LUND UNIVERSITY

Cassava Processing: Safety and Protein Fortification

Tivana, Lucas

2012

[Link to publication](#)

Total number of authors:
1

Cassava Processing:

Safety and Protein Fortification

Lucas Daniel Tivana

Department of Food Technology, Engineering and Nutrition

Faculty of Engineering, LTH

Lund University, Sweden

Doctoral Thesis

Cassava Processing:

Safety and Protein Fortification

Lucas Daniel Tivana

2012

Department of Food Technology, Engineering and Nutrition

Faculty of Engineering, LTH

Lund University, Sweden

Cassava Processing:

Safety and Protein Fortification

Doctoral Thesis

Abstract

Cassava (*Manihot esculenta* Crantz) is an important and cheap source of carbohydrate in tropical regions, particularly in Sub-Sahara Africa. Cassava as a human food is a good source of energy as it has a comparable high energy density of about 610 kJ/100 g fresh root. The crop has growth advantages and production can take place in soil where other crops such as maize, sorghum and sweet potatoes cannot grow. In the region, cassava is used mainly by the farmers themselves as a subsistence crop because the fresh cassava roots have a short shelf-life (1 to 2 days) after harvest. Consequently, the manufacture of cassava-based industrial products may be of interest as a potential way of boosting the economic value of the crop.

Producing a valuable, nutritional and safe food from cassava involves certain challenges. Cassava has a poor protein content (1% fresh root weight) and contains cyanogenic glucosides that may cause intoxication. This thesis reports on work aimed at overcoming these negative aspects of cassava. The report begins by describing how cassava can be detoxified through processing. Secondly, we propose a new method to facilitate the control of residual toxic compounds in cassava products, and finally we describe a methodology for protein fortification of cassava products while preserving consumer-perceived textural properties.

The cyanogens in cassava are hydrolyzed into volatile free cyanide by allowing contact between the cyanogenic substances localized in the vacuoles of the cells with hydrolyzing enzymes in the cell walls. This can be achieved by damaging the cells mechanically or by fermentation. Studies carried out in Nampula, Mozambique showed that heap fermentation of cassava roots may reduce toxic compounds by up to 96%. Another process used for the removal of toxic compound in cassava roots is shredding of roots, which is also an efficient detoxification method (98% reduction).

Assessment of toxic compounds in cassava products is of crucial importance but most existing methodologies have been shown to depend either on analytical equipment or on laborious and slow procedures. In this study, a simple cyanide detection sensor synthesized from Vitamin B12 is proposed. The new reagent detected the cyanide in seconds and has the advantages of being simple and not toxic.

If the economic value of cassava is to be increased, industrial production of cassava-based products is essential. In this study we chose to process cassava into the traditional product known as rale (a dry, pre-gelatinized and granulated product). Protein was added to this product to improve its nutritional value. Addition of protein to shredded cassava roots before roasting resulted in hard agglomerates, a feature that is undesirable for the product. Pre-treatment of protein suspension such as heating and reducing the pH changes the microstructure in such a way that a protein-fortified product could be obtained without properties that the consumer perceives as negative.

Popular summary

Cassava roots are quite rich in starch, like potatoes. It is produced mainly in tropical regions of the world, but as food crop it is of particular importance in Sub-Sahara Africa. Cassava has advantages as a crop; production can take place in low fertile soil, the yield is high, it is resistant to pests and survives droughts. However, cassava has also some disadvantages; fresh cassava roots deteriorate rapidly (2 day) after harvest, some type of cassava contains toxic substances and has poor protein content. If cassava is eaten without adequate pre-treatment, people may get intoxicated. In Africa cassava is used mainly by the farmers themselves as a subsistence crop. The farmers know the properties of their type of cassava and they commonly prefer to produce the cassava with high toxicity because it is more resistant to pests and wild animals cannot eat it. Usually cassava with toxic substances is bitter.

It is of interest to develop the agro-based economy in the rural areas to improve the people living conditions. Cassava-based industrial food products have a potential of boosting the local economy. For industrialization of cassava, it is necessary to know all the stages of cassava treatment for removal of toxic substances. Also it is important to have simple methods to control these substances. Due to low protein content in cassava it is also important to add protein from other crop to cassava products.

This thesis describes a possible development of the cassava processing in Mozambique, Africa. Several processes to remove toxic substances from cassava are described, including boiling, heap fermentation, extraction, sun drying and grating followed by roasting. All methods using grating appear to be most effective.

The methods of analysing toxic substances in cassava are complex and expensive, particularly in countries like Mozambique where technology is still developing. Thus, in this work we developed a method that would facilitate the analyses. The method is based on a reagent produced from vitamin B_{12}. The method can be used in all type of cassava products.

Another aspect of this thesis was to add soy protein to cassava product as a way to increase its nutritional value. The designed way by adding soy flour rich in protein to grated roots prior to roasting. The product obtained at first

place was hard and un-pleasent. It was found that this malfunction was due to a special starch in protein microstructure. Then the soy protein was treated in such way that this microstructure was avoided and replaced by a protein in starch structure and a softer and more attractive roasted cassava product was obtained.

Populär sammanfattning

Kassava är ett stärkelserikt livsmedel, jämförbart med potatis. Det produceras främst i jordens tropiska regionerna och är ett viktigt livsmedel i Afrika söder om Sahara. Kassava har stora fördelar som gröda eftersom avkastningen är hög, den har god motståndskraft mot sjukdomar, den växer även på mager jord och den är motståndskraftig mot torka. Emellertid, kassava har också nackdelar: färskt kassava har kort hållbarhet (2 dagar), kassava kan innehålla giftiga substanser och kassava har ett lågt innehåll av protein. Giftig kassava är förhållandevis bitter medan kassava med låg halt giftiga substanser är mer eller mindre söt. Bönderna föredrar ofta bitter kassava med hög halt av de giftiga substanserna eftersom detta gör dem mer motståndskraftig mot sjukdomar och för att det förhindrar vilda djur från att angripa grödan. Om man äter kassava utan rätt förbehandling kan man bli förgiftad. I Afrika är kassava främst konsumerad inom det självförsörjande jordbruket. Bönderna känner sin kassavas egenskaper och vet hur de skall behandla den.

Det är intressant att utveckla en landsbygdsindustri för förädling av kassava till livsmedel för avsalu eftersom lokalt företagande kan stärka ekonomin för fattiga människor på landsbygden. För att industrialisera tillverkning av livsmedel som bygger på kassava behöver man känna till effektiva metoder för avgiftning och man behöver kunna kontrollera effektiviteten av avgiftningen. Det är också bra om man kan tillverka livsmedel där kassavan kombineras med proteinrika livsmedelsingredienser.

Denna avhandling beskriver och jämför olika metoder att reducera halten toxiska substanser. Metoderna inkluderar kokning, extraktion, fermentering med mögel, soltorkning och rivning med rostning. Alla metoder som inkluderar en effektiv sönderdelning av den färska kassavaroten ger en effektiv avgiftning.

Många av analysmetoderna är komplexa och dyra, särskilt i ett land som Mocambique. Därför ar vi arbetat med en ny enklare metod som bygger på ett regent utvecklat från vitamin B12. Metoden kan användas på olika produkter från kassava.

När man tillsätter protein till kassava erhåller man en produkt med betydligt bättre näringsvärde. Receptet vi undersökte var ett kassava gryn som erhölls genom rostning av riven kassava. I receptet med hög proteinhalt tillsattes

soja protein till den sönderdelade kassavan före rostningen. Den produkt som erhölls var hård och mindre attraktiv som livsmedel. Vi kunde identifiera att mikrostrukturen var av typ stärkelse i protein. Genom att ändra processen kunde vi erhålla en produkt med den mer lämpliga mikrostrukturen protein i stärkelse vilket gav en mjukare och mer attraktiv produkt.

Contents

List of Publications

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals.

I.   L. D. Tivana, J. M. Bvochora, A. N. Mutukumira, J. D. Owens (2007). **A Study of Heap Fermentation Process of Cassava Roots in Nampula Province, Mozambique**. *Journal of Root Crops*, **33** (2), 119-128

II.   L. D. Tivana, J. Da Cruz Francisco, B. Bergenståhl, P. Dejmek (2009): **Cyanogenic Potential of Roasted Cassava (*Manihot esculenta* Crantz) Roots Rale from Inhambane Province, Mozambique**. *Czech Journal of Food Sciences,* **27**, s375-s378

III.   L. Tivana, J. Da Cruz Francisco, F. Zelder, B. Bergenståhl, P. Dejmek. **Straightforward rapid spectrophotometric quantification of total cyanogenic glycoside in fresh and processed cassava products**. Manuscript

IV.   L. D. Tivana, P. Dejmek, B. Bergenståhl (2010). **Characterization of the agglomeration of roasted shredded cassava (*Manihot esculenta* crantz) roots**. *Starch/Stärk Journal*, **62** (12), 637-646

V.   L. D. Tivana, P. Dejmek, B. Bergenståhl. **Effect of pH and soy flour heat treatment on texture and color of fortified roasted sheared cassava (*Manihot esculenta* Crantz) roots**. Submitted to the *Starch/Stärk Journal*

The author's contribution to the papers

I. Lucas Tivana designed the study, performed the fieldwork, designed the laboratory experiment with co-workers, evaluated the results and wrote the paper.

II. Lucas Tivana designed the study, performed the fieldwork then, with co-workers, evaluated the results and wrote most of the paper.

III. Lucas Tivana designed the study with co-workers, performed the experimental part, evaluated the results and wrote most of the paper.

IV. Lucas Tivana designed the study with co-workers, performed the experimental part and wrote most of the paper.

V. Lucas Tivana designed the study with co-workers, performed the experimental part, evaluated the results and wrote most of the paper.

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. Cassava is tolerant to drought; it is productive in poor soil where other staple crops cannot grow without intensive inputs (Bradbury and Holloway, 1988; Leihner, 2002). In Africa, the continent with the largest cassava production (53% of world production of 230 million tonnes in 2010; FAO, 2012), about 93% of the production is used as food (Nweke *et al.*, 2002). The crop is an important source of carbohydrate for humans and animals, having higher energy density (610kJ/100g fresh weight) than other root crops, such as sweet potatoes (460 kJ/100g) and taro (490 kJ/100g) (Bradbury and Holloway, 1988). However, cassava has certain drawbacks: its tissues contain toxic cyanogenic compounds, it has a very low protein content (1-2% dw) and a very short shelf-life in fresh form of 1-3 days (Booth *et al.*, 1974, Rickard, 1985; Westby, 2002). Regarding the toxic compounds, there are several plants that contain cyanogenic glycosides, for instance *Rosaceae* (including almonds, apples, cherries etc) and in some *Graminaceae* (sorghum), however cassava and sorghum are the most important staple foods that contain cyanogenic glycosides in eatable parts. It is believed that cyanogens protect the plant from attacks by some worms, arthropods and mammals, which is the main reason why the majority of farmers in Southern Africa prefer to grow the variety with high level of cyanogens, known, as bitter varieties (Bellotti and Riis, 1994, Vetter, 2000; Chiwona-Karltun and Mkumbira, 2000). 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* (Rosling, 1986; Rosling, 1988; Nzwalo and Cliff, 2011), endemic goitre and cretinism (Delange *et al.*, 1994). These problems have been reported in the Democratic Republic of Congo, Nigeria and Mozambique (Bonmarin *et al.*, 2002; Ernesto *et al.*, 2002; Nhassico *et al.*, 2008, Mlingi *et al.*, 2011; Ciglenecki *et al.*, 2011). Such illnesses occur when there are prolonged cyanide exposures associated with food shortage, social instability, under-nourishment and deficiency in some essential nutrients such as sulphur amino acids and iodine (Delange *et al.*, 1994; Teles, 1995; Nzwalo and Cliff, 2011). The cassava roots need to be processed if these

negative aspects are to be overcome, and experienced cassava producers are aware of different ways and methods of processing cassava (Nyirenda *et al*., 2011). Scaling up of cassava processing into a food industry in Africa is still in its infancy, and investors are more interested in using cassava for bioethanol production (Haggblade *et al*., 2012). One of the aims of this project is to contribute to the work on developing cassava processing to an industrial scale in Africa and particularly in Mozambique. The project first describes some methods of cassava processing applied in Mozambique for detoxifying cassava roots, then reports on development of a method for rapid detection of cyanide, and finally develops a method of protein fortification in a well-known local cassava product.

