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Cyanide Content of Cassava and Fermented Products with Focus on Attiéké and Attiéké Garba

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TABLE OF CONTENTS

I ABBREVIATIONS

cfu	Colony forming units
CIAT	Centro Internacional de Agricultura Tropical (International Center for Tropical Agriculture)
COSCA	Collaborative study of cassava in Africa
CSRS	Centre Suisse de Recherches Scientifiques
d	Day
dwt	Dry weight
FAO	Food and Agriculture Organization
fwt	Fresh weight
HCN	Hydrocyanic acid
IITA	International Institute of Tropical Agriculture
JECFA	Joint FAO/WHO Expert Committee on Food Additives and Contaminants
KCN	Potassium cyanide
n	Number of samples
SCN	Thiocyanate
TAN	Tropical ataxic neuropathy
TMS	Tropical Manioc Selection
WHO	World Health Organisation
y	Year

II SUMMARY

Cassava (*Manihot esculenta* Crantz) is an important root crop, providing energy for about 500 million people. The roots contain large quantities of the antinutrient factor cyanide, which occurs in the form of two cyanogenic glycosides, linamarin and lotaustralin. Processing of cassava roots will bring the endogeneous enzyme linamarase into contact with the glycosides and will thus release free cyanide and reduce the glycosides' content of the final product.

Attiéké is a fermented product popular in the Côte d'Ivoire. Preparation of attiéké includes the following steps: The roots are peeled, cut to pieces, mixed with a traditional inoculum, ground and left in bags to ferment for about 15 hours. The fermented intermediate is then pressed, dried and steamed to get the final product attiéké. Few years ago, the ethnic group Ebrié began to market a second, lower quality attiéké, called attiéké garba. The preparation of attiéké garba is substantially simpler than attiéké, some steps being shortened or omitted. The influence of abbreviated production on the cyanide content of the end product was studied.

The cassava variety IAC normally used for attiéké preparation in Côte d'Ivoire is a high-cyanogenic variety containing more than 100 mg HCN/kg fresh weight. The present study shows the importance of extensive preparation for varieties with high initial cyanide content for the preparation of attiéké and attiéké garba. The traditional preparation of attiéké can be regarded as safe concerning the cyanide content. A mean value of 5.9 mg HCN/kg dry weight in the end product of 19 attiéké preparations was measured. This is below the tolerated cyanide content of 10 mg/kg dry weight for flour (Codex Alimentarius). A shortening in the preparation process as seen in attiéké garba, does not lead to a significant increase in cyanide content in the end product. In 20 attiéké garba preparations, a mean cyanide content of 9.2 mg/kg dry weight was found.

Three important steps with regard to cyanide removal were identified: fermentation, pressing and steaming. The milling prior to fermentation brings the endogeneous enzyme linamarase into contact with linamarin, starting the decomposition to cyanide. During fermentation, all cyanogenic glycosides are degraded to cyanohydrin and glucose and, subsequently, to HCN. The fermentation time of 15 hours allows the HCN to escape. Total cyanide content is reduced during fermentation by about 30 to 50%. Pressing removes about 20% ot total cyanide for attiéké preparation and about 30% for attiéké garba preparation. By the final steaming, approximately 15% of total cyanide are evaporated. In attiéké garba preparation, the steaming removes about

30% of total cyanide, since more cyanogenic compounds are left in the product before steaming. The cyanide content in the end product is about 2% of the intitial cyanide content in attiéké and about 3% in attiéké garba.

The preparation of attiéké was also carried out with new varieties introduced from the International Institute of Tropical Agriculture (IITA) in Nigeria. The aim of introducing these varieties is to broaden the number of available varieties for farmers and possibly replace the local variety IAC in a few years. The following varieties were used in this study: TMS 92/00057, 92/00061, 92/00067, 92/00325, 92/00326, 92/00398, 92/00427, 92/01425, 92/02327, Anader1, Anader2, Bonoua, IAC, TME1, Okolyawo (TME7) and Olekanga (TME9).

In several varieties such as TMS 92/00061, 92/00067, 92/00325, 92/00326, 92/00398, 92/01425, Anader1, Bonoua, TME1 and Okolyawo, the cyanide content during attiéké production was similiar to the cyanide content of the traditional variety IAC and the remaining cyanides in the end product were below the recommendations of Codex Alimentarius of maximal 10 mg HCN/kg dry weight. However, in some varieties such as TMS 92/00057, 92/00427, 92/02327, Anader2 and Olekanga, the decomposition of cyanogenic compounds was not completed after fermentation and linamarin was recovered in the end product. Cyanide contents between 18 and 140 mg HCN/kg dry weight with a mean value of 65 mg/kg dry weight were found. Results in this study show that in these varieties, linamarase activity was lower than for varieties with low cyanide content in the end product. Therefore, varieties with low linamarase activity should not be used for attiéké preparation.

This study contributes to deepen the knowledge of cyanogenic compounds and their degradation, helping to select new varieties with desired attributes such as yield, resistance against pests and diseases, starch content, cyanide content and quality of attiéké. The enlarged knowledge improves the safety of the end products attiéké and attiéké garba.

III ZUSAMMENFASSUNG

Maniok ist eine wichtige Kulturpflanze und liefert Nahrung für mehr als 500 Millionen Menschen. Die Wurzel enthält grosse Mengen toxischer Blausäure, welche in Form zweier cyanogener Glycoside, Linamarin und Lotaustralin, vorliegt. Die Verarbeitung der Wurzel führt zur Freisetzung von endogener Linamarase, welche die Glycoside zu freien Cyaniden abbaut und so den Blausäuregehalt des Endproduktes reduziert.

Attiéké ist das wichtigste fermentierte Maniokprodukt der Côte d'Ivoire. Die Herstellung umfasst die folgenden Schritte: Die Wurzeln werden geschält und zu Stücken geschnitten, mit einem Inokulum versetzt und vermahlen. Der Brei wird in Säcke gepackt und während 15 Stunden fermentiert. Der Brei wird anschliessend gepresst, zu Körnern geformt und getrocknet. Durch Dämpfen der getrockneten Körner erhält man das Endprodukt Attiéké. Seit ein paar Jahren stellt die Ethnie Ebrié ein zweites, qualitativ schlechteres Produkt her, das sogenannte Attiéké Garba. Bei dessen Herstellung werden einige Schritte abgekürzt oder weggelassen. Diese Arbeit soll klären, ob sich die verkürzte Herstellung negativ auf den Blausäuregehalt des Endproduktes auswirkt.

Für die Herstellung von Attiéké wird normalerweise die lokale Sorte IAC verwendet, eine Sorte mit hohem Blausäuregehalt von mehr als 100 mg HCN/kg Frischgewicht. Die vorliegende Arbeit zeigt, dass die aufwändige Verarbeitung für Sorten mit hohem Blausäuregehalt wichtig ist für den Cyanidgehalt der Endprodukte Attiéké und Attiéké Garba. Die traditionelle Herstellung kann bezüglich Blausäuregehalt als sicher angesehen werden. Ein Durchschnittsgehalt von 5.9 mg HCN/kg Trockenmasse wurde in 19 Attiéképroben gemessen. Dieser Wert liegt unter den Empfehlungen des Codex Alimentarius von maximal 10 mg HCN/kg Trockenmasse für Maniokmehl. Die verkürzte Herstellung von Attiéké Garba führt nicht zu einem signifikant höheren Blausäuregehalt im Endprodukt. Der Durchschnittsgehalt von 20 Attiéké Garba Proben lag bei 9.2 mg HCN/kg Trockenmasse.

Drei wichtige Prozessschritte in Bezug auf Blausäureabnahme wurden identifiziert: Fermentation, Pressen und Dämpfen. Die endogene Linamarase wird bei Mahlen vor der Fermentation freigesetzt, der Abbau von Linamarin zu freien Cyaniden beginnt. Während der Fermentation wird Linamarin vollständig zu Cyanohydrin und Glukose und anschliessend zu HCN abgebaut, welches aus dem Brei freigesetzt wird. Der Gesamtgehalt an Blausäure wird während der Fermentation um 30 bis 50% reduziert, während das Pressen rund 20% der Blausäure bei Attiéké und 30% bei Attiéké Garba entfernt. Beim abschliessenden Dämpfen verdampfen rund 15% der Cyanide bei Attiéké.

Bei Attiéké Garba werden rund 30% entfernt, da der Gehalt an Blausäure vor dem Dämpfen höher liegt als bei Attiéké. Im Endprodukt finden sich rund 2% des ursprünglichen Gehaltes an Blausäure bei Attiéké und rund 3% bei Attiéké Garba.

Die Herstellung von Attiéké wurde auch mit neu eingeführten Sorten des International Institute of Tropical Agriculture (IITA) in Nigeria durchgeführt. Diese Varietäten sollen die Auswahl an verfügbaren Sorten ausweiten und unter Umständen die lokale Sorte IAC ersetzen. Folgende Sorten wurden in dieser Arbeit verwendet: TMS 92/00057, 92/00061, 92/00067, 92/00325, 92/00326, 92/00398, 92/00427, 92/01425, 92/02327, Anader1, Anader2, Bonoua, IAC, TME1, Okolyawo (TME7) and Olekanga (TME9).

In mehreren Sorten (TMS 92/00061, 92/00067, 92/00325, 92/00326, 92/00398, 92/01425, Anader1, Bonoua, TME1 und Okolyawo) entsprach die Abnahme der Blausäure bei der Attiékéherstellung derjenigen der traditionellen Sorte IAC. Im Endprodukt Attiéké lag der Endgehalt der Blausäure unter den Empfehlungen des Codex Alimentarius von maximal 10 mg HCN/kg Trockenmasse. In einigen Sorten hingegen (TMS 92/00057, 92/00427, 92/02327, Anader2 und Olekanga) war der Abbau von Linamarin zu Cyanohydrin und Glukose nach der Fermentation nicht abgeschlossen, und Linamarin konnte im Endprodukt Attiéké gefunden werden. Gehalte zwischen 18 und 140 mg HCN/kg Trockenmasse mit einem Durchschnittsgehalt von 65 mg HCN/kg Trockenmasse wurden gefunden. In dieser Arbeit wurde gezeigt, dass die Aktivität der endogenen Linamarase bei diesen Sorten tiefer ist als bei Sorten mit niedrigem Blausäuregehalt im Endprodukt. Sorten mit niedriger Linamaraseaktivität sollten daher nicht zur Attiékéherstellung verwendet werden.

Diese Arbeit trägt zum besseren Verständnis von Cyaniden und deren Abbau bei und hilft bei der Auswahl neuer Sorten mit erwünschten Eigenschaften wie Erntemenge, Krankheitsresistenz, Stärkegehalt, Blausäuregehalt und Attiékéqualität. Das erweiterte Wissen verbessert auch die Produktsicherheit von Attiéké und Attiéké Garba.

1 INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is an important root crop in Africa, Asia, South America and India, providing energy for about 500 million people (Padmaja, 1995). It counts as one of the leading crops with respect to the energy produced per hectare and year (Bokanga, 2001). The tuber consists of 20 to 25% starch but very limited quantities of proteins, fats, vitamins and minerals (Moorthy and Mathew, 1998). Additionally, the roots contain considerable quantities of the antinutrient factor cyanide. Cyanide occurs in cassava in the form of two cyanogenic glycosides, linamarin and lotaustralin. Hydrolytic enzymes which are capable of breaking down these cyanogenic glycosides to free cyanide (hydrocyanic acid, HCN) are also present in the plant. However, under normal conditions, they are separated from the substrate. Any process that ruptures the cell walls will bring the enzymes into contact with the glycosides and will thus release free cyanide and reduce the glycosides' content of the final product.

Traditionally, cassava roots are processed in a number of ways that vary from region to region, leading to many different products like gari, agbelima, fufu, lafun, farinha, pande yucca etc. Processing steps can include soaking, fermentation, cooking, steaming, chipping, drying and roasting in varying order. A very popular product in the Côte d'Ivoire is called attiéké. Preparation of attiéké includes the following steps: The roots are peeled, cut to pieces, mixed with a traditional inoculum, ground and left in bags to ferment for about fifteen hours. The fermented intermediate is pressed, dried and steamed to get the final product attiéké.

Cassava toxicity in humans is a well-documented problem. Cassava tubers vary widely in their cyanogen content, although most varieties contain 15 to 400 mg HCN per kg fresh weight (Padmaja, 1995). Cyanide doses of 50 to 100 mg are reported to be lethal to adults (Halstrom and Moller, 1945). Whereas acute poisoning occurs scarcely, chronic toxicity is a significant problem. Several diseases are associated with the consumption of inadequately processed cassava roots, such as tropical ataxic neuropathy, endemic goiter, spastic paraparesis and Konzo (Adewusi and Akintonwa, 1994). Sublethal doses of cyanogenic compounds are usually detoxified by the body to the low toxic metabolite thiocyanate, which is excreted in the urine. A chronic overload of thiocyanate in conjunction with low iodine intake, however, results in goiter and, in extreme cases, in cretinism in children (Oke, 1983).

The different techniques of processing cassava roots have one common goal: The reduction of cyanogenic compounds in order to obtain a safe food. The traditional methods usually include chipping, soaking, fermentation, cooking, steaming, drying and roasting. They all permit the enzyme linamarase to interact with the cyanogenic

compounds to release HCN. The HCN then either dissolves in water or escapes into the air. However, it is often impossible to remove all the cyanogenic compounds through processing.

Different methods are available for the quantitative determination of cyanogenic compounds (linamarin, cyanohydrin and free cyanide). The majority require three steps. The first step, extraction of cyanogens, is normally carried out in dilute acid (Bradbury *et al.*, 1994) to stop the degradation of cyanogenic compounds. The second step, degradation of linamarin to cyanohydrin and glucose and, subsequently, to HCN, can be achieved either by autolysis, which relies on the endogeneous linamarase (Cooke and de la Cruz, 1982), by enzymatic hydrolysis by adding exogenous linamarase (Rao and Hahn, 1984) or by acid hydrolysis (Bradbury *et al.*, 1991). For the third step, determination of HCN, various methods have been developed, such as titration with $AgNO_3$ (AOAC, 1990), reaction with alkaline picrate (Egan *et al.*, 1998), and, most widely used, the photometric method based on the König reaction (Cooke, 1978; O'Brian *et al.*, 1991; Essers *et al.*, 1993). Several other methods are available and have been reviewed by Bradbury *et al.* (1994). Since the method based on the König reaction is suitable for a laboratory with limited equipment and for field analysis, it was choosen for this study.

Cyanogenic compounds in cassava and the fermented product attiéké and attiéké garba have not yet been studied in detail. Therefore, this thesis will aim at understanding the degradation mechanism of cyanogenic compounds during preparation of attiéké and attiéké garba and thus help to improve food safety. The first step will be to adapt a simple and sensitive method, which is suitable for a laboratory with limited equipment and for field analysis. In the second step, the content of cyanogenic compounds in cassava, intermediate products and the fermented products attiéké and attiéké garba will be studied. This aims at identifying relevant steps during the processing of cassava with regard to the removal of cyanogenic compounds. In a last step, causes responsible for cyanide removal (micro-organism, drop in pH, heat, leaching etc.) will be indentified.

2 LITERATURE REVIEW

2.1 Overview on cassava (*Manihot esculenta* Crantz)

Cassava (*Manihot esculenta* Crantz) is a perennial shrub grown for its edible tubers and leaves. It was introduced to Africa by Portuguese settlers in the 16th century from Brazil, and is now grown throughout the tropics (Cock, 1985). On a worldwide basis it counts as the sixth important source of energy in human diet; in developing countries it is ranked as the fourth supplier of energy after rice, sugar and maize (Bokanga, 2001).

Cassava is mainly grown as a subsistence crop by small farmers, thanks to its ease of cultivation, cheap production and its ability to tolerate drought and poor soils. Moreover it requires little labour and attention, suffers from few serious pests and diseases and is available throughout the year. Disadvantages are the high moisture content which leads to a fast rottening and expensive transport and the high cyanogenic glycosides content. To increase storage life and to detoxify the products, cassava roots are nearly always processed before consumption.

