corms and its quality in corms that are stored as planting material or consumption has not been fully explained. Scanning electron microscopy was used to examine the changes that occur to the surface morphology of starch granules in corms of taro landraces, *Dumbe-dumbe*, *Mgingqeni* and *Pitshi,* stored at cool (12ºC) and ambient (20ºC) temperatures. Alpha-amylase activity and sprouting were used as indicators of changes in the starch granules, and hence corm quality, during storage in polyethylene bags, card boxes, and mesh bags. The degradation of starch granules, alpha amylase activity, and sprouting increased over storage time and varied with landraces, storage material, and temperature. Overall, there was 23% more alpha-amylase activity and 67% more sprouting at 20ºC compared with 12ºC. With respect to storage material, polyethylene bags showed the highest alpha-amylase activity (0.18 EU 0.1 g^{-1}) followed by card boxes (0.15 EU mg^{-1} 0.1 g^{-1}) and mesh bags (0.14 EU mg^{-1} 0.1 g^{-1}). A similar, but more pronounced, trend was observed for sprouting. The findings have implications for selection of storage material for food and propagule storage.

Keywords: *Colocasia esculenta,* Electron microscopy, Landrace, Starch granules.

INTRODUCTION

Taro is cultivated to a limited extent in South Africa due to two major reasons. Firstly, the crop requires high rainfall (> 1,600 mm $annum^{-1}$) for dryland production, and South Africa has an average rainfall of 450 mm $annum^{-1}$ (Smith, 2006). However, the eastern seaboard of the country, mainly the KwaZulu-Natal coast land, has the highest rainfall (up to 2,000 mm $annum^{-1}$) and this is where the majority of taro is produced (Mabhaudhi and Modi, 2013). Secondly, there has not been research to produce hybrid taro varieties in South Africa. This makes the farmers rely on landraces that can be differentiated on morphological basis, although recently, Mabhaudhi and Modi (2013) attempted to provide genetic characterization. This study is an attempt to provide more data with respect to germplasm preservation and possibly nutritional value of the crop during term storage. Farmers store the crop for a period of up to three or four months while it is used for subsistence and later as a source of planting material. The ability of corms to sprout during storage is an important consideration for availability of the crop as a foodstuff and when it is required for planting purposes (Modi, 2004)

The surface morphology of starch granules changes during storage (Cottrell *et al*., 1993). This was observed by various patterns of erosion and associated with starch degradation. Extensive surface

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erosion was shown to indicate a high degree of hydrolysis, whereas less surface erosion indicated less degradation (Zhang and Oates, 1999). The normal visible response to degradation *in vivo* appeared to be varying degrees of roughness of the granule surface (Silva and Luh, 1978; Wetzstein and Wetzstein, 1981) or holes protruding through the granule (Dronzek *et al*., 1972). The same results were found by (Cottrell *et al*., 1993), who reported pitting and indentations in potatoes, and erosion in legumes observed by Valetudie *et al*. (1993) who reported that roughening of the surface with pitting and fissures along the edges of the planes of contact of the starch granules in tannia, sweet potato and cassava and external pitting on only one face of the granule to produce an internal cavity in yams. In chickpea, on the other hand, no difference in character of starch granules could be seen during starch degradation (Fernandez and Berry, 1989).

The erosion is believed to be brought about by alpha-amylase activity, because cereal starch granules showed holes in the surface and corroded channels into the interior when subjected to *in vitro* digestion with alpha amylase (Dronzek *et al*., 1972; Sreenath, 1992). The activity of the enzyme was reported to increase during storage (Cochrane *et al*., 1991; Cottrell *et al*., 1993). Previously, it was reported that degradation is initiated by synthesis of new alpha amylase in wheat and barley (Fincher, 1989; MacGregor and Balance, 1980). The surface of potato granules showed resistance to enzyme attack with a few shallow pits and ridges initially, but it degraded rapidly once the enzyme reached the interior of the granule (Gallant *et al.*, 1972; 1973).

