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# Straightforward rapid spectrophotometric quantification of total cyanogenic glycosides in fresh and processed cassava products

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ABSTRACT

In this study, we extend pioneering studies and demonstrate straightforward applicability of the corrin-based chemosensor, aquacyanocobyrinic acid (ACCA), for the instantaneous detection and rapid quantification of endogenous cyanide in fresh and processed cassava roots. Hydrolytically liberated endogenous cyanide from cyanogenic glycosides (CNp) reacts with ACCA to form dicyanocobyrinic acid (DCCA), accompanied by a change of colour from orange to violet. The method was successfully tested on various cassava samples containing between 6 and 200 mg equiv. HCN/kg as verified with isonicotinate/1,3-dimethylbarbiturate as an independent method. The affinity of ACCA sensor to cyanide is high, coordination occurs fast and the colorimetric response can therefore be instantaneously monitored with spectrophotometric methods. Direct applications of the sensor without need of extensive and laborious extraction processes are demonstrated in water-extracted samples, in acid-extracted samples, and directly on juice drops. ACCA showed high precision with a standard deviation (STDV) between 0.03 and 0.06 and high accuracy (93–96%). Overall, the ACCA procedure is straightforward, safe and easily performed. In a proof-of-concept study, rapid screening of ten samples within 20 min has been tested.



## 1. Introduction

Cassava (*Manihot esculenta* Crantz) is a staple food in most tropical regions, and is grown over a range of climates and altitudes and on a wide variety of soils. The estimate total world cassava production in 2012 was 256 million tonnes according to FAO (2013), which is an increase of 40% since 2000. Africa represents the continent with the largest cassava production of more than 50% of the annual world production. The crop is one of the most important staple food with about 93% of the production used for human consumption (Nweke, Spencer, & Lynam, 2002). The roots of this important carbohydrate source are eaten both fresh and as processed products (Westby, 2002). However, in its tissue, cassava contains cyanogenic glycosides, mainly linamarin, that are enzymatically hydrolysed to glucose, acetone and hydrogen cyanide during cell rupture (Conn, 1994). The released cyanide is highly toxic for humans and is a threat to the cassava consumer (Rosling, 1988). The presence of cyanogenic glycosides in cassava tissues is related to illnesses that occur in populations where cassava is the staple food. These illnesses include tropical ataxic neuropathy, epidemic spastic paraparesis, also known as *konzo* (Cliff, Muquingue, Nhassico, Nzwalo, & Bradbury, 2011; Rosling, 1988). These problems have been reported in the Democratic Republic of Congo, Nigeria, Tanzania and Mozambique (Ciglenecki et al., 2011; Mlingi, Nkya, Tatala, Rashid, & Bradbury, 2011; Nhassico, Muquingue, Cliff, Cumbana, & Bradbury, 2008).

Monitoring of cyanogenic potential (CNp) of cassava is therefore of utmost importance due to the following reasons: determination of safeness of different cassava products, evaluation of the efficiency of different existing methods of cassava processing on the removal of cyanogenic glycosides and determination of level of CNp of released new cassava varieties in breeding programmes. Unfortunately, all existing methodologies for the determination of CNp have been shown to be dependent either on analytical equipment or on laborious and slow procedures.

Various methods are used to determine cyanogens in cassava. Probably the most common analysis involves three main steps: (i) extraction of cyanogens from cassava, (ii) hydrolysis of CNp to cyanide and (iii) analysis of the latter (Borges, Fukuda, & Caldas, 1993; Bradbury, Bradbury, & Egan, 1994; Cook, 1978). Extraction of cyanogens from the plant material is normally carried out using dilute acid such as 0.1 M phosphoric acid in order to stop

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endogenous linamarase activity and to stabilise the cyanohydrins (Cooke, 1978; Essers, Bosvel, van der Grift, & Voragen, 1993). Cyanogens can be hydrolysed to cyanide by acid hydrolysis (Bradbury, Egan, & Lynch, 1991), by autolysis (AOAC, 1990), or by enzymatic hydrolysis (Cook, 1978; Essers et al., 1993; O'Brien, Taylor & Poulter, 1991). The acid hydrolysis method involves hydrolysis of cyanogens in 2 M $H_2SO_4$ at 100 °C for 50 min, which is laborious and slow. Autolysis, the hydrolysis of linamarin by endogenous linamarase, is more suitable for fresh cassava material, but the reaction time can take up to 24 h for certain samples like sun-dried cassava flours (Bradbury & Bradbury, 1994). Another disadvantage of this method is that it cannot be applied to cooked or roasted products since the endogenous enzyme is permanently inactivated. In enzymatic hydrolysis, linamarase is added to the acid-extracted sample after pH adjustments (pH 5–6). Enzymatic breakdown of cyanogenic glycosides is rapid at around 30 °C and takes less than 15 min depending on enzymatic activity (Cooke, 1978; Essers et al., 1993; Haque & Bradbury, 1999; Nambisan, 1999). Linamarase can be easily isolated from cassava latex (Haque & Bradbury, 1999; Nambisan, 1999). Both acid and enzymatic hydrolysis methods convert linamarin to cyanohydrins, which further decompose to cyanide and acetone at alkaline pH levels (Bradbury & Bradbury, 1994).