2.1.1 Botany and cultivation

Cassava is a short-day plant, belonging to the family of Euphorbiaceae, and is mainly grown for its starchy roots. In Africa, the crop is planted preferably between 10°N and 15°S, where the rainfall is higher than 750 mm/y. The highest production can be achieved in tropical low-land where temperature is 25-29°C (Conceição, 1979) and hence about 85% of cassava production is within this climatic zone (Carter and Jones, 1989). Yet, cassava is well adapted to a broad range of climates, and can tolerate temperatures between 16 and 38°C (Cock, 1984). Cassava is well adapted to poor soils with marginal nutritional status and pH from 4 to 9 (Tewe, 2004). The plant cannot withstand frost, so it can only be grown in regions which are frost-free. In favoured areas, the roots can be harvested as early as six to seven months after planting, but most of the local varieties attain maximum yield after 18 months. Improved varieties reach their maximum starch content after 12-15 months (Hillocks, 2002). The cassava roots can remain in the ground for up to three years, but there is a quality loss mainly due to increased lignification (Bokanga, 2001).

Among major food producing plants, cassava is one of the most drought resistant. The crops can be grown in areas with an annual rainfall as low as 500 mm. At the onset of a dry period, the plant reduces its leaf area by shedding some of its older leaves and by ceasing growth. The tolerance of drought up to 6 months mostly depends on the efficient use of water (Onwueme and Charles, 1994). Once established, no critical period with lack of rainfall will cause crop failure.

Connor *et al.* (1981) withheld rainfall for twelve weeks from two cassava varieties ten weeks after planting. After this stress period, they let the plants grow normally until they were ten months old. In one variety, M Col 22, the major storage root bulking occurred during the drought period and storage root growth was significantly reduced. In the other variety, M Mex 59, with later bulking, the growth of storage roots was only slightly reduced. After the stress period the root bulking in M Mex 59 was even higher than growth in the control. Biomass production during the stress period was significantly reduced in both varieties, particularly in plant leaves and stems compared with roots. After ten weeks of stress, both plants rapidly expanded their leaves but the allocation of putting more biomass in roots than stems did not change. In the variety M Mex 59 this led to yields advantage for the stressed plant over the non-stressed control.

Cassava is mostly grown by small-scale farmers. According to Hillocks *et al.* (2002), the main reason for farmers to produce cassava is in response to drought, famine and hunger. The second most important reason is the resistance to pests and diseases. A large investigation, the Collaborative Study of Cassava in Africa (COSCA), revealed that the increased land use for cassava, as cited by farmers, is due to famine and hunger, market demand and population growth (Nweke, 1992a). Hence, cassava is mainly planted as a subsistence crop, since the roots can be left in the ground until needed. Intercropping with other food plants such as maize, sorghum and pigeonpea is very common, more than half of the cassava in Africa is raised under intercropping.

The COSCA study also showed the importance of cassava as a cash crop (Nweke, 1992b). An average of 40% of cassava in Côte d'Ivoire, Ghana, Nigeria, Zaire, Tanzania and Uganda is grown for sale. This proportion is related to market access. In remote areas with little market access, cassava is mainly planted for home consumption. Access to post-harvest handling facilities, such as graters and mills, encourages producing cassava as a cash crop. Cassava is therefore not only important as a food security crop but also as a major source of income for farmers.

Cassava can be grown on soils with low fertility to give reasonable yields but fertilizers are often needed to reach the maximum production potential. However, due to high costs and lack of availability, application of chemical fertilizers is limited. Only about 3% of cassava fields in Africa are fertilized compared to 2% of banana/plantain, 11% of rice, 15% of maize and 20% of yam (Nweke, 1994). Yields are usually low under unfertilized conditions, about 9.0 t ha^{-1} in Africa and about 11.2 t ha^{-1} in South America (Henry and

Hidayat, 1995). Long-term trials over 30 years showed that cassava yields remain constant over time on the same plot of land without fertilizer (Nweke *et al.*, 2004). With improved varieties it is possible to attain 67 t ha^{-1} under optimal conditions using fertilizer, optimal planting time, healthy cuttings and sufficient weeding (Nweke, 1994).

2.1.2 Pest and diseases

Although cassava suffers from fewer pests than other major food plants, some diseases can cause significant damage. Cassava mealy bug and cassava green mites have infested almost all cassava-growing countries in Africa. There are two groups of cassava pests, one group that has co-evolved with cassava and a second group of generalist feeders (see page 21). The latter will mainly attack cassava during dry seasons when cassava is one of the few feed available.

The **cassava mealybug (*Phenacoccus manihoti*)** was introduced into Africa in the early 1970ies from South America. It spread rapidly throughout Africa. This pest can induce severe defoliation (Nwanze *et al.*, 1979), causing substantial yield losses. The control of cassava mealybug by resistant varieties was not successful, since only low levels of resistance could be identified in the more than 3000 varieties at CIAT (Porter, 1988). It was, however, possible to introduce the parasitoid *Apoanagyrus lopezi* from South America.

The **cassava green mite (*Mononychellus tanajoa*)** was also introduced from South America into Africa in the 1970ies. It can cause up to 60% of chlorophyll depletion and a reduction of leaf area of about 50% (Ayanru and Sharma, 1984).

The **African cassava mosaic virus** destroyed 80% of Uganda's crop within six years (Nweke *et al.*, 2004). Thresh *et al.* (1994) reported that about 28 to 40% of the production was lost in 1990 due to African cassava mosaic virus. According to Wydra and Verdier (2002) control of this virus is possible using an integrated approach of weeding and planting of mixed varieties.

Cassava bacterial blight (*Xanthomonas campestris* pv. *manihotis*) is a major constraint to cassava cultivation, and crops loss can reach 50 to 75% when highly susceptible varieties are grown (Wydra and Verdier, 2002). CIAT (1996) estimated yield losses caused by cassava bacterial blight up to 7.5 million tons. To reduce cassava bacterial blight, weeding, mixing varieties, crop rotation and intercropping with maize are recommended (Wydra and Verdier, 2002). Additionally, several resistant cassava varieties are available, which are also resistant to the cassava mosaic disease (Onwueme and Charles, 1994).

2.1.3 Economic importance

For more than 500 million people in Africa, Asia and South America, cassava provides income, employment and food security. The world production has steadily increased, in the last 35 years, the worldwide cassava production doubled (Plucknett *et al.*, 2001). In 2002, about 186 million tons of cassava were produced. More than half of that amount was produced in Africa (55%), the rest in Asia (28%) and South America (17%) (Table 1). About two thirds of the production is grown in Nigeria, Democratic Republic of Congo, Thailand, Indonesia and Brazil. In Côte d'Ivoire, the production of 1.7 million tons is quite low, compared with that of yam, another important root crop with an estimated yield of 3 million tons in 2002 (FAO Stat, 2005).

Tab. 1: Production, imports and exports (in million tons) of cassava in selected countries in 2002 (stock change, waste and other uses not included) (FAO Stat, 2005)

	Production	Domestic supply	Exports	Imports	Food	Feed	Post harvest losses
World	186	185	14.1	16.4	100	47.8	26.8
Africa	102	102	0.02	0.04	64.6	17.0	19.0
Nigeria	34.5	34.5	0.01	0	14.8	8.7	11.0
Congo D.R.	15.0	16.1	0	0	14.7	8.7	0.6
Ghana	9.7	9.7	0	0	4.4	0.2	2.9
Côte d'Ivoire	1.7	1.7	0	0	1.5	0.09	0.01
Asia	51.7	45.7	13.0	10.4	23.8	10.5	4.2
Thailand	16.9	2.1	11.3	0	0.7	0	1.3
Indonesia	17.0	16.6	0.4	0.1	11.8	0.3	2.1
South America	31.4	31.3	0.1	0.1	11.3	15.3	3.2
Brazil	23.1	23.0	0.01	0	7.8	11.6	2.3
Europe	0	4.8	0.7	5.5	0	4.7	0
USA	0	0.2	0.01	0.2	0.03	0.2	0

Cassava is primarily used as food. In Africa, the whole production is used for private consumption, 63% is used as food and only 17% for animal feed. Post-harvest losses of cassava are quite high, for example, almost one fifth of the African production is lost during storage, transportation and processing. In 2002, Thailand exported about 70% of its production and was the leading exporter of cassava products.

Since cassava is produced by many small scale farmers, it is difficult to estimate production in some countries. The above estimates should therefore be interpreted with appropriate caution.

In Africa, the contribution of cassava to total energy intake is greater than for maize or sorghum (Hillocks, 2002). Table 2 summarizes the per capita supply for selected countries in Africa. In the Democratic Republic of Congo, 54% of the total energy intake comes from cassava; in Mozambique it corresponds to about 36% and in Angola to 32%. The average energy supply from cassava in Africa is about 8.5%.

Tab. 2: Cassava per capita supply 2002 (FAO Stat, 2005)

	per capita supply			
	kg/y	**kJ/d**	**total kJ/d**	**% of total kJ/d**
Africa	77.9	867	10153	8.5
Congo, D.R.	286.5	3580	6695	53.5
Mozambique	240	3010	8704	36.4
Angola	242	2763	8721	31.7
Ghana	212.9	2659	11166	23.8
Côte d'Ivoire	93.4	1176	11011	10.7

2.1.4 Composition

The mature cassava plant consists of 6% leaves, 44% stem and 50% storage roots (Tewe, 2004). Only the roots and leaves are nutritionally valuable parts, whereas the stems are usually used for propagation or are burned. Cassava roots consist of 60-66% moisture, 32-35% carbohydrate, 0.4-0.6% protein and 0.1-0.3% fat (Table 3). Cassava starch contains 83% amylopectin and 17% amylose (Rawel and Kroll, 2003). Root bulking usually begins between 45 and 60 days after planting and the starch content reaches a maximum after eight to twelve months. Thereafter lignification leads to an increase in fibre formation and a decrease in starch content. Roots also contain small amounts of maltose, fructose, glucose and sucrose (Tewe, 2004).

Tab. 3: Composition of fresh cassava roots and leaves, yam and potatoes [per 100g fresh weight (fwt)]

Content per 100g	**Cassava tuber**[1]	**Cassava leaves**[1]	**Yams**[2]	**Potatos**[2]	**RDI**[3]
Moisture (g)	60.7 - 66.2	74.8-81	71.2	77.8	
Dry weight (g)	33.8 - 39.3	19 - 25.2	28.8	22.2	
Carbohydrate (g)	32.4 - 35	7	22.4	15.4	
Protein (g)	0.4 - 1.5	5.1 - 6.9	2.0	2.04	63
Fat (g)	0.1 - 0.3	1.0 - 2.0	0.13	0.11	
Dietary fibre (g)	1.43 - 1.57	2.1 - 5.1	3.3	2.51	
Minerals (g)	0.4 - 0.9[2]		1.0	1.02	
Vitamin C (mg)	14.9 - 36	80 - 200	10.0	17	90
Vitamin A (µg)	5.0 - 35.0	8300 - 11800[4]	10.0	10.0	1000
Riboflavin (mg)		0.3			1.3
Calcium (mg)	19 - 22	145 - 350	25.0	6.16	1000
Energy (kJ)	528 - 611	209 - 251	411	298	12000

[1] Bradbury and Holloway, 1988; [2] Souci, 1994; [3] recommended daily intake (Subcommittee on the 10th edition of the RDAs, 1989); [4] Lancaster *et al.*, 1982

2.1.5 Processing into different products

Two factors limit the direct utilisation of fresh cassava. First, the high moisture content leads to rapid deterioration in a few days and poor shelf life. Second, some cassava varieties [see chapter 2.2 (p. 15)] contain high amounts of cyanide and can not be consumed directly. Only cassava with low amounts of cyanide can be safely consumed with little or no processing. Moreover, processing not only helps to improve shelf life and decreases the amount of cyanide but also reduces the bulk of the roots, reducing transportation costs.

The deterioration starts two to three days after harvesting. The blue to black discoloration is referred to as «vascular streaking» and caused by enzymatic processes. The discoloration leads to a loss of acceptability by the customer. Vascular streaking can be slowed down by dipping cassava tubers in warm water (53°C for 45 min), storing under anaerobic conditions, waxing, submerging in water or refrigeration (Onwueme and Charles, 1994). A second deterioration process takes place five to seven days after harvest and is induced by micro-organisms. This rotting and fermentation also causes

discoloration (Wheatley *et al.*, 1984). It is possible to store cassava roots for up to eight weeks after harvest by putting them in boxes with moist sawdust or storing them in clamps.

Cassava processing is normally done by women as confirmed by the COSCA study (Nweke, 1992a), which reported that about 92% of processing is done by women, often together with children. However, the percentage of men involved in cassava processing increases with the extended use of grinders and mills and post-harvest operations becoming more mechanised (Nweke and Bokanga, 1994). Industrial processing using peeling and chipping machines and grinders is still limited and only common in Brazil, Thailand and Indonesia (Rawel and Kroll, 2003).

Cassava is consumed in a number of ways and a myriad of products that differ from region to region have been developed. Processing techniques can include peeling, chipping, soaking, fermentation, cooking, steaming, drying and roasting in varying orders resulting in many different products. Processing sequences may have similar starting steps, then diverging to very different end products. On the contrary, different processes can lead to similar products. Product names are sometimes confusing, since different products may have the same name or similar products may have different names. A general review has been compiled by Lancaster *et al.* (1982).

Gari

In West Africa, gari is by far the most popular form of cassava consumed by about 200 million people daily (Okafor *et al.*, 1998). The tubers are peeled and washed, grated, packed in cloth bags, and dewatered by placing heavy stones on the bags. After two to five days of spontaneous fermentation, which gives the product a characteristic sour flavour, the mash is sieved and then heated in a steel pan. The grains are dried to about 10% moisture to get the final dry granular product gari. The low moisture content permits a long storage period for up to one year in normal atmospheric conditions. Gari is consumed in a variety of ways. The granules can be added to soup or stew, or like in Nigeria, a product called eba can be prepared by mixing gari with hot water to obtain a thick paste (Bokanga, 2001).

Foutou

Foutou refers to a non-fermented sticky dough prepared from starchy roots such as cassava, yam, plantain or cocoayam. These roots may also be mixed together. To prepare foutou, roots are boiled and then pounded in a mortar to achieve a homogeneous dough, to be eaten with vegetables or meat.

Fufu

The term fufu covers very different products depending on the region. In Nigeria, the name refers to a product from fermented roots that are mashed and cooked into a dough (Westby and Twiddy, 1992). In Central Africa, fufu is obtained by mixing cassava flour with hot water. The flour is made either by sun-drying whole roots or chips and then milling, or by soaking roots in water for three to five days, where fermentation occurs, then milling and drying.

Lafun

Lafun is a fermented, pulverised cassava product, known only in Nigeria (Oyewole and Odunfa, 1988). Tubers are peeled, washed and fermented for about three to four days in water. After crumbling, the small pieces are sun-dried and sieved to eliminate woody fibres.

Chickwangue

Chickwangue (or Kwanga) is a fermented cassava bread that is popular in the Congo, in Cameroon and in the Democratic Republic of Congo. To prepare chickwangue, cassava roots are fermented by steeping them in water for three to five days. Then the soft roots are mashed to a pulp and fibres are removed. After kneading, the smooth dough is pre-cooked, wrapped in leaves and steamed to get the final product. Its shelf life is about three to seven days (Regez, 1989)

Farinha

Farinha is a widely commercialised cassava product from Brazil; its production has been mechanised for several decades. Small-to-medium size factories, which are fully mechanised, have an output of 10 to 50 tons per day (Bokanga, 2001). Peeled cassava roots are washed, grated and pressed for a few hours and then sieved. The pulp is then roasted by stirring it in heated trays. The preparation of farinha is similar to gari production, the only difference is that the fermentation time for farinha is much shorter than for gari.

A different product, farinha d'agua, is obtained by soaking roots in water for about three to eight days. Spontaneous fermentation leads to a softening of the roots, which are grated, dewatered and dried like farinha. Farinha d'agua differs in texture, taste and colour from farinha.

Agbelima

Agbelima, a fermented cassava dough, is an important product in terms of production and consumption in Ghana, Benin and Togo. The production of agbelima is similar to the first steps in attiéké production [see chapter 2.1.6 (p. 12)] as an inoculum is used to start the fermentation. The inoculum is prepared by first peeling and cutting of cassava tubers into small chunks. The pieces are then either boiled, roasted on a fire, partially sun-dried or air-dried and wrapped in a piece of cloth previously used for inoculum preparation. The sack is left to ferment in a warm place for two days. Agbelima is then prepared by grating peeled cassava tubers with about 3% of inoculum, packed in sacks and fermented for two to three days. The final product is a smooth sour meal. Amoa-Awua *et al.* (1997) identified four main activities in an inoculum-initiated cassava product:

- The texture of the grated cassava mash is broken down by microbial cellulase.
- The souring is caused by lactic acid bacteria producing predominantly lactic and acetic acid.
- Reduction in cyanide level is enhanced in the presence of an inoculum. This can either be attributed to the endogeneous enzyme linamarase, which is more effective when the cassava tissue is broken down by cellulase, or to the lactic acid bacteria, yeasts and moulds, many of which are able to produce β-glucosidase as well.
- Lactic acid bacteria and yeasts are responsible for aroma production.