According to Biemelt *et al*. (2000) starch degradation is not a prerequisite for the initiation of sprouting. These authors observed no change in starch degrading enzymes prior to visible sprout growth and stimulated alpha-amylase activity by approximately 30% was observed after the onset of sprouting in potatoes. On the other hand, it was found (Lorenz *et al*., 1981; Revedin *et al*., 2010) that the production of modified starches was possible by sprouting corn, barley, and triticale grains. These authors also reported that the starch susceptibility to the enzyme increased with sprouting. It was indicated (Sun and Henson, 1990) that alpha-amylase was synthesized during germination in barley, hence, it might be the case in taro that alpha-amylase is synthesized during sprouting. Germination sharply increased the enzymatic digestibility of starch granules from the chickpea and lentil (Fernandez and Berry, 1989; Frias *et al*., 1998), but no change in the appearance of scanning electron micrographs could be attributed to germination (Fernandez and Berry, 1989).

With the hypothesis that there is no significant difference between taro local varieties in their response to short term storage, which is normally three to four months, the objective of this study was to investigate taro corm quality as determined by changes in starch morphology and degradation during short term storage between cropping seasons. Starch is a major nutrient storage compound in taro and its degradation by alpha amylase and associated sprouting response should be useful indicators of corm quality.

MATERIALS AND METHODS

Plant Material

Corms used in this study were of the three taro [*Colocasia esculenta* (L.) Schott] local landraces, *Dumbe-dumbe*, *Mgingqeni* and *Pitshi,* which were produced under field conditions at Umbumbulu (29° 59' 0" South, 30° 42' 0" East) in KwaZulu-Natal, South Africa (Mare and Modi 2012). The landraces differed in that *Dumbe-dumbe* is a large tall plant (up to 1.2 m) that forms one large (~75g) round corm varying in size depending on environmental conditions; *Mgingqeni* is a large tall plant (up to 1.2 m) that forms two to four medium size (~50g) oval-shaped corms; and *Pitshi* is a smaller

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plant (0.5 to 0.8 m) that forms many (4 to 6) small oval shaped corms (~25g) Mabhaudhi and Modi (2013). The landraces were planted (37,000 plants ha^{-1}) in late spring (October) and harvested in June under dryland conditions (Average rainfall= 1,600 mm, Average temperature= 18°C), using 1.2 t ha^{-1} of 2:3:2 (22) fertilizer according to soil analysis results. The soil was a well-drained Avalon Blackmoor (Soil Classification Working Group, 1991), 120 mm deep. The crop was weeded manually twice at two and four months after planting and harvested 8 months after planting.

Scanning Electron Microscopy

Scanning electron microscopy was performed at harvest and monthly for four months starting one month after storage for corms of the three taro cultivars which were stored at ambient temperature. The freeze-dried corm materials were mounted on the brass stubs using double-sided cellotape, and then coated with gold palladium, using a Polaron E5100 sputter coater (Klein *et al.*, 2012). Samples were viewed at 10 kW using a Hitachi S-570 Scanning Electron Microscope (SEM). Starch grains were spot viewed and the area for starch grain viewing was selected randomly. The micrographs were used to compare the morphology (form and structure) of the starch granules.

Alpha-amylase Activity

Alpha-amylase activity was determined at harvest and monthly for four months starting one month after storage according to Modi and Cairns (1994) with modifications. Only alpha amylase activity of corms stored at ambient temperature was used to study the relationship between the changes in the surface morphology of three taro starch granules, alpha-amylase activity, and sprouting of corms. Freeze-dried corm material was ground to pass through a 0.5 mm motor mill sieve. Approximately 0.1 g was weighed into a 100 mL Erlenmeyer flask and incubated in 10 mL of the extraction buffer [5g NaCl: 0.2g Ca $(C_2H_3O_2)_2$, mass/mass]. Alpha-amylase was extracted by shaking the contents for 20 minutes in a water bath at 30°C. Contents were then filtered through a Whatman No.1 filter paper into a test tube. One milliliter of the supernatant was diluted 10 times in the extraction buffer and one Phadebas tablet (Pharmacia Diagnostics AB, Uppsala, Sweden) was introduced into the test tube. Phadebas tablet is composed of a substrate made by cross-linking partially hydrolyzed starch, using 1,4 butandiol-diglycidether as a cross-linking agent. The soluble starch is transformed into a three-dimensional insoluble lattice network which expands in water. The number of cross-linking bridges regulates the degree of expansion and susceptibility of the substrate to alpha-amylase attack. The substrate is labeled with Cibachron Blue by covalent bonds. Each tablet contains 45 mg of blue starch polymer, and 25 mg buffer salt to give 0.2 mol dm^{-3} phosphate buffer, pH 7, and 0.05 mol dm^{-3} NaCl when appropriately dissolved. The tablets also contain bovine serum albumin. The contents of the test tube were mixed for 30 seconds on a vortex mixer and then incubated in the shaking water bath at 50°C for 10 minutes. The reaction was then terminated by adding 1 mL of 0.5M NaOH and the contents were filtered through Whatman No.1 filter paper. Absorbance of the supernatant was determined at 620 nm in a spectrophotometer (Mare and Modi, 2012).

