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SHORT COMMUNICATION

EFFECTS OF POSTHARVEST STORAGE ON PROXIMATE COMPOSITION AND CYANIDE CONTENTS OF BITTER CASSAVA (*MANIHOT UTILISSIMA*) TUBERS IN OKITIPUPA, ONDO STATE OF NIGERIA.

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Abstract

composition of bitter cassava tubers. The proximate composition of freshly harvested tubers (FHT), Tubers Stored for 4 days (TS4D) and Tubers Stored for 8 days (TS8D) were determined using standard methods while the cyanide content was determined by linamarase digestion method using an autoanalyzer. The proximate analysis for FHT showed carbohydrate (20.6 ± 1.27 %), crude protein (2.61 ± 0.01%), ash (3.0 ± 1.41 %), crude fat (7.0 ± 4.24 %), total solid (29.75± 0.35%),and moisture contents for FHT were 31.45 ± 0.77% respectively. The values obtained for TS4D are ash content (4.5 ± 0.71)%, moisture (25.1 ± 0.82)%, Fat (7.0 ± 1.41)%, total solid (23.2 ± 1.13)%, protein (1.21 ± 0.07)% and carbohydrate (36.8 ± 1.13)% while TS8D are ash content (2.0 ± 0.00)%, moisture (23.27 ± 0.81)%, Fat (9.0 ± 1.41)%, total solid (18.4 ± 0.49)%, protein (0.66 ± 0.01)% and carbohydrate (41.8 ± 0.35)%. The cyanide content for FHT was 32.86 ± 0.13 mg/kg while TS4D and TS8D are 30.17 ± 0.40 mg/kg and 27.9 ± 2.43 mg/kg respectively. In conclusion, the postharvest storage of bitter cassava had minimal effects on the proximate composition while the cyanide content reduced significantly with storage. This implies that postharvest storage before processing of cassava tubers might be a cheap way of reducing cyanogenic glycosides in bitter cassava.

Key words: Postharvest; Cyanogenic glycosides; Cyanide; Linamarase; Proximate; Autoanalyzer.

Introduction

Cassava (*Manihot esculenta Crantz,* Euphorbiaceae), a remarkable source of carbohydrate is a starchy storage root that is grown and exploited in the tropics with Africa having the highest percentage production. Besides its use as livestock feed, it serves as staple food for over 200 million Africans (Aro, 2008; FAO, 2012). The commonly grown varieties in west - Africa include the sweet and the bitter types. Sweet cassava has low cyanide content (≤ 50 mg HCN per kg) compared to bitter cassava and could be consumed freshly fried, baked, boiled or through other processing methods (Ifeabunike *et al.,* 2017). Bitter cassava (*Manihot utilissima*) undoubtedly contains large amount (≥ 100 mg HCN/kg) of cyanogenic glycosides, which can be hydrolyzed to hydrocyanic acid (HCN) by the endogenous enzyme linamarase when the plant tissue is damaged during harvesting, processing or other mechanical processes

(Oboh and Akindahunsi, 2003; Mckey *et al.,* 2010).

Despite the enormous role of bitter cassava cultivars in providing food security by daunting foraging rodents and pests from feasting on the crop, consumption of it products with high cyanide levels may cause acute intoxications, aggravate goiter and, in critical cases, induce paralytic diseases (Milena *et al.,* 2013). To avoid dietary cyanide exposure, it must be removed by processing before consumption. Adopted processing methods include a combination of procedures, such as peeling, slicing, fermentation, boiling, drying, pounding or milling and sieving. (Njoku and Obi, 2010; Oke *et al.,* 2015).

Post-harvest handling of cassava begins the moment it is harvested. It ranges from simple uprooting (i.e lifting of the roots from the ground), transporting them to the house for immediate cooking and consumption or subjected to complex preparatory methods of processing into high quality products (Mlingi and Ndunguru, 2007).

Effective removal of cyanogenic glycosides from cassava remains top priority of ensuring cassava-based products is free from cyanide poisoning. It is plausible to assume that introduction of postharvest storage of tubers could help alleviate the cyanide contents without affecting the nutritional status of the bitter variety of cassava. This research is aimed at investigating the proximate composition and cyanide contents of freshly harvested and stored bitter cassava (*Manihot utilissima*) samples.

Materials and Methods

Materials

Linamarinase, cold orthophosphoric acid, Isonicotinate and dimethyl barbiturate, Boric acid, Markham distillation apparatus, Steam distillation apparatus, Kjeldahl apparatus.