Leaf consumption

In some parts of Africa, particularly in the Democratic Republic of Congo, Sierra Leone, Tanzania and Gabon, cassava leaves are used as a green vegetable. The use of cassava leaves as human food has been reviewed by Lancaster and Brooks (1983). Leaves provide a good source of protein, vitamin C, β-carotene (provitamin A) and riboflavin. Rogers (1959) analysed over 100 leaf samples and found a protein content of 20.6 - 36.4 g/100 g dry weight (dwt) [corresponding to 7-12.6 g/100 g fresh weight (fwt)]. Vitamin C contents of 231 mg (in light-coloured leaves) and 482 mg (dark-coloured leaves) per 100 g fwt have been reported (Lancaster and Brooks, 1983). However, considerable losses occur during the preparation of cassava leaves for consumption. For example, boiling for 10 min reduces the vitamin C content by about 60% (Lancaster and Brooks, 1983).

The common way of preparing cassava leaves is by pounding and boiling for a period of 30 min to several hours. Other methods of preparation include sun-drying and grounding into a flour. Cassava leaves normally contain higher amounts of cyanogenic glycosides than roots. Levels of 100 - 1100 mg/kg fwt have been reported (Lancaster and Brooks, 1983). Leaf maturity seems to have the highest impact on cyanide content, which was shown by several authors to decrease between just full-grown leaves and older leaves (Cooke and de la Cruz, 1982; Williams, 1979; Lancaster and Brooks, 1983). However, nutritional status of the plant also seems to influence cyanide levels (Lancaster and

Brooks, 1983). Using traditional processing techniques will reduce cyanide content of the product to low levels (Lancaster and Brooks, 1983). Maduagwu and Umoh (1982) measured the reduction of cyanogenic compounds in a traditional vegetable sauce «mpondu» in Zaire. The sauce is prepared by blanching young cassava leaves for 10 min, mashing and then boiling for 80 min. The initial cyanide content of 325 mg/kg fwt was reduced by this procedure to 25 mg/kg fwt, which corresponds to a reduction of 92%.

The harvest of leaves will, however, decrease tuber yield. Reductions of 50%, 33% and 24% are to be expected when leaves are harvested at intervals of one, two or three months (Lancaster and Brooks, 1983). An optimal leaf harvest with little reduction in yield can be achieved by harvesting at two to three-months intervals.

2.1.6 Attiéké and attiéké garba

Attiéké is a fermented product mainly consumed in Côte d'Ivoire (Firmin, 1998). It is a slightly sour, couscous-like product which is consumed two to three times a day, either with meat, fish or vegetables. It was initially prepared by three ethnic groups, Ebrié, Adjoukrou and Alladjan, in the south of Côte d'Ivoire, but its preparation has extended throughout the country. The largest amounts of attiéké are prepared by these three ethnic groups, which supply the city of Abidjan (Assanvo, 2000). Increasingly, attiéké preparation is spreading to other countries in West Africa (Firmin, 1995), it is also exported to Europe as a dehydrated product (Firmin, 1998). The traditional attiéké preparation changed during the adaption in other regions (see p. 14). However, the ethnic group Ebrié also modified the traditional attiéké production to obtain a second, cheaper product called attiéké garba. The preparation of attiéké garba and the differences between attiéké and attiéké garba is further discussed in the results part in chapter 4.8 (p. 68).

Preparation of attiéké

An extensive study on the traditional attiéké preparation by the ethnic groups Ebrié, Adjoukrou and Alladjan was carried out by Coulin (2004). Attiéké is one of the few products, whose fermentation is not spontaneous but initiated by the addition of a traditional inoculum. The use of an inoculum allows to shorten the fermentation time to 15 h compared with other fermented products like gari or chickwangue, where the fermentation lasts for two to five days.

Preparation of inoculum: The traditional inoculum is prepared by peeling about 10 kg of roots and cooking them for 10 min until they gain a smooth and adhesive sticky surface. After cooling to 30°C, the roots are placed in a jute sack, which is repeatedly used without cleaning for inoculum preparation. The roots are left to ferment for three days at ambient temperature. During this spontaneous fermentation, the roots become soft, sour in taste and smell, and overgrown by a white fungus. After removal of the fungus, the inoculum is submerged in water for 1 h. Before using the inoculum, it is squeezed by hand to remove excessive water. According to the producer, the traditional inoculum is important for the final quality of attiéké (Coulin, 2004).

Peeling: In Côte d'Ivoire, attiéké is mainly prepared from the local variety called «Improved African Cassava» (IAC), which is bitter, pest-resistant and produces high yields. About 50 to 100 kg roots are peeled and cut into pieces to ease milling and eliminate foul spots and the central fibre. The pieces are then washed three times with fresh water to remove dirt.

Milling and fermentation: The milling takes place in a cooperative mill located in the village. Before milling, 5-10% (w/w) of inoculum, 10% (v/w) water and about 0.1% (v/w) of palm oil is added and the pieces are ground to a fine paste, which is placed in large bowls. The mash is left to ferment for about 12 to 15 h at ambient temperature. Women control the end of fermentation by sensorially assessing the texture of the fermented pulp.

Pressing and sieving: After fermentation, a 10 kg-aliquot of mash is placed in a jute sack and pressed continuously in a hand press for an hour. The starchy juice is discarded. The press cake is then passed through two sieves with a mesh size of 5 and 2 mm, respectively, to obtain a fine powder and remove hard fibres.

Granulation and drying: The grains are formed by shaking and rotating the powder in a large bowl. The small particles agglomerate into round grains of up to 5 mm diameter. The grains are sun-dried on black plastic canvas or flat bowls for a time period ranging from a few min up to half an hour depending on heat and grain size. Drying is important for removing fibres but also for reducing the stickiness of grains during the final steaming.

Removing fibres and steaming: After drying, fibres and dirt are removed by sprinkling the grains from a height of about 1.5 m into bowls. Fibres and dirt are blown away by the air draft.

Steaming: If desired, the grains are passed again through a sieve to separate large (> 2 mm) and small grains. Attiéké with large granules («agbodjama») is particularly produced for special occasions such as celebrations, whereas attiéké with small granules («attiéké petits grains») are for everyday consumption. A metallic sieve is attached on a cauldron filled with boiling water, and the grains are poured onto the sieve up to a height of 15 to 20 cm. Steaming lasts for about 20-25 min and causes the grains to turn glassy due to water absorption. The grains are then stirred and steamed for another 5 min.

Packaging and selling: The hot attiéké is now filled into plastic bags, sealed airtight and sold on the market or eaten at home, either with tomato sauce, meat or fish. Hot packaging helps to extend shelf life, which is approximately two weeks without cooling. The shelf life of attiéké is largely determined by its preparation in terms of careful manipulations and good hygiene. Spoilage of attiéké is caused by micro-organisms, which lead to changes in taste and colour, and increase pH (Firmin, 1998). The women need to produce large amounts of attiéké (> 50 kg) several times a week to cover all the needs of the family.

Active micro-organisms during attiéké fermentation: The composition of micro-organisms in the inoculum as well as during fermentation was studied in detail by Coulin *et al.* (2006). They concluded that the traditional inoculum provides the main source of micro-organisms for attiéké production. Final steaming of attiéké leads to a reduction in all vegetative micro-organisms below the detection limit of 100 cfu (colony forming units)/g. Both the acidification and the heat treatment contribute to the long shelf life at ambient temperature.

Adaptation of attiéké production to attiéké garba and other attiéké variants

The production of attiéké is laborious and time consuming. Due to high market demand, the ethnic group Ebrié began to market attiéké of lower quality, called attiéké garba, which is less laborious and less time consuming to produce. Profits are higher, despite the lower price.

The production of attiéké garba is simpler than the one leading to attiéké, some steps being shortened or omitted. The fermentation of attiéké garba is initiated by an inoculum, which is left to ferment for only one day compared to attiéké, where always a two-days inoculum is used. Additionally, less inoculum is added to the milling process, only about 3% of inoculum is used. Compared with attiéké preparation, the pore size in the milling disc is wider for attiéké garba yielding coarser grains in the resulting mash. After fermentation, the mash is pressed, sieved and steamed directly. The granulation and the drying step of the traditional attiéké preparation are omitted. Steaming time is about 10 min shorter than for the attiéké production. The resulting attiéké garba is a sticky and mealy product with a lot of unwanted fibres and provides a high energy source at a low cost. The preparation of attiéké garba is discussed in detail in the results part in chapter 4.8 (p. 68).

Attiéké production is not limited to Côte d'Ivoire. It has also spread to neighbouring countries such as Ghana, Benin and Burkina Faso. The traditional method of preparation has been simplified many times and is now more similar to attiéké garba than to the traditional attiéké.

Obilie *et al.* (2004) describe the following procedure to produce akyeke, an other attiéké variant, which is consumed in south western Ghana. The fermentation of akyeke is also initiated by an inoculum. It is prepared by cutting peeled cassava roots into pieces and submerging them in water for three days. The fermented roots are then sun-dried for three days to serve as inoculum. Akyeke is prepared by peeling and cutting cassava roots, mixing with about 2.5% (w/w) inoculum and grating. The mash is packed into polyethene bags and left to ferment for five days. After pressing, the dough is sieved to obtain granules, which are sun-dried for two hours. The dried granules are steamed to obtain the final product akyeke.

2.2 Cyanogenic compounds in plants

The production of hydrocyanic acid (HCN) from plant tissue containing cyanogenic glucosides was first investigated by Wöhler and Liebig (1837). These cyanides do not exist in the free form as HCN, which is a volatile gas (boiling point 25.7°C; Nweke and Bokanga, 1994), but usually as β-glucosides of α-hydroxynitriles (cyanohydrins). About 3'000 to 12'000 plant species produce considerable amounts of cyanogenic compounds (McMahon *et al.*, 1995). These secondary plant metabolites can also be found in various species consumed by man and animals including cassava, linseed, lima beans, bamboo shots, almonds, sorghum seeds, white clover foliage, passion fruit, flax and macadamia nuts (McMahon *et al.*, 1995; Conn, 1979; Jones, 1998). Interestingly, among the 24 major food crops, at least 16 plants are cyanogenic, although the parts consumed are not necessarily cyanogenic. This is not true for the plants mentioned above, whose cyanogenic parts are consumed by man and animals. If cyanogenesis was evenly distributed among species, one would expect about 3% of all species to be cyanogenic (Jones, 1998). Jones (1998) provided data in support of the function of cyanides as chemical defence of cassava against herbivores such as wild pigs, porcupines, baboons, rats, game, and grasshoppers. Not all researchers agree on this view. In a general overview on the function of cyanogenesis in plants, Kakes (1990) concluded that the proof of the defensive function is difficult to establish. There appears to be few advantages of cyanogenesis and limited knowledge about the preference of large herbivores for cyanogenic or acyanogenic species.

Nevertheless, when plants were domesticated, farmers may have preferred varieties which were eaten less by animals and suffered fewer attacks by pests due to their chemical defence either due to cyanogenesis or other defence systems. This would therefore compensate for the danger associated with eating toxic plants. Additionally, unlike animals, humans have the ability to process food and remove cyanogenic compounds before consumption.

Different terms are used in the literature to describe cyanide content. The terms «total cyanide», «cyanide» and «HCN equivalent» are used for the sum of cyanogenic glycosides, cyanohydrin and HCN. The term «free cyanide» is used for the sum of cyanohydrin and HCN.

The ability to produce HCN from cyanogenic compounds is called cyanogenesis. Two factors are required for the production of HCN: HCN precursors and catabolic enzymes in the cyanogenic plant. The precursors in cassava and many other unrelated plant families are linamarin (2-hydroxyisobutyronitrile-β-D-glucopyranoside) and lotaustralin (2-hydroxy-2-methylbutyronitrile-β-D-glucopyranoside). These two compounds are always present in plants but not necessarily in the same ratio. In cassava linamarin is the dominant cyanogenic glycoside, accounting for 95% (Conn, 1979; Cock, 1985).

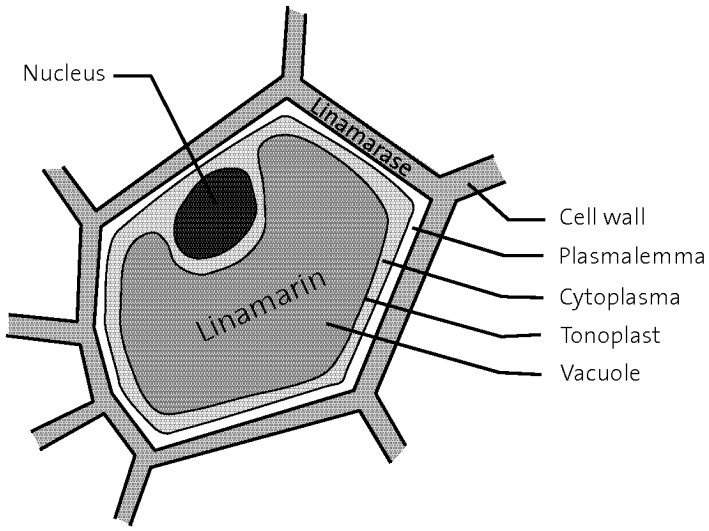


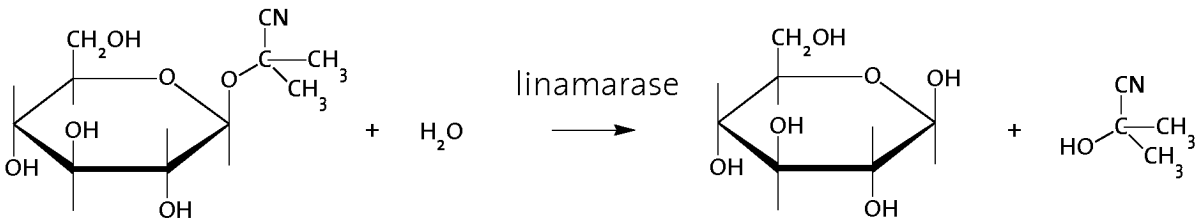
Fig. 1: Distribution of linamarin and its hydrolysing enzyme linamarase (adapted from Kakes, 1990)

Cyanogenesis is catalysed by linamarase (EC 3.2.1.21), a β-glucosidase and by an α-hydroxynitrile lyase (EC 4.1.2.39). Linamarin is stored in the vacuoles of cassava cells (Fig. 1; Kakes, 1990) and hydrolysed by linamarase to cyanohydrin and glucose. Linamarase is stored in the cell wall, separate from linamarin (Mkpong *et al.*, 1990). Any process that ruptures the cell wall brings linamarin into contact with linamarase, thus releasing cyanohydrin and glucose (Fig. 2). Cyanohydrin then breaks down non-enzymatically to

HCN and acetone, at pH above 4 or temperatures above 35°C (McMahon *et al.*, 1995). It can also be degraded by a hydroxynitrile lyase, an enzyme that is mainly expressed in the leaves but with low level

in the roots as well (White *et al.*, 1998). The resulting HCN either evaporates into the air or dissolves in the soaking water and is removed by pressing.

reaction 1



reaction 2

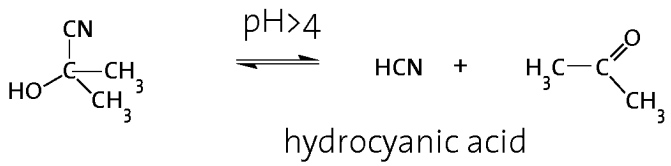


Fig. 2: Catabolism of linamarin to produce cyanohydrin and hydrocyanic acid (HCN) (adapted from McMahon *et al.*, 1995)

Since cyanohydrin decomposes spontaneously at the above mentioned conditions, it is generally assumed that linamarase activity is the limiting factor in cyanide degradation (McMahon *et al.*, 1995). However, the low expression of hydroxynitrile lyase in roots is believed to be an important factor in a disease referred to as Konzo (see page 23). Low activity of hydroxynitrile lyase may also contribute to the accumulation of high amounts of cyanohydrin in certain cassava flours (White *et al.*, 1998).