1.1 **Objectives:**

To understand the mechanism of cassava root detoxification in local traditional processing of cassava roots as applied in Mozambique. The process of heap fermentation was monitored and simulated in laboratory scale (Paper I).

To evaluate the level of cyanogenic potential (CNp) in roasted shredded cassava roots, known locally as rale and similar to the product named garri in other parts of the world, using samples collected in Inhambane Province, Mozambique (Paper II).

To evaluate and develop a new method for determinations of cyanogenic potential in cassava and cassava products using a recently proposed cyanide sensor (Paper III).

To understand the microstructure formation and physical properties of rale agglomerate (Paper IV).

To identify and understand the effect of soy protein fortification on texture properties of rale (Paper V).

2. Production and importance of cassava

2.1 Ecology of cassava plant

Cassava is a tropical crop, a perennial shrub of the *Euphorbiaceae* family, distributed between latitudes 30° N and 30° S (Costa and Silva, 1992; Alves 2002). The ideal growth temperature range is 24 to 30^oC (IITA, 1990) but it can tolerate temperatures ranging from 16 to 38^oC. Cassava can grow in the semi-arid tropics with an annual rainfall less than 800 mm, but the ideal rainfall is 1000 to 1500 mm per year (Alves, 2002). Cassava can grow in low-nutrient soils where cereals and other crops do not grow. It grows well in sandy to light soils where the storage roots can develop easily. Cassava can tolerate soils with low pH (Islam *et al*., 1980). Soils with a superficial hard layer or with many stones are not suitable for cassava growth.

2.2 Production and utilization of cassava

The estimate total world cassava production in 2010 was 230 million tonnes according to FAO (2012), which is an increase of 25% since 2000. Table 1 summarises the most important cassava producing countries. In 2010, Nigeria produced 37 million tonnes, making it the world's largest producer. In Africa, Mozambique was fifth in cassava production in 2010 with 5.7 million tonnes, after Nigeria, Democratic Republic of Congo, Angola and Ghana (FAO, 2012). The average world cassava yield in 2010 was estimated at 12.4 tonnes per ha. African countries present the lowest yields and Asian countries present the highest yields. Maximum yield was reported to be 34 tonnes per hectare in India (FAO, 2012). Data of cassava production per capita show how cassava is important in Africa. In 2010, Angola led the production per capita with 726 kg/person followed by Ghana with 563 kg/person, sufficient quantities to completely satisfy the need for food carbohydrate in the population of those countries.

According to Nweke *et al.* (2002), cassava plays five important roles in African development: famine-reserve crop, rural staple food, cash crop for both rural and urban households and, to a minor extent, raw material for feed and chemical industries. In South America, it is used mainly for animal feed (about one-third) followed by human consumption then starch production. In Asia, consumption of fresh roots and exportation to the European Union for use in animal feed are important, but its use for biofuel production is increasing (Almeida, 1995; Westby, 2002; Jansson et al., 2009). According to FAO statistics for 2010, cassava is the primary crop

produced in Mozambique, contributing more than 40% of the total food energy requirement (FAO, 2012).

Table 1. The major cassava producing countries in 2010 (data from FAO, 2012)

Countries	Root production (x1000 tonnes)		Yield (tonnes/ha)		Production per capita (kg fresh weight)
	Fresh roots	dry weight*	Fresh roots	dry weight*	
Nigeria	37 500	13 100	12	4.2	230
Brazil	24 500	8 600	14	4.9	126
Indonesia	23 900	8 400	20	7.0	100
Thailand	22 000	7 700	19	6.7	314
D. R. Congo	15 000	5 300	8	2.8	231
Angola	13 800	4 800	15	5.3	726
Ghana	13 500	4 700	13	4.6	563
Vietnam	8 500	3 000	17	6.0	97
India	8 000	3 000	34	12.0	67
Mozambique	5 700	2 000	6	2.1	248
Uganda	5 300	1 900	12	4.2	161
China	4 700	1 600	17	5.9	34
Tanzania	4 400	1 500	6	2.1	64
Cambodia	4 200	1 500	20	7.0	300
Malawi	4 000	1 400	21	7.4	267

* Estimated dry matter in fresh cassava roots was 35%

3. Chemical and nutritional composition of cassava

Cassava roots and cassava leaves are used for human consumption and animal feed (Buitrago 1990, Dahniya, 1994). The edible part of the root comprises about 85% of the total weight of the root (Alves, 2002). The general chemical composition of cassava roots and leaves is shown in **Table 2**.

Table 2. Chemical composition of cassava roots and leaves (Buitrago, 1990; IITA, 1990)

Nutrient	Storage root		Leaves	
	Fresh weight basis (%)	Dry weight basis (%)	Fresh weight basis (%)	Dry weight basis (%)
Dry matter	35.00	100.00	28.00	100.00
Starch	30.21	85.10	16.23	39.00
Crude protein	1.10	3.10	6.80	24.00
Fat	0.47	1.30	1.80	6.50
Crude fibre	1.10	3.10	5.80	20.60
Ash	0.70	1.90	1.70	6.20
Calcium	0.10	0.33	0.43	1.50
Phosphorus	0.15	0.44	0.08	0.27

Cassava roots are rich in digestible carbohydrates, mainly starch. Cassava starch granules are mainly composed of two polysaccharides, amylose (20%) and amylopectin (80%) (Sandoval, 2008). Cassava starch is simple to extract from the roots, is a pure white in colour and is free from other components than other root and tuber crops (Moorthy, 2002). There is a large variation in sucrose content between cassava genotypes. In certain sweet varieties, sucrose constitutes up to 17% of total carbohydrates (Hendershot, 1972) but, generally, cassava roots have less than 1% free

sugars (Bradbury & Holloway, 1988). Cassava roots are low in protein and fat.

A comparison of the protein composition of cassava roots and other foods is shown in **Table 3**. Cassava root has less than the recommended minimum limit of almost all essential amino acids, except tryptophan. Cassava roots should be eaten along with other crops rich in essential amino acids to supplement the deficiency, such as vegetables, cereals, fish and meat. Cassava leaves are much richer in protein than the roots, although the leaf contains a lower proportion of methionine than the root protein (Eggum, 1970). The levels of all other essential amino acids in leaf protein exceed the FAO's recommended reference patterns (Eggum, 1970; Okigbo, 1980; FAO, 1990). Cassava leaves are also consumed in many African countries (Achidi *et al*., 2005), but the cassava plant does not have leaves during the dry season and at low temperature (lower than 15°C) due to dormancy.

Table 3. Amino acids and protein content in cassava and other foods

Amino acid	(g/ 100 g crude protein)				
	Cassava roots*	Potato*	Soyabean† (seeds)	cowpea*	FAO Reference patterns*
Lysine	4.14	6.00	6.24	6.83	5.50
Threonine	2.64	3.90	3.68	3.60	4.00
(Tyrosine+ phenylalanine)	4.10	7.80	8.64	7.78	6.00
Valine	3.34	5.10	4.8	4.53	5.00
Tryptophan	1.15	1.40	1.26	1.09	1.00
Isoleucine	2.80	3.90	4.8	3.82	4.00
(Methionine +cysteine)	2.77	3.00	3.00	2.26	3.50
Leucine	4.00	5.90	7.52	7.74	7.00
Total EAA	24.94	36.30	39.94	37.65	36.00
Total protein (% dw)	1.8	10	35	23	-

EAA- essential amino acids

*   Modification from FAO, 1990

†   (Endres, 2001)

# 4. Cyanogenic glycosides in cassava and implications for human health

The major constraint in cassava roots as human food is the presence of toxic cyanogenic glycoside compounds in the tissues. Cassava tissues also contain the enzyme linamarase, which can hydrolyse cyanogenic glycoside but the enzyme is not located in the same cell compartments as the cyanogenic glycosides (Bruijn, 1971; Nweke, 1994; Teles, 1995). Cyanogenic glycosides are located inside vacuoles and the enzyme linamarase in the cell wall as shown in Figure 1 (Conn, 1994).

Disruption of cassava tissues initiates the hydrolysis of cyanogenic glycosides. Cyanogenic glycoside are leached from vacuole and come into contact with linamarase, a β-glucosidase, to produce acetone cyanohydrin from linamarin and 2-butanone cyanohydrin from lotaustralin (Conn, 1994). These cyanohydrins are unstable and decompose spontaneously to the corresponding ketones and hydrogen cyanide (HCN) at pH values above 5 and temperatures above 30°C. Cyanohydrin degradation (Figure 2) can also be catalysed by α-hydroxynitrile lyase, located in apoplastic space (White *et al*., 1994).
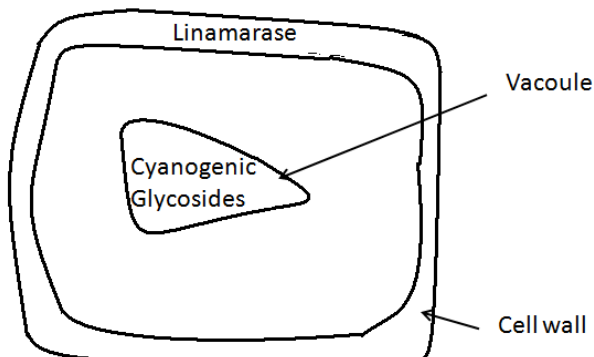
Figure 1. Location of cyanogenic glycoside and the enzyme linamarase in the cell plant, adapted from Conn (1994)

In all the tissues, with the exception of the seeds, cassava contains 4 to 5 cyanogenic glycosides (McMahon *et al.*, 1995). The main ones are linamarin and lotaustralin in a ratio 97:7 (Teles, 1995). The concentrations of cyanogens vary in different varieties, between tissues in the same plant and even between compartments of the same tissue (Barrios and Bressani, 1967; Bruijn, 1971; Nambisan and Sundaresan, 1994; Wheatley and Chuzel, 1993, Burns *et al.*, 2012) (Table 4). According to Bruijn (1971), cyanogen concentration in cassava roots, in the longitudinal direction, increases from insertion point on the plant to the root terminal and in the transverse direction, cyanogenic glycosides levels decrease from the external area to centre of the root. The knowledge of the distribution of cyanogenic glycosides in cassava roots is important for the sampling of roots.
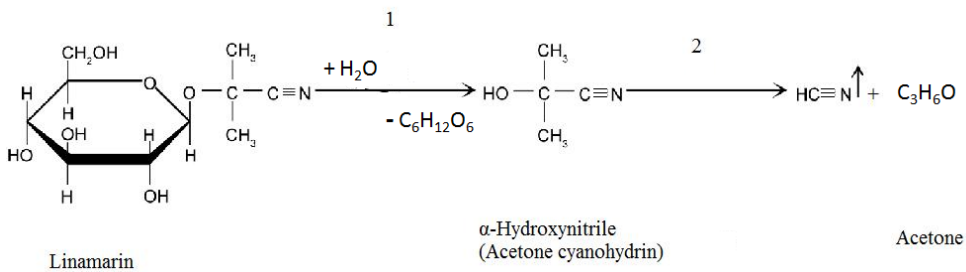
Figure 2. Hydrolysis of linamarin, in 1) is β-glucosidase (linamarase)(pH=5.5), in 2) is α-hydroxynitrile lyase (pH≥5, Temp. ≥30). Adapted from Conn (1994)

Table 4. Total cyanogenic glycosides (CNp) in different tissues of the cassava plant

Plant tissues	CNp (mg HCN/kg) DW	Source
Roots parenchyma (pulp)	30-1200	Barrios and Bressani (1967)
	30-1300	Wheatley and Cruzel (1993)
	160-700	Burns *et al*. (2012)
	81-500	Nambisan and Sundaresan (1994)
Roots cortex (peel)	50-770	Barrios and Bressani (1967)
	60-550	Wheatley and Cruzel (1993)
	800-1600	Burns *et al*. (2012)
	1800-1900	Nambisan and Sundaresan (1994)
Leaves	10-940	Barrios and Bressani (1967)
	400-800	Burns *et al*. (2012)
	1700-1900	Nambisan and Sundaresan (1994)

DW: dry weight

Cassava varieties are often described as being bitter or sweet by reference to the taste of fresh roots and this partly correlates with cyanogen concentrations (Chiwona-Karltun *et al*., 2004). Bitter varieties are associated with high concentrations of cyanogenic glycosides (> 100 mg/kg fresh weight) (Sundaresan *et al*., 1987; Nambisan and Sundersan, 1994; Chiwona-Karltun *et al*., 2004). Sweet varieties have a high concentration of free sugars but it does not always follow that they have low concentrations of cyanogenic glycoside (Borges and Fukuda, 1989; King and Bradbury, 1995). However, bitter taste and high level of cyanogens can also be related to environmental stress conditions, such as drought, low soil fertility and pest attack (Bruijn, 1971).