Methods

Sample collection

The bitter cassava tubers used in this research were collected from a local farm along Irele road, Okitipupa, Ondo State. The bitter cassava was firstly identified by a local farmer who tasted the tuber and critically scrutinizes the stem and leaves. It was further authenticated by Mr. Ojo Samuel in the Herbarium unit, Ondo State University of Science and Technology (OSUSTECH). The bitter cassava was collected in a polythene bag and transported to the Chemical Sciences Laboratory, OSUSTECH for further analysis.

Treatment of Samples

The method of Ndunguru *et al.* (1998) was adopted for the storage process. Selected samples without damage (mechanical or physical damage) were used for this research. They were thoroughly cleaned and rinsed in a running tap water. The bitter cassava samples were grouped into three (3) categories: Freshly harvested tubers (FHT), Tubers stored for four (4) days (TS4D) and Tubers stored for eight (8) days (TS8D). The stored samples were daily dipped once into water for about five (5) minute and kept at ambient room temperature.

Sample Analysis

Proximate Analysis

The proximate composition (ash, crude fat, total solid and moisture contents) of the freshly and stored bitter cassava tubers were determined using AOAC (1990) method. Carbohydrate was estimated using the method of Anthrone. Protein content was determined using the Kjeldahl method of protein analysis.

Cyanide Content Estimation

Cyanide contents were estimated by an enzymatic assay that uses the enzyme linamarinase to digest the cyanogenic glycosides lotaustralin and linamarin in the

cassava to form cyanide ions which reacts with chloramines T, isonicotinate and dimethyl barbiturate to produce a dye colour that is read by the auto-analyzer (Rao and Hahn, 1984).

Statistical Analysis

The results of the two best replicates were pooled and expressed as mean ± standard error of mean (SEM). A one-way analysis of variance (ANOVA) and the least significance difference (LSD) were carried out with level of significance accepted at $p < 0.05$.

Results

Physical Appearance

After postharvest treatment for consecutive periods of four (4) and eight (8) days, samples were investigated for some physical changes. Observations from figures 1 and 2 showed the skin of unpeeled tuber samples having brown discoloration probably due to polyphenol oxidase. Dissecting the stored samples (i.e TS4D and TS8D) into sections revealed intactness of the parenchyma tissues with the released clean whitish fluid (sap) and no physical sign of infestation and damage.

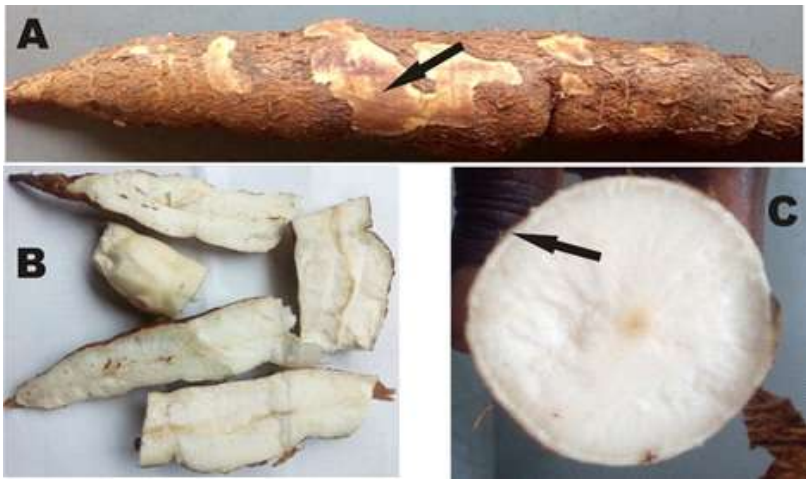

Figure 1: Tubers stored for 4 days, (A) Unpeeled tuber showing discolouration of skin probably due to polyphenol oxidase, (B) Longitudinal sections showing the internal part of the tuber, (C) transverse section showing the presence of clean whitish sap.

