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Changes in Glucose Level and Activities of Acid and Alkaline Phosphatases of *Solanum incanum* L. During Storage

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ABSTRACT: *Solanum incanum* L. is an eggplant that is usually cooked before consumption. This study was designed to determine the glucose level, activities of non-specific acid and alkaline phosphatases in *S. incanum* stored under ambient condition (30°C) and refrigeration (10°C) for 22 days. Glucose level was found to decrease significantly (P<0.01)(from 0.588 ± 0.006 to 0.389 ± 0.002 % fresh weight) in *S. incanum* stored in ambient condition and(from 0.588 ± 0.006 to 0.413 ± 0.002 % fresh weight) under refrigeration. However, the decrease in glucose level of *S. incanum* (33.8%) stored under ambient condition was higher (26.7%) compared to that stored under refrigeration. Acid phosphatase activity also increased significantly (P<0.01) in *S. incanum* stored both under ambient condition and refrigeration. The increase(from 0.0036 ± 0.0002 to 0.0293±0.0004 µmol/min/g fresh weight) was higher in *S. incanum* stored under ambient condition compared to *S. incanum* stored under refrigerated condition (from 0.0036 ± 0.0002 to 0.0240 ± 0.0004 µmol/min/g fresh weight).
Alkaline phosphatase activity decreased (from 0.029±0.0006 to 0.008±0.0002 µmol/min/g fresh weight) significantly (P<0.01) in *S. incanum* stored under ambient condition. Under refrigeration, there was a decline in alkaline phosphatase activity of *S. incanum* from day 1 to day 4 and a subsequent increase from day 4 to day 22. Storage reduced the level of glucose in *S. incanum*, thereby making it a suitable diet in weight reducing programmes and in the management of diabetes mellitus. Under refrigeration, the increase in the activity of alkaline phosphatase could be attributed to enhanced alkaloid biosynthesis that occurs at low temperature.

Keywords: *Solanum incanum*, Storage, Alkaline phosphatase, Acid phosphatase, Glucose

Introduction

Several species of eggplants (*Solanum spp*.) are cultivated domestically throughout Africa for their nutritive and medicinal values (Grubben and Denton, 2004 & Sodipo, 2009). *Solanum incanum* L. is one of the species that is cultivated for its nutritive value. It is usually cooked before consumption; it is not eaten raw like some species such as *Solanumm elongena*, *Solanum aethiopicum* and *Solanum gilo*. *S. incanum*is used as a vegetablein sauces, stews, soups and mixed vegetable dishes (Aliyu, 2006). *S. incanum*is green, long and ovoid in shape and turns yellow during storage before it deteriorates.
Glucose is considered to be one of the main sugars in fruits, hence contributes to their flavour and taste. Reducing sugar such as glucose infruits like apples, bananas, tomatoes, and muskmelonsincreaseduring storage due to the breakdown of polysaccharides to sugars(Ali *et al*. 2004, Adão and Glória, 2005, Znidarcic and Pozrl, 2006, Menon and Ramana Rao, 2012).Cells use glucose as aprimary source of energyand as fuel for cellular respiration (Fairclough*et al*.2004). In humans, the maintenance of glucose level in the blood is an efficiently regulated system.Maintenance of blood glucose level is important because it allows continuous supply of glucose to the brain and red blood cells (Vasudevan and Sreekumari, 2007). Disruption to the maintenance of blood glucose level can occur as a result of the metabolic disease, diabetes mellitus. The management of diabetes mellitus therefore puts great emphasis on dietary control; this is achieved by the intake of diet low in glucose and high in dietary fibre (Okolie*et al*. 2009).
Phosphatases are classified as acid and alkaline phosphatases due to their optimum pH for catalysis, which may be below or above pH 7.0 (Sharma*et al*.2004). In plants, these phosphatases play a crucial role in the supply and metabolism of inorganic phosphate for the maintenance of cellular metabolism (Tabaldi*et al*. 2007, Mishra and Dubey, 2008).
Turner and Plaxton (2001) reported that acid phosphatase is important for the maintenance of adequate transport of inorganic phosphate in banana during ripening. Agoreyo (2010) also reported that acid phosphatase is the main non – specific phosphatase that is responsible for the production and supply of inorganic phosphate during ripening in plantain fruits.
Alkaline phosphatase has also been reported to be involved in catabolism and mobilization of starch and sucrose for synthesis of essential oil in lemongrass (Ganjewala*et al*. 2010).
Fruits and vegetables are living tissues with continuing metabolism and are subject to respiration, water loss and cell softening throughout the post harvest system. Their rate of respiration doubles for every 10°C rise in temperature. Reduced temperature however decreases the physiological, biochemical and microbiological activities of fruits and vegetables (Workneh and Woldetsadik, 2004, Worknehe*t al*. 2011).
This study was carried out to determine the effect of storage at ambient condition (30°C) and refrigeration (10°C) on the glucose level, activities of acid and alkaline phosphatases in *S. incanum*.