The liberated HCN through the hydrolysis of cyanogenic glycosides is toxic. The HCN blocks the reduction of oxygen in the respiratory pathway (Nelson and Cox, 2008). The acute oral lethal dose of hydrogen cyanide (HCN) is 3 mg/kg body weight (Borges and Fukuda, 1989). The FAO/WHO (1991) recommended limit value for the safety of consumption

of cassava products is 10 mg HCN/kg and the acceptable limit in Indonesia is 40 mg HCN/kg (Damardjati *et al*., 1993). The acceptable intake can be compared with the lethal dose. Here it seems like the acceptable intake at meal is about 3 to 10% of the lethal dose assuming that a person with 50 kg of body weight would ingest 300g of cassava in one meal. Cases of fatal and acute intoxication related to cassava consumption have been reported. In 1887, the crew of Stanley's remarkable expedition through central Africa (today the Democratic Republic of Congo), suffered from acute and fatal poisonings when they consumed bitter cassava roots without the extensive soaking as normally applied by local inhabitants (Manning, 1985). In Nampula-Mozambique, Ceara-Brasil and Nigeria, acute toxic effects after consumption of cassava meals have been reported (Mozambique Ministry of Health, 1984; Akintonwa *et al*., 1994; Teles, 1995).

Most common illness related to cassava consumption is due to prolong exposure to comparable low concentrations of cyanogens in ingested cassava products (Rosling, 1988; Nzwalo and Cliff, 2011. Residual cassava cyanogens, when ingested, are hydrolysed in the human digestive system. It is assumed that intestinal microbes hydrolyse the cyanogens (Teles, 1995). The HCN in the human body is metabolised to thiocyanate (McMahnon and Birnbaum, 1990). Figure 3 shows a scheme of the metabolism of cyanide to thiocyanate in the human body. The conversion of HCN to thiocyanate is catalysed by rhodanese and 3-mercaptopyruvate sulphur transferase (Westley, 1981; Vazques *et al*., 1987). These enzymes require sulphur, which is supplied from sulphur amino acids. The thiocyanate (SCN^-) is eliminated from the human body through urine and saliva (Figure 3). Deficiency in essential sulphur amino acids may enhance the risks in illnesses such as tropical ataxic neuropathy, epidemic spastic paraparesis, also known as *konzo* (Rosling, 1986; Rosling, 1988; Casadei *et al*., 1990; Nzwalo and Cliff, 2011).

Excessive levels of SCN may under a long term exposure lead to reduced iodine uptake, which in an iodine-deficient region may contribute to endemic goitre and cretinism (Bradbury and Holloway, 1988; Delange *et al*., 1994).

The cyanogenic glycoside levels can be reduced to acceptable levels that would not impose hazard to the consumer when the cassava roots are adequately processed. Also the diet pattern influences the risks. When cassava is eaten with other foods balancing the nutritional value by being rich in sulphuric amino acids, there is only a limited risk of intoxication.
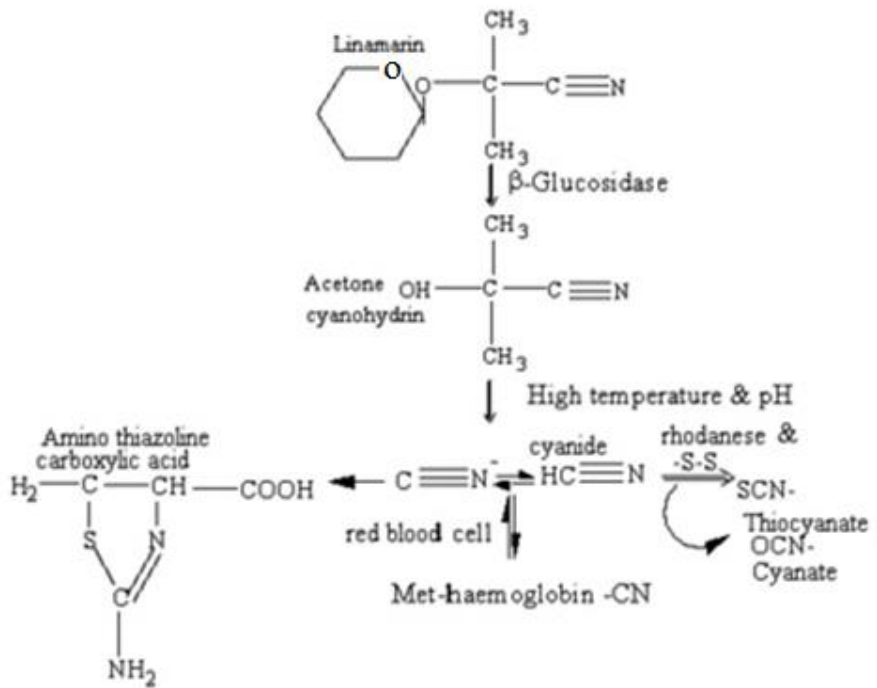
Figure 3 Metabolism of cyanide to thiocyanate in the human body (from Rosling, 1994)

5. Cassava processing

Cassava roots are used for human food and animal feed in a large number of different products. Various processing methods are used to produce different food products, depending on locally available processing resources, local customs and preferences, and the most common are summarized in **Table 5**. Cassava processing improves palatability, increases shelf-life, facilitates transport and, most importantly, detoxifies cassava roots by removing cyanogens (Nweke, 1994; Westby, 2002; Nyirenda *et al.*, 2011).

5.1 Boiling of the roots

Boiling is used in the processing of cassava roots in almost all countries where cassava is used as food. The cell structure during heating remains more or less intact until the cell membranes are destroyed around 60-70 °C. Then there is a small window until the enzyme is deactivated, between 60 and 80 °C. Thus, the enzymatic detoxification using boiling is limited. Thereby the main elimination way is extraction. The efficiency of cyanogen removal due to extraction is proportional to the ratio of cassava to water (Nambisan and Sundaresan, 1985). As consequences of this inefficiency boiling alone can only be used for sweet varieties (< 100 mg HCNeq/kg fresh weight) (Alves, 2002; Nyirenda *et al.*, 2011). Boiled roots are usually eaten directly, fried or stewed with some spice to improve the taste.

5.2 Sun drying

Sun-dried products are the most common types of processed cassava products in Africa (Westby, 2002). The process involves peeling the cassava roots, chipping (or grating) and spreading on dried grass or on the roof of the houses for sun drying. The efficiency of removal of cyanogenic glycosides in this process depends somehow on the rate of moisture loss. Fast drying results in lower cyanogen removal, while slower rates of drying result in a higher reduction. More important is the degree of disruption of cellular tissues of the cassava roots. The disruption of the tissue and the cells is creating contact between cyanogenic glycosides and hydrolytic enzymes (Nambisan & Sundaresan, 1985; Essers *et al.*, 1996). Processes

that promote high disruption of cassava roots such as grating and crushing before sun drying remove much of the cyanogenic glycoside (Cardoso *et al*., 2005). Dried roots are then milled to make a sticky porridge known as *karakata* in Mozambique.

Table 5. Most important cassava processing methods for food

Processing method	Countries of use	Estimate CNp removal (%)	Sources
Boiling of fresh roots	All country that use cassava as food	25-65	Nambisan & Sundaresan(1985) Cardoso *et al*. (2005)
Sun drying after chipping	Mostly African country	65-75	Cardoso *et al*. (2005) Mlingi and Bainbridge (1994)
Soaking in water (fermentation)/sun drying	Malawi, Tanzania, Zambia, Uganda, Democratic Republic of Congo	97-98	Nyirenda *et al*. (2011) Cardoso *et al*. (2005)
Heap fermentation/ sun drying	Uganda, Tanzania, Mozambique	83-95	Essers *et al*. (1995) Zvauya *et al*. (2002)
Grating/ferment-ation/roasting	West African countries, Mozambique	97-98	Cardoso *et al*. (2005) Westby and Choo, (1994)

5.3 Soaking of roots in water/Sun drying

Peeled cassava roots are soaked in water for 3-5 days, followed by sun drying. The process is reported to be the best for cyanogen removal (Westby, 2002; Cardoso *et al*., 2005). Nyirenda *et al*. (2011) reported that in Zambia, pre-sundried roots are also soaked in water for 3 to 14 days.

This type of fermentation is mostly used in areas where there is a sufficient supply of water, such as near a river or lake, and is common in countries such as Nigeria, Democratic Republic of Congo, Tanzania, Zambia and Malawi (Westby, 2002; Nyirenda *et al.*, 2011). In soaked roots, microbial growth is essential because it disrupt the cellular tissues of roots, which has the combined effect of allowing cyanogenic glycosides to come in contact with linamarase (Westby and Choo, 1994). The fermentation of soaked roots to produce *fufu* is dominated by lactic acid bacteria with a decrease of pH during the fermentation (Westby and Twiddy, 1992).

5.4 Heap fermentation/Sun drying

Heap fermentation of cassava root products is common in Tanzania (Ndunguru *et al.*, 1999), Uganda and Mozambique (Essers *et al.*, 1995a). The process involves peeling of cassava roots, sun drying for 1 to 3 days, heaping and covering, fermentation, scraping off the mould mycelia, crushing into crumbs, sun drying, pounding and sieving into flour. Essers *et al.* (1995a) observed that the heap fermentation was dominated by mould mycelium growth of *Neurospora sitophila*, *Geotrichum candidum* and *Rhizopus oryzae*. Essers *et al*. (1995a) observed that the growth of moulds softens the roots by enzymatic degradation of cell wall of cassava tissues (Essers *et al*., 1995b). The degrading of tissue structures in the cassava roots enables contact between cyanogenic glycosides and linamarase. Heap fermentation of cassava roots followed by sun drying is capable of reducing the cyanogen levels up to 95% (Essers *et al*., 1995a).

