Snapshot Survey of Cyanogen levels in Cassava for Retail Sale in a South-east Asian Region Context. A Singapore Study.

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Introduction

Cassava (*Tapioca),* is a starchy root vegetable commonly grown in tropical countries such as Malaysia and Thailand where they are consumed either roasted, fried or boiled (FAO, 1990). There are 2 main types of cassava, the 'sweet' cultivars which have been found to contain low cyanogenic glucosides and the 'bitter' cultivars which contain higher levels of cyanogenic glucosides (OECD, 2015). Sweet cassava cultivars are usually consumed by humans while the bitter cultivars are usually used in the industry for purposes like livestock feed (OECD, 2015). *Linamarin* is a cyanogen found naturally in cassava roots that can be broken down into 'free cyanide'[as hydrogen cyanide (HCN)] by the enzyme *linamarase*, also found naturally in the root, when sliced/chopped (Essers et al., 1992). Exogenous purified *linamarase* extracted from cassava peel used in colourimetric assays to determine cyanogenic potential (CNp) in cassava roots, requires enzymatic activity of 3EU/ml or more, for full function of the assay (Cooke, 1979). Maintaining a pH environment of more than 4.0 in the assay is also required so that the *linamarin* can be completely broken down into 'free cyanide' for measurement (Conn, 1969). Therefore, a *linamarase* activity test must be done first to ensure the enzyme to have an enzymatic activity of 3EU/ml or more, before running the CNp assay for the cassava roots.

Aims & Objectives

1. To determine the cyanogenic potential of fresh cassava roots sold in Singapore.
2. Setting up a suitable experimental method for the determination of cyanogenic potential on cassava roots
3. Apply experimental method on cassava roots purchased from the North-East of Singapore. [Ang Mo Kio (AMK) & Toa Payoh (TP)]

Method

Potassium cyanide (KCN) standard curve

1. 1.0ml of KCN stock solution was diluted to 100ml with pH 6 phosphate buffer.
2. 0.2ml, 0.4ml, 0.6ml, 0.8ml and 1.0ml KCN/pH 6 solution added to tubes and made up to 4.0ml each with pH 6 phosphate buffer.
3. Each KCN/pH 6 solution proceeds with a colourimetric procedure: 0.1ml chloramine T + 0.6ml Barbiturate Isonicotinate reagent. Absorbance read at λ 605 nm.

Linamarase activity test

(A) Preparation for 'neat' *linamarase* 1 & 2:

1. Freeze dried purified *linamarase* 1 and 2 [both enzymes given by Centro Internacional de Agricultura Tropical (Columbia)] were thawed and added to clean plastic tubes.
2. 8ml for *linamarase* 1 & 10ml for *linamarase* 2[A] of pH 6 phosphate buffer were added into two tubes containing the different enzyme.
3. 50ml (for *linamarase* 2[B]) of pH 6 phosphate buffer was pipetted into another separate tube containing *Linamarase* 2.

(B) Dilute *Linamarase* solution: 0.1ml of 'neat' *linamarase* solutions were diluted to 100ml with pH 6 phosphate buffer.

(C) *Linamarase* activity assay:

1. 0.1ml dilute *Linamarase* solution was added to a tube containing 0.5ml of 5mM *linamarin*, prepared in quadruplicates.
2. The tubes were incubated at 30ºC for 30min.
3. 0.6ml of 0.2M NaOH followed by 2.8ml of pH 6 phosphate buffer were added to each tube before proceeding to the colourimetric procedure (point 3 of KCN standard curve).

CNp assay of cassava roots

1. "Heads and tails" of cassava roots were removed before slicing into three parts: top, middle and end.
2. From each root, 10g (a mix of all three parts) were dried in a drying oven (105ºC) for 24h before re-weighing (Moisture determination).
3. The remainder of the roots were chopped & weighed (Approx. 50g each root) before blending in 200ml of 0.1M phosphoric acid (pH<4.0) followed by sieving to obtain 50ml extracts for each root.
4. 0.1ml *Linamarase* solution was added to tubes containing 0.1ml extracts (buffered to pH 7 with phosphate buffer) of each root and then kept in a water bath (30ºC) for 15mins. After which, step 3 of (C) '*Linamarase* activity assay' is applied.

Results

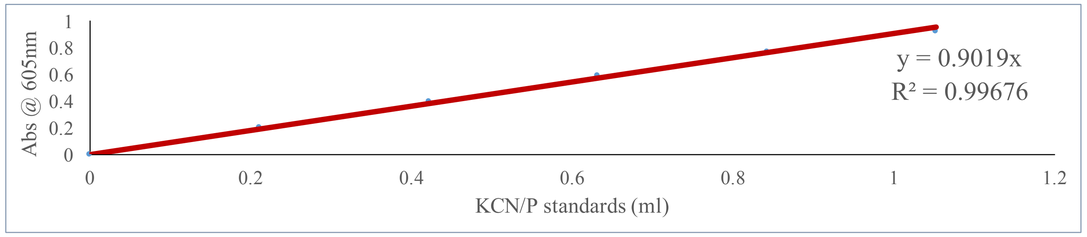

Figure 1. *KCN Standard Curve* (***1ml KCN/pH 6 solution exist as 1µg HCN**)

Table 1: *Linamarase* activity values

Linamarase no.	Enzyme activity (EU/ml)	
1	9.668	
2[A]/2[B]	19.0	3.72

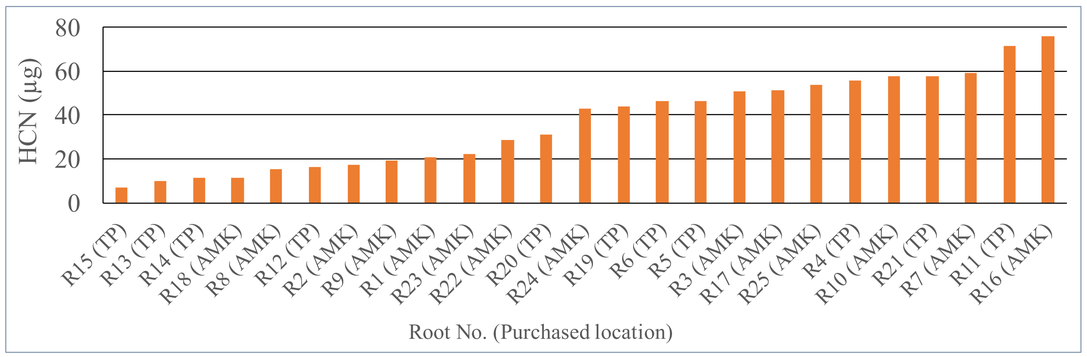

Figure 2. Cyanogenic potential (Fresh weight basis) of cassava roots

Discussion & Conclusion

- The CNp of the cassava roots varied from 6.95mg/kg HCN to 75.96mg/kg HCN (Fresh weight basis) which average to 36.91mg/kg HCN for all 25 roots.
- This value complied with the CODEX Alimentarius description on 'Sweet cassava' to not exceed 50mg/kg HCN (Fresh wt) in cassava meant for consumption.
- However, 9 roots had CNp ranged 50.8–75.9mg/kg and this indicates that some cassava roots sold in Singapore have CNp exceeding safe limits.
- Thus, a follow up study on accessing more roots/roots of specific country origin (where possible), from different retail outlets located throughout Singapore, can provide a more conclusive reflection of the CNp of cassava roots sold in Singapore.

References

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