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Materials and Methods

Plant material
Freshfruits of *Solanum incanum* L. were purchased in New Benin market, Benin City, Nigeria. The eggplants were divided into two groups of 30 and stored for 22 days in order to give time for effective study of the parameters. The first group wasstored under ambient condition
(30^oC), while the second group was stored under refrigeration (10^oC). Samples were collected from each group at aninterval of 3 days to allow enough time for detectable changes to occur between the days of storage. Analyses of glucose and activities of acid and alkaline phosphatases were then carried out.

Glucose extraction
Glucose was extracted from *S.incanum* by water extraction according to the method of Ogiwara *et al* (1999) but with slight modification. 5g of the sample was weighed and homogenized with 15ml of distilled water in a mortar and pestle containing acid washed sand. The mixture was then centrifuged at 4000 rpm using 80-2 centrifuge for 10min. The supernatant was filtered with a double layer cheese cloth. The filtrate was boiled for 5min and allowed to cool. The extract was used for glucose assay.

Glucose assay
Glucose analysis of the extract was carried out using a glucose kit (Randox). 1.0ml of glucose reagent was added to 0.1ml each of the standard and extract. The reaction mixtures were incubated for 10 min at 37^oC in a water bath. The absorbance was measured at 546nm and distilledwater was used as control. The assay was performed in triplicate and glucose level expressed as g/100g (%) fresh weight (Agoreyo and Oghene, 2011).

Enzyme extraction for non-specific acid phosphatase (EC 3.1.3.2)
The enzyme extraction was done according to the method of Murray (1980). 5 g of *S. incanum* were ground using chilled pestle and mortar with acid washed sand and 20ml of chilled 50mMTris-HCl buffer (pH 7.6) containing 1 mM EDTA. The homogenate was then filtered through a double layer of cheese cloth and centrifuged at 20,000g using 80-2 centrifuge for 20min. The supernatant was used as the crude extract for the enzyme assay.

Enzyme assay for non-specific acid phosphatase (EC 3.1.3.2)
Acid phosphatase activity was assayed by adding 1.0ml of 3mM α-naphthyl phosphate in 60mM sodium citrate buffer pH 5.3 to 0.1ml of the enzyme extract. The reaction mixture was incubated at 37^oC for 5min after which the absorbance was read at 405nm for 5min to determine ΔA/minute. The assay was performed in triplicate and acid phosphatase activity expressed as µmol α-naphthol released $min^{-1}g^{-1}$ fresh weight. (Agoreyo, 2010).

Enzyme extraction for non-specific alkaline Phosphatase (EC 3.1.3.1)
The enzyme extraction was done according to the method of Murray (1980).
5g *S. incanum* were homogenized in chilled mortar and pestle containing acid washed sand with 20ml of chilled 0.05M sodium carbonate buffer (pH 10).The homogenate was filtered through double layers of cheese cloth and centrifuged at 20,000g using 80-2 centrifuge for 20min. The supernatant was then used as the crude extract for enzyme assay (Agoreyo, 2010).

Enzyme assay for non-specific alkaline Phosphatase (EC 3.1.3.1)
Alkaline phosphatase activity was assayed by adding 0.05 ml of enzyme extract to 0.5 ml of 3.6 mM sodium thymolphthalein monophosphate in 0.2 M 2–Amino–2–methyl–1– propanol buffer (pH 10.2) containing 1 mM magnesium chloride. The reaction mixture was incubated for 10 min at 37^oC and the reaction was terminated by the addition of 2.5 ml of 0.1 M sodium hydroxide containing 0.1 M sodium carbonate. Absorbance was read at 590 nm and the amount of sodium thymolphthalein released was estimated. The assay was performed in triplicate andalkaline phosphatase activity expressed as µmol sodium thymolphthalein released min^{-1} g^{-1} fresh weight (Agoreyo, 2010).

Statistical analysis
Analysis of variance of data was evaluated by the statistical analysis system (INSTAT SOFTWARE). Turkey-Kramer multiple comparison test was employed (SPSS Software version 20) to determine the statistical differences among the mean.

Results

At ambient condition (30°C), the glucose level of *Solanum incanum* L. decreased significantly ($P<0.01$) by 33.8%; while under refrigeration (10°C), the glucose level of *S.incanum*also decreased significantly ($P<0.01$) by 26.7% from day 1 to day 22. The percentage decrease in the glucose levels of *S.incanum* was higher in all the storage days at ambient condition (30°C) compared to those stored under refrigeration (10°C) (Tables 1 & 2).

