Relationship between antioxidant capacity and storability of sweetpotato storage roots

Report prepared by Eric Dery and submitted to RTB Postharvest

Introduction

The world's population is expected to reach 9 billion by the year 2050, which will require increases in food production estimated to be at least 70% of current production (FAO, 2011). Therefore, food crops with shorter growing cycle, high nutritional value, tolerance to environmental stresses, disease and pest resistances and longer shelf life will be critical in meeting food security goals. Sweetpotato is an untapped food crop with huge potential as a food security crop (Sugri et al., 2012). It is reported to be rich in carbohydrate, β-carotene, vitamins, and minerals (Curaya et al., 2019). Despite the potential, sweetpotato's limitation as a major crop in the food industry lies with its taste (Dery et al., 2020; Kays et al., 2005) and high perishability (Sugri et al., 2017; Rees et al., 2000). Availability is largely seasonal (Sugri et al., 2017) with peak seasons characterized by gluts, often leading to huge postharvest losses. Hence consumption and marketing patterns have been adapted to its short shelf life, which reduces opportunities for increased consumption and marketing. Recommendations have been made to explore opportunities to increase sweetpotato shelf-life.

Different storage systems have been exploited to extend the shelf-life of sweetpotatoes. Sand, pit and straw storage methods have been recommended to be effective for traditional homes in Africa (Abidin et al., 2012; Rees et al., 2000; Sugri et al., 2017). Storage conditions such as temperature, humidity, moisture and microbial infections can greatly affect spoilage of the crop. Ideal storage conditions for sweetpotato have been suggested to be between 10-15^{o}C at 80-85% which are generally lacking in average tropical countries (Tang et al., 2019). Therefore, postharvest storage in the tropics is often under ambient conditions and results in relatively rapid senescence of storage roots. In response to stress, storage roots may elicit stress signals such as reactive oxygen species (ROS) (H_2O_2, O^{-}_2) which accelerate senescence and decay. The destructive ability of these ROS is generally averted by enzymatic and non-enzymatic antioxidant systems such as flavonoids and phenolic acids, ascorbate peroxidase (APX), superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) (Song et al., 2021; Tang et al., 2019) through the mechanism of free radical scavenging, metal chelation and enzyme inhibitions (Oloniyo et al., 2021). Therefore, some correlations between these antioxidant systems and storability of sweetpotatoes has been reported. Tang et al., (2019), working on four sweetpotatoes with varying storage ability reported that genotypes with low storability (Yanshu 19 and Sushu 16) had higher lipoxygenase (LOX) activity than genotypes with higher storability (Shangshu 19 and Xushu 32), but lower ascorbate peroxidase (APX), peroxidase (POD), catalase, superoxide dismutase (SOD) and polyphenol peroxidase (PPO) when stored under recommended conditions (11-15^{o}C). Reactive oxygen species (ROS) (H_2O_2, O^{-}_2) and malondialdehyde (MDA) were highest among the poorer storing genotypes. Antioxidative enzymes and ROS metabolites in leaves were also observed to follow similar trends as in the storage root. A similar conclusion was made by Song et al., (2021) when 10 sweetpotato genotypes were evaluated. Tang et al., (2019) and Far and Taie, (2009) also indicated that sugars such as sorbitol and sucrose increased antioxidant activity to improve shelf life. However, the relationship of antioxidant activities to storability may be influenced by temperature and relative humidity. Hence, the conclusion made by these authors

under temperate production conditions and controlled temperature storage might not hold in tropics under less optimal storage conditions.

Ghana is a tropical country characterized by high temperature and relative humidities which pose storage challenges for most fruits and vegetables. These conditions increase rates of respiration and metabolism that cause sweetpotato postharvest losses through weight loss, sprouting and root rots when in storage or during marketing (Rees et al., 2000). Relative humidity increases during sand or pit storage can lead to moisture accumulation with a corresponding increase in microbial attacks (Rees et al., 2000). Conversely, roots lack of protection for roots during marketing or storage reduces relative humidity leading to high water loss through the skin, and hence higher weight loss (Rees et al., 2000). Therefore, sweetpotato shelf life needs to be improved under tropical conditions. Sweetpotato breeders thus aim to select consumer preferred varieties with improved storability to improve the potential for sweetpotato to contribute to food security in the tropics. This research therefore seeks to evaluate the relationship between the antioxidant potential of sweetpotatoes and their storability under non-temperature-controlled conditions in the tropics.