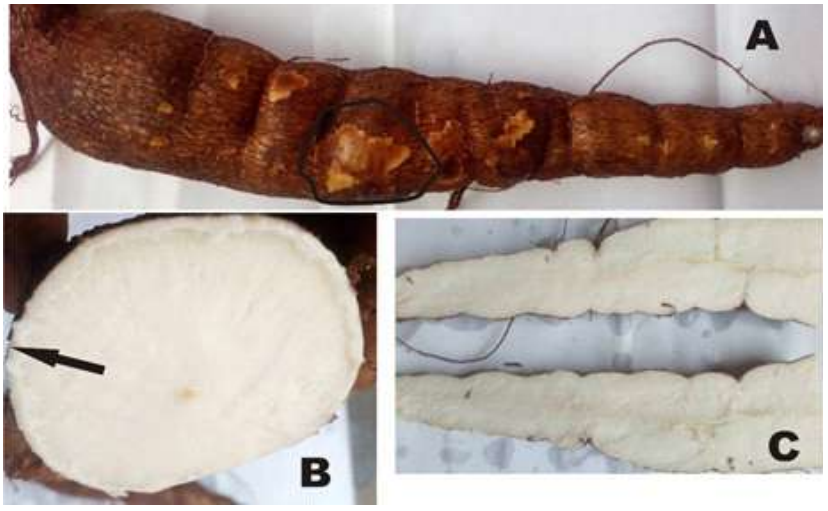

Figure 2: Tubers stored for 8 days, (A) Unpeel tuber showing skin discolouration, (B) Transverse section of internal part showing the presence of clean white sap, (C) Longitudinal section showing an intact parenchyma tissue.

Proximate analysis

The results of the proximate composition of freshly harvested tubers (FHT), tubers stored for four (4) days (TS4D) and tubers stored for eight (8) days TS8D are presented in Table 1.

The carbohydrate content of FHT was 20.6 ± 1.27 % while 36.8 ± 1.13 % and 41.8 ± 0.35 were obtained for TS4D and TS8D respectively. There were significant increases ($p < 0.05$) in the carbohydrate content of TS4D and TS8D compared to FHT. Total protein contents obtained from FHT was 2.61 ± 0.01 %, while TS4D and TS8D days were 1.21 ± 0.07 % and 0.66 ± 0.01 % respectively. There was significant decrease ($p < 0.05$) in the protein content with postharvest storage days. The % moisture content in the freshly harvested tubers, tubers stored for 4 days and tubers stored for 8 days were 31.45 ± 0.77 %, 25.1 ± 0.82 % and 23.27 ± 0.81 % respectively. During postharvest storage of bitter cassava, there was significant decrease in moisture at $p < 0.05$. The results obtained for % ash contents and total solid showed non-significant difference during the period of storage (TS4D and TS8D) compared to the freshly harvested sample. The values obtained for percentage fat contents from FHT, TS4D and TS8D showed non-significant ($P > 0.05$) difference

Cyanide Content

The result of cyanide content is presented in Table 2. The cyanide content of freshly harvested tuber (FHT) was 32.86 ± 0.13 mg/kg while cyanide content from tuber stored for 4 days (TS4D) and tuber stored for 8 days (TS8D) were 30.17 ± 0.40 mg/kg and 20.35 ± 2.43 mg/kg respectively. There was significant ($p < 0.05$) decrease in cyanide content as postharvest storage of bitter cassava progressed.

Table 1: The proximate composition

	% Carbohydrate	% Protein	% Ash	% Crude fat	% Total Solid	% Moisture
FHT	20.6 ± 1.27^a	2.61 ± 0.01^b	3.0 ± 1.41^a	7.0 ± 4.24^a	29.75 ± 0.35^a	31.45 ± 0.77^b
TS4D	36.8 ± 1.13^a	1.21 ± 0.07^b	4.5 ± 0.71^b	7.0 ± 1.41^b	23.2 ± 1.13^a	25.1 ± 0.82^b
TS8D	41.8 ± 0.35^a	0.66 ± 0.01^b	2.0 ± 0.00^c	9.0 ± 1.41^c	18.4 ± 0.49^a	23.27 ± 0.81^b

Values are expressed as mean ± standard error of mean of three determinations. Values with the same alphabet along the same column are significantly different ($p < 0.05$). FHT = Freshly Harvested Tuber, TS4D = Tuber Stored for 4 Days, TS8D = Tuber Stored for 8 Days

Table 2: Cyanide content

Group	Cyanide concentration (mg/kg)
FHT	32.86 ± 0.13
TS4D	30.17 ± 0.40
TS8D	27.9 ± 2.43

Values are expressed as mean ± standard error of mean of three determinations.
FHT = Freshly Harvested Tuber, TS4D = Tuber Stored for 4 Days, TS8D = Tuber Stored for 8 Days