The activity of linamarase varies significantly between leaves, cortex and parenchyma of roots. The activity in root parenchyma is about 5 to 50 times lower than in leaves and root cortex (Nambisan and Sundaresan, 1994; Yeoh, 1989). The highest linamarase activity was observed at temperatures between 40 and 45°C and at pH 5.5 to 6.0 (Nambisan, 1994), but other authors found an optimal pH of 6.0 to 7.3 (Yeoh, 1989). An activity of 85% of the maximum activity was found at pH 5 and 8 (Yeoh, 1989), but Ampe and Braumann (1995) reported that linamarase activity was still very high at pH 4.0. Linamarase is inactivated at temperatures above 70°C (Nambisan, 1994).

2.2.1 Cyanogenic compounds in cassava and their function

Cyanogenic compounds in cassava are found in all tissues of the plant, with the exception of seeds. The highest quantities are encountered in the leaves and the cortex (peel) of the tuber (Cook, 1985; Brimer and Daalgard, 1984; Balagopalan, 2002; Bradbury and Egan, 1992). In addition, the amount of cyanogenic glycosides not only depends on the variety but also on climatic and cultivation conditions (Cook, 1985). Most reported values for root parenchyma lie between 15 and 400 mg/kg fwt (Dufour, 1988). A few exceptions have been reported, for example: total cyanide level of 617 mg/kg fwt was found in Tanzania (Mlingi and Bainbridge, 1994); 1100 and 780 mg/kg fwt in India (Nambisan, 1994); and 311 to 583 mg/kg fwt (Dufour, 1994) for 13 varieties used by Tukanoan Indians. Higher amounts of cyanide than in the parenchyma have been reported for the root cortex. Values of 730 mg/kg fwt (King and Bradbury, 1995) and a mean value of 1390 mg/kg fwt (Dufour, 1994) for the root cortex of 13 varieties have been found.

Bolhuis (1954) classified cassava based on its cyanogenic potential as «innocuous» (< 50 mg/kg fwt), «moderately poisonous to poisonous» (50 - 100 mg/kg fwt) or «very poisonous» (> 100 mg/kg fwt). This classification was based on the assumption that the minimal poisonous dose for an adult of 50 kg was 50-60 mg HCN (Bolhuis, 1954). At that time, it was only possible to analyse total hydrolysable cyanide as HCN. This approach is still being used today, although it is now possible to separately analyse cyanogenic glycosides, cyanohydrin and HCN. However, the effects of ingesting intact cyanogenic glycosides are still not fully understood.

Additionally, cassava is also grouped into «bitter» and «sweet» varieties. Varieties with a cyanide content of less than 100 mg/kg fwt are considered to be sweet varieties, those with a higher content than 100 mg/kg are bitter varieties. This also refers to the taste of raw roots, which is used by the farmers to distinguish between sweet and bitter varieties. In older literature, cassava (*Manihot esculenta* Crantz) was divided into a bitter species (*Manihot palamata*) and a sweet species (*Manihot aipi*) (Rawel and Kroll, 2003), despite the fact that there is no morphological difference (Mkumbira *et al.*, 2003). This division into sweet and bitter varieties is also used by the farmers, since sweet varieties not need to be processed before eating. Often these roots are just peeled and boiled or may also be eaten raw. Bitter varieties must be processed before consumption to reduce the amount of cyanide.

Only a few publications exist regarding safety levels of cyanide in cassava products. In 1993 the Joint FAO/WHO Expert Committee on Food Additives and Contaminants (JECFA) tried to estimate a safe level of cyanogenic glucosides intake (Speijers, 1993). The committee concluded that in the absence of quantitative toxicological and epidemiological information, a general safe level could not be determined, but a level of

up to 10 mg HCN equivalent/kg dwt, as defined by the Codex Alimentarius (FAO/WHO, 1991), was considered not to be acutely toxic. In Indonesia, the acceptable limit for cassava flour has been set to 40 mg/kg fwt (Damardjati *et al.*, 1993).

Cultivating cassava with low cyanide content is a challenge that has been going on for decades. At the International Institute of Tropical Agriculture (IITA) in Nigeria, several varieties with low cyanide content, giving high yields and with resistance to bacterial blight and mosaic virus disease have been developed (Hahn, 1985). These improved varieties were introduced throughout Africa to increase yields and decrease losses due to pests and diseases.

Varieties with very low cyanide content have been identified, for example levels of 4 mg/kg fwt (Bradbury *et al.*, 1991), 6 mg/kg fwt (de Bruijn, 1983) and 5 mg/kg fwt (Bourdoux *et al.*, 1982). However, variations in cyanide content can be high, probably due to environmental differences. For example, in a variety with a cyanide level of 4 mg/kg fwt, levels of 13-27 mg/kg were reported when the plant was grown in a different location (Bradbury *et al.*, 1991). It has also been possible to generate transgenic cassava plants with a 99% reduction of linamarin compared to the wild type (Siritunga and Sayre, 2003). Significantly, an acyanogenic variety has yet to be identified (Iglesias *et al.*, 2002; Vetter, 2000).

Relationship between bitter taste and cyanogenic glucosides of roots

In practice, taste of cassava roots is used by farmers to distinguish between bitter and sweet cassava varieties and as an indicator for the need of elaborate processing. It would therefore be important that a correlation between bitterness in taste and cyanide content of roots would exist in every case. A positive correlation has been observed by various workers. Sundaresan *et al.* (1987) grouped 38 varieties into non-bitter, bitter and very bitter according to taste. In non-bitter varieties the cyanide content ranged from 27.5 to 77.5 mg/kg fwt, from 100 to 180 mg/kg fwt in bitter varieties and from 320 to 1100 mg/kg fwt in very bitter varieties. Boiling pieces of cassava in water for 30 min rendered bitter varieties non-bitter, reducing the glucosides content to 50 - 80 mg/kg fwt. Cyanide content in very bitter varieties was reduced to 220 - 530 mg/kg fwt, the taste remained bitter.

The farmers ability to predict cyanide levels in cassava has been studied by Chiwona-Karltun *et al.* (2004). In the study, farmers were asked to taste root tips of cassava and score them for bitterness on a five point scale. The correlation coefficient was 0.65, indicating a reasonable relationship between taste scores and cyanide levels of the roots. In parallel, a trained panel also tasted the roots and recorded bitterness on a five point scale. The correlation coefficient of 0.82 was higher than for the farmers. This led the authors to conclude that it is highly probable, that linamarin is responsible for the bitter taste in cassava.

King and Bradbury (1995) identified other compounds than linamarin such as IAG[1], phenylalanine and tryptophan, which contribute to bitterness. These compounds were present in the parenchyma in small quantities, contributing little to bitter taste. However, higher amounts in the cortex contributed significantly to bitterness. Moreover, sugars, citrate and malate were also believed to influence the perception of bitterness. As concluded by King and Bradbury (1995) bitterness of cassava may not always correlate with cyanide content.

Relationship between leaf and root cyanide content

Cassava leaves normally contain higher amounts of cyanide than roots. Some authors have tried to correlate cyanide content of leaves and roots, enabling screening for cassava with low cyanide content by analysing only the leaves. Moh (1976) found a correlation coefficient of 0.59 between the HCN levels in the leaves and roots of 26 cassava varieties, although he used root peels to determine cyanide content of roots instead of root parenchyma. A low positive correlation (correlation coefficient of 0.36) was also found by Cooke *et al.* (1978) by screening 108 varieties and by Mahungu (1994) in four groups of genotypes (correlation coefficients of 0.28, 0.20, 0.23 and 0.30, respectively).

However, Ayanru and Sharma (1984) found no relationship between cyanide content of leaves and roots. They concluded that high or low cyanide content of leaves are not necessarily related to high or low cyanide content in the roots. In another study, Ayanru (1985) observed that cyanide content of leaves increased, decreased or remained unchanged with leaf age, varieties and plant diseases such as mosaic virus disease. This prevents using cassava leaves for screening purposes.

Relationship between linamarase activity and cyanide content

A high linamarase activity would lead to a more efficient elimination of cyanogenic glycosides during processing. Several studies have tried to correlate total cyanide levels with linamarase activity in root parenchyma. Iglesias *et al.* (2002) tried to correlate total cyanide content with linamarase activity of 113 cassava clones derived from six cassava varieties. They confirmed previous studies showing no correlation (Bradbury and Egan, 1992; Nambisian and Sundaresan, 1994). However, they found many clones with low cyanide content and high linamarase activity. Since the addition of exogeneous linamarase can enhance cyanide degradation (Ikediobi and Onyike, 1982), clones with a high linamarase activity may complement the search for varieties that are more suitable for human consumption.

1. isopropyl-β-D-apiofuranosyl-(1->6)-β-D-glucopyranoside

Defence mechanism against pests and pathogens

To date, there is no consensus on the exact role of cyanides in cassava as a defence mechanism against pests and pathogens. It seems that pathogens and pests, such as hornworm (*Erinnyis ello*) and the green mite (*Mononychellus tanajoa*), which have co-evolved with cassava, are not affected by cyanide levels in the plant (Bellotti and Riis, 1994). However, certain generalist feeders such as the African grasshopper (*Zonocerus variegatus*) avoid feeding on cassava with high cyanide content (Bernays *et al.*, 1977). Young nymphs of grasshopper prefer to die rather than feed on cassava. Older nymphs will only feed on cassava when deprived from other feed sources for a considerable period of time. Even adult females fail to gain weight and 3 out of 5 grasshoppers died when they had only access to growing cassava (Bernays *et al.*, 1977). Bernays *et al.* (1977) attributed this to the cyanide levels in cassava, since grasshoppers will feed on diseased spots or yellowing leaves which produce less HCN. Also, when healthy plant leaves are damaged, it is normally the lower leaves, which produce less HCN, that are eaten first. The same study also showed that the rejection is due to the fast HCN gas release after biting a leaf. Wilted leaves, which contain similar amounts of cyanides but do not rapidly produce HCN gas, are eaten without hesitation by grasshoppers.

Nevertheless, grasshoppers can severely defoliate a cassava field in the latter part of a dry season. Shortage of alternative feed sources can lead to (unestimated) production losses (Bellotti and Riis, 1994).

Reduced animal destruction

Farmers often justify the practice of growing bitter varieties, because they are less spoiled by animals (Chiwona-Karltun *et al.*, 1998). Although this has been shown for other plants (Jones, 1998), only a few studies have provided support for reduced destruction of cassava crops. Lal and Maini (1977) reported that varieties with low concentrations of HCN in the root cortex are highly susceptible to rodent attacks. Varieties with high HCN content were shown to suffer less damage and varieties with an HCN content higher than 290 mg/kg fwt remained completely intact.

Farmers preferences

Chiwona-Karltun *et al.* (1998) studied the selection of cassava varieties by Malawi farmers and their motivation to use certain preferred varieties. Normally, farmers grow only a small portion of sweet varieties in their fields, which is intended to be used as a ready to eat snack. The larger part of cassava grown are bitter varieties. Different reasons were given by the farmers for preferring bitter varieties over sweet ones: to discourage human theft, to reduce spoilage by animals, to avoid an unplanned harvest, to obtain higher yields and higher quality of end-products. Protection from human theft was the most important factor, especially in zones, where people tended to steal sweet varieties

to ease their hunger. Bitter varieties were not as attractive since they require a considerable amount of processing to reduce HCN concentrations to safe levels. Another major reason was reduced destruction by animals such as wild pigs, monkeys, baboons, moles and goats. Farmers have reported that animals seem to dig up only sweet varieties. Bitter varieties have also been reported to deter from unwanted harvesting, for example, by family members who try to earn money by selling sweet varieties especially during seasons with scarce amounts of cassava on the market.

Farmers have also stated that bitter varieties will give higher yields. This view is supported by some studies (Nweke and Bokanga, 1994; Nye, 1991), but another study disagrees (Mahungu, 1994). A connection between root yield and bitterness is likely to exist, since farmers have selected bitter varieties for centuries.

Taking into account the possible defence mechanism of cyanide against some pests and the farmers preference for high cyanogenic varieties (Mkumbira *et al.*, 2003), the breeding for low-cyanide varieties might not be a priority for farmers.

Several authors have tried to compare cyanide content of cassava roots with other traits such as yield, number of roots per plant, length or diameter, dry matter or starch content, but to date no significant correlation has been found (Cooke *et al.*, 1978; Mahungu, 1994).

2.2.2 Toxic effects and diseases associated with cyanide exposure

Cassava toxicity in humans is a well-documented problem, however opinions on its importance vary greatly. On one hand, cyanide exposure can cause diseases in humans that may occasionally be serious or fatal. On the other hand, considering the fact that bitter cassava varieties are extensively used, reports on cyanide poisonings in humans are scarce (Akintonwa *et al.*, 1994; Akintonwa and Tunwashe, 1992). This inconsistency may be explained by the various processing methods used to reduce cyanide to low levels [see chapter 2.4 (p. 25)]. Toxicity only seems to be a problem in a few cases. Cyanide poisoning could occur, if a population uses bitter varieties for the first time, without sufficient knowledge on processing, or if short-cuts in processing are introduced due to increased market demand or food shortages. Increased cyanide levels in the roots due to drought may also lead to increasing cyanide exposure (Rosling *et al.*, 1992).

Cyanide is a naturally occurring toxin (Bolhuis, 1954) that has been present throughout human evolution. Hence, the human body developed a certain tolerance towards cyanide exposure. Two defence mechanisms are present. Firstly, the methemoglobin fraction in the red blood cells can temporarily neutralise about 10 mg (0.4 mmol) of cyanide by a reversible reaction (Lundquist *et al.*, 1985). Secondly, an enzyme called rhodanase, which is present in most tissues, converts cyanide to the less toxic

thiocyanate (SCN) by a reaction with sulphans (Rosling, 1994). Normally, about 50 mg cyanide is converted into SCN per day in healthy human tissues (Schulz, 1984). The SCN is then gradually excreted in the urine.

The sulphur required to convert cyanide into SCN is derived from two amino acids, cysteine and methionine. The detoxification reaction is therefore limited by the availability of sulphur-containing amino acids. Since cassava roots contain only small amounts of protein of poor quality, this also contributes to the deficit. Cassava leaves are rich in protein (5-7%) but also deficient in sulphur amino acids (Diasolua *et al.*, 2003).

The fate of ingested cyanogenic glucosides was investigated by Carlson *et al.* (1999). They reported that ingestion of linamarin can lead to a cyanide exposure, although only half of the ingested linamarin was converted to cyanide and then to SCN. One quarter was found unchanged in the urine and one quarter was transformed into an unknown compound. Hence, cyanohydrin is believed to contribute most to the toxicity of cassava products, as it is easily degraded to HCN in the alkaline environment of the gut. Tylleskär *et al.* (1992) also showed that cyanohydrin accounted for most of the cyanogenic compounds in poorly processed cassava roots.

As discussed above, the detoxification of cyanide requires the amino acids cysteine and methionine, causing a depletion of essential sulphur-containing amino acids in the body. However, this is not the only negative consequence. There are also several diseases associated with a high cyanide intake (McMahon *et al.*, 1994):

- hyperthyroidism and goitre, from SCN influencing the iodine metabolism
- tropical ataxic neuropathy
- Konzo, a paralytic disease.

Acute and chronic toxicity

The acute lethal dose of HCN for humans is reported to be 0.5 - 3.5 mg/kg body weight and the acute oral dose of HCN was stated to be 60 mg for an adult (Jones, 1998). Signs of poisoning include vomiting, nausea, headache, dizziness, diarrhoea, mental confusion and stupor followed occasionally by death (Conn, 1979; Mlingi *et al.*, 1992). Indeed, poisoning by cassava and its products containing large amounts of cyanides have been occasionally reported (Mlingi *et al.*, 1992; Akintowa and Tunwashe, 1992; Akintowa *et al.*, 1994; ProMED-mail, 2005).

Konzo: Konzo is a disease that leads to a rapid and permanent paralysis of the legs (Tylleskär *et al.*, 1992). The disease has only been reported in poor, rural areas in Africa. Children and women are predominantly affected, since they may have less access to animal protein and a sufficiently large diversity of food (Boivin, 1997). The disease is caused by the simultaneous occurrence of several facts. Food shortage due to war or a rapid increase in population, low intakes of sulphur-containing amio acids and

shortening of traditional processing methods leading to a high intake of cyanide for several weeks. Reduced processing times are believed to be a major factor leading to high cyanide loads from cassava flour (Tylleskär *et al.*, 1992). Flours consumed in Konzo-affected villages contained a mean cyanide content of 32 mg/kg in rural Zaire and 26 to 186 mg/kg in northern Mozambique (Tylleskär *et al.*, 1992).