Various methods have been developed to determine endogenous cyanide liberated from cyanogenic glycosides. In the titration method, endogenous cyanide is precipitated with $AgNO_3$ (AOAC, 1990) after steam distillation of hydrogen cyanide from autolysed cassava samples (AOAC, 1990). A disadvantage of this method is the loss of cyanide during the distillation process (Borges et al., 1993). The alkaline picrate method is a semi-quantitative method in which cyanide reacts with alkaline picrate paper, and the change of colour is matched against a colour chart, the resulting chromophore can also be dissolved from the picrate paper for more accurate quantitative determination using spectrophotometer (Bradbury, 2009; Bradbury, Egan, & Bradbury, 1999). Although the picrate method is easy to use, it has certain disadvantages. The reaction is very slow (~16 h), the chemical needs special handling and storage, and the response is sometimes imprecise. Cyanide detection by picrate was also used in a micro diffusion method, where the change of colour of picrate pre-coated in ion-exchange sheet is read in a reflectometer (Saka, Mhone, & Brimer, 1998). The method, which is based on the König reaction in which $CN^-$ is oxidised to a cyanogen halide by chloramine T (Cook, 1978; Essers et al., 1993; O'Brien et al. 1991), is the most important and most accurate colorimetric method. The cyanogen halide reacts with pyridine or a related compound to produce a dialdehyde, which is then coupled with primary amines or compounds with active methylene groups such as pyrazolone or barbituric acid to yield a coloured complex (Lambert, Ramasamy, & Paukstelis, 1975). The following combination of reagents have been developed to produce colours: pyridine/pyrazolone (Cooke, 1978), pyridine/barbituric acid (Bradbury et al., 1991; Mendoza, Kojima, Iwatsuki, Fukuda, & Uritani, 1984), isonicotinic acid/barbituric acid (Nagashima, 1978) and isonicotinic acid/1,3-dimethylbarbituric acid (Essers et al., 1993; Meeussen, Temminghoff, Keizer, & Novozamsky, 1989). All these multi-step reactions are relatively complex and the methods can only be carried out by trained personnel. Most recent published work related to the determination of cyanide in cassava products have used the picrate sensor (Banea et al., 2012; Bradbury & Denton, 2010; Burns, Bradbury, Cavagnaro, & Gleadow, 2012; Burns, Gleadow et al., 2012), despite its above mentioned disadvantageous (Bradbury et al., 1999, 2009). This behaviour can be rationalised by the lack of a suitable alternative.

Consequently, development of new, straightforward and rapid tests for the detection of cyanide in cassava products is crucial. Recently, the development of metal-based chemosensors for detecting endogenous cyanide has attracted much attention and future applications in food safety control have been proposed. The aquacyanocobyrinic acid chemosensor (ACCA), a Co(III) metal complex (a derivate of vitamin $B_{12}$), seems to be advantageous compared to other colorimetric reagents since it reacts with cyanide within seconds and detection does not interfere with common anions or biological material, as demonstrated by Zelder (2008) and Männel-Croisé, Probst, and Zelder (2009). Furthermore, the chemicals are non-toxic and the system is easy to handle (Zelder, 2008).

In this study, we extend the first studies and demonstrate straightforward applicability of the corrin-based chemosensor for the instantaneous detection of endogenous cyanide in various cassava products such as fresh cassava roots, boiled fresh cassava roots and dried cassava roots, and describe a spectrophotometric method for the rapid quantification of total cyanogenic glycosides (CNp).

## 2. Materials and methods

### 2.1. Samples

All samples of cassava products were collected in Mozambique. Samples from fresh roots were harvested in two different forms: two roots from unnamed bitter cassava, identified as roots 1 and 2, and two roots from an unnamed sweet variety, identified as roots 3 and 4. The pulp and cortex of the fresh roots were used in the analysis. CNp were also measured in the boiled pulp of roots 3 and 4. Two samples of cassava flour from sun-dried bitter cassava roots and one sample of roasted shredded cassava roots (garri) were also used for CNp analysis.

### 2.2. Chemicals

The following chemicals were used: sodium hydroxide (Sigma–Aldrich, CAS 1310-73-2), potassium cyanide (Fluka Biochemika, CAS 151-50-8), orthophosphoric acid (Sigma–Aldrich CAS 7664-38-2), tri-sodium phosphate (Sigma–Aldrich CAS 7601-54-9), 1,3-dimethylbarbituric acid (Aldrich, CAS 769-42-6), isonicotinic acid (Aldrich, CAS 55-22-1), chloramine T (Sigma–Aldrich CAS 7080-50-4), glycine (Merck, CAS 56-40-6), α-amylase (Sigma–Aldrich CAS 9000-85-5) and Milli-Q water.

Aquacyanocobyrinic acid (ACCA) was synthesised as described elsewhere (Männel-Croisé & Zelder, 2009).

### 2.3. Chemical solutions

Stock solutions of NaOH (0.2 M), orthophosphoric acid (0.1 M), tri-sodium phosphate (0.1 M), phosphate buffer pH 6 (mixture of equal volume of phosphoric acid (0.1 M) and tri-sodium phosphate (0.1 M)) were prepared. Chloramine T was prepared freshly each day by dissolving (0.5 g) in water (25 ml). Glycine buffer (0.1 M) was prepared and the pH was adjusted to 9.5 with NaOH (1 M). For the standard curve, a potassium cyanide (300 μM) stock solution was prepared in NaOH (0.2 M; pH 12.4) or in glycine buffer (0.1 M, pH 9.5).

The stock solution of the aquacyanocobyrinic acid (ACCA) reagent was prepared by dissolving aquacyanocobyrinic acid (4 mg) in water (10 ml). The exact concentration of the ACCA sensor was calculated after diluting an aliquot (100 μl) with water (650 μl) followed by the full conversion of ACCA to DCCA with excess cyanide. From the absorbance of the diluted solution, $A_{366nm} = 1.015$ and the characteristic absorbance of dicyano-corrinoids at 366 nm $\epsilon_{366nm} = 30400\ M^{-1}\ cm^{-1}$, the molar concentration of ACCA in the stock solution was found to be 248 μM.