Sprouting

The post-harvest storage experiment was arranged with two temperatures (12 and 20°C) as main plots, packaging (polyethylene bag, card box and mesh bag) as sub-plots and cultivars as sub-sub-plots. The experiment was replicated three times. All packaging materials were kept under light conditions in an open room at 60% RH

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(Robertson, 2012). Each packaging material contained 30 corms of *Dumbe-dumbe*, *Mgingqeni* and *Pitshi,* respectively. Corms were randomly sampled, peeled, cut into slices, immediately frozen in liquid nitrogen and freeze-dried monthly to produce four samples representing harvest stage, one, two, and three months after harvest for chemical analysis (Ramanatha *et al*., 2010).

The number of sprouted corms was counted monthly for three months and expressed as percentages. Corms were considered sprouted when they had developed a shoot of about 3.0 mm in length.

Statistical Analysis

Data collected were subjected to analysis of variance (ANOVA) using GenStat® Version 16 (VSN International, UK) at the 5% level of significance. Means of significantly different variables were separated using Duncan's test in GenStat® at the 5% level of significance.

RESULTS

Scanning Electron Microscopy

The appearance of starch granules changed considerably during storage. The starch granules of *Dumbe-dumbe and Pitshi* appeared smooth at harvest (Figures 1 and 3). For *Mgingqeni*, some granules were smooth while some were rough (Figure 2). After one month in storage, all cultivars showed signs of degradation, although the severity varied between them. *Dumbe-dumbe* and *Mgingqeni* were showing some indentations whereas *Pitshi* was showing some depressions. The indentations appeared shallow and did not penetrate deep into the body of the granules.

By the second sampling after one month (Figure 4), granules from all cultivars showed evidence of erosion. Granules from *Dumbe-dumbe* showed deep indentations, but they were not as deep as those showed by *Mgingqeni*. After two months in storage, *Mgingqeni*, as it happened earlier, showed

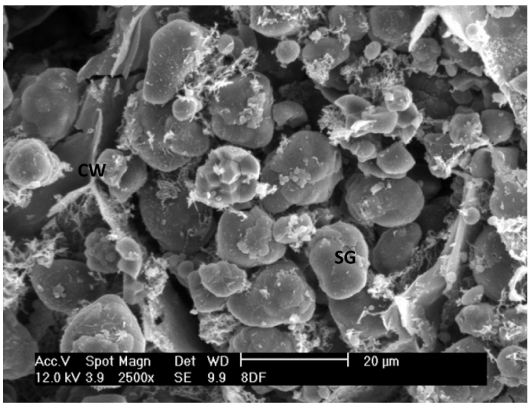

Figure 1. Scanning electron micrographs showing Starch Granules (SG) of *Dumbe-dumbe* taro variety at harvest in corm cells (CW= Cell Wall).