Iodine deficiency disorder and goitre: High amounts of SCN in the blood slows down iodine uptake by the thyroid gland (Delange, 1994). If iodine uptake is low, this may worsen iodine deficiency disorders. However, an iodine supply of more than 60 µg per day can compensate for the goitrogenic effect caused by high SCN load. In populations with an adequate iodine uptake, goitre does not develop (Delange, 1994).

Tropical ataxic neuropathy: Similar to Konzo, tropical ataxic neuropathy (TAN) has been attributed to cyanide intake from insufficiently processed cassava roots. TAN is a paralytic disease with a slow onset, which is observed in elderly people who follow a monotonous cassava diet. Symptoms include balance disturbance due to degeneration of the spinal cord, deafness and loss of vision (Osuntokun, 1994; Howlett, 1994)

Other diseases: Exposure to cyanide may also be linked to other diseases such as protein malnutrition or diabetes. High cyanide intake may contribute to protein malnutrition, since essential amino acids are required for detoxification of cyanohydrin to SCN. The low protein content of cassava further contributes to low protein intake (Rosling *et al.*, 1992).

2.3 Methods to measure cyanogenic compounds

Different methods are available for qualitative, semi-quantitative and quantitative determinations of cyanogenic compounds. Several methods have been developed for direct quantification of cyanogenic glycosides (Vetter, 2000). However, the majority of methods use an indirect approach following three separate steps:

- extraction of cyanogens from plant material,
- hydrolysis of cyanogens to cyanohydrin and subsequently to HCN,
- determination of HCN.

The first step, extraction of cyanogens, is normally carried out in dilute acid (Bradbury *et al.*, 1994) to stop the degradation of cyanogenic compounds. The second step can be achieved either by autolysis, which relies on the endogeneous linamarase (Cooke and de la Cruz, 1982), by enzymatic hydrolysis by adding exogeneous linamarase (Rao, 1984, 108) or by acid hydrolysis (Bradbury *et al.*, 1991). Various methods have been developed for the

third step, such as titration with $AgNO_3$ (AOAC, 1990), reaction with alkaline picrate (Egan *et al.*, 1998), and, most widely used, the photometric method based on the König reaction (Cooke, 1978; O'Brian *et al.*, 1991; Essers *et al.*, 1993). Several other methods are available and have been reviewed by Bradbury *et al.* (1994). Since the methods based on the König reaction and on alkaline picrate are suitable for a laboratory with limited equipment and for field analysis, they were chosen for this study.

The determination of the individual cyanogenic compounds, glycosides, cyanohydrin and HCN, respectively, was not possible until Cooke (1978) developed an enzymatic method to analyse the degradation products. Because of its medical significance, most reported data is presented as cyanogenic potential or HCN equivalents rather than cyanogenic glycosides, cyanohydrin and HCN itself. Normally all data is expressed as HCN equivalents per kg fresh or dry weight.

2.4 Processing methods to reduce levels of cyanogenic compounds

The aim of processing of cassava roots is normally to increase shelf life and to remove cyanide. Health problems caused by cyanide exposure as reported on page 22 arise only from insufficient processing of bitter varieties (Padmaja, 1995).

Several studies on remaining cyanides in traditionally prepared foods are available and summarised in table 4. The differences may be explained by the variations in cyanide content of varieties used and by differing processing methods for similar products.

Tab. 4: Total cyanide levels in different ready-to-eat products

product	region	processing method	total cyanide level	standard deviation	fwt / dwt[1]	reference
			mg/kg	mg/kg		
gari	Nigeria	n.a.[3]	25.4	5.0	fwt	Adindu *et al.* (2003)
fufu	Nigeria	n.a.[3]	20	6.0	fwt	Adindu *et al.* (2003)
tapioca	Nigeria	n.a.[3]	17.5	6.2	fwt	Adindu *et al.* (2003)
root slices	south-east Asia	n.a.[3]	53.3	14.8	fwt	Yeoh and Sun (2001)
cassava flour	south-east Asia	n.a.[3]	40.1	15.5	fwt	Yeoh and Sun (2001)
grated root	south-east Asia	n.a.[3]	7.3	6.6	fwt	Yeoh and Sun (2001)
flour	Mozam-bique	sun-drying	59	43	fwt	Cardoso *et al.* (1998)
flour	Mozam-bique	heap fermen-tation	32	26	fwt	Cardoso *et al.* (1998)
flour	Zaire	soaking for 3 days	32	3	fwt	Tylleskär *et al.* (1992)
gari	Nigeria	n.a.[3]	20.5	1.4	fwt	Edijala *et al.* (1999)
gelled gari	Nigeria	gari gelled in boiling water	n.d.[2]	0	fwt	Edijala *et al.* (1999)
fufu	Nigeria	n.a.[3]	24.3	0.2	fwt	Edijala *et al.* (1999)
cassavita	Nigeria	n.a.[3]	5.5	0.03	fwt	Edijala *et al.* (1999)
tapioca	Nigeria	n.a.[3]	11.8	1.5	fwt	Edijala *et al.* (1999)
makopa	Tanzania	sun-dried flour	133	71	dwt	Mlingi and Bainbridge (1994)
chinyanya	Tanzania	sun-dried flour	144	18	dwt	Mlingi and Bainbridge (1994)
akyeke	Ghana	fermented cassava	1.4		dwt	Obilie *et al.* (2004)

[1]fwt: fresh weight / dwt: dry weight; [2] n.d.:not detectable; [3] n.a.: not available

2.4.1 Boiling

Boiling of cassava pieces to remove cyanide was studied by Nambisan and Sundaresan (1985). Samples of different size (A: 6x3x3 cm, B: 3x2x1 cm, C: 1x0.5x0.5 cm) were boiled in water for 30 min. Reduction was highest for the smallest size C, 69-75% of the inital level, followed by size B and size A, where reductions were 50% and 25%, respectively. Most of the cyanide removed was recovered in the water, confirming that cyanide dissolves in water. The effects of different water volumes were also studied by extracting cyanide from cassava pieces at ratios of 1:1, 1:2, 1:5, and 1:10 cassava weight to water volume. An increase in cyanide reduction was observed from 30% (1:1) to 64% (1:2) and 76% (1:5). An increase of water volume to 1:10 did not further decrease cyanide content in cassava pieces.

Free cyanide (cyanohydrin and HCN) was rapidly removed in a cooking experiment by Cooke and Maduagwu (1978), while bound cyanide required 25 min of boiling to achieve a reduction of 55%. In the same study, blanching of thin pieces for 5 to 10 min reduced cyanogenic glycosides by about 50%.

2.4.2 Sun- and oven-drying

To produce makopa, a Tanzanian cassava meal, longitudinally cut cassava roots are sun-dried for 17 d and pound into a flour. Cyanide removal in makopa processing was studied by Mlingi (1995), after an incident of acute intoxication in 1988 (Mlingi *et al.*, 1992). Fresh roots and cassava pieces, sun-dried for 8 and 17 d, were analysed. Cyanide levels, initially 1090 mg/kg dwt, remained high. Only 46% and 63% were eliminated after sun-drying for 8 d and 17 d, respectively. The final cyanide level after 17 d was 401 mg/kg dwt, mostly in the form of cyanogenic glucosides (96%). In a second batch using roots with a lower cyanide content (493 mg/kg dwt), the elimination was 71% after 8 d and 73% after 17 d, resulting in 132 mg/kg dwt in the final product. Products from the market showed similar amounts of cyanides (145 mg/kg dwt).

From multiple stage sampling, the authors concluded that enzymatic hydrolysis stops when the moisture content reaches 13%, the border level for enzymatic hydrolysis. If roots are cut into smaller pieces, the drying will be faster, resulting in even higher levels of cyanogenic glucosides (Mlingi *et al.*, 1992). Since cyanohydrin and HCN are removed when the product is completely dry, the residues in the final product will be cyanogenic glucosides. The conversion of cyanogenic glucosides to cyanohydrin is therefore the limiting step, determining the cyanide content of the final product (Mlingi *et al.*, 1994).

The effects on cyanide removal of different drying temperatures was studied by Nambisan (1985). Samples were cut into 3 and 10 mm thick chips and oven-dried at 50°C or 70°C for 24 h or sun-dried for 18 h. The reduction in cyanide content was 36-40% in 3

mm chips and 50-54% in 10 mm chips dried at 50°C. Drying at 70°C reduced the cyanide content by 20-26% in 3 mm chips and by 40-47% in 10 mm chips. The reduction in sun-dried chips was 42-48% in 3 mm chips and 67-73% in 10 mm chips. These results show that the retention of cyanides is higher for thin chips dried at higher temperature. A minimal reduction of 15 - 20% was observed when chips were baked or fried. This agrees with Cooke and Maduagwu (1978) who found a reduction in cyanogen levels of 29% drying at 46°C and of 10% drying at 80°C.

Based on the above studies, cyanide degradation appears to be affected by chip thickness and drying temperature. Drying chips at higher temperature leads to lower linamarase activity due to a fast removal of water and inactivation of linamarase.

2.4.3 Fermentation

Fermentation is one of the most common practices in cassava processing, about 75% of harvested roots in Africa are processed to fermented products (Westby, 1991), leading to improvements in shelf life, taste and flavour. It is generally accepted that fermentation plays an important role in the reduction of cyanides. We distinguish between two fermentation types:

- fermentation of grated roots, in products such as gari, attiéké and agbelima,
- fermentation of soaked roots, in products such as fufu, chickwange and lafun.

Both types involve lactic acid bacteria, but their contribution to cyanide removal is different.

Fermentation of grated roots: Westby and Choo (1994) reported that in grated roots bacteria play little or no role in cyanide reduction. They compared spontaneously fermented grated roots with irradiated grated roots. In both treatments, about 95% of the linamarin content was hydrolysed in the first three hours after grating. No clear improvement in cyanide reduction was found in the fermented mash, although 64% of the detected lactic acid bacteria were able to hydrolyse linamarin. On the contrary, decreases in pH during fermentation led to a stabilisation of cyanohydrin, slowing down its degradation to HCN. The authors concluded that grating is the important process to bring linamarase into contact with linamarin and that bacteria had a negligible effect on cyanide content. This finding was also reported by Giraud (1993), who showed that a *Lactobacillus plantarum* species with high linamarase activity did not increase the reduction of cyanogenic glucosides in grated roots.

The view that bacteria do not contribute to cyanide reduction was already showed in an earlier work by Maduagwu (1983). In a cassava medium treated with sodium iodoacetate to prevent microbial growth, the rate of cyanide degradation was not affected. On the contrary, when the cassava medium was mixed with 1,5-gluconolactone to inhibit

linamarase activity, cyanide degradation was substantially reduced, even during an ongoing fermentation. The author suggests that the contribution of micro-organisms to cyanide degradation is minimal. However, Amoa-Awua *et al.* (1996) reported that cyanide reduction was enhanced in the presence of an inoculum, used for agbelima preparation (see page 11). In that study cyanogenic compounds in fresh cassava were measured at the start of the fermentation and in the final product after a fermentation time of 72 hours. The cyanide content was reduced by 60% and 82% using a roasted or a thatched inoculum, respectively. The reduction in a control sample without inoculum was only 24%. Based on these findings, the authors concluded that the cellulase present in the inoculum breaks down the cassava tissue, leading to a more intimate contact of cyanogenic compounds and endogeneous linamarase. Cyanogenic compounds in the resulting product agbelima were present in the form of cyanohydrin,which was possibly stabilised by the low pH.

Fermentation of soaked roots: The role of micro-organisms in the fermentation of soaked roots was studied by Westby and Choo (1994). They immersed roots in either untreated water or in water containing an antibiotic to prevent microbial growth. The cyanide reduction exceeded 90% after 3 d in the untreated water, where fermentation took place, whereas a reduction of less than 50% was found in the absence of microbial growth. The reduction strongly correlated with root softening and release of linamarin into the surrounding water. In view of these findings, growth of micro-organisms appears to be important for the loss of cell structure of cassava and subsequently leaching of cyanogens, although other mechanisms, such as β-glucosidase activity of micro-organisms, may also intervene.

Other authors attribute the decrease in cyanogenic compounds to β-glucosidase activity of many micro-organisms in spontaneously fermented products (Kimaryo *et al.*, 2000; Okafor and Ejifor, 1986; Okafor and Ejifor, 1990; Padmaja *et al.*, 1993; Ikediobi and Onyike, 1982). However, the experiments do not show whether the decrease was due to β-glucosidase activity or to root softening, which also leads to a more intimate contact between cyanogenic glycosides and endogeneous linamarase (Westby and Choo, 1994; Ampe and Braumann, 1995). This has been demonstrated by Yeoh (2001), who immersed intact cassava slices in cellulase and linamarase. The addition of β-glucosidase to cassava chips enhanced the release of linamarin and thus improved linamarin hydrolysis.

A high linamarase activity would lead to a more efficient elimination of cyanogenic glucosides during processing. Some studies (Idediobi and Onyike, 1982; Yeoh and Sun, 2001) have demonstrated that addition of exogeneous linamarase could enhance cyanide removal during processing. Ikediobi and Onyike (1982) prepared gari and gradually added exogeneous linamarase to the fermenting pulp each morning. In one batch of gari no exogeneous linamarase was added, one received one dose, one received two doses, and so forth. On the fifth day, the five batches were transformed into gari and

analysed for cyanide content. A considerable improvement in cyanide detoxification was observed in the batch receiving four linamarase additions compared to the batch without exogeneous linamarase. Cyanide content in the final gari ranged from 62 mg/kg fwt with no added linamarase to 33, 29, 22, 20 mg/kg fwt with one, two, three and four additions of linamarase, respectively. Addition of linamarase therefore seemed to improve the rate and extent of cyanide detoxification of gari.

2.4.4 Complex processing methods

As suggested by different authors, the combination of several cassava processing steps can lead to further reduction of cyanide levels. A decrease in cyanide content by 96-99% was found by crushing cassava pieces and sun-drying for 8 h (Nambisan and Sundaresan, 1985). First, crushing permits the enzyme linamarase to come into contact with linamarin resulting in an effective hydrolysis of cyanogenic glucosides. Then, sun-drying removes cyanohydrin and HCN to very low levels of 1.6 - 16.7 mg/kg fwt.

Table 5 summarises processing methods and cyanide reduction for several products with complex prossessing methods and the processing steps required for these products. All studies clearly indicate that the cyanide content of final products not only depend on the processing steps, but also on the sequence of the steps performed.

Tab. 5: Complex processing methods and resulting cyanide reduction in various cassava products

product	processing steps	initial cyanide content	cyanide content of final product	cyanogenic glycosides	cyanohydrin	HCN	reduction in %	reference
		mg/kg dwt	mg/kg dwt	mg/kg dwt	mg/kg dwt	mg/kg dwt		
cassava leaves	pounding of leaves, boiling for 30 min	35.9 - 107.5	0.3 - 1.9				96 - 99	Diasolua *et al.* (2003)
mpondu	Blanching of young cassava leaves, grinding, boiling in water	325	24.8	19.5	5.3		92.4	
makopa	longitudinal quater sections of peeled cassava, sun-dried for 8 and 17 d, pounded and sieved into flour (batch 1)	1090	591 (after 8 days) 401 (after 17 days)	548 383	25 8	18 10	46 63	Mlingi *et al.* (1995)
makopa	longitudinal quater sections of peeled cassava, sun-dried for 8 and 17 d, pounded and sieved into flour (batch 1)	493	145 (after 8 days) 132 (after 17 days)	109 123	31 5	5 4	71 73	Mlingi *et al.* (1995)
chinyanya	peeled roots, pounded into small pieces, sun-dried for 2-3 h, then pounded and sieved into flour (batch 1)	1553 (batch 1) 494 (batch 2) 513 (batch 3)	226 (batch 1) 73(batch 2) 48 (batch 3)				87 (mean)	Mlingi *et al.* (1995)
agbelima	peeled roots, mixed with an inoculum (roasted), grated and left to ferment for 2 d	123.1	49	0	41	8.1	60.2	Amoa-Awua *et al.* (1996)
agbelima	peeled roots, mixed with an inoculum (thatched), grated and left to ferment for 2 d	123.1	22.5	0	22	0.5	81.7	Amoa-Awua *et al.* (1996)
akyeke	peeled roots are mixed with an inoculum, fermented for 5 d, dewatered and sieved and steamed	69.3	1.4	0	1.3	0.1	98.0	Obilie *et al.* (2004)

3 Materials and Methods

3.1 Location and sample collection

The fieldwork was carried out in the village Adiopodoumé near Abidjan in the South of Côte d'Ivoire. For attiéké preparation, the producer used only the bitter variety IAC. The roots originated from the surroundings of Adiopodoumé, the source of the roots changed every day. Attiéké preparation followed a strict time schedule. Roots were purchased by the producer and processing started normally on the same day. On the afternoon of the first day, the roots were peeled, cut to pieces and washed three times with cold water. In the evening, the roots were ground together with the inoculum, water and palm oil and placed in large bowls. The following morning, the mash was filled in jute sacks and pressed for about one hour. The press cake was then sieved and grains were formed, which were sun-dried on black plastic canvas for about half an hour. In the afternoon, the fibres were removed and the grains were steamed and filled into plastic bags. These attiéké bags were sold on the market either directly after packaging or on the following morning.