Table 1: Glucose level of *S. incanum* at different days of storage under ambient condition (30°C)

Days of Storage	Glucose level (% fresh weight)	% Decrease
1	0.5880 ± 0.0060^{a}	0.00
4	0.5472 ± 0.0036^{b}	6.94
7	0.5256 ± 0.0018^{c}	10.61
10	0.5004 ± 0.0018^{d}	14.89
13	0.4673 ± 0.0016^{e}	20.52
16	0.4507 ± 0.0016^{e}	23.35
19	0.4224 ± 0.0012^{f}	28.16
22	0.3889 ± 0.0024^{g}	33.86

Values are mean ±SEM (n=3)
Means with the same letter(s) are not significantly different at p< 0.01.

Table 2: Glucose level of *S. incanum* at different days of storage under refrigeration (10°C)

Days of storage	Glucose level (% fresh weight)	% Decrease
1	0.5880 ± 0.0060^{a}	0.00
4	0.5690 ± 0.0015^{b}	3.23
7	0.5401 ± 0.0022^{c}	8.15
10	0.5152 ± 0.0042^{d}	12.38
13	0.4898 ± 0.0024^{e}	16.70
16	0.4619 ± 0.0021^{f}	21.44
19	0.4385 ± 0.0018^{g}	25.42
22	0.4129 ± 0.0021^{h}	29.77

Values are means ±SEM (n=3)
Means with the same letter(s) are not significantly different at P< 0.01

Under ambient condition (30^{o}C), the activity of acid phosphatase in *S. incanum* increased significantly (P<0.01) by 8.14 fold; while the acid phosphatase activity of the samples stored under refrigeration (10°C) increasedsignificantly (P<0.01) by 6.67 fold from day 1 to day 22. The increase in the activity of acid phosphatase of *S. incanum* was also higher in all the storage days at ambient condition (30°C) compared to those stored under refrigeration (10°C) (Figs 1 &2).

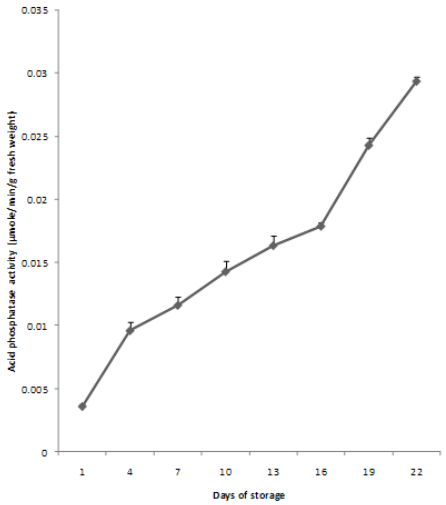

Fig. 1: Acid phosphatase activity of *S. incanum* at different days of storage under ambient condition (30°C)

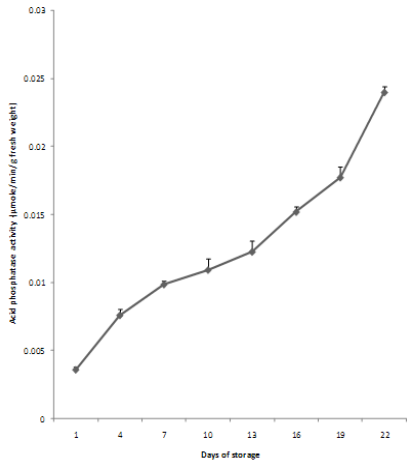

Fig. 2: Acid phosphatase activity of *S. incanum* at different days of storage under refrigeration (10°C)

Alkaline phosphatase activity of *S. incanum* decreased significantly (P<0.01) by 3.59 fold at ambient condition (30°C) from day 1 to day 22. In *S. incanum* stored under refrigeration (10°C), the activity of alkaline phosphatase decreased significantly (P<0.01) by 2.33 fold from day 1 to day 4 and then increased significantly (P<0.01) by 2.21 fold from day 4 to day 22 (Figs 3& 4).

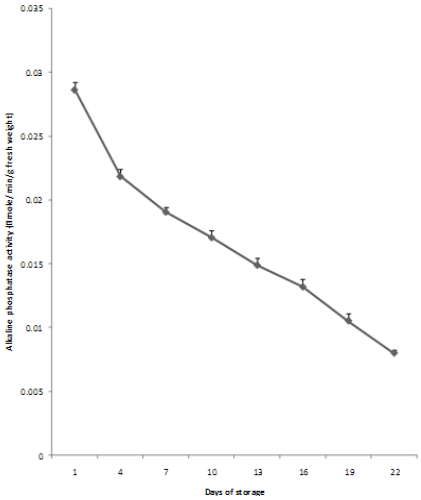

Fig. 3: Alkaline phosphatase activity of *S. incanum* at different days of storage under ambient condition (30°C)