Materials and Methods

Chemicals

Trolox (2, 5, 7-tetramethylchroman-2-carboxylic acid), Folin-ciocalteau reagent, chlorogenic acid, 2,2-diphenyl-1-picryl hydrazyl (DPPH), 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfoni acid) (ABTS) and potassium persulfate were obtained from sigma Aldrich (St. Louis, MO).

Storage Samples

Seven sweetpotato genotypes with varying flesh colours (cream, orange and purple) and storability (low, moderately and highly) (Table 1) were grown at the CSIR-Crops Research Institute, Fumesua-Kumasi. Storage root and leaf samples were taken from the crops at harvest, 4 months after planting. Genotypes for evaluation were selected based on work done by Samba (2021) where sweetpotatoes were evaluated based on their shelf-life duration under sand and shelf storage systems. In the work, storability was evaluated based on weight loss, sprouting and root rot rate. We used root rot rate under sand storage system was used as the main criterion for rating storability, due to the importance of this system to farmers in rural communities in Ghana. Sweetpotatoes with root rot rate (RRR) below 20% (Mother's delight and CRI-Ligri) after 12 weeks of sand storage were considered as highly storable; those with RRR between 21-60% were considered moderately storable (CRI-Okumkom and SARI-Nyumingre); and those with RRR above 60% (CRI-Apomuden and PGA14351-4) were classified as low storable (Table 1). Due to prior knowledge about antioxidant capacity of purple fleshed varieties, CRI-Diedi was also evaluated. Orange-fleshed varieties (CRI-Apomuden and Mothers delight) were also selected to provide an insight into comparison between the various flesh colours. Planting was done using a randomized complete bock design and subsequently followed during the sampling for lab testing. Due to uneven distribution of antioxidants in plants, sampling was done to include the different maturity stages (the meristematic young leaves, the medium broad leaves and old leaves) and root sizes (small, medium and bigger roots).

Table 1. Genotypes selected for antioxidant capacity evaluation

Varieties	Flesh colour	RRR	Storability
CRI-Apomuden	Orange	87	Low
CRI-Ligri	Cream	13	High
CRI-Okumkom	Cream	60	Moderate
Mothers Delight	Orange	13	High
PGA14351-4	Pale yellow	93	Low
SARI-Nyumingre	Cream	43	Moderate
CRI-Diedi	Purple	57	Moderate

RRR=Root rot rate (Samba, 2021)

Preparation of raw storage roots and leaves

Storage roots and leaves were harvested during the early hours of the morning (6-8am) and after sunset to minimize degradation of highly volatile antioxidants. Harvested roots were immediately transported in an ice packed cold chest to the nearby laboratory, for processing. Processing was done in an enclosed room with limited illumination to prevent breakdown of antioxidants. Sliced root samples (50 g) were immediately dipped in liquid nitrogen before freezing for freeze drying for 72 hrs. Sweetpotato leaves were dipped into liquid nitrogen immediately after harvesting on the field before transporting to the laboratory for freeze drying. Freeze dried samples were milled using a hammer mill to pass a 40 mesh screen. Samples were then bagged in a ziplock and stored in a freezer (-20 ^{o}C) before analysis.

Extraction of lipophilic and hydrophilic fractions

Milled samples were vortexed for 2 min in 25 ml hexane and the mixture was filtered using a Buchner funnel. The hexane extraction was repeated twice and the combined lipophilic extracts were evaporated to dryness at 50^{o}C using a vacuum evaporator. The residue after hexane extraction was then extracted with 25ml of acidified methanol (7% acetic acid in 80% methanol) to obtain the hydrophilic fraction. The final volume of the hydrophilic fraction was made to 50ml with acidified methanol.

Assay of DPPH radical scavenging activity

A modified version of Brand-Williams et al., (1995) was used for DPPH assay. Aliquots of the hydrophilic fractions were diluted (1:10) with ethanol and the assay was performed. The diluted sample of 0.1ml was pipetted into 3.9ml of DPPH solution to initiate the reaction. The color change in DPPH assay was determined by the UV spectrophotometer shown in eight different concentrations of Trolox (0, 100, 200, 300, 400, 500, 600, and 700 μg/mL) were used to prepare a standard curve. The test sample solution or Trolox (0.1 mL) was transferred into a cuvette and mixed with DPPH (0.1mM/L, 1.9 mL). The absorbance at 517 nm was recorded at 0 and 30 min, respectively. The free radical scavenging activity was expressed as mg Trolox equivalent/mL based on the standard curve.