Samples were taken during the following steps of attiéké production: traditional inoculum, fresh cassava pieces, mash after grinding, mash after fermentation, press cake, grains before and after drying and final attiéké. The samples size was between 100 and 500 g and they were collected in flasks containing 0.1 M phosphoric acid and stored for no more than 2 h at ambient temperature before further treatment. Analytical work was done at the Centre Suisse de Recherches Scientifiques (CSRS) in Abidjan (Côte d'Ivoire) and at the ETH in Zurich (Switzerland).

Cassava roots used for experiments at the ETH were purchased from «Da Leo - siempre fresco» (Zurich, Switzerland). According to the vendor, the roots originated from Costa Rica. The variety of the roots was unknown.

3.2 Sampling and sample preparation

The following cassava varieties were grown at the site in Bringakro, a field station village of CSRS, approximately 200 km north of Abidjan: TMS 92/00057, TMS 92/00061, 92/00067, 92/00325, 92/00326, 92/00398, 92/00427, 92/01425, 92/02327, Anader1, Anader 2, Bonoua, IAC, TME1 and Olekanga and Okolyawo. After harvesting of the roots, they were packed in plastic bags and transported to the CSRS in Abidjan. The bags were stored at ambient temperature in the shade and used within 5 d after harvest. The stability of cyanide content with this procedure has been tested by Dufour (1988).

Since cyanide content varies both longitudinally and radially (Bradbury *et al.*, 1991), a homogeneous sample was obtained by removing both the stem end and the distal end of the tuber and cutting the root into cubes of 1 cm. The cubes were mixed and a sample was taken randomly.

For further sample analysis in Zurich, roots were cut to small pieces, freeze-dried and ground to a fine powder.

3.3 Determination of dry matter and pH

Determination of dry weight

Cassava roots were peeled, chopped into small pieces and portions of about 10 g were dried for 24 h at 95°C in an oven (Heraeus, type FB420, Hanau, Germany). About 10 g were also taken from the samples of the attiéké production and dried for 24 h at 95°C. All samples were then left to cool down in an exsiccator. The loss in weight was used for the calculation of dry weight (Iglesias *et al.*, 2002).

Determination of pH

Ten grams of sample were homogenised with 90 ml of distilled water (Polytron, Kinematica, Littau, Switzerland), and the pH of the sample was measured using a pH meter model 744 (Metrohm AG, Herisau, Switzerland) according to the procedure described in the Swiss Food Manual (Schweizerisches Lebensmittel-Buch, 2001).

3.4 Determination of cyanogenic compounds

3.4.1 Chemicals

Chemicals used for the determination of cyanogenic compounds were: linamarase (VWR International AG, Dietikon, Switzerland, BDHA391172R), barbituric acid (Fluka AG, Buchs, Switzerland, 11710), trisodiumphosphate hexahydrate (Fluka, 71906), isonicotinic acid (Fluka, 58930), sodium hydroxide (Fluka, 71690), phosphoric acid 85% (Riedel-de Haën Laborchemikalien GmbH & Co KG, Seelze, Germany, 30417), acetone cyanohydrin (Siegma-Aldrich Chemie GmbH, Steinheim, Germany, A1000-0), chloramin T (Riedel-de Haën, 31224), ammonium sulphate (Fluka, 9982), ethanol (Fluka, 02860), hydrochloric acid (J.T. Baker, Deventer, The Netherlands, 6081), sodium dihydrogenphosphate (Fluka, 71506), disodium hydrogenphosphate (Fluka, 71639), sodium carbonate (Fluka, 71350), linamarin (Toronto Research Chemicals, North York, Canada, L466000). All chemicals were of analytical grade.

3.4.2 Extraction of cyanogenic compounds

Solutions

For the extraction of cyanogenic compounds, the following solutions were prepared:

acid extraction medium: 0.1 M phosphoric acid in distilled water

ethanol/acid extraction medium: 75% (v/v) of acid extraction medium and 25% (v/v) ethanol.

Extraction procedure

(i) **Fresh cassava and intermediate products**: Samples of 10 g were homogenised in 30 ml of acid extraction medium (Polytron). The amount of sample varied, but the ratio of sample to extraction medium was always approximately 1 : 3. The homogenised samples were left to stand for 10 min and then centrifuged at 10'000 g for 10 min (Beckman, J-25i, Fullerton, USA). The supernatant was stored at 4°C until assayed for cyanogenic compounds.

(ii) **Attiéké and other cooked products with gelatinised starch**: Samples of 10 g were homogenised in 30 ml of ethanol/acid extraction medium and further treated as in (i).

3.4.3 Enzymatic procedure

The procedure for the enzymatic hydrolysis was developed by Cooke (1978) and allows to determine total cyanide, free cyanide and HCN. The method was further improved by O'Brien *et al.* (1991) by introducing an ethanol/acid extraction step, which simplified extraction of cyanide from cooked cassava products containing gelatinised starch. The photometric procedure used in this work was developed by Essers *et al.* (1993), who has replaced the toxic pyridine/pyrazolone colour reagent used by Cooke (1978) by a less toxic isonicotinic/chloramin T reagent.

The linamarase used in the assay was either purified from cassava root cortex as described in chapter 3.5 (p. 40) or purchased from VWR International AG.

Solutions

Phosphate buffer pH 7.0, 6.0 and 4.0 were prepared from 0.1 M H_3PO_4 and 0.1 M Na_3PO_4. Linamarase from BDH was dissolved in phosphate buffer pH 6.0 to give an activity of 5 enzyme units (EU)/ml (hydrolysis of 5 µmol of linamarin per min at 30°C in phosphate buffer pH 6.0). Chloramin T reagent was prepared by dissolving 0.5 g of chloramin T in 100 ml water. The isonicotinic acid / barbituric acid reagent was prepared by dissolving 3.5 g barbituric acid and 2.85 g isonicotinic acid in 0.5 M NaOH solution. The pH of this reagent was adjusted between 7 and 8 with 2 M HCl or NaOH, respectively.

Acetone cyanohydrin, used to calibrate the samples, was prepared as follows: A stock solution of 628 mg cyanohydrin per litre in 0.1 M phosphoric acid (corresponding to 200 mg HCN/l) was diluted in 0.1 M phosphoric acid so that the standard solutions contained 3.1, 9.4, 15.7, 25.1, 31.4, 47.1 and 62.8 mg/l cyanohydrin (corresponding to 1, 3, 5, 8, 10, 15 and 20 mg HCN/l).

Assay procedure

(i) **Total cyanide** (cyanogenic glycosides + cyanohydrin + HCN): In a stoppered 1.5 ml tube 0.1 ml extract and 0.05 ml linamarase were added to 0.45 ml phosphate buffer pH 7.0. After incubation at 37°C for 30 min (Julabo VC/3, Julabo Labortechnik GmbH, Seelbach, Germany), the mixture was transferred to a 15 ml tube containing 0.6 ml 0.2 M NaOH. After 5 min, the sample was diluted with additional 2.8 ml phosphate buffer pH 6.0 and analysed in the photometric procedure.

(ii) Free cyanide (cyanohydrin + HCN): An amount of 0.1 ml extract was mixed with 0.4 ml phosphate buffer pH 4.0 in a 15 ml tube, and 0.6 ml 0.2 M NaOH was added. After 5 min, 2.9 ml phosphate buffer pH 4.0 was added and the mixture was analysed photometrically.

(iii) HCN: In a 15 ml tube, 0.1 ml extract and 3.9 ml phosphate buffer pH 4.0 were mixed and analysed photometrically.

(iv) Calibration standards: Standard solutions were assayed as described in (ii). The calibration curve was established at least once a day.

To the samples, 0.1 ml chloramin T reagent was added and mixed on a shaker (Vortex, GMB Glasmechanik AG, Therwil, Switzerland). After 5 min, 0.6 ml colour reagent (isonicotinic acid / barbituric acid reagent) was added and mixed well. The absorbance was measured photometrically after 20 min at 600 nm. In Zurich, a Varian, Cary 100 photometer (Varian, Palo Alto, USA) was used, in Côte d'Ivoire a Photolab S12 WTW photometer (Photolab, Weilheim, Germany). Duplicate analyses for samples and standard solutions were performed. A flow sheet of the assay procedure is shown in Fig. 3.

Calculation of the cyanide content

Total cyanide, free cyanide and HCN contents of the samples were calculated as mg HCN equivalent / kg dwt using the formulas I to IV.

$$\text{Extraction factor} = \frac{\text{weight}_{\text{sample}}(g) + \text{extraction media (g)}}{\text{weight}_{\text{sample}}(g)} \quad \text{(I)}$$

$$\text{Cyanide content}_{\text{sample solution}}(mg/l) = \frac{\text{absorption}_{\text{sample}} - \text{y-intercept}_{\text{standard}}}{\text{slope}_{\text{standard}}} \quad \text{(II)}$$

$$\text{Cyanide content (mg/kg fwt)} = \text{dilution factor}_{\text{sample}} \times \text{extraction factor} \times \text{cyanide content}_{\text{sample solution}} \quad \text{(III)}$$

$$\text{Cyanide content (mg/kg dwt)} = \frac{\text{cyanide content}_{\text{fwt}} \times 100}{\text{dry weight}_{\text{sample}}} \quad \text{(IV)}$$

Cyanogenic glycosides were calculated as (total cyanide minus free cyanide) and cyanohydrin as (free cyanide minus HCN). Because cyanogenic glycosides and cyanohydrin were calculated by subtraction, negative values were sometimes obtained, when the concentration of cyanogenic glycosides or cyanohydrin were very low in the samples.

In this study, we generally use HCN equivalent per kg dwt to account for different water levels in products.

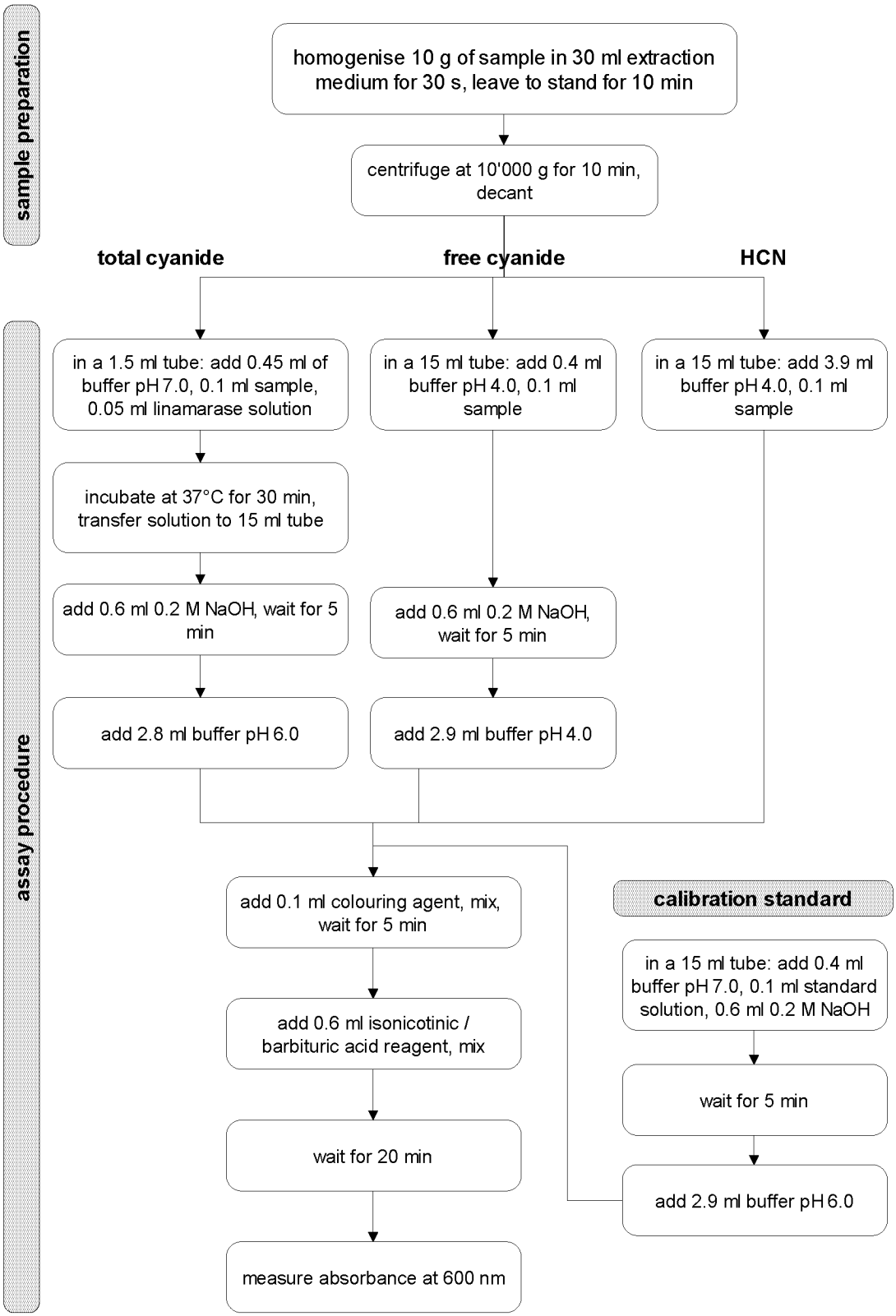


Fig. 3: Flow sheet of the assay procedure to determine cyanogenic compounds according to O'Brien *et al.* (1991) and Essers *et al.* (1993)

3.5 Purification of linamarase from cassava root cortex

3.5.1 Isolation of linamarase

Cassava linamarase for use in the cyanide determination reaction [see chapter 3.4.3 (p. 36)] was purified from cassava root cortex using the method described by Cooke *et al.* (1978) as adapted by Nambisan (1999). Linamarase was prepared by homogenising about 25 g cassava root cortex in 200 ml 0.1 M phosphate buffer pH 6.0. Extracts were centrifuged at 10'000 g for 15 min, the pellet was discarded and the supernatant was brought to 60% saturation with ammonium sulphate and stirred overnight (IKA Labortechnik, RTCbasic, Staufen, Germany). The precipitate obtained by centrifuging at 10'000 g for 30 min was dissolved in 5 ml 0.01 M phosphate buffer pH 6.0 and dialysed (Servapor dialysis tubing, 44145, Serva Electrophoresis GmbH, Heidelberg, Germany) against 300 ml 0.01 M phosphate buffer pH 6.0. After 4 to 5 h, dialysis buffer was replaced with fresh 0.01 M phosphate buffer pH 6.0. Dialysed extracts were centifuged at 10'000 g for 15 min and the supernatant was stored at -20°C until used.

3.5.2 Determination of linamarase activity in root cortex

The activity of the linamarase from root cortex was compared to the activity of the commercial linamarase purchased from BDH. In a 1.5 ml tube, aliquots of 10, 20 and 50 µl were added to 490, 480 and 450 µl 0.1 M phosphate buffer pH 7.0, respectively. After addition of 100 µl linamarin standard solution (200 mg linamarin/l), samples were incubated for 15 and 30 min respectively and transferred to a 15 ml tube containing 0.6 ml 0.2 M NaOH. After 5 min, the sample was diluted with 2.8 ml 0.1 M phosphate buffer pH 6.0, then 0.1 ml chloramin T reagent was added and the samples were mixed on a shaker. After 5 min, 0.6 ml colour reagent was added and mixed well. The absorbance was measured after 20 min at 600 nm. The activity of the linamarase from root cortex had to be equal or higher than the activity of the commercial linamarase to be used as substitute for the commercial linamarase in the assay procedure.