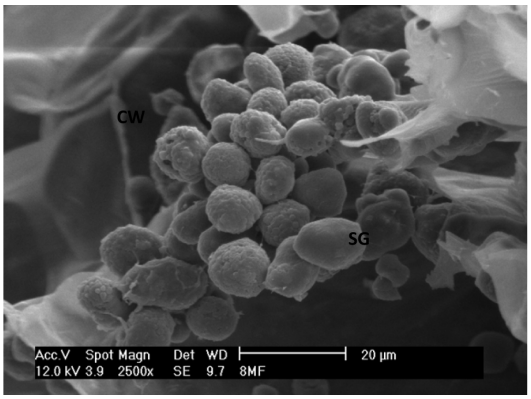

Figure 2. Scanning electron micrographs showing Starch Granules (SG) of *Mgingqeni* taro variety at harvest in corm cells (CW= Cell Wall).

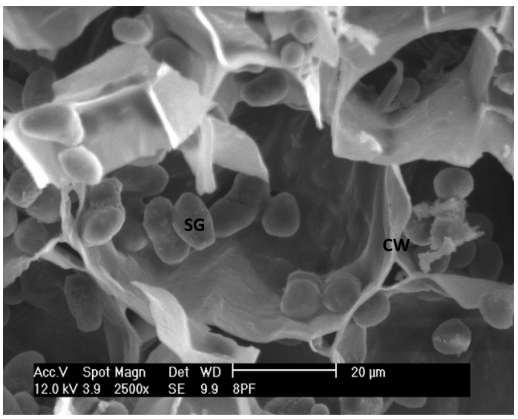

Figure 3. Scanning electron micrographs showing Starch Granules (SG) of *Pitshi* taro variety at harvest(CW= Cell Wall).

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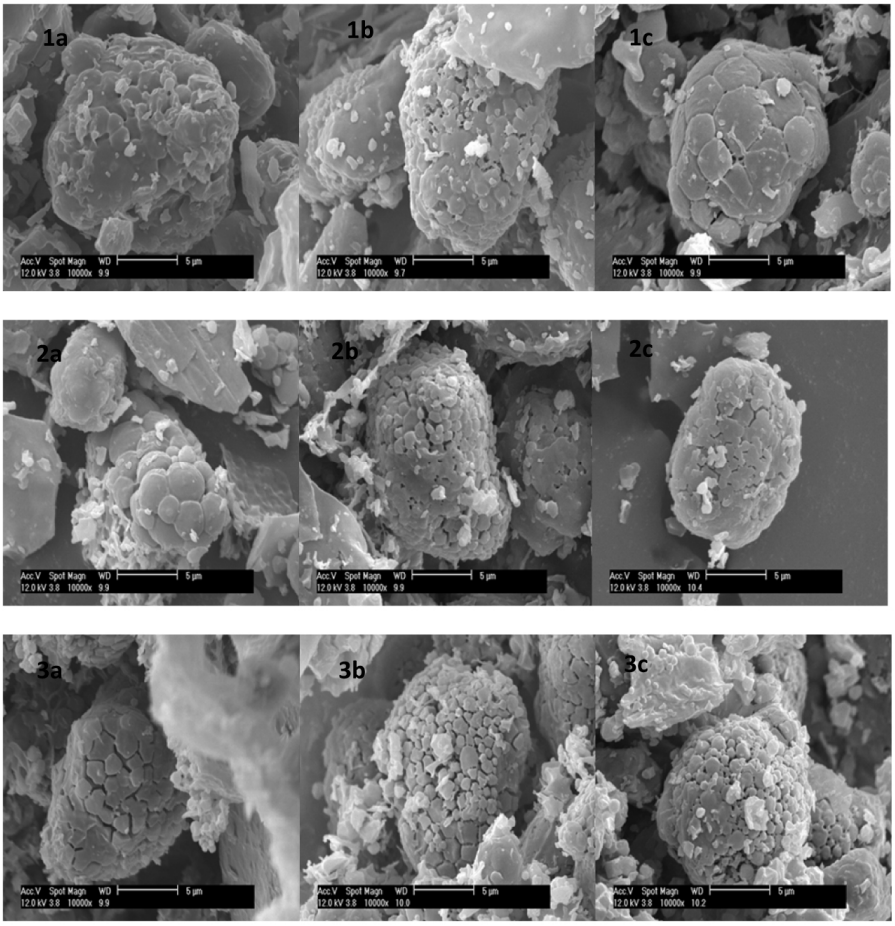