5.5 Grating/fermentation/roasting

A combined process of grating (shredding) and fermentation of cassava roots is important in the processing of roots for many West African products, including roasted granules (*garri*), steamed granules (*Iattieke*) from Côte d'Ivoire and some of the fermented pastes (*agbelina* and *placali* from Ghana and Côte d'Ivoire respectively) (Westby and Choo, 1994, Westby, 2002; Obilie *et al*., 2004). Shredded roots are also roasted in the south of Mozambique to produce *rale* (Francisco *et al.*, 1992). The shredded cassava roots are allowed to ferment in sacks for 1-7 days, which encourages lactic acid fermentation. The pH after 3 days decreases from 6 to 4. The fermentation is dominated by lactic acid bacteria (Westby and Twiddy, 1992). Grating is important for the disruption of cellular tissues of

cassava roots. The process allows the reduction of roots to particle size of between 0.1 to 1 mm. The reduction of cassava tissues maximizes the hydrolysis of cyanogenic glycosides as they come in contact with linamarase. Lactic acid bacteria play only a limited role in cyanogen reduction (Westby and Choo, 1994). Lactic acid fermented products are reported to have significant remninant concentrations of cyanohydrin because pH decreases during fermentation and cyanohydrin is stable at low pH (Vasconcelos *et al*., 1990) (Figure 2).

6. Methods for the analysis of cyanogenic glycosides

The determination of total cyanogenic glycosides (cyanogenic potential) (CNp) in cassava products is of crucial importance. The development of new cassava varieties, new methods of cassava processing and new applications of cassava in food need the monitoring of cyanogenic potential. Cyanogenic potential (CNp) is defined as the concentration of cyanogenic glycosides and their break down products (cyanohydrins and hydrogen cyanide)

Many different methods have been developed to determine cyanogens in cassava. All methods of analysis of cyanogens in cassava involve mainly three steps: extraction of cyanogens from cassava, hydrolysis to cyanide and analysis of cyanide (Borges *et al*., 1993; Bradbury *et al*., 1994). Some of the most commonly used methods are described in Paper III. Unfortunately, until today all existing methodologies have been shown to be dependent either on analytical equipment and expensive reagents (Cook, 1978; O'Brien *et al*., 1991; Essers *et al*., 1993), or being laborious (Bradbury *et al.*, 1991) and slow procedures (Saka *et al*., 1997; Bradbury *et al*., 1999).

Table 6. Common methods to measure the cyanogenic potential of cassava.

| Method	Extraction process of Cyanogenic glycoside (CNp)	Conversion of CNp to cyanide	Detection of cyanide	Advantage/disadvantage
Titration method (De Bruijn, 1971, AOAC, 1990))	Material crushed and added to water. After 8 to 24 hours the sample is steam-distilled	Hydrolysis of linamarin by endogenous linamarase (Autolysis)	Titration of the distilled HCN with $AgNO_3$.	The use of endogeneous enzyme limit the method to fresh tissue. The steam distilation takes long time and is laborous. Risk of loss of gas cyanide during steam distilation
Enzymatic assay by Cooke (1978)	In 0.1M of H_3PO_4, filtration/centrifugation	Hydrolysis by exogenous enzyme	Based on the König reaction. CN^- converted to halide with Chloramine T. The halide reacts with complex sensor pyridine/pyrazolone (λ=620 nm)	Complex and expensive sensor. Pyridine is toxic compound.
Enzymatic assay (O'Brien et al. (1991)	In 0.1M of H_3PO_4, filtration/centrifugation. Addition of ethanol (25%) in gelatinized samples		CN^- reacts with complex sensor pyridine/pyrazolone (λ=620 nm)	
Enzymatic assay (Essers et al., (1993)	In 0.1M of H_3PO_4, filtration/centrifugation		The halide reacts with sensor complex of isonicotinic acid/1,3-dimethylbarbituric acid (λ=606 nm)	
Acid hydrolysis (Bradbury et al. (1991)	In 0.1M of H_3PO_4, filtration/centrifugation	Hydrolysis of cyanogens with 2M H_2SO_4 at 100°C for 50 min	The halide reacts with sensor complex of pyridine/barbituric acid. (λ=583 nm)	
Picrate kits (Bradbury et al., 1999)	Small amount of sample is used (no extraction)	Autolysis (fresh roots) hydrolysis by exogenous enzyme (processed)	Cyanide gas reacts with picrate adsorbed in paper and the colour change is matched against a colour chart or read in a colorimeter ((λ=510 nm)	Picric acid is toxic and explosive if not handled adequately. Slow detection of cyanide.
Microdiffusion method (Saka et al., 1998)	In 0.1M of H_3PO_4, filtration/centrifugation	Hydrolysis by exogenous enzyme. Evaporisation at 40°C during 5 h.	Cyanide reacts with picrate adsorbed in pre-coated ion-exchange sheet. The concentration is obtained using reflectometry ($\lambda \approx$500-550 nm)	

7. General discussion of the value of this work

7.1 Study of heap fermentation in Mozambique (Paper I)

Heap fermentation of cassava roots was investigated in Uganda by Essers *et al*., (1995a). They observed that heap fermentation may be an efficient route for detoxification of cassava roots. Then Zvauya et al., (2002) showed that the results may vary. According to Essers *et al*., (1995a) observations, mould is important for the enzymatic degradation of cell walls of the cassava roots and thereby enabling the hydrolysis of cyanogenic glycosides. They also reported that the pH during the heap fermentation was between 5.5 and 6.3, which is optimum for linamarase activity (Nok and Ikediobi, 1990). The high pH at the end of heap fermentation enhances the breakdown of cyanohydrin into volatile HCN, resulting in about 96% CNp removal. However, in Mozambique, Zvauya *et al*. (2002) reported that about 24% of 71 samples of cassava flour produced from roots that were heap fermented had values above 40 mg HCNeq/kg. Some samples had unacceptable values of about 140 mg HCNeq/kg and, in one of the locations, average values were 59±48 mg HCNeq/kg in heap-fermented samples.

In an attempt to understand the process of heap fermentation in Mozambique, the author of this report monitored the process carried out by three farmers located in Nacaroa district, Nampula Province, Mozambique (Paper I). CNp values were determined in flour made from dried cassava roots with different time periods of heap fermentation. The average CNp concentration in cassava flour that was not heap fermented was about 160 mg HCNeq/kg while for flour from roots that were heap fermented for 72 hours was 23 mg HCN/kg. Both flours were prepared from fresh cassava roots with initial average CNp content of about 260 mg HCNeq/kg fresh weight. The CNp figures presented in Paper I are calculated on a fresh weight basis. During the drying process, about 60% of water evaporated, so the estimated CNp retention after a combination of heap fermentation and sun drying was about 4%, which is similar to results reported by Essers *et al*. (1995a).

Observation of the heap fermentation of batches showed that the fermentation is dominated by moulds. Mould growth starts on the surface

of root pulp that has been sliced or damaged. Two moulds were successfully isolated and identified (Figure 4). The isolated moulds were then used in the laboratory, where their spores were harvested and inoculated in cassava roots that were sliced differently, as shown in Figure 5.
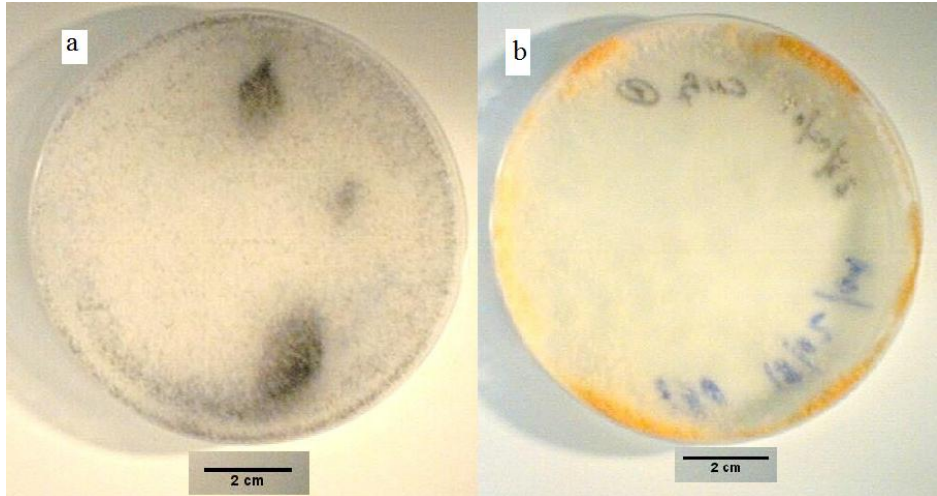
Figure 4. Isolated moulds in heap fermentation batches in rural farmhouses. *Rhizopus stolonifer* (a) and *Neurospora sitophila* (b). Scale bar corresponds to 2 cm

Results from inoculated roots confirm the observation from heap fermentation batches that the moulds grow better in the damaged cassava root pulp than on the surface of the root pulp. Mycelia of moulds can be observed in Figure 5, where the sliced roots show more mould mycelia on the cross-sectional surface of root pulp (internal part of the pulp) than in the external surface of the roots (where the peel was removed). Root pulp that was sliced longitudinally promoted rapid growth of mould mycelia due to the exposure of larger cross-sectional surface than root sliced only in transversal section. It is important to note that slicing the roots longitudinally before heap fermentation increases the level of CNp removal by heap fermentation. The reported high CNp values of cassava flour of heap fermented flour collected in Mozambique may result in weak growth of moulds in heaps due to the use of large cassava roots that were not sliced

properly. The heap fermentation batches that were monitored and gave the 96% removal of CNp may not be representative of the method usually applied by the majority of famers. The poor practice is especially common when the product is aimed for the market.
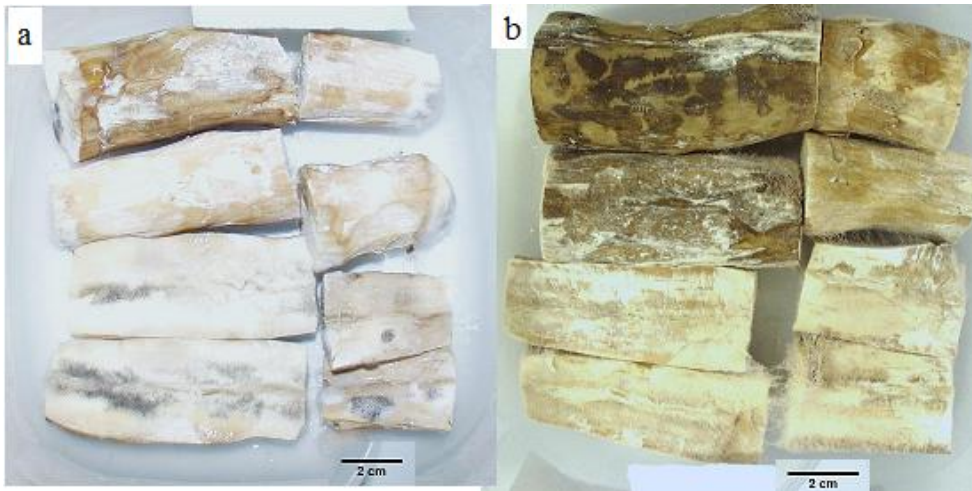

Figure 5.   Cassava roots inoculated with *Rhizopus stolonifer* (a) and *Neurospora sitophila* (b). Upper roots (not sliced longitudinally), lower roots (sliced longitudinally). Scale bar corresponds to 2 cm.

7.2 Determination of cyanogenic potential of *rale*, a roasted shredded cassava root, in Inhambane Province, Mozambique (Paper II)

Roasted shredded cassava root, rale, is important food product in the South part of Mozambique. Assessment of cyanogenic potential (CNp) in roasted shredded cassava root (*rale*) in Mozambique is limited, possibly because the product is produced in the southern part of Mozambique where no cases of illness relating to cassava consumption have been reported. This study presents CNp results for some rale samples collected from homes and local markets in three districts of Inhambane Province, Mozambique, namely Inhambane, Inharrime and Maxixe.