3.6 Determination of linamarase activity in root parenchyma

Activity of linamarase extracted from root parenchyma was measured as follows: In a 15 ml tube, 1.2 g of lyophilised root parenchyma and 10 ml of 0.1 M phosphate buffer pH 6.0 were mixed on a shaker (Vortex) and left to stand for 20 min. Extracts were centrifuged at 10'000 g for 15 min and the supernatants were used in the following assays:

Total HCN present in the extract plus HCN liberated by the extract: In a stoppered 1.5 ml tube 0.1 ml linamarin standard (200 mg linamarin/l) and 0.05 ml extract were added to 0.45 ml of 0.1 M phosphate buffer pH 7.0.

HCN content of the extract: In a stoppered 1.5 ml tube 0.05 ml extract was added to 0.55 ml of 0.1 M phosphate buffer pH 7.0.

HCN content in the linamarin standard (blank): In a stoppered 1.5 ml tube 0.1 ml linamarin standard (200 mg linamarin/l) was added to 0.5 ml of 0.1 M phosphate buffer pH 7.0.

All samples were analysed for total cyanide [see chapter 3.4.3 (p. 36)]. At the beginning and after incubation at 37°C for 4 h, the mixture was transferred to a 15 ml tube containing 0.6 ml 0.2 M NaOH. After 5 min, the sample was diluted with 2.8 ml phosphate buffer pH 6.0 and assayed in the photometric procedure described in chapter 3.4.3 (p. 36).

HCN liberated by the linamarase in the extract was calculated by subtracting HCN content of the extract (ii) and the blank (iii) from total HCN (i).

3.7 Small scale preparation of attiéké and fermentation experiments

Attiéké preparation at small scale: To follow the process more closely, attiéké preparations were carried out with different cassava varieties at the CSRS in Abidjan by two producers from the village Adiopodoumé. They followed the traditional method of attiéké preparation except that the batch size for all varieties was lowered. About 15 kg of cassava roots were peeled, cut to pieces (circa 3 x 3 x 3 cm) and washed tree times with water, about 10% (w/w) of inoculum, 10% (v/w) of water and about 0.1% (v/w) of palm oil was added. The pieces were ground to a fine paste, placed in large bowls and left to ferment at an ambient temperature of about 30°C for about 12 to 15 h. The rest of the procedure was carried out as for the tradtitional attiéké preparation described in chapter 2.1.6 (p. 12). Samples were taken identically to the traditional attiéké preparations for the steps: fresh roots, mash after grinding, mash after fermentation, press cake, grains before and after drying and end product attiéké. All samples were analysed for dry weight [see chapter 3.3 (p. 34)], total cyanide, free cyanide and HCN [see chapter 3.4 (p. 35)].

Fermentation experiments: Fermentation experiments were carried out at the CSRS in Abidjan with different cassava varieties by peeling and cutting to pieces (circa 3 x 3 x 3 cm) of about 5 kg cassava roots. Pieces were washed three times with water and mixed with 10% (w/w) traditional inoculum, obtained in the village Adiopodoumé, and 10% (v/w) of water. After grinding in a large meat mincer, the mash was placed in large bowles and stored at an ambient temperature of about 30°C. Samples were taken at 0, 30, 60, 90, 120, 180, 300 and 600 min and analysed for dry weight [see chapter 3.3 (p. 34)], total cyanide, free cyanide and HCN [see chapter 3.4 (p. 35)].

3.8 Model to produce attiéké and attiéké garba

A standardised attiéké preparation model was used to understand cyanide degradation and allowed to change parameters (time, amount of inoculum, steaming time etc.) and to study their influence on the cyanide content of the end product. The model developed by Coulin (2004) was used to produce attiéké.

Preparation of inoculum

Inoculum was prepared by cutting about 2 kg of peeled cassava roots in large pieces (circa 10 cm long) and cooking them in water (water: cassava = circa 5:1) about 10 min until they were soft. After cooling down, the pieces were wrapped in a jute sack previously used for inoculum preparation and incubated at 30°C for 3 d in a incubater (Heraeus, type FB420, Hanau, Germany). After removal of the mycelia, the inoculum was washed in water and squeezed by hand to remove excessive water. The inoculum was stored at 4°C for up to one week until needed.

Attiéké production

The model to prepare attiéké in small quantities included the following steps: about 3 kg of roots were peeled, cut into small pieces (circa 3x3x3 cm)and washed twice with water. The washed pieces, 10% (w/w) of inoculum and about 0.1 % (v/w) of palm oil were milled using a meat mincer (type 49.1, Rotel, Aarburg, Switzerland). After addition of 10% (v/w) of water, the mash was mixed and left to ferment at 30°C overnight in an incubator. The fermented mash was filled in a cloth and pressed using a hand press. The press cake was then sieved through two sieves with a mesh size of 5 and 2 mm to obtain a fine powder and remove hard fibres. The grains were formed by shaking and rotating the powder in a large bowl. The granules were dried in a dryer / dehydrator (Dörrex, type L0075, Stöckli, Netstal, Switzerland) for about 15 min. Removing of fibres was not necessary, since roots bought in Switzerland normally contain only small amounts of fibres. The grains were steamed for about 20 min until they were glassy using a household steamer (Vitasteam Interstar Deluxe, JP689-T, Interdiscount, Jegenstorf, Switzerland).

The contribution of the inoculum to the breakdown of linamarin was studied as follows: About 3 kg of roots were peeled, cut to pieces (circa 3x3x3 cm) and washed three times with water. One part was mixed with 10% (w/w) inoculum and 10% (v/w) water, the other part only with 10% (v/w) of water. Samples were taken at an interval of 30 min and analysed for total cyanide, free cyanide and HCN, according to chapter 3.4 (p. 35).

Attiéké garba production

To produce attiéké garba at laboratory scale, the model to produce attiéké was altered in the following steps:

- More palm oil [about 1.5% (v/w)] was added.
- Fermentation time was shortened to 3 to 5 h.
- The press cake was sieved only once through the 5 mm mesh.
- The sieved flour was neither formed to grains nor dried.

The end product attiéké garba had a strong yellow colour.

3.9 Preparation of various cassava products

Bonoua raw and cooked

To determine cyanide reduction during cooking, peeled cassava pieces of the variety Bonoua were cut to pieces of about 10 cm length and cooked in salted water for about 20 min (water:cassava = circa 5:1). Cyanogenic compounds and dry weight were determined before and after treatment as described in chapter 3.4.3 (p. 36).

Placali and placali flour

Placali and placali flour were purchased on the market in Adiopodoumé. Placali is prepared similar to the first steps in attiéké production. In fact, the mash after fermentation of an attiéké production will occasionally be used to prepare placali. Preparation runs as follows: Roots are peeled, cut to pieces, ground together with an inoculum and left to ferment overnight. The mash is then pressed and sieved to remove fibres. The resulting powder is cooked in water to gain a sticky, highly viscous dough. Placali flour (pressed fermented mash) can also be purchased on the market and is then prepared at home. Cyanogenic compounds and dry weight were determined before and after treatment as described in chapter 3.4.3 (p. 36).

Foutou manioc and foutou banane

To prepare foutou, cassava pieces (for foutou manioc) or cassava pieces and plantaine (foutou banane) were cooked. The soft pieces were ground in a mortar to obtain foutou, a sticky and highly viscous dough. Cyanogenic compounds and dry weight were determined before and after treatment as described in chapter 3.4.3 (p. 36).

4 Results and Discussion

4.1 Preservation of samples at different temperatures

Samples taken during different processing steps in attiéké and attiéké garba production had to be stored in diluted phosphoric acid until assayed for cyanogenic compounds as described in chapter 3.1 (p. 33). The use of dilute phosphoric acid, as suggested by Cooke (1978), has various advantages. The endogeneous enzyme linamarase is inhibited by the low pH and cyanohydrin is stabilised and not further broken down to HCN.

The preservation of samples and the inhibition of the degradation of cyanogenic compounds to free cyanide is a prerequisite for the study of changes in cyanogenic compounds during processing and storage of cassava products. This was particularly important for samples obtained from products prepared in the village Adiopodoumé, which could not be analysed immediately and had to be stored for some time.

To check the inhibition of linamarase and the stability of phosphoric acid extracts, the cyanogenic compounds from freshly ground cassava pieces were extracted and determined as described in chapter 3.4 (p. 35). Aliquots were stored at -25°C, at 4°C, at room temperature and at 37°C, respectively. After 7 d, cyanogenic compounds were determined and all samples were stored at 4°C for 21 d. The results are summarised in Table 6.

The initial cyanide content was 12.16 mg/kg fwt linamarin, 0.41 mg/kg cyanohydrin and 0.79 mg/kg HCN. Linamarin content was stable over the storage period of 28 d and changed only slightly in all samples. Storage at room temperature or 37°C resulted in a higher decrease in linamarin than storage at 4°C or at -25°C. Cyanohydrin and HCN were less stable, however, since these compounds were present in low concentrations in these samples, the results should be interpreted with care.

Tab. 6: Cyanogenic compounds (in mg/kg fwt) of phosphoric acid extracts of freshly ground roots stored at various temperatures.

storage temperature	after 7 d			7 d treatment, then 21 d at 4°C			change in % after 28 d		
[°C]	linamarin	cyano-hydrin	HCN	linamarin	cyano-hydrin	HCN	linamarin	cyano-hydrin	HCN
-25°C	12.73	0.33	0.76	12.45	0.31	0.58	2.4	-24.4	-26.6
4°C	12.19	0.41	0.74	11.79	0.41	0.52	-3.0	0	-34.2
20-25°C	12.18	0.49	0.69	11.14	0.46	0.51	-8.4	12.2	-35.4
37°C	11.94	0.40	0.75	11.41	0.30	0.62	-6.2	-26.8	-21.5

Initial cyanide content: 12.16 mg/kg linamarin, 0.41 mg/kg cyanohydrin, 0.79 mg/kg HCN

To check the stability of phosphoric acid extracts, a second experiment was carried out. Cyanogenic compounds of freshly ground roots and mash before fermentation were extracted and stored at 4°C for 14 d. Changes in cyanogenic compounds are listed in Table 7. After 14 days, linamarin content in ground roots increased on average by 14.6% and by 24.1% in the mash before fermentation. The increase may be due to a reverse reaction of cyanohydrin to linamarin, however, this assumption has to be proven with further experiments. O'Brien *et al.* (1991) also reported an increase in total cyanogens of 4.9% after storing samples at 4°C for 15 d. Free cyanide decreased by 8.1% in the same period and a total increase of linamarin of 13% was reported.

In this study, cyanohydrin content increased by 7.1% in the ground roots and decreased by 3.4% in the mash before fermentation. HCN content decreased by 8.1% and 4.7% after two weeks in the ground roots and the mash before fermentation, respectively. The high volatility of HCN at low pH may explain the decrease. Changes in cyanide content were also reported by Essers (1993), who stored cassava samples extracted with phosphoric acid at different temperatures [room temperature (21-28°C), 2-6°C and -18°C]. Total cyanide content remained constant after 28 d in the different treatments, free cyanide, however, decreased by 7%.

Tab. 7: Cyanide content (in mg/kg dwt) of ground roots and mash before fermentation, stored at 4°C for 14 d.

storage time	fresh			14 d			change in % after 14 d		
sample	linamarin	cyano-hydrin	HCN	linamarin	cyano-hydrin	HCN	linamarin	cyano-hydrin	HCN
ground roots	91.8	3.6	5.7	103.2	3.9	5.2	12.3	10.3	-7.5
ground roots	113.5	5.2	6.0	132.7	5.4	5.5	16.9	4.0	-8.7
mash	53.1	55.0	15.8	70.6	53.4	14.9	33.1	-2.8	-5.7
mash	83.7	27.8	9.1	97.0	27.0	8.7	15.9	-2.9	-4.6
mash	49.1	48.0	24.4	60.4	45.9	23.5	23.2	-4.4	-3.8

From the above experiments we concluded that collecting samples in remote villages was possible without cooling (Table 6). Cyanogenic compounds changed only slightly even after 7 d of storage at 37°C. Changes in cyanogenic compounds were expected to be smaller than reported since the storage time for the samples collected in Adiopodoumé was less than one week. The smallest changes in linamarin and cyanohydrin content occurred during the storage at 4°C (Table 6), therefore that treatment was used throughout this study.

4.2 Standards to measure cyanogenic compounds

The amount of cyanogenic compounds in cassava and the degradation products of these cyanogenic compounds were measured by comparing the absorption at 600 nm to a calibration curve. This curve can be obtained by using different cyanide containing substances, such as linamarin, cyanohydrin or potassium cyanide (KCN). These substances were tested for their suitability to serve as standards. The calibration curve with linamarin was assayed as «total cyanide» as described in chapter 3.4.3 (p. 36). The calibration curves with cyanohydrin and KCN were assayed as «free cyanide» as described in chapter 3.4.3 (p. 36).

The three calibration curves obtained with linamarin, cyanohydrin and KCN were compared in Fig. 4. The curves were almost identical, standard deviations were small, indicating a good reproducibility. The correlation coefficients were excellent and each calibration curve could be used to determine cyanogenic compounds in samples. However, the slope for linamarin is slightly lower than for cyanohydrin and KCN. This finding is discussed in detail by Essers *et al.* (1993). They suggest to use a linamarin calibration curve for products, where cyanogenic compounds are almost exclusively present as cyanogenic glycosides. For processed roots, where cyanogenic compounds are present as cyanohydrin and HCN, a cyanohydrin or KCN calibration curve should be used. However, the proportion of linamarin, cyanohydrin and KCN are not known beforehand for most products. Considering that pure linamarin is difficult to obtain and the solid KCN is more difficult to handle than the liquid cyanohydrin, the latter was chosen as standard throughout this study. Since samples taken from processing steps normally contain cyanides as cyanohydrin and HCN, a calibration curve with cyanohydrin is optimal.

As the calibration curve changed slightly form day to day, calibration curves were run at least once a day to calculate appropriate conversion factors for samples.

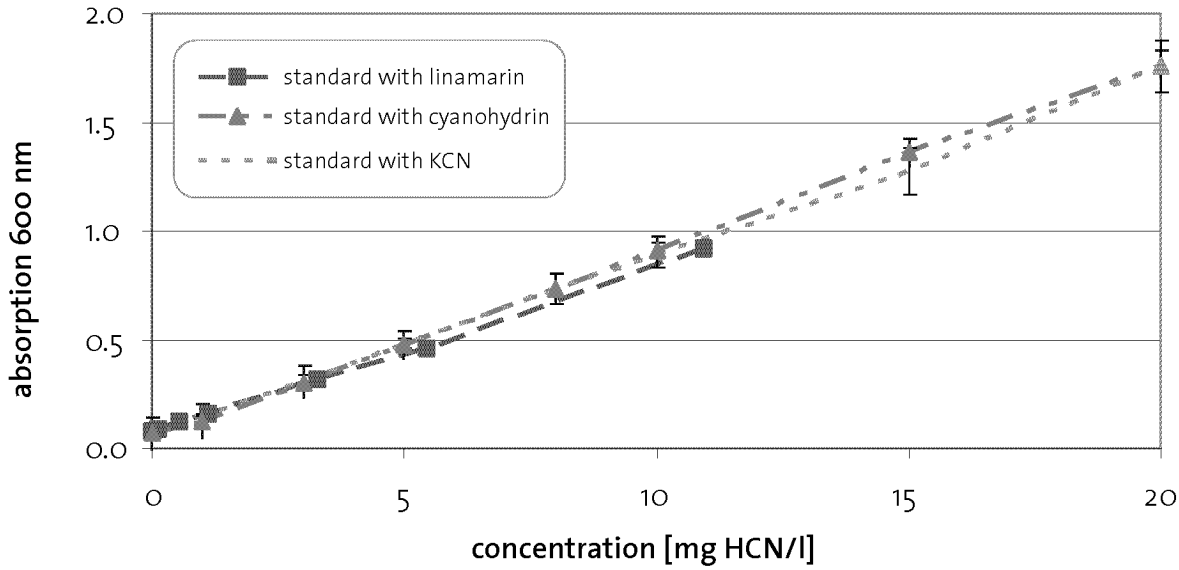


Fig. 4: Calibration curves using linamarin (n=2), cyanohydrin (n=16) and KCN (n=4). Values for all calibration curves are listed in the appendix (p. 105)

4.3 Distribution of cyanogenic compounds

Cyanide contents of cassava roots do not only vary among varieties but also among plants of the same variety and within the roots of the same plant (Cooke *et al.*, 1978). In this study, the distribution of cyanogenic compounds in the root as well as the distribution between roots from the same plant and between different plants were determined.