Figure 4. Scanning electron micrographs showing starch granules of taro cultivars stored in polyethylene bags for four months at ambient temperature (a, b and c represent *Dumbe-dumbe, Mgingqeni* and *Pitshi,* respectively and 1, 2 and 3 represent the sampling time in months after harvesting).

more degradation compared to *Dumbe-dumbe* and *Pitshi*, which were still showing some indentations, although they seemed to be deeper than after two months. By the final sampling, more degradation was evident and it was more pronounced for *Mgingqeni*, followed by *Pitshi* and *Dumbe-dumbe*, respectively (Figure 4). The smaller pieces were starting to break and fall from the starch granules.

Alpha-amylase Activity

The cultivars were different from each other in average alpha-amylase activity at harvest and there was a highly significant (P= 0.001) interaction between storage temperature, packaging method and landrace (Figure 5). Alpha-amylase activity of corms generally increased with time and it was also significantly (P= 0.04) more pronounced at 12^{o}C compared with 20^{o}C (Figure 5). *Dumbe-dumbe* and *Pitshi* generally showed higher alpha-amylase activity than *Mgigqeni* at all temperatures (Figure 5). Within temperature, there was a significantly (P= 0.04) higher alpha-amylase activity in corms stored in polyethylene bags, compared with mesh bags and boxes (Figure 5). At 12^{o}C, there was no significant difference between mesh bags and card boxes, whereas at 20^{o}C card boxes showed a significantly (P= 0.01) higher alpha-amylase activity than mesh

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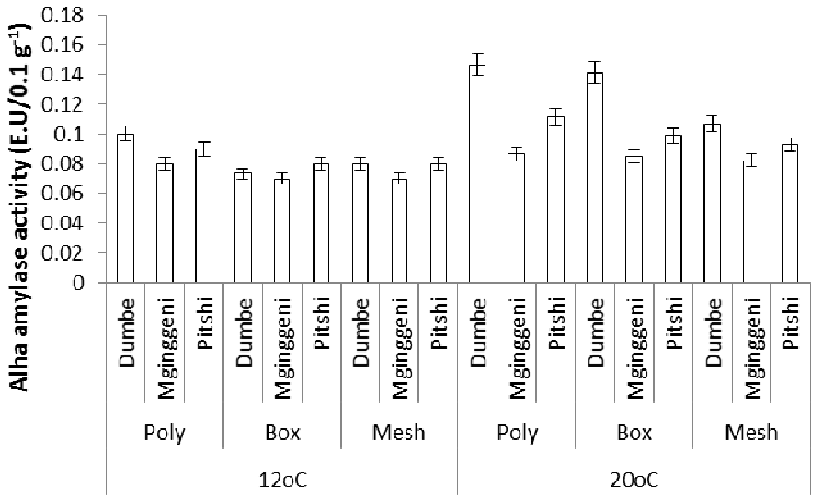

Figure 5. Average alpha-amylase activity in taro corms of three landraces *Dumbe-dumbe* (Dumbe), *Mgingqeni* and *Pitshi* over a period of three months in storage in different packaging materials (Poly= Polyethylene bags; Box= Card box, Mesh= Mesh bags) at 12 and 20°C.

boxes, mainly due to *Dumbe-dumbe* displaying the highest alpha-amylase activity (P= 0.03).

Sprouting

There was a highly significant (P= 0.001) difference between temperatures with respect to average sprouting, with 20°C displaying higher levels of sprouting than 12°C (Figure 6). There was also a very clear pattern showing that polyethylene bags cause the greatest sprouting followed by card boxes and mesh bags, respectively (Figure 6). The three cultivars did not show a consistent pattern within temperature and in polyethylene bags and card boxes, but within mesh bags, the pattern from highest to lowest sprouting was *Dumbe-dumbe, Mgingqeni and Pitshi,* respectively, at both temperatures (Figure 6).