The production of *rale* in Mozambique comprises of grating of peeled roots (Figure 6a) followed by pressing the mass to remove the excess water (fermentation may occur) for 1 to 5 days (Figure 6b) and roasting of the shredded roots (Figure 6c). Shredding of the roots plays a very important role in disruption of cellular tissue of cassava roots and thereby reducing the CNp content (Westby and Choo, 1994). The process is faster than that of heap fermentation and most of the producers that commercialise their product combine pressing and fermentation in one day. From 56 rale samples collected, the CNp contents obtained were in the range 20 to 110 mg HCNeq/kg with an average of 41±16 HCN mg/kg. Figure 7 shows the distribution of CNp results for all samples. The results are higher than the average figures for CNp of 25±5 mg/kg in 30 samples in similar products in Port Harcourt, Nigeria reported by Adindu *et al*. (2003). Although the average figures for CNp in rale samples collected in Mozambique were higher than those of heap-fermented samples (34±33 mg/kg) reported by Zvauya *et al.* (2002), the process of grating and roasting results in faster detoxification than the heap fermentation process and it can be carried out hygienically without risk of contamination by pathogens such as fungus that can produce mycotoxins. *Rale* production processes in Mozambique differ from those of garri in West African countries in that the grated mass is not subjected to a long period of fermentation, sometimes not at all. The recent development of small-scale equipment such as an improved motorised press has shortened the process of *rale* production to less than a day. The disintegration of cellular tissue has strong impact than the fermentation. Westby and Choo (1994) stated that 95% of initial linamarin is hydrolysed within 3 hours after grating. Furthermore, the results showed that the concentration of residual cyanogens is highly dependent on the cassava varieties. CNp figures for rale from sweet varieties were about half

of those from bitter varieties. Some processors have produced rale from a mixture of sweet and bitter varieties, resulting in intermediate values.
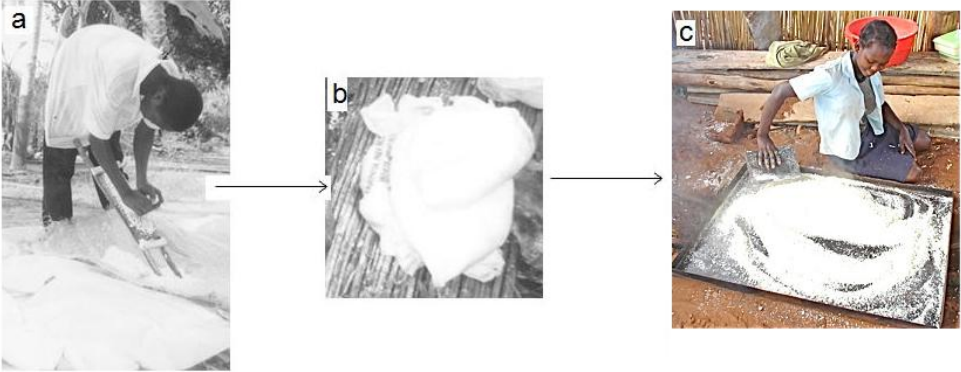
Figure 6. Some steps of rale production. Shredding of the cassava roots (a), pressing of cassava root mass (b) and roasting of the shredded mass (c).
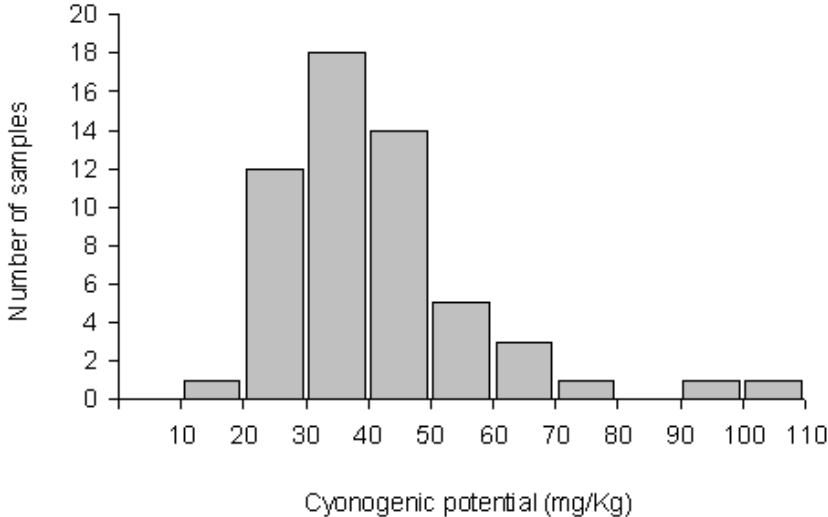

Figure 7. Distribution of the cyanogenic potential of all garri samples from Inhambane Province, Mozambique (Paper II).

7.3 Straightforward rapid spectrophotometric quantification of total cyanogenic glycoside in fresh and processed cassava products (Paper III)

Paper III described the development of procedures for rapid detection of cyanide by modifying the enzymatic method described by Essers *et al*. (1993). The modification is focused on the extraction procedure and the use of a new sensor for cyanide detection, but the hydrolysis process of cyanogens is similar to the traditional method for processed cassava products.

Recently Zelder (2008) developed a Co (III) -based (aquacyanocobyrinic acid) chemosensor (a derivate of vitamin B_{12}) that allows the detection of endogenous cyanide in plants and it was successfully evaluated in fresh cassava roots (Männel-Croisé *et al.*, 2009). The aquacyanocobyrinic acid chemosensor (ACCA) is rapid (seconds) and selective to cyanide, with a colour change from orange (ACCA) to violet (dicyano-cobyrinic acid (DCCA) (Figure 8)

The (ACCA) sensor seems to be advantageous compared to other colorimetric reagents since it reacts with cyanide within seconds and detection does not interfere with common anions and biological material as demonstrated by Zelder (2008) and Männel-Croisé *et al.* (2009). Furthermore, the chemicals are non-toxic and the system is easy to handle (Zelder, 2008). In Paper III, we describe how the ACCA can be used in different cassava samples as a substitute for the complex sensor of Essers *et al*. (1993) but with easy and simple extraction procedures such as squeezing out the juice of cassava pulp into water.
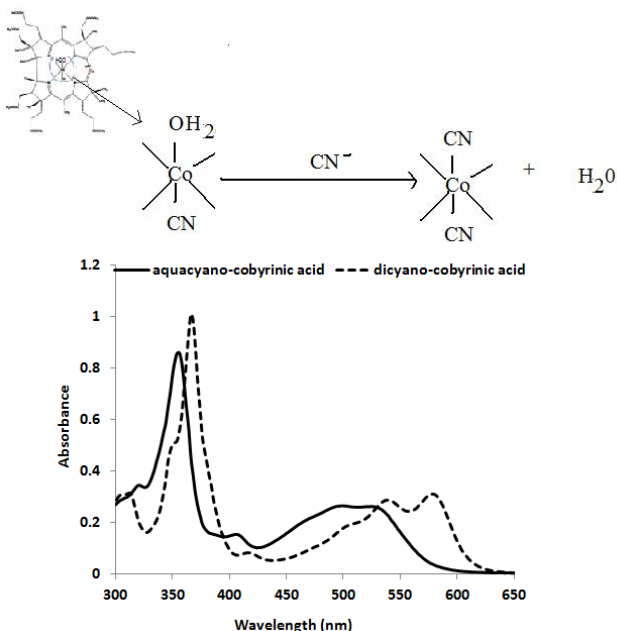
Figure 8. Coordination mechanism of aquacyanocobynic acid to dicyano-cobyrinic acid adapted from Zelder (2008). Spectrum from experiment carried out in this study (modified from Paper III).

For samples such as fresh roots, where the endogen enzyme is active, the squeezing process means that the whole procedure takes only 20 minutes, and the reagents used are cheap buffers such as 0.1M of glycine (pH 9.5) and the ACCA sensor. For processed cassava roots such as rale or dried cassava flour, an external enzyme is required and can be easily obtained from the latex of cassava leaves (Haque & Bradbury, 1999; Nambisan, 1999) that is suspended in water with no need for further purification. A small sample of cassava flour is dispersed in water with an enzyme, hand shaken and then incubated for 5 minutes at a temperature 30 °C. Any appropriate aliquot can be used with a colour sensor. The only drawback is that the method still requires a spectrophotometer. The results of the ACCA sensor using samples extracted in either phosphoric acid (O.1M) or water had a strong correlation with results obtained from the phosphoric acid-extracted samples using the method of Essers *et al.* (1993).

7.4 Characterisation of the agglomeration of roasted shredded cassava (*Manihot esculenta* crantz) roots (Paper IV)

Another aim of this thesis was to formulate a nutritious product based on cassava roots using a well-known traditional form of cassava processing. This involved studying the physical properties in the microstructure of shredded and roasted cassava roots. The fermentation and its role for the detoxification as part of the processing of cassava roots into garri has been studied previously (Westby and Twiddy, 1992, Westby and Choo, 1994). However, the mechanism that drives the formation of rale agglomerates and the physical behaviour of rale agglomerate when reconstituted in water is little known. The understanding of these features may be important if the product needs to be fortified with other nutrients such as protein or in the design of processes for commercial production of rale. Samples of rale processed as described in Paper IV were used in the study.

The roasting of shredded cassava roots results in a pre-gelatinized product with properties such as significant swelling capacity, softness and without disintegration of the agglomeration neither when reconstituted in cold (room temperature) or hot water (90°C). These properties can be considered important for consumer appreciation, so it is important to preserve them if *rale* of greater nutritious quality is developed. The parameter used for the determination of these features was the texture. Along with taste, texture is a sensory property of largest importance in food and plays important role in the acceptability of a new product by specific groups of the population (Nishinari, 2004).

The measurement of texture of *rale* agglomerates was not an easy task using available texture probes, since the *rale* is made up of coarse particles. A type of specially designed extrusion cell to be used in the Stable Micro System Texture Analyser (SMS TAXTA2i) was designed as shown in Figure 9a. The force of extrusion was considered as a measure of the agglomerate strength of the *rale*. The measurement was carried out after the *rale* agglomerates had been dispersed and allowed to swell in distilled water at room temperature. The method was verified by suspending the *rale* in absolute alcohol and measuring the texture. As shown in Figure 9b, *rale* suspended in water could pass the net probe, while the *rale* that was

suspended in alcohol did not swell and resulted in a hard coarse substance that could not pass through the probe net.
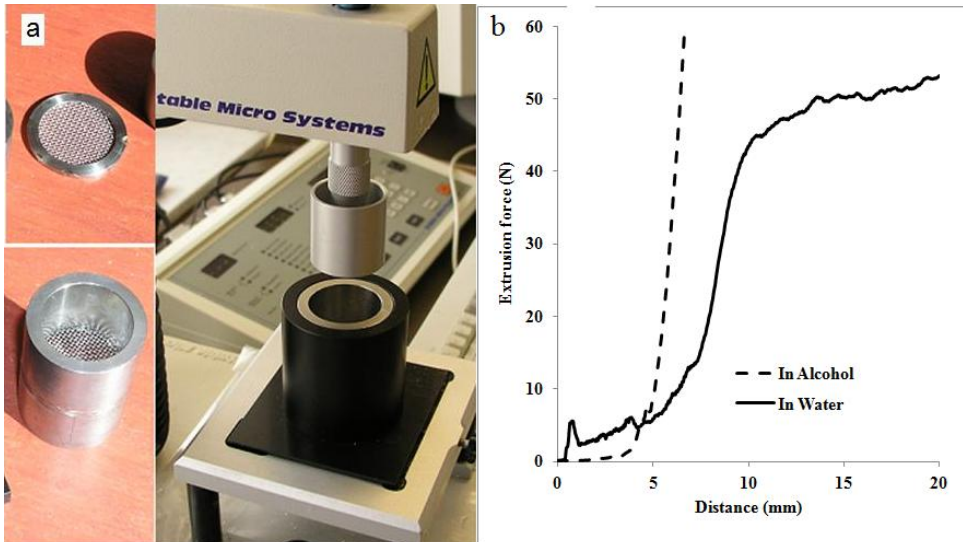
Figure 9. Apparatus system (a) for the determination of extrusion force (b) of swollen *rale* samples.