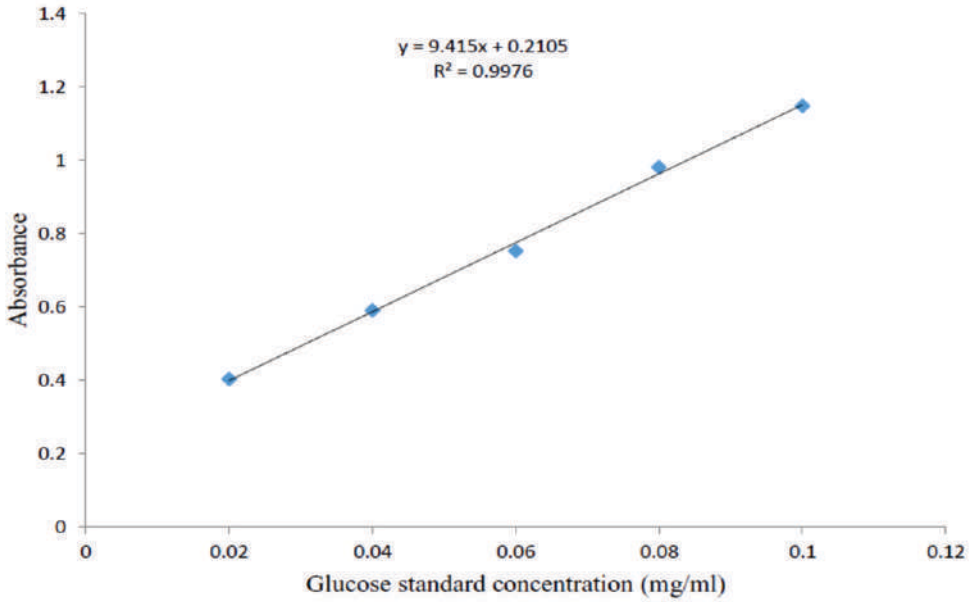

Figure 3: Glucose concentration standard curve

Discussion

The stored tuber samples (TS4D and TS8D) showed no streaking of xylem tissues and sign of deterioration (Figures 1 and 2). The five (5) minutes soaking of root samples in water could apparently increase the relative humidity resulting in improved storage life and the observed physical features. Fadebiyi (2012) revealed that tubers of cassava with cut responds to curing process for about four (4) days under factors such as ambient temperature of 30–40 °C and high relative humidity between 90–100 % relative humidity

In this research, there was significant increase ($p < 0.05$) in carbohydrate content of TS8D as compared to FHT and TS4D. This may be due to the increase in the activity of Linamarase which metabolize cyanogenic glycosides to glucose and cyanohydrins with the free glucose adding to the carbohydrate pool. Glucanase and related enzymes associated with carbohydrate metabolisms could also play plausible roles during storage (Iyer *et al.,* 2010).

Several studies have shown that cassava root (either sweet or bitter variety) is particularly poor source of protein compared to cereals (IITA, 2012). The significant decrease in protein contents as observed in this study could be due to increasing activities of some proteolytic enzymes such as phenylalanine ammonia lyase (PAL), proteinase and polyphenol oxidase (Opara *et al.,* 2015).

As the length of storage days progressed, there was reduction in the % moisture content suggesting that there was continuous transpiration of roots. Ifeabunike *et al.* (2017) reported a moisture content value of 38.20 ± 2.69% for the bitter cassava variety. Physiological deterioration in cassava root is attributed to its high moisture level (60 to 75 %) (Salcedo *et al.,*2010). While a high moisture content in cassava sample revealed its inability to be stored for a long period of time, low moisture content improved the lifespan by hindering the growth of micro-organisms.

Ash content is an indication of the mineral

content in a sample. From this study, the implication of a non-significant difference in the ash content could be attributed to the ability of bitter cassava roots to retain their mineral contents during postharvest storage.

The significant reduction in cyanide contents with days of storage was presumably due to activity of endogenous enzymes (such as β cyanoalanine – synthase and rhodanese) that is responsible for cyanogenic glycosides metabolisms.

Conclusion

The gradual fading and disappearance of bitter cultivars of cassava from circulation may be due to the loss of interest in its cultivation by most farmers. The reasons might be due to certain limitations associated with its cultivation such as prolonged period of stay in the soil prior to harvesting, and high concentration of cyanogenic glycosides as compared to sweet cassava. The research thus has shown that post-harvest storage has minimal effect on the nutritional status of bitter cassava while reducing the cyanide content. This suggested post-harvest storage technique as a cheap and promising method of reducing the toxic cyanogenic glycosides of bitter cassava before its processing into edible based products.

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