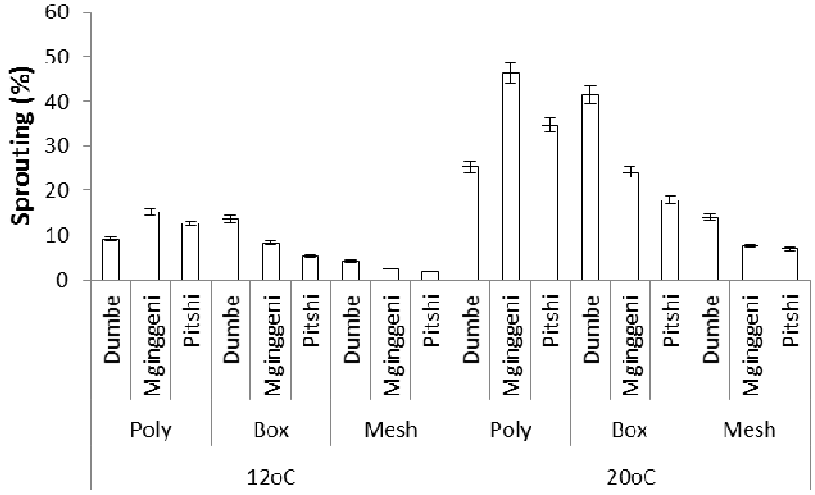

Figure 6. Average sprouting of taro corms of three landraces *Dumbe-dumbe* (Dumbe), *Mgingqeni* and *Pitshi* over a period of three months in storage in different packaging materials (Poly= Polyethylene bags; Box= Card box, Mesh= Mesh bags) at 12 and 20°C.

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It was necessary to correlate average alpha-amylase activity and sprouting during the period of storage across all landraces, temperatures, and storage materials. The results showed that there was a highly significant correlation (R^2= 0.897). Although the corms had an average of about 0.05 EU 0.1 mg^{-1} at harvest, they showed no sprouting (Figures 7). However, there was 38% increase in alpha-amylase, which was associated with 150% increase in sprouting (Figures 7). The positive pattern of increase in both alpha amylase and sprouting was evident until the third month, when the last sampling was done.

DISCUSSION

Scanning electron micrographs showing the changes in surface morphology of starch granules of taro cultivars during storage