The results for texture of *rale* agglomerates were compared with the results of shredded flour that had been sun dried (without pregelatinization of starch). *Rale* agglomerates suspended in water at a temperature range of 30 to 90 °C swelled and the agglomerates could be extruded through probe net. The softness of *rale* was temperature depedent (Figure 10i). Non-gelatinized shredded flour could only be extruded when dispersed at temperature above 70 °C (Figure 10ii). When both sample dispersions were heated at 90 °C, *rale* agglomerate remain coarse while non gelatinized flour was almost solubilised. Since the starch comprises amylose and amylopectin, which both are leaches out during the roasting process, the remaining question was which molecule that was responsible for binding the starch granulates and rendering them resistance against disintegration even when dispersed in hot water (90°C). A modelling experiment using agglomerated glass beads as a model of the *rale* and using either amylose

or amylopectin as a binder. The results showed that the amylose agglomerated glass beads resisted the disintegration while the amylopectin agglomerated glass beads did not provide stable agglomerates when they were re-suspended in hot water. Consequently, the model experiment suggests that in the *rale*, amylose was the component responsible for binding and stabilizing the *rale* starch agglomerates when dispersed in hot water.
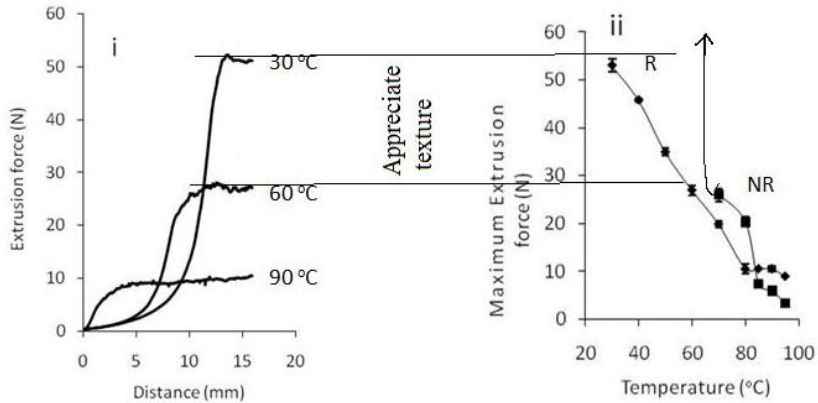
Figure 10. Texture analysis. i) extrusion force curves of *rale* swollen in water, ii) comparison of maximum force in extrusion of *rale* (R) and non-pre-gelatinized shredded flour (NR). Adapted from **Paper IV**

7.5 Effect of pH and soy flour heat treatment on texture and colour of fortified roasted sheared cassava (*Manihot esculenta* Crantz) roots (Paper V)

Fortification of *rale* with protein may enhance the nutritious quality of the product by changing the starch protein ratio since cassava roots are very poor in protein (about 1% fresh weight). However, this may also affect the physical properties, so in this thesis the influence of protein fortification on *rale* was studied. The reason for using *rale* is that the product has advantages over other products, e.g. the processing procedure is relatively more hygienic and detoxifies the cassava; the roasting process of the shredded mass allows fast drying of material and at the same time the material is pre-gelatinized, making the product ready to eat. Another advantage of the process is the long shelf-life of *rale* (Falade and Akingbala, 2010); if it is hygienically stored, *rale* can be preserved for more than a year without dramatic change in its sensory properties (Ikenebomeh, 2005,). Focusing on protein fortification of *rale* the study described in Paper V was carried out. The source of protein used to fortify *rale* was soybean flour.

Protein is usually added to food to improve the texture rather than to provide nutritional fortification, but in recent years, protein or certain amino acids are added to food, particularly cereals, in order to enhance nutritional value. Soya beans (*Glycine max*) are recognised as the main source of protein for food fortification as it is highly available, relatively cheap and that the production is sustainable (Hoogenkamp, 2005; Subbulakshmi and Udipi, 2006). Soybean protein has slightly less digestibility compared to animal proteins (Sugano, 2005). The proteins are mostly globulins, the storage proteins, and are divided into two proteins: glycinin, also called 11S, and β-conglycinin also known as 7S (Sugano, 2005). Glycinin comprises about 60 to 70% of the globulin protein (Kinsella, 1979, Sugano, 2005). Glycinin is more nutritionally valuable than β-conglycinin because it has more methionine and cysteine (Sugano, 2005). Soybean protein has a higher nutritional value than cassava root protein, so it can be used as protein fortification in cassava products. Several studies have reported on protein fortification of cassava products using soy flour, e.g. cassava flour (Muoki *et al*. 2012) and garri (Sanni and Sobamiwa, 1994; Osho, 2003; Ugwu and Odo, 2008). Muoki *et al*., 2012 reported that a complementary porridge made through extrusion of a

mixture of cassava flour and soy flour had acceptable sensory properties. Others studies, based in sensory evaluation by panels, show that the acceptability of garri fortified with soy flour is not statistically different from that of commercial garri (Osho, 2003; Ugwu and Odo, 2008). However, the effect on microstructure and texture by soy protein fortification in *rale* has not yet been studied. It is known that the addition of soybean protein in common starch food affect the rheological properties of the food and a weakening of the starch gel as a consequence of presence of proteins has been observed (Ribotta, et al., 2007).

Sun-dried shredded cassava root flour (CF) was used to simulate *rale* fortified with soy protein. The preparation of CF is described in Paper IV. Three types of soy flour were used: toasted soy flour (TSF), isolate soy protein (ISP) and defatted soy flour (DSF). First the soy flour was dispersed in water using a planetary mixture, and then CF was added to the soy dispersion until agglomerate was formed that was then roasted in a pan. The ratio of soy flour to CF was adjusted so that the final mixture contained about 15% protein, bearing in mind that cassava contains only 1% protein. The main parameter measured to characterize the protein-fortified *rale* was texture, by measurement of the extrusion force as described in Paper IV.

The main finding was that adding protein to *rale* resulted in a harder *rale* agglomerate, as shown in Figure 11a. Light microscopy observation of *rale* stained for protein with Coomassie Brilliant Blue, showed that the *rale* fortified with soy flour without any pre-treatment appeared to be surrounded by protein at the natural pH (pH 6.5). However pre-treatment of soy flour dispersion to promote maximal aggregation of protein reducing the solubility of protein, such as pre-boiling of soy flour at pH 4.5 resulted in *rale* with a similar texture to that of a commercial product, as shown in Figure 11b. The microscopy observation shows that the protein patches appeared to be surrounded by starch. These results support the finds by Quiroga and Bergenståhl (2008) that in the mixture of amylopectin and protein may switch from protein in amylopectin to amylopectin in protein depending on the mixing ratios and solubility. Figure 10c illustrates models of the *rale* system based on light microscopic observations, demonstrating a matrix of hard *rale* where starch granules are surrounded by non-granular starch leached from granules with more continuous protein domains and soft *rale* where continuous non-granular starch leached from starch granules with isolated dense protein domains.

Regarding to colour, there was a slight difference between the colour of the CF and the *rale* as a result of the Maillard reactions that occur during roasting. The brown colour of fortified *rale* was more pronounced with soy flour fortification, but the colour seemed to be more affected by the colour of the soy flour than by reactions during roasting.
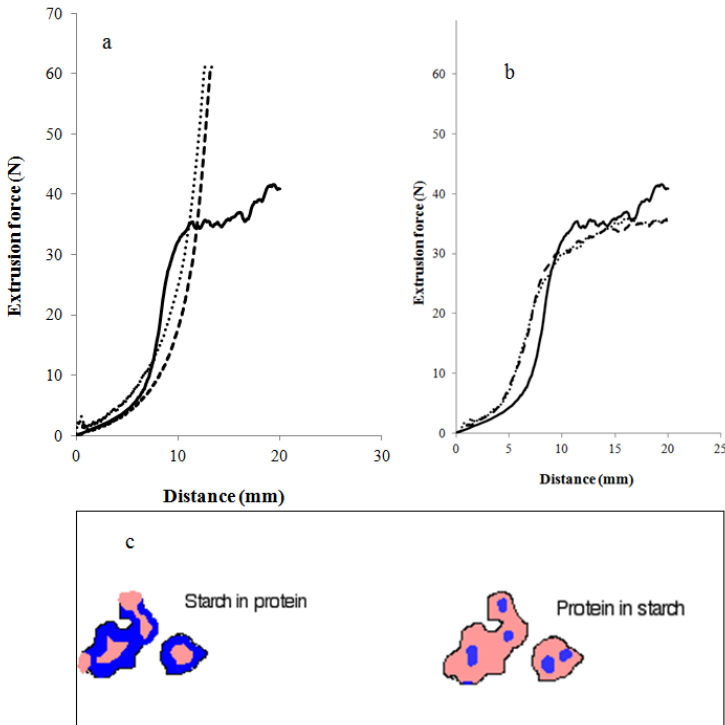
Figure 11. Effect of soy flour addition on the extrusion force of swollen *rale* in water at 30°C. a) Soy flour dispersion at pH 6.5, b) soy flour dispersion at pH 4.5, boiled. Commercial *rale* (———), TSF-*rale* (– –), ISP-*rale* (········), DSF-*rale* (---). C) Proposed system model for hard *rale* (starch in protein) and soft *rale* (protein in starch). Adapted from Paper V

8. Conclusions

A conclusion from the observations of heap fermentation of cassava roots in Mozambique is that the moulds responsible for breaking down the cassava cellular tissues, allowing the cyanogenic glycosides in the vacuoles to be hydrolysed by the enzymes in the cell walls. Inoculation of cassava roots with isolated moulds resulted in faster softening of longitudinally-sliced cassava roots compared to the roots only cut transversally. The longitudinally slicing increase the surface of the cross-section of the roots, which promote the growth of moulds. The degree of softness depends on the type of mould strain; in this study, *Rhizopus stolonifer* softened the cassava roots more than *Neurospora sitophila*, so further research is necessary under controlled fermentation conditions, which can be easily adopted at rural household level.

Assessment of the cyanogenic potential of *rale* produced in Mozambique, shows that the levels are higher than those recommended by FAO/WHO. The level of cyanogen in *rale* is significantly influenced by cassava variety. Further studies aiming to improve the removal of cyanogenic glycoside during *rale* production in Mozambique is highly recommended.

The method of detecting cyanide by spectrophotometer, using aquacyano-cobyrinic acid as a sensor, is simple. It can be used for water-extracted samples for immediate analyses as well as for phosphoric acid-extracted samples if the samples need to be stored. The cyanide measurement can also be carried out using a drop of juice squeezed from cassava pulp or small amount of cassava flour in water, without need for centrifugation. The usual enzymatic activity of the cassava leaf latex extract is sufficient to perform the assay on processed cassava

A study of the physical characterization of *rale* could conclude that the product may be described as pre-gelatinized and agglomerated cassava flour with 95% of starch gelatinized. Roasting is needed to agglomerate the structure. *Rale* has a rapid swelling and the structure remains stable, avoiding complete disintegration in water at high temperature. The softness of swollen *rale* is temperature dependent. In the *rale,* amylopectin contributes to the swelling of starch. Leaching of amylose, followed by retrogradation, leads to limited swelling of *rale* and the formation of agglomerates stable up to $90^{o}C$. Comparisons with model experiment using

glass beads coated with amylose and amylopectin indicated that retrograded amylose is necessary for formation of agglomerates stable in hot water. The textural properties of starch are important for consumer appreciation of the product. The rapid softening as well as the non-disintegration of agglomerates particles during swelling is usually appreciated by the *rale* consumers, thus we can expect that these features need to be preserved when *rale* is reformulated in order to improve its nutritional value.