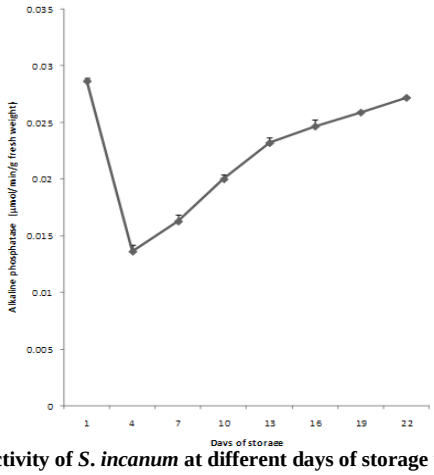

Fig. 4: Alkaline phosphatase activity of *S. incanum* at different days of storage under ambient condition (10°C)

Discussion

Glucose levels under both ambientcondition and refrigeration in S*olanum incanum* L. showed a significant decrease ($p<0.01$) from Day 1 to Day 22. However, the rate of decrease in glucose level of *S. incanum* stored under ambient condition was higher (33.8%) compared to that stored under refrigeration (26.7%). Inapples, bananas, tomatoes, and muskmelons, increase in reducing sugarduring storage has been reported (Ali *et al*. 2004, Adão and Glória, 2005, Znidarcic and Pozrl, 2006, Menon and Ramana Rao, 2012). Aydin and Kadioglu (2001) also reported an increase in glucose level in medlar fruits during storage. Melkamu *et al.* (2008) reported that during storage of tomato reducing sugar content initially increased and then decreased during later stages of storage. Increase in reducing sugars such as glucose during storage has been attributed to the breakdown of polysaccharides to sugars (Ganjewala, 2010). Normally, reducing sugar decreases faster if there is high respiration rate as occurs in climacteric fruits (Getinet *et al*.2011).

Tembo*et al*. (2008) reported that the rate of change in total sugar could be an indication of rate of respiration in the fruit. Hence, the respiration rate gives an indication of the rate of breakdown of respiratory substrates such as starch, sugars and organic acid. Starch andsucrose are hydrolyzed to fructose and glucose during storage inhoneydew melon and muskmelon toserve as a substrate for respiratory energy production (Lester2008, Menon and Ramana Rao, 2012). In this study, increasein the rate of breakdown of respiratory substrateswithout a corresponding increase in the rate at which starch and sucrose are hydrolyzed to fructose and glucose will lead to a decrease in glucose level asobserved in S. *incanum*. Also the decrease observed in the glucose level of *S.incanum*stored under ambient condition could be associated with the higher rates of hydrolysis of polysaccharides under higher temperature without a corresponding increase in the rate atwhich starch and sucrose are hydrolysed (Getinet *et al*. 2011).

Acid phosphatase activity of *S. incanum* stored under ambient conditionand refrigerationshowed a significant increase ($P<0.01$) from day 1 to day 22. However, increase in acid phosphatase activity of *S. incanum* stored under ambient condition was higher compared to that stored under refrigeration. The increase in acid phosphatase activity in *S. incanum* during storage correlates with the finding of Agoreyo (2010) who reported an increase in the activity of acid phosphatase in plantain (*Musa paradisiacal* L.) during ripening. It has also been reported that the activity of acid phosphatase increased in indole acetic acid treated tomato fruits (Olaiya, 2010). Acid phosphatase activity also increased in *Pisum sativum* and alfafa (*Medicago sativa* L.) under drought and salt stress respectively (Ehsanpour and Amini, 2003).

Alkaline phosphatase activity decreased significantly ($P<0.01$) in *S. incanum* stored under ambient condition. However, under refrigeration, alkaline phosphatase activity decreased from day 1 to day 4 and subsequently increased gradually until day 22. The result of this study also correlates with the finding of Agoreyo (2010) where the activity of alkaline phosphatase decreased from the hard green stage (unripe plantain) to the yellow with green tip stage and subsequently increased until the plantain was overripe. Increase in alkaline phosphatase activity in *S. incanum* during storage also correlates with what has been observed in pearl millet seeds in which alkaline phosphatase activity increased during storage (Jain *et al*.2004). Under refrigeration, alkaline phosphatase activity increase from day 4 to day 22. This increasedactivity of alkaline phosphatase could be attributed to enhanced alkaloid biosynthesis that occurs at low temperature. Machado *et al*.(2007) reported a higher rate of glycoalkaloids synthesis in potato tubers stored under refrigeration than those stored under ambient condition. Hence, in *S. incanum*, refrigeration could also be responsible for the change in pH of the fruit making it more alkaline and thus, favouring the activity of alkaline phosphatase.

In conclusion, the findings of this study showed that storage reduces the level of glucose in *S. incanum*, thereby making it a suitable diet in weight control programmes and in the management of diabetes mellitus in diabetic patients.

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