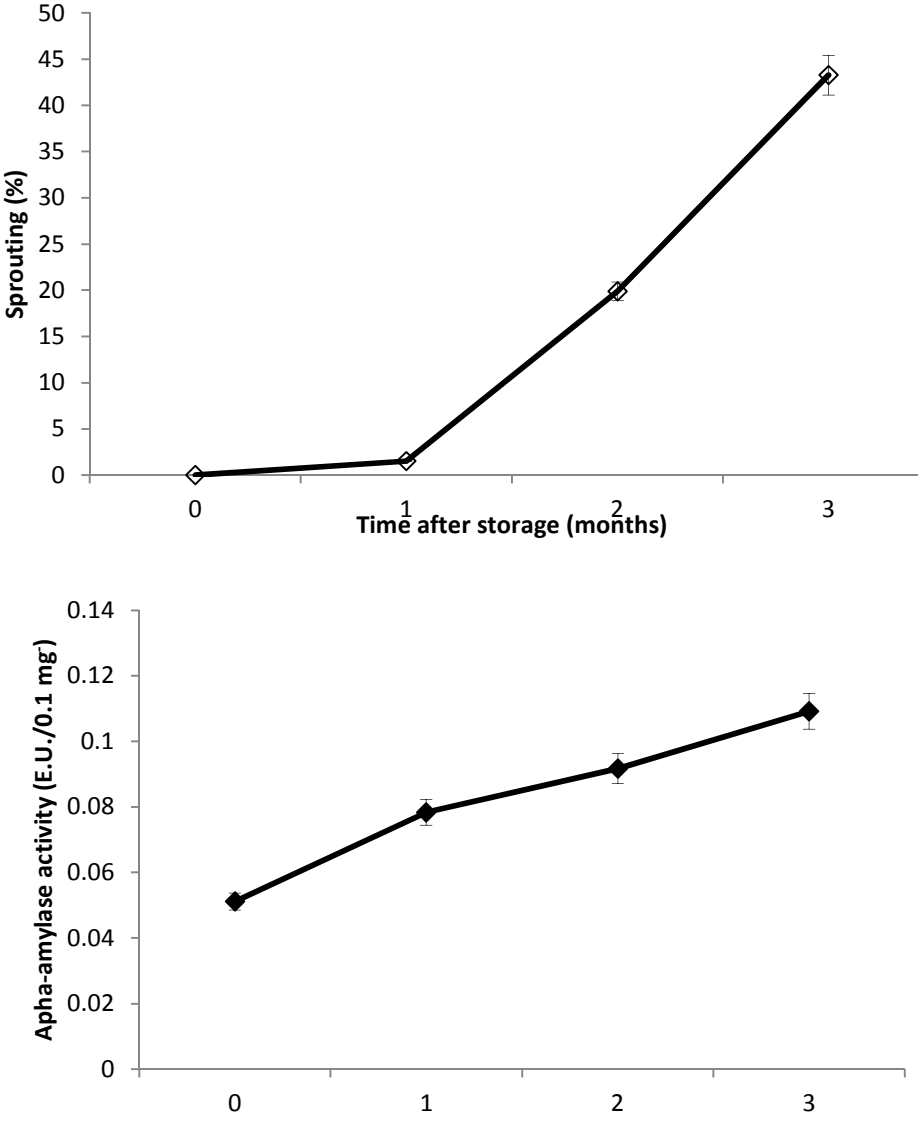

Figure 7. Comparison of sprouting (top) with alpha-amylase activity (bottom) for taro corms of three landraces (*Dumbe-dumbe*, *Mgingqeni* and *Pitshi*) over a period of three months in storage in different packaging materials (polyethylene bags; card boxes, and mesh bags) at 12 and 20°C. The correlation between sprouting and alpha-amylase activity was R^2= 0.897.

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demonstrate that starch breakdown occurs early in storage, but the extent varies with cultivars. The starch granule breakdown started with roughening of the surface followed by development of small pits and indentations which became deeper with time and caused the granules to open up and breakdown onto smaller granules. The same results were previously reported in legumes, potatoes, tannia, sweet potato and cassava (Hoover and Sosulki, 1991; Cottrell *et al*., 1993; Valetudie, 1993). Extensive surface erosion was displayed by *Mgingqeni,* and according to Zhang and Oates (1999) that might suggest a higher degree of hydrolysis compared with the other cultivars. That this landraces showed more starch degradation while its alpha-amylase activity was lowest suggests that the effect of alpha amylase is linked to genotype, whereby some genotypes may be more sensitive than others.

The alpha-amylase increase during corm storage was previously reported (Modi, 2004). This increase was also reported by Panneerselvam *et al*. (2007) in *Dioscorea esculenta* who showed that alpha-amylase activity was lower up to 35 days after harvest, and afterwards it increased to a rapid phase. The activity of the enzyme was also reported to increase in potatoes during storage (Cochrane *et al*., 1991; Cottrell *et al*., 1993; Maki *et al*., 2012). Corm sprouting was associated with alpha-amylase activity which enhanced starch mobilization (Aien *et al.*, 2014; Modi, 2004). This is possible considering the increase in alpha-amylase activity which correlates with the starch granules break down and sprouting (R^2= 0.897) (Figure 7) and possibly fiber content in starchy foods (Majzoobi *et al*., 2015). This finding suggests that the enzyme is responsible for the breakdown of starch granules and sprouting; moreover, because of a report that, at the time of sprouting, alpha-amylase activity increased to a higher level when compared with early storage period (Panneerselvam *et al*., 2007). The increase in alpha-amylase was positively correlated with germination in cherry tomato (Modi and White, 2004).

The starch granules breakdown was only monitored at ambient temperature, but it is believed that the same process takes place at a lower temperature although it might be slower since alpha-amylase activity and sprouting were lower at lower temperature. This was in contrast to what was earlier reported (Nielsen *et al*., 1997) that alpha-amylase was not affected by low storage temperature. Findings of the previous study (Modi, 2004) suggested that air and temperature conditions may influence taro corm sprouting.