Antioxidant activity = [(control absorbance-sample absorbance) / control absorbance] X 100

Assay of ABTS radical scavenging activity

ABTS radical-scavenging activity of the hydrophilic fractions was determined by using Miller and Rice-Evans (1997). The $ABTS^+$ solution was prepared by mixing 8 mM of potassium persulfate in 25 ml of distilled water. The solution was held at room temperature in the dark for 16 hrs before use. The $ABTS^+$ solution was diluted with 95% ethanol (approximately 600 µl ABTS to 40 ml 95%). Fresh $ABTS^+$ solution was prepared for each. Antioxidant or standard solutions, 20 µl, were mixed with 1 ml of diluted $ABTS^+$ and incubated at 30^{o}C. The absorbance at 517nm was read for 30min using UV spectrophotometer. Ethanol (95%) was used as a blank. Trolox with concentrations from 0 to 250 µM was used as a standard. The free-radical-scavenging activity was expressed as mM of Trolox per gram of samples (mM TE/g dw).

Statistical Analysis

All gathered data were analyzed using Analysis of Variance (ANOVA) using SAS-JMPro, version 2014 with separation of means done by tukey test at 5% significance level. Pearsons correlation matrix was then used for correlations between antioxidant capacity and root rot rate to establish the relationship between storage and antioxidant activity.

Results and Discussions

Antioxidant activity in sweetpotato leaves

Plant nutrients are generally produced in the leaves and stored in the roots. Plant pigments such as anthocyanins and phenolic compounds located in the leaves play critical role in the production of these plant nutrients. These pigments are highly volatile and unstable and hence tend to degrade with time and under unfavorable temperatures. Their quantities and compositions are therefore important in human nutrition as well as plant protection. Antioxidant activity (AA) has therefore become critical in evaluating the quantities of these pigments and their function in protecting plants. Two different assays (DPPH and ABTS) were employed in the current research to evaluate the antioxidant activities in the different sweetpotato genotypes. The results showed a general decrease of antioxidant activity from young leaves to older matured leaves for all genotypes under the DPPH and ABTS assays (Tables 2 and 3). Young leaves possess highly dividing meristematic cells capable of producing additional pigments and hence will tend to possess more pigments. This phenomenon was also observed by (Padda and Picha, 2007) when investigating antioxidant activity in different leaf age and root sizes of Beauregard sweetpotato variety. In terms of genotypes, Mother's Delight, CRI-Apomuden, which are orange fleshed varieties, and CRI-Okumkom, a cream fleshed variety, recorded the highest antioxidant activities with SARI-Numingri leaves having the least activity for young leaves under DPPH assay. PGA14351-4 which produces pale yellow tuberous roots however had higher antioxidant activity in medium leaves with CRI-Apomuden (an orange fleshed variety) having the least antioxidant activity. A similar trend was observed in older leaves as was recorded in the medium leaves. The range of values observed were found to be comparable to Padda and Picha, (2007). For ABTS assay (Table 3), there was no significant differences among genotypes for young leaves. However, Mother's Delight was observed to have highest antioxidant activity in medium leaves with CRI-Apomuden having the least. In the older matured leaves for ABTS assay, CRI-Diedi recorded the highest antioxidant activity with CRI-Apomuden still recording the least. This data indicates that, the antioxidant activity in leaves has limited influence on the flesh colour of the roots but may depend on the

composition and type of antioxidant present. Generally, DPPH and ABTS data are highly correlating (Teow et al., 2007). However, ABTS and DPPH differ in the sense that, ABTS can be used over a wider pH range, operates much faster and is not significantly affected by colour interference (Teow et al., 2007: Prior et al., 2005)