Cassava roots can be fortified by adding soy flour during the processing of cassava into *rale*. However, our results shows that the fortification with soy protein leds to changes in colour and texture of the swollen *rale* agglomerates. The protein tends to interact with the cassava starch in different ways, depending on the source and pre-processing, which had different effects on the hardness of the swollen *rale* agglomerates. Conditions expected to cause maximal aggregation of the protein, either during soy flour processing or in pre-treatment before roasting, such as boiling at pH 4.5, had the least detrimental effect on *rale* texture. All kinds of fortification had discernible effects on the colour of *rale*, converting it towards darker, brownish hues. Based on these results, we believe that soy protein fortification of *rale* can be achieved with minimal effects on texture by a suitable choice of pre-treatment of the raw soy material, but the choice of raw material should be guided by consumer preferences regarding colour of the finished product.

Acknowledgements

I would like to express my personal thanks to:

My colleagues in Lund from Mozambique, Eulalia Chiau, Telma Magaia and Rui Raice, from Bolivia, Deysi Perez, Alejandra Castro, Claudia Lazarte and Leslie Tejeda, from Botswana Lesedi Lebogang for contributing to a creative and pleasant atmosphere in Lund.

Karin Petersson for all guidance relating to printing this thesis.

Aniko Wolf for helping me find an office at the Department of Food Technology

Ann-Christin Wikander for providing accommodation during my stay in Lund.

Yvonne Granfeldt, Ingegerd Sjöholm, Kerstin Skog, Andreas Håkansson for preparing some special Swedish dishes.

People who taught me how to play *innebandy*. I will really miss it.

All the wonderful people at Department of Food Technology, Lund University for their support

Special thanks for my family

My mummy, Atalia Manjate and my dad, Guguro Tivana for their patience during my long time away from home.

My wife, Anabela Casimiro Chambule, my two daughters, Júlia and Sara Tivana and my son Jack Tivana for their patience and moral support. I love you all.

Financial support

Swedish International Development Cooperation Agency

Eduardo Mondlane University, Mozambique

References

Achidi, A. U., Ajayi, O. A., Bokanga, M., and Maziya-Dixon, B. 2005. The Use of Cassava Leaves as Food in Africa. *Ecology of Food and Nutrition*, **44**, 423-435.

Akintonwa, A., Tunwashe O. and Onifade, A. 1994. Fatal and non-fatal acute poisoning attributed to cassava-based meal. In Bokanga, M., Essers, A. J. A., Poulter, N., Rosling, H. and Tewe, O. (eds), Proceedings of the international workshop on cassava safety, March 1-4, 1994, Ibadan, Nigeria, *Acta Horticulturae*, **375**, 285-288.

Almeida, J. M. R. 1995. Manual de mandioca. Cultivar, Porto, Portugal.

Alves, A. A. C. 2002. Cassava botany and physiology. In Hillocks, R. J., Thresh, J. M. and Bellotti, A. C. (Eds), Cassava biology, production and utilization. CABI Publishing, Wallingford, UK, 67-89

AOAC, 1990. Hydrocyanic acid in beans, alkaline titration method. In Offcial Methods of Analysis, 15th Ed, AOAC, Inc, Arlington, Va, USA, 1213.

Barrios, E.A. and Bressani, R. 1967. Composición química de la raíz y de la hoja de algunas variedades de yuca *Manihot*. *Turrialba*, **17**, 314–320.

Bellotti, A. C. and Riis, L. 1994. Cassava cyanogenic potential and resistence to pests and diseases. In Bokanga, M., Essers, A. J. A., Poulter, N., Rosling, H. and Tewe, O. (eds), Proceedings of the international workshop on cassava safety, March 1-4, 1994, Ibadan, Nigeria, *Acta Horticulturae*, **375**, 141-151.

Bonmarin, I., Nunga, M. and Perea, W. A. 2002. Konzo Outbreak, in the South-west of the Democratic Republic of Congo, 1996. *Journal of Tropical Pediatrics*, **48**, 234-238.

Booth, R. H. and Coursey, D. G. 1974. Storage of cassava roots and related postharvest problems. In Araullo, E. V., Nestel, B. and Campbell, M. (eds) *Cassava Processing and Storage. Proceedings of an interdisciplinary Workshop*. IDRC, Ottawa, 43-49.

Borges, M. F. and Fukuda W. M. G. 1989. Teor de cinetos em raízes frescas e processadas de mandioca. *Revista Brasileira de mandioca*, **8**, Bahia, Brasil, 71-76.

Borges, M. F., Fukuda, W. M. G. and Caldas, R. C. 1993. Avaliação de três métodos para determinação de cianeto de mandioca. *Revista Brasileira de mandioca*, **12**, Bahia, Brasi, 75-80.

Bradbury, J. H. and Holloway, W. D. 1988. Chemistry of tropical root crops: significance for nutrition and agriculture in pacific. ACIAR Monograph No 6.

Bradbury, J. H., Egan, S. V. and Lynch, M. J. 1991. Analysis of cyanide in cassava using acid hydrolysis of cyanogenic glycosides. *Journal of the Science of Food and Agriculture*, **55**, 277-290.

Bradbury, J. H., Bradbury, M. G. and Egan S. V. 1994. Compararison of methods of analysis of cyanogens in cassava. In Bokanga, M., Essers, A. J. A., Poulter, N., Rosling, H. and Tewe, O. (eds), Proceedings of the international workshop on cassava safety, March 1-4, 1994, Ibadan, Nigeria, *Acta Horticulturae*, **375**, 87-96.

Bradbury, M. G., Egan, S. V. and Bradbury, J. H. 1999. Picrate paper kits for determination of total cyanogens in cassava roots and all forms of cyanogens in cassava products. *Journal of the Science of Food and Agriculture*, **79**, 593-601.

Bruijn, G. H. 1971. Étude du caractère cyanogénetique du manioc. *Meded Landb Hogesch Wageningen* **71**, Wageningen, The Netherlands.

Buitrago, J. A. 1990. La yucca em alimentacion animal. Publicacion 85, CIAT, Cali, Colombia.

Burns, A. E., Gleadow, R. M., Zacarias, A. M., Cuambe, C. E., Miller, R. E. and Cavagnaro, T. R. 2012. Variations in the Chemical Composition of Cassava (Manihot esculenta Crantz) Leaves and Roots As Affected by Genotypic and Environmental Variation. *Journal of Agricultural and Food Chemistry*, **60**, 4946-4956.

Cardoso, A. P., Mirione, E., Ernesto, M., Massaza, F., Cliff, J., Haque, M. R. And Bradbury, J. H. 2005. Processing of cassava roots to remove cyanogens. *Journal of Food Composition and Analysis*, **18**, 451-460.

Casadei, E., Cliff, J. and Neves, J. 1990. Surveillance of urinary thiocyanate concentration after epidemic spastic paraparesis in Mozambique. *Journal of Tropical Medical Hygiene*, **93**, 257-261.

Chiwona-Karltun, L., Brimer, L., Kalenga Saka, J. D., Mhone, A. R., Mkumbira, J., Johansson, L., Bokanga, M., Mahungu, N. M. and Rosling, H. 2004. Bitter taste in cassava roots correlates with cyanogenic glucoside levels. *Journal of the Science of Food and Agriculture*, **84**, 581-590

Chiwona-Karltun, L. and Mkumbira, J. 2000. Low dietary cyanogen exposure from frequent consumption of potentially toxic cassava in Malawi. *International Journal of Food Sciences & Nutrition*, **51**, 33-43

Ciglenečki, I., Eyema, R., Kabanda, C., Taafo, F., Mekaoui, H. and Urbaniak, V. 2011. Konzo outbreak among refugees from Central African Republic in Eastern region, Cameroon. *Food and Chemical Toxicology*, **49**(3), 579-582.

Conn, E. E. 1994. Cyanogenesis- a personal perspective. In Bokanga, M., Essers, A. J. A., Poulter, N., Rosling, H. and Tewe, O. (eds), Proceedings of the international workshop on cassava safety, March 1-4, 1994, Ibadan, Nigeria, *Acta Horticulturae*, **375**, 31-43.

Cooke, R. D. 1978. An enzymatic assay for the total cyanide content of cassava. *Journal of the Science of Food and Agriculture*, **29**, 345-352.

Costa, I. R. S. and Silva, S. O. 1992. Coleta de germoplasma de mandioca no Nordeste do Brasil. *Revista Brasileira de mandioca*, **11**, Bahia, Brasil, 19-27.

Dahniya, M. T. 1994. An overview of cassava in Africa. *Crop science journal*, Makerere University, Uganda, **2**, 337-343.

Darmardjati, D. S., Widowati, S. and Racim, S. 1993. Cassava flour production and consumers acceptance at village in Indonesia. *Indonesian Agricultural Research and Development*, **76**, 39-48.

Delange, F., Ekpechi, L. O. and Rosling, H. 1994. Cassava cyanogenesis and iodine deficiency disorders. In Bokanga, M., Essers, A. J. A., Poulter, N., Rosling, H. and Tewe, O. (eds), Proceedings of the

international workshop on cassava safety, March 1-4, 1994, Ibadan, Nigeria, *Acta Horticulturae*, **375**, 289-293.

Eggum, B. O. 1970. The protein quality of cassava leaves. *British Journal of Nutrition*, **24**, pp 761-768

Endres, J. G. 2001. Soy Protein Products: Characteristics, Nutritional Aspects, and Utilization. AOCS Press. USA.

Ernesto M., Cardoso A. P., Nicala D., Mirione E., Massaza F., Cliff J., Haque M. R. and Bradbury J. H., 2002. Persistent *konzo* and cyanogen toxicity from cassava in northern Mozambique. *Acta Tropica.*, **82**, 3, 357-362.

Essers, A. A., Bosvel, M., van der Grift R. and Voragen, A. 1993. Studies on the quantification of specific cyanogens in cassava products and introduction of a new chromogen. *Journal of the Science of Food and Agriculture*, **63**, 287-296.

Essers, A. A., Ebong C., van der Grift, R. Nout, M. R. Otim-Nape, W. And Rosling, H. 1995a. Reducing Cassava Toxicity by Heap-Fermentation in Uganda. *International Journal of Food Science and Nutrition*, **46**, 125-136.

Essers, A. A., Jurgens, C. M. G. A. and Nout, M. J. 1995b. Contribution of selected fungi to the reduction of cyanogen levels during solid-substrate fermentation of cassava. *International Journal of Food Microbiology* **26**, 251-257.

Essers, A.J.A., Van der Grift, R.M. and Voragen, A.G.J. 1996. Cyanogen removal from cassava root during sun-drying. *Food Chemistry*, **55**, 319-325.

Falade, K. O. and Akingbala, J. O. 2010. Utilization of Cassava for Food. *Food Reviews International*, **27**, 51-83.

FAO, 1990. Roots, tubers, plantains and bananas in human nutrition. FAO, Rome, Italy.

FAO, 2012. http://faostat.fao.org. Agricultural Statistics. Food and Agricultural Organization of the United Nations. Rome [15 September 2012].

FAO/WHO 1991. Joined FAO/WHO Food Standards Programme, Codex Alimentarius Commission, **XII**, supplement 4. FAO/WHO, Rome, Italy.

Francisco, J. C., Jorge, M. A. B. and Zacarias, A. M. 1992. A cultura da mandioca em Moçambique: estado actual e suas potencialidades. *Revista Brasileira de Mandioca*, **11**, Bahia, Brasil, 156-163.

Haggblade, S., Djurfeldt, A., Nyirenda, D. B., Lodin, J. B., Brimer L. Et al. 2012. Cassava commercialization in Southeastern Africa. *Journal of Agribusiness in Developing and Emerging Economies*, **2,** pp 4-40

Haque, M. R. and Bradbury, J. H. 1999. Preparation of linamarase solution from cassava latex for use in the cassava cyanide kit. *Food Chemistry*, **67**, 305-309.