Packaging is the technology of enclosing or protecting products for distribution, storage, sale, and use (Yam, 2009; Yoxall *et al*., 2006). In this study, the three packaging materials were compared for their ability to preserve corms. It is clear from the findings of the study that while polyethylene bags made corms more susceptible to starch degradation by alpha amylase compared with card box and mesh bags, there was an interaction between packaging material, storage temperature, and landraces that cannot be ignored (Figures 5 and 6).

CONCLUSIONS

The present study demonstrated that taro starch granules were degraded within the first two months of storage at 12 and 20^{o}C. This degradation was evidenced by morphological changes in starch granules over time in storage. The increase in alpha amylase activity provided physiological explanation for the degradation. This enzyme activity led to early sprouting. The results also showed that alpha-amylase activity and sprouting increased with storage temperature and this suggests that starch degradation increases with increase in storage temperature. There was a significant interaction between landrace, temperature, and packaging material. Corms stored in polyethylene bags showed the highest alpha-amylase activity as well as sprouting and

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hence starch degradation. However, there was no evidence of moisture accumulation in polyethylene bags. It is suggested that for the purposes of preserving taro corms for food security after harvest, card boxes and mesh bags would be more appropriate than ethylene bags, because they delay starch degradation and sprouting. However, if a farmer wants to enhance sprouting before planting, so that plant emergence in the field can occur faster and possibly more uniformly, storage in polyethylene bags would be advisable. Hence, it is concluded that the findings of this study will be useful in consideration of storage of taro corms for use as future propagules and processing quality for nutritional purposes.

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فعالیت آلفا آمیلاز و جوانه زنی پداژه گیاه Taro در طی انبار داری کوتاه مدت

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چکیده

هدف این پژوهش بررسی کیفیت پداژه (corm) گیاه taro (*Colocasia esculenta* (L.) Schott] در اثرتغییرات در شکل نشاسته و تخریب آن در طی انبارداری بعد از برداشت بود. نشاسته جزء اصلی عناصر غذایی پداژه گیاه تارو است و کیفیت آن در پداژه هایی که به عنوان بذر برای کاشت یا مصرف خوراکی انبار می شوند به طور کامل تشریح نشده است. در این تحقیق، در بررسی تغییراتی که در شکل سطح دانه های نشاسته درپداژه های کالتیوارهای گیاه تارو شامل *Dumbe-dumbe*، *Mgingqeni*، و *Pitshi* در شرایط خنک (۱۲ درجه سلسیوس) و درحرارت اطاق (۲۰ درجه سلسیوس) رخ می دهد از اسکن الکترن میکروسکپی استفاده شد. همچنین، در طی انبار داری، پداژه ها در کیسه های پلی اتیلن، جعبه های کارتن، و کیسه های توری نگهداری شدند و فعالیت آلفا آمیلاز و جوانه زنی به عنوان نشانگر تغییرات در دانه های نشاسته (و در نتیجه تغییرات کیفیت پداژه ها) در نظر گرفته شد. در دوره انبار داری، تخریب دانه های نشاسته، فعالیت آلفا آمیلاز، و جوانه زنی افزایش یافت و مقدار آن با نوع کالتیوار، جنس وسیله نگهداری، و درجه حرارت تغییر نشان میداد. به طور کلی، در حرارت ۲۰ درجه سلسیوس فعالیت آلفاآمیلاز ۲۳٪ و جوانه زنی ۶۷٪ نسبت به درجه حرارت ۱۲ درجه سلسیوس افزایش نشان داد. در رابطه با جنس وسیله نگهداری در انبار، کیسه های پلی اتیلن بیشترین فعالیت آلفا امیلاز(برابر E.U/mg 0.1g ۰/۱۸) را نشان دادند وبعد از آن جعبه های کارتونی (E.U/mg 0.1g ۰/۱۵) و کیسه های توری (E.U/mg 0.1g ۰/۱۴) قرار گرفتند. در مورد جوانه زنی نیز روندی مشابه ولی شدیدتر مشاهده شد. این نتایج برای استفاده در انتخاب جنس وسیله نگهداری مواد غذایی یا مواد افزونه (propagule) در انبار کاربرد دارند.

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