Table 2. DPPH Antioxidant capacity of leave samples from storage genotypes

Varieties	Young leaves mM Trolox/ g DW	Medium leaves mM Trolox/ g DW	Old leaves mM Trolox/ g DW
CRI-Apomuden	170.90±3.78a	101.53±0.83d	111.29±5.09c
CRI-Ligri	157.46±5.30ab	166.60±12.96ab	110.33±21.14c
CRI-Okumkom	172.57±4.47a	130.02±12.04cd	121.44±3.25bc
Mothers Delight	173.39±4.97a	161.52±12.04abc	153.85±9.67a
PGA14351-4	163.92±5.92ab	176.80±3.49a	116.37±1.97c
SARI-Nyumingre	132.70±2.49b	135.35±26.02bcd	149.25±11.58ab
CRI-Diedi	150.86±33.82ab	130.31±6.88cd	134.70±12.95abc
p-value	0.0227	0.0001	0.0009

Means with similar letters in the same column are not statistically significant at 5% significance level

Table 3. ABTS Antioxidant capacity of leave samples from storage genotypes

Varieties	Young leaves mM Trolox/ g DW	Medium leaves mM Trolox/ g DW	Old leaves mM Trolox/ g DW
CRI-Apomuden	146.07±8.47	100.64±34.19ab	74.94±28.77c
CRI-Ligri	150.53±7.92	126.50±10.67ab	103.48±14.08bc
CRI-Okumkom	178.75±75.04	133.30±27.17ab	129.03±8.14abc
Mothers Delight	145.71±8.29	159.22±14.45a	157.99±36.47ab
PGA14351-4	149.37±14.60	126.13±10.00ab	136.03±11.78abc
SARI-Nyumingre	124.70±20.19	97.65±20.60b	154.77±43.06ab
CRI-Diedi	137.30±15.79	104.38±18.85ab	181.35±18.57a
p-value	0.5563	0.0318	0.0036

Means with similar letters in the same column are not statistically significant at 5% significance level

Antioxidant activity of sweetpotato storage roots

Antioxidant activity in the roots under DPPH assay increased from smaller roots to medium sized roots and declined in bigger sized roots (Table 4). This could be due to breakdown of these antioxidants after certain stage of root maturity. Observations made on field shows uniform distribution of pigmented colours in smaller root flesh than bigger roots. CRI-Diedi, which is purple fleshed consistently had the highest antioxidant activities followed by orange fleshed genotypes like CRI-Apomuden and Mother's Delight, and then light fleshed genotypes. A similar trend was also observed by Teow et al. (2007) in their research using DPPH, ABTS and ORAC methods. Reports suggest that purple fleshed varieties are highly rich in anthocyanins which are major components of antioxidants (Oloniyo et al., 2021; Curayag et al., 2019; Vizzoto et al., 2017; Teow et al., 2007). Orange fleshed varieties are also known to be highly rich in β-carotene with quantities correlating to their flesh colour intensity (Curayag et al., 2019). DPPH

antioxidant activity values ranged between 54.67±2.44 mMTrolox/g DW and 323.67±12.03 mMTrolox/g DW which comparable those reported by Curayag et al., (2019) and Teow et al., (2007). Even though the dynamics were similar to DPPH for the root genotypes, ABTS assay generally showed a higher antioxidant activity (Tables 4 and 5). Antioxidant activity was highest in smaller roots for PGA14351-4, SARI-Nyumingre and CRI-Diedi. Antioxidant activity under ABTS ranged between 155.34±29.76 mMTrolox/g DW and 323.08±26.44 mMTrolox/g DW.

Table 4. DPPH Antioxidant capacity of tuberous root samples from storage genotypes

Varieties	Small size mM Trolox/ g DW	Medium size mM Trolox/ g DW	Big size mM Trolox/ g DW
CRI-Apomuden	83.71±1.84bc	128.38±3.11b	123.35±1.66b
CRI-Ligri	59.25±0.80d	93.45±1.19de	54.67±2.44d
CRI-Okumkom	61.03±3.52d	104.83±8.67cd	81.88±1.47c
Mothers Delight	90.05±5.28b	116.08±1.94bc	110.84±13.16b
PGA14351-4	71.60±3.65cd	76.66±1.06f	69.19±0.73cd
SARI-Nyumingre	80.88±7.49bc	85.71±2.40ef	84.74±1.47c
CRI-Diedi	285.51±4.93a	323.67±12.03a	319.47±7.49a
p-value	<0.0001	<0.0001	<0.0001