Hendershott, C. H. 1972. A literature review and research recommendations on cassava (*Manihot esculenta*, Crantz). University of Georgia, Georgia. 336p

Hoogenkamp, H. W. 2005. Soy protein and formulated meat products. CAB International. 247p

IITA (International Institute of Tropical Agriculture). 1990. Cassava in tropical Africa. Reference Manual, IITA, Ibadan, Nigeria

Ikenebomeh, M. J. 2005. Extension of shelf life of garri by hygienic handling and sodium benzoate treatment. *African journal of biotechnology*, **4**, 744-748.

Islam, A. K. M. S., Edwards, D. G. and Asher, C. J. 1980. pH optima for crop growth: Results of flowing culture experiment with six species. *Plant and soil*, **54**, 339-357

Jansson, C., Westerbergh, A., Zhang, J., Hu, X. and Sun, C. 2009. Cassava, a potential biofuel crop in (the) People's Republic of China. *Applied Energy*, *86, Supplement* **1**, S95-S99.

King, N. L. R. and Bradbury, J. H. 1995. Bitterness of cassava: identification of new apiosyl glycoside and other compounds that affect its bitter taste. *Journal of the Science of Food and Agriculture*, **68**, 223-230.

Kinsella J. E. 1979. Functional Properties of Soy Proteins. J. AM. Oil Chemists' Soc, 56, 242-258

Leihner, D. 2002. Agronomy and Cropping Systems. In Hillocks, R. J., Thresh, J. M. and Bellotti, A. C. (Eds), Cassava biology, production and utilization. CABI Publishing, Wallingford, UK, 91-113.

Männel-Croisé, C., Probst, B. and Zelder, F. 2009. A Straightforward Method for the Colorimetric Detection of Endogenous Biological Cyanide. *Analytical Chemistry*, **81**, 9493-9498.

Manning, O. 1985. The remarkable expedition: the story of Stanley's rescue of Emin Pasha from Equatorial Africa. Weidenfeld & Nicilson, London.

McMahnon, T. and Birnbaum, L. 1990. Age-relted changes in toxicity nd biotransformation of potassium cyanide in male C57BL 6N mice. *Toxicology and Applied Pharmacology*, **105**, 305-314.

McMahon, J. M., White, W. L. B., and Sayre, R. T. (1995). REVIEW ARTICLE. *Journal of Experimental Botany*, **46**, 731-741

Ministry of Health Mozambique, 1984. Mantakassa: an epidemic of spastic paraparesis associated with chronic cyanide intoxication in cassava staple area in Mozambique. *Bulletin of the World Health Organization*, **62**, 477-492.

Mlingi, N. L. V. And Bainbridge, Z. 1994. Cassava processing, moisture levels, cyanogens removal. In Bokanga, M., Essers, A. J. A., Poulter, N., Rosling, H. and Tewe, O. (eds), Proceedings of the international workshop on cassava safety, March 1-4, 1994, Ibadan, Nigeria, *Acta Horticulturae*, **375**, 233-240.

Mlingi, N. L. V., Nkya, S., Tatala, S. R., Rashid, S., and Bradbury, J. H. 2011. Recurrence of konzo in southern Tanzania: Rehabilitation and prevention using the wetting method. *Food and Chemical Toxicology*, **49**, 673-677.

Moorthy, S. N. 2002. Physicochemical and functional properties of tropical tuber starches: A review. *Starch-Starke*, **54**, 559-592.

Muoki, P. N., de Kock, L. H. And Emmambux M. N. 2012. Effect of soy flour addition and heat-processing method on nutritional quality and consumer acceptability of cassava complementary porridges. *Journal of the Science and Food Agriculture*, **92**, 1771-1779

Nambisan, B. 1999. Cassava latex as a source of linamarase for determination of linamarin. *Journal of Agriculture and Food Chemistry*, **47**, 372-373.

Nambisan, B. and Sundaresan, S. 1985. Effect of processing on the cyanoglycoside content of cassava. *Journal of the Science of Food and Agriculture*, **36**, 1197-1203.

Nambisan, B., and Sundaresan, S. 1994 Distribution of linamarin and its metabolising enzymes in cassava tissues. *J. Sci. Food Agric.*, **66**, 503-507

Nelson, D. L. And Cox, M. M. 2008. Lehninger principles of biochemistry. W H Freeman. 5th Edition, USA. 1100 p

Nhassico, D., Muquingue, H., Cliff, J., Cumbana, A., and Bradbury, J. H. 2008. Rising African cassava production, diseases due to high cyanide intake and control measures. *Journal of the Science of Food and Agriculture*, **88**, 2043-2049.

Ndunguru, G. T., Thomson, M., Waida, T. D. R., Rwiza, E., Jeremiah, S. and Westby, A. 1999. Relationship between quality and economic value of dried cassava products in Mwanza, Tanzania. In Akoroda, M. O. and Terri, J. (eds) *Food Security and Crop Diversification in SADC Countries. The Role of Cassava and Sweet potato*. SARRNET, Malawi, 408-414.

Nishinari, K. 2004. Rheology, food texture and mastication. *Journal of Texture Studies*, **35**, 113-124.

Nweke, F. I. 1994. Cassava distribuition in Africa. Study of cassava in Africa. IITA, Ibadan, Nigeria, work paper no 12.

Nweke, F. I., Spencer, D. S. C. and Lynam, J. K. 2002. The cassava transformation: Africa's best-kept secret. Michigan University Press, USA.

Nyirenda, D. B., Chiwona-Karltun, L., Chitundu, M., Haggblade, S., and Brimer, L. 2011. Chemical safety of cassava products in regions adopting cassava production and processing - Experience from Southern Africa. *Food and Chemical Toxicology*, **49**, 607-612.

Obilie, E. M., Tano-Debrah, K. and Amoa-Awua, W. K. 2004. Souring and breakdown of cyanogenic glycosides during the processing of cassava into akyeke. *International Journal of Food Microbiology*, **93**, 115-121.

O'Brien, G. M. O., Taylor, A. J., and Poulter, N. H. 1991. Improved enzymic assay for cyanogens in fresh and processed cassava. *Journal of the Science of Food and Agriculture*, **56**, 277-289

Okigbo, B. N. 1980. Nutritional implications of projects giving high priority to the production of staples of low nutritive quality: the case for cassava in the humid tropics of West Africa. *Food and Nutrition*, **2,** 4, 1-10.

Osho S. M. 2003. The processing and acceptability of a fortified cassava-based product (gari) with soybean. *Nutrition and food science*, **33**, 278-283

Quiroga, C. C. and Bergenståhl, B. 2008. Rheological Behavior of Amylopectin and β-lactoglobulin Phase-Segregated Aqueous System. Carbohydrate Polymers, **74**, 358-365

Ribotta P. D., Colombo A., León A. E. and Anón M. C. 2007. Effects of soy protein on physical and rheological properties of wheat starch. *Starch/Stärke*, **59**, 614-623

Rickard, J. E. 1985. Physiological deterioration of cassava roots. *Journal of the Science of Food and Agriculture*, **36**, 167-176.

Rosling, H. 1986. Cassava, cyanides and epidemic spastic paraparesis, a study in Mozambique on dietary cyanide exposure. Uppsala University.

Rosling, H. 1988. Cassava toxicity and food security – A review of health effects of cyanide exposure from cassava and of ways to prevent such effects. Uppsala, International Child Health Unit, Uppsala University.

Rosling, H. 1994. Measuring effects in humans of dietary cyanide exposure from cassava. In Bokanga, M., Essers, A. J. A., Poulter, N., Rosling, H. and Tewe, O. (eds), Proceedings of the international workshop on cassava safety, March 1-4, 1994, Ibadan, Nigeria, *Acta Horticulturae*, **375**, 271-283.

Saka, J. D. K., Mhone, A. R. K., and Brimer, L. 1998. An improved microdiffusion method with solid-phase detection for the determination of total cyanogens in fresh cassava. Comparison to the method of Cooke. *Journal of the Science of Food and Agriculture*, **76**, 334-340

Sandoval, E. R., Quintero, A. F., Cuvelier, G., Relkin and P., Pérez, L. A. 2008 Starch Retrogradation in Cassava Flour From Cooked Parenchyma. *Starch/Stärke*, **60**, 174-180.

Sanni, M. O. and Sobamiwa, A. O. 1994. Processing and characteristics of soyabean fortified *gari*. *World of Microbiology and Biotechnology*, **10**, 368-378.

Subbulakshmi, G. and Udipi, S. A. 2006. Food processing and preservation. India, 310p

Sugano, M. 2005. Nutritional Implications of Soy. *Soy in Health and Disease Prevention* (pp. 1-16): CRC Press.

Sundaresan, S., Nambisan, B. and Easwari, A. C. S., 1987. Bitterness in cassava in relation to cyano-glycoside content. *Indian Journal of Agricultural Science*, **57**, 34-40.

Teles, F. F. F. 1995. Toxicidade crônica da mandioca na África e América Latina. *Revista Brasileira de mandioca* **1**, CNPMF, Bahia, Brasil. 107-116.

Ugwu F. M. and Odo M. O. 2008. Effect of cassava variety on the Quality and Shelf Stability of Soy-Barri. *Pakistan Journal of Nutrition*,**7**, 381-384

Vasconcelos, A. T., Twiddy, D. R., Westby, A. And Reilly, P. J. A. 1990. Detoxification of cassava during *gari* preparation. *International Journal of Food Science and Technology*, **25**, 198-203.

Vazques, E., Buzaleh, A., Wider, E. and Batlle, A. 1987. Red blood cell rhodanese: its possible role in modulating aminolaevulinate synthetase activity in mammals. *International Journal of Biochemistry*, **19**, 217-219.

Vetter, J. 2000. Plant cyanogenic glycosides. *Toxicon*, *38*(1), 11-36.

Westby, A. 2002. Cassava utilization, storage and small-scale processing. In Hillocks, R. J., Thresh, J. M. and Bellotti, A. C. (Eds), Cassava biology, production and utilization. CABI Publishing, Wallingford, UK, 281-300.

Westby, A. and Choo, B. K. 1994. Cyanogen reduction during lactic fermentation of cassava. In Bokanga, M., Essers, A. J. A., Poulter, N., Rosling, H. and Tewe, O. (eds), Proceedings of the international

workshop on cassava safety, March 1-4, 1994, Ibadan, Nigeria, *Acta Horticulturae*, **375**, 209-215.

Westby, A. and Twiddy, D. R. 1992. Characterization of *gari* and *fu-fu* preparation procedures in Nigeria. *World Journal of Microbiology and Biotechnology*, **8**, 175-182.

Westley, N. S. 1981. Cyanide and sulfane sulphur. In Vennesland, B., Conn, E. E., Knowles, C. J., Westley, J. and Wissing, F. (eds), Cyanide in Biology. London, Academic Press, 61-76.

Wheatley, C. C. and Chuzel, G. 1993. Cassava: the nature of the tuber and use as raw material. In Macrare, R., Robinson, R. K. and Sadler, M. J. (eds) *Encyclopedia of Food Science, Food Technology and Nutrition*. Academic Press, San Diego, California, 734-743.

White, W. L. B., McMahon, J. M. and Sayre, R. T. 1994. Regulation of cyanogenesis in cassava. In Bokanga, M., Essers, A. J. A., Poulter, N., Rosling, H. and Tewe, O. (eds), Proceedings of the international workshop on cassava safety, March 1-4, 1994, Ibadan, Nigeria, *Acta Horticulturae*, **375**, 69-77.

Zelder, F. H. 2008. Specific Colorimetric Detection of Cyanide Triggered by a Conformational Switch in Vitamin B12. *Inorganic Chemistry*, **47**, 1264-1266.

Zvauya, R., Ernesto, M., Bvochora, T., Tivana, L., and Francisco, J. C. 2002. Effect of village processing methods on cyanogenic potential of cassava flours collected from selected districts of Nampula Province in Mozambique. *International Journal of Food Science & Technology*, **37**, 463-469.