Means with similar letters in the same column are not statistically significant at 5% significance level

Table 5. ABTS Antioxidant capacity of tuberous root samples from storage genotypes

Varieties	Small size mM Trolox/ g DW	Medium size mM Trolox/ g DW	Big size mM Trolox/ g DW
CRI-Apomuden	226.69±22.17b	272.56±21.43ab	257.05±0.94ab
CRI-Ligri	155.34±29.76c	233.00±12.80bc	202.53±18.14bc
CRI-Okumkom	181.37±12.99bc	259.98±33.96ab	227.91±28.99
Mothers Delight	235.07±11.08b	246.99±16.77	257.58±28.31ab
PGA14351-4	217.30±40.88bc	215.86±17.54bc	168.15±24.68c
SARI-Nyumingre	233.68±3.25b	191.46±16.44c	190.81±23.43c
CRI-Diedi	323.08±26.44a	314.57±28.19a	297.29±23.69a
p-value	<0.0001	0.0003	<0.0001

Means with similar letters in the same column are not statistically significant at 5% significance level

Relationship between antioxidant activity and storability

Pearson's correlation matrix showed a lack of correlation between antioxidant activity in root rot rate under sand storage system (Table 6). This contradicts reports by Tang et al., (2019) and Song et al., (2021) where significant correlations were found between storage and antioxidant capacity. This could be because their storage was carried out under cool temperatures (11-15oC) in a temperate region while this research was in done in the tropical regions in sand where temperatures and humidity are high. Under tropical conditions, roots stored in sand experience higher relative humidity and temperatures which

increases moisture making them susceptible to rots such as *Aspergillus niger, Rhizospus stolonifer, Botryodiplodia theobroma, Fusarium oxysporum* and *Penicillin sp* (Olaitan et al., 2012; Sugri et al., 17) and spongy texture (Rees et al., 2000). Hence, storage roots with higher moisture content such as CRI-Apomuden might deteriorate faster than low moisture genotypes with even less antioxidant activity. Sugars such sucrose, sorbitol, fructose and glucose have been reported to influence antioxidant content through their stimulation of these secondary metabolites (Far and Taie, 2009). These simple sugars derived as a results of starch breakdown through heat and enzyme (amylase) action. Hence genotypes such as PGA14351-4, a non-sweet genotype with limited amylase activity might experience high rot rate under storage. Rees et al., (2000) also suggested that storability under tropical conditions was a function the periderm which influences wound healing. Hence genotypes with high antioxidant ability but weak periderm may still be susceptible to rotting. Furthermore, plants respond to stress by releasing stress signal agents such as reactive oxygen species (ROS) (H_2O_2 and O^{2-}) which are injurious to cells and cause decay (Song et al., 2021; Tang et al., 2019). These cell destructions are mitigated by antioxidants through a series of enzyme pathways. More work may be needed to conclude on the relationship between storability and antioxidant potential of sweetpotato under tropical conditions.

Table 6. Correlation matrix between antioxidant activity and root rot rate (RRR)

Variables	RRR
DPPH Young Leaf	0.148
DPPH Medium Leaf	0.430
DPPH Old Leaf	-0.463
DPPH Young roots	0.066
DPPH Medium roots	0.003
DPPH Old roots	0.112
ABTS Young Leaf	0.122
ABTS Medium Leaf	-0.470
ABTS Old Leaf	-0.251
ABTS Young roots	-0.060
ABTS Medium roots	-0.214
ABTS Old roots	-0.317

Conclusion

It can be concluded that, purple fleshed sweetpotatoes roots have high antioxidant activity, followed by orange fleshed genotypes and yellow/cream fleshed genotypes. Young meristematic leaves also possess higher antioxidant activity than matured aging leaves while medium sized roots were higher in antioxidant activity than smaller and bigger roots. Storability of sweetpotato roots may be influenced by wound healing ability, sugars, periderm thickness, antioxidant scavenging capacity, antioxidant content and quantity, antioxidant enzymes and their complex interactions rather than just the antioxidant activity of leaves. Future work could include the evaluation of the relationship of antioxidant activity in sweetpotato

genotypes to root storability under controlled temperature conditions to determine if the intriguing reports from Song et al. (2021) and Tang et al. (2019) can be replicated in Ghana.

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