4.3.1 Distribution in the roots

Cyanogenic compounds in cassava roots are not evenly distributed but vary along the length of the tuber (Bradbury *et al.*, 1991; Bokanga and Otoo, 1994; Cooke *et al.*,1978b). According to Cooke (1978), the cyanide content of the central disc is usually within 15-20% of the mean cyanide content of the root. To verify these findings, cassava roots of an unknown variety were purchased in Zurich and the distribution of cyanogenic compounds in cassava roots was measured. The mean distribution (n=3) of total cyanide and linamarin in different parts of the root is summarised in Table 8.

Tab. 8: Distribution of cyanogenic compounds in cassava roots in mg/kg fwt (n = 3)

part	cyanide content in mg/kg fwt				in % of peeled root	
	total cyanide	s.d.[1]	linamarin	s.d.[1]	total cyanide	linamarin
central disc	71.9	12.0	63.0	11.3	100	87.6
root tip basal	60.3	19.2	49.6	15.7	83.8	69.0
root tip apical	104.8	28.2	94.5	22.6	145.7	131.3
peripheral area / outer radial part	134.8	46.5	124.7	46.9	187.4	173.3
middle part of central disc	36.7	21.2	25.5	15.6	51.0	35.4
root cortex	281.8	87.2	248.2	79.2	391.8	345.0

[1]standard deviation

Total cyanide contents ranged from 37 mg/kg fwt for the middle part of the central disc to 282 mg/kg fwt for the root cortex. Generally, parts closer to the root cortex and closer to the basal root tip contained more cyanides than parts near the centre of the root. The cyanide content of the apical root tip was about 60% higher than in the basal root tip. This corresponds to the results by Bradbury (1991), who also found the proximal root end to contain about 55% higher cyanide content than the distal end.

The radial gradient was considerably high, total cyanide increased from 37 mg/kg fwt for the centre of the root to 135 mg/kg fwt for the peripheral part of the root parenchyma. These results confirm those obtained by Cooke (1978) who reported a sharp decrease in the radial cyanide gradient towards the centre of the root.

Most of the cyanogenic compounds found in the roots are present as linamarin. The mean percentage of free cyanide in the root parenchyma is 15%, two thirds are present as cyanohydrin. The percentage of free cyanide is higher than those found by Cooke (1978), who reported a range of 2- 5 % free cyanide in the root parenchyma. This might be explained by the fact that the roots measured in this study were not recently harvested but waxed and stored for an unknown period of time.

4.3.2 Distribution in plants and varieties

As shown in the previous chapter, the variation of cyanide contents both longitudinally and radially may produce misleading results of the mean cyanide content of a root. However, not only the variation in the root itself is a problem. Cyanide content of the roots not only varies along the length of the tuber (Bradbury *et al.*, 1991; Bokanga and Otoo, 1994; Cooke *et al.*, 1978b), there is also a variation between roots of the same plant and between plants of the same variety (Cooke *et al.*, 1978). To estimate the variation of cyanide content of roots from the same plant and roots from the same variety but different plants, 27 root samples were analysed (Table 9).

Tab. 9: Variation in cyanide content (mg/kg dwt) of cassava roots from the same plant and from different plants of the same variety.

variety	Plant	Number of roots	Mean cyanide content	standard deviation in %
92/0061	1	5	217.2	62.5
	2	3	336.0	21.3
Mean	1 + 2	8	261.8	48.0
92/00067	1	3	258.8	50.4
	2	14	203.9	50.5
Mean	1 + 2	17	215.7	49.3
92/00427	1	3	130.6	55.3
	2	4	204.4	22.2
Mean	1 + 2	7	172.7	38.0
Anader2	1	7	498.1	41.5
	2	2	320.4	2.8
Mean	1 + 2	9	458.6	42.6
Olekanga	1	4	96.3	22.1

There is a high variation between roots from the same plant, but also between roots from the same variety, although all roots were of the same age and grown on the same plot of land. The highest variation between roots from the same plant was found in the variety 92/0061 (plant 1) with 63%, the lowest variation in Anader2 (plant 2) with 3%.

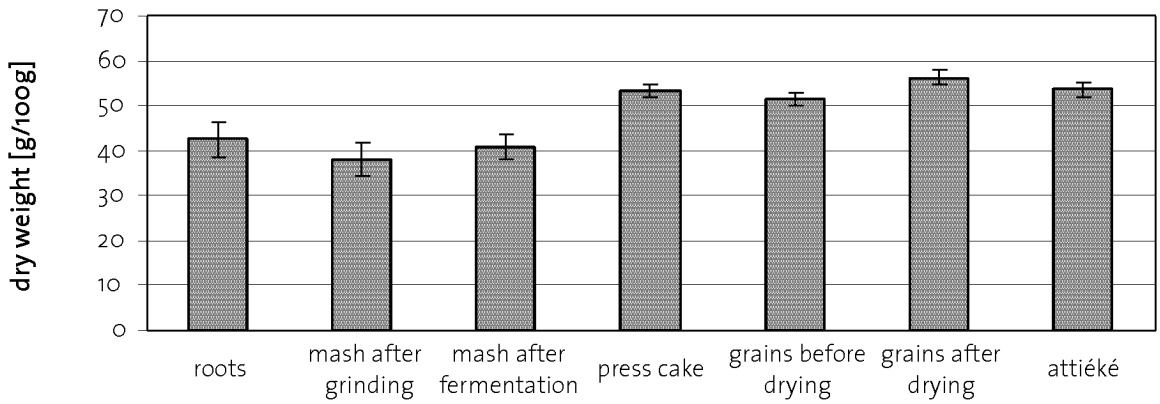
There is also a significant variation between roots of the same variety but from different plants. The mean cyanide content for the variety Anader2 was 498 (plant 1) and 320 mg/kg dwt (plant 2), respectively. Taking into account the large variations observed for the mean cyanide content in one root, roots were chipped to pieces and and a random sample was analysed. Root samples from the attiéké preparation were always taken randomly.

4.4 Cyanide reduction during traditional production of attiéké using the local variety IAC

To study the degradation of cyanogenic compounds during the traditional attiéké preparation, 20 attiéké productions by two producers from the village Adiopodoumé were closely followed. Samples were taken at the following steps: fresh cassava pieces, mash after grinding, mash after fermentation, press cake, grains before and after drying and end product attiéké. Cyanogenic compounds were determined as described in chapters 3.4.2 (p. 35) and 3.4.3 (p. 36).

4.4.1 Dry weight and pH

Since dry weight of the samples varies at the different stages of attiéké preparation, all results were calculated on a dry weight basis. Dry weight was measured as described in chapter 3.3 (p. 34). In Fig. 5 dry weights for the traditional attiéké prepraration steps are shown. The number of samples changes, since samples were not always taken for all steps.



	roots	mash after grinding	mash after fermentation	press cake	grains before drying	grains after drying	attiéké
number of samples	16	14	19	21	21	21	20
dry weight [g/100g]	42.4	37.9	40.9	53.1	51.4	56.2	53.6
standard deviation	3.8	3.6	2.9	1.4	1.3	1.5	1.6

Fig. 5: Dry weight in g/100g of attiéké preparations at different processing steps

Dry weight of roots varied between 35.7 and 49.4 g/100g, with a mean value of 42.4 g/100g. Grinding lowered dry matter through addition of water and inoculum to 37.9 g/100g. The value then slightly increased during fermentation, probably due to water evaporation. Pressing led to an increase in dry matter to 53.1 g/100g. Granulation slightly decreased dry matter to 51.4 g/100g. This may be explained by the difficulty of taking a representative sample from the press cake, since it is firmer in the centre and softer in the outer parts. Samples might have been taken more frequently from the inner part with less water. After sun-drying, the dry matter increased to 56.2 g/100g due to water evaporation. The water absorption during steaming of the grains lowered dry matter to 53.6 g/100g. Whereas the differences in water content of the roots are considerable, varying between 35.7 and 49.4 g/100g, dry matter variation in the final product attiéké was narrow. The producers seemed to adapt to differences in the raw material, especially during pressing, since variations in samples after pressing were low. This adaptation led to a rather homogeneous and constant end product with regard to water content. These results were confirmed by analysing additional attiéké samples purchased on the market. Overall, 37 samples were analysed. Dry matter for all attiéké samples was 52.6 g/100g ± 2.1. Variation was slightly higher for the samples from the market, since samples were purchased from different producers.

The pH of the end product (n=9) was determined as 4.17 with a low variation of 0.13. These results are similar to those reported by Coulin (2004), who found a pH of 4.2 - 4.4 for attiéké.

4.4.2 Total cyanide content

Total cyanide of roots, intermediate products and the end product attiéké was determined for 20 preparations as described in chapter 3.4 (p. 35). Results for all preparations are listed in Table A3 in the Appendix (p. 106). The decrease in cyanide content was higher for roots with high initial cyanide content compared to roots with low initial cyanide content. Therefore two groups were formed. One group comprised roots with an initial cyanide content of <400 mg/kg dwt, the other group contained roots with an initial cyanide content of >400 mg/kg dwt. The total cyanide content for these two groups are shown in Fig. 6.

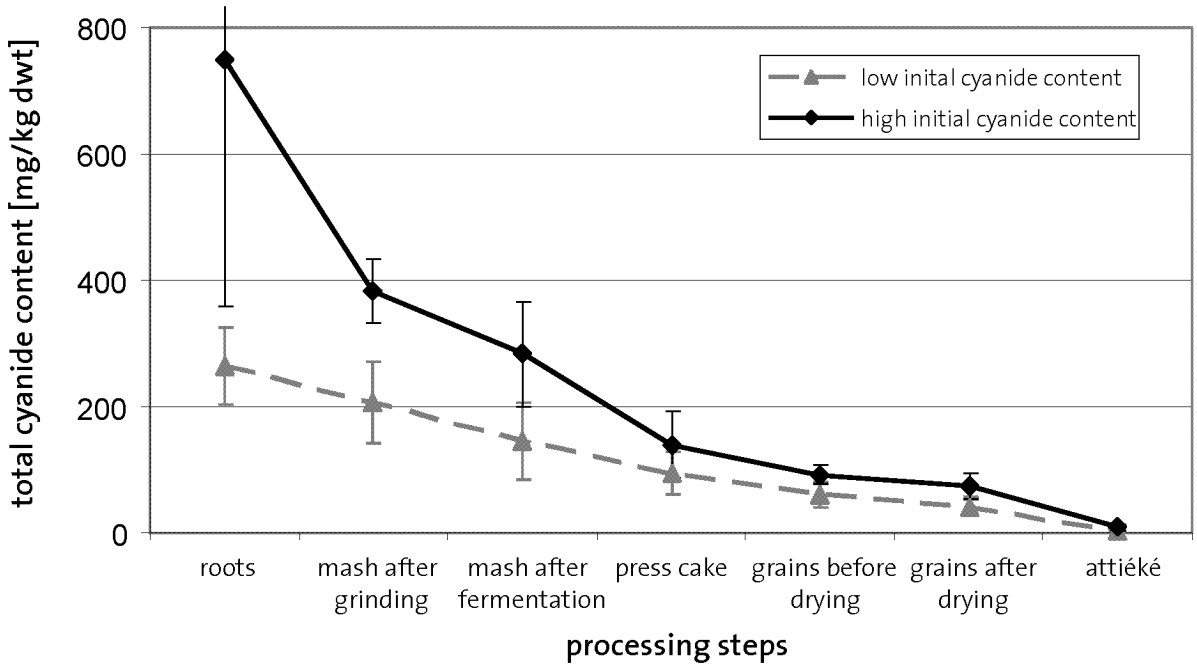


Fig. 6: Total cyanide content (mg/kg dwt) of samples from different steps during attiéké production with low and high initial cyanide content in the roots (n=20)

The initial cyanide content of the roots varied largely between 159 and 1612 mg/kg dwt. Fluctuations in cyanide content of the roots can be explained by the different source of roots as well as other reasons discussed in chapter 4.3 (p. 48).

Group with low cyanide content: For roots with an initial cyanide content of <400 mg/kg dwt, total cyanide content was reduced from a mean value of 264mg/kg dwt to 146 mg/kg dwt during fermentation. Pressing of the fermented mash further reduced cyanide content to 95 mg/kg dwt by leaching cyanide containing water. Drying and granulation possibly reduced the total cyanide content through evaporation of cyanohydrin and HCN. The final steaming sharply reduced total cyanide to levels of 4 mg/kg dwt. This value lies within the tolerated limit of 10 mg/kg dwt for cassava flour recommended by Codex Alimentarius (FAO/WHO, 1991).

Group with high cyanide content: Results for roots with high initial cyanide content (>400 mg/kg dwt) showed a similar pattern of cyanide reduction. However, the decrease during fermentation from 751 mg/kg dwt to 284 mg/kg was more pronounced. The cyanide content of 9 mg/kg dwt for the final attiéké was just within the recommendations of Codex Alimentarius.

Table 10 summarises the relative cyanide reduction of each step during attiéké preparation for the two groups. Fermentation considerably reduced cyanide content for both the low and the high cyanide group. However, in the high cyanide group, the decrease of 58% was much higher than for the low cyanide group with 41%. Possibly, linamarase activity for the high cyanide group was higher than for the low cyanide group and more cyanohydrin and HCN were produced. The pressing step reduced cyanide content for both groups by about 20%. The cyanide reduction during granulation and drying was not pronounced. However, the final steaming played an important role in reducing cyanide content to low levels. For both groups the cyanide content of the end product was 1.6% of the initial cyanide content of the roots.

Tab. 10: Relative cyanide reduction of each step of attiéké preparation

	low initial cyanide content reduction in %	**high initial cyanide content reduction in %**
Fermentation	40.9	58.1
Pressing	20.7	20.6
Granulation	13.9	6.8
Drying	7.6	2.5
Steaming	15.1	10.5
Attiéké (remaining cyanides)	3.9 mg/kg dwt	9.3 mg/kg dwt

4.4.3 Linamarin

Cyanide reduction was further studied in 13 attiéké preparations by analysing the cyanogenic compounds linamarin, cyanohydrin and HCN. The division into two groups with low (n=7) and high (n=6) initial cyanide content was retained.

Tab. 11: Mean content of cyanogenic compounds (mg/kg dwt) during attiéké preparation in samples with low initial cyanide content (n=7) and with high inital cyanide (n=6) content of the roots

steps	total cyanide	s.d.	linamarin	cyanohydrin	HCN
low cyanide group (<400 mg/kg dwt in the roots)					
roots	257.1	46.9	250.3	18.3	-13.1
mash after grinding	224.0	55.5	148.4	79.7	2.1
mash after fermentation	154.2	57.4	-12.0	90.0	76.2
press cake	114.5	43.2	-12.7	62.9	64.3
grains before drying	66.1	19.2	-5.3	69.1	2.2
grains after drying	41.0	19.8	-3.6	45.2	-0.6
attiéké	5.1	2.2	0.5	4.9	-0.3
high cyanide group (>400 mg/kg dwt in the roots)					
roots	851.0	429.5	811.3	25.6	11.9
mash after grinding	400.6	31.5	313.0	74.1	13.5
mash after fermentation	313.1	64.5	-32.0	159.2	185.9
press cake	160.3	39.5	-15.9	79.5	96.7
grains before drying	93.6	16.0	-7.4	96.3	4.6
grains after drying	74.2	22.5	-2.2	73.2	3.2
attiéké	11.5	5.1	0.5	8.6	2.3

As shown in Table 11 and in Fig. 7, in the roots almost all cyanogenic compounds were present as linamarin, only little cyanohydrin and HCN were found. In the samples after grinding, linamarin content was already reduced by 41% in the low cyanide group and by 61% in the high cyanide group. This decrease may be explained by the fact that sampling immediately after grinding was not always possible and linamarin was quickly degraded to cyanohydrin after grinding [see chapter 4.7 (p. 67)]. During fermentation, linamarin was completely degraded to cyanohydrin. Negative values result from the indirect determination of linamarin [as discussed in chapter 3.4.2 (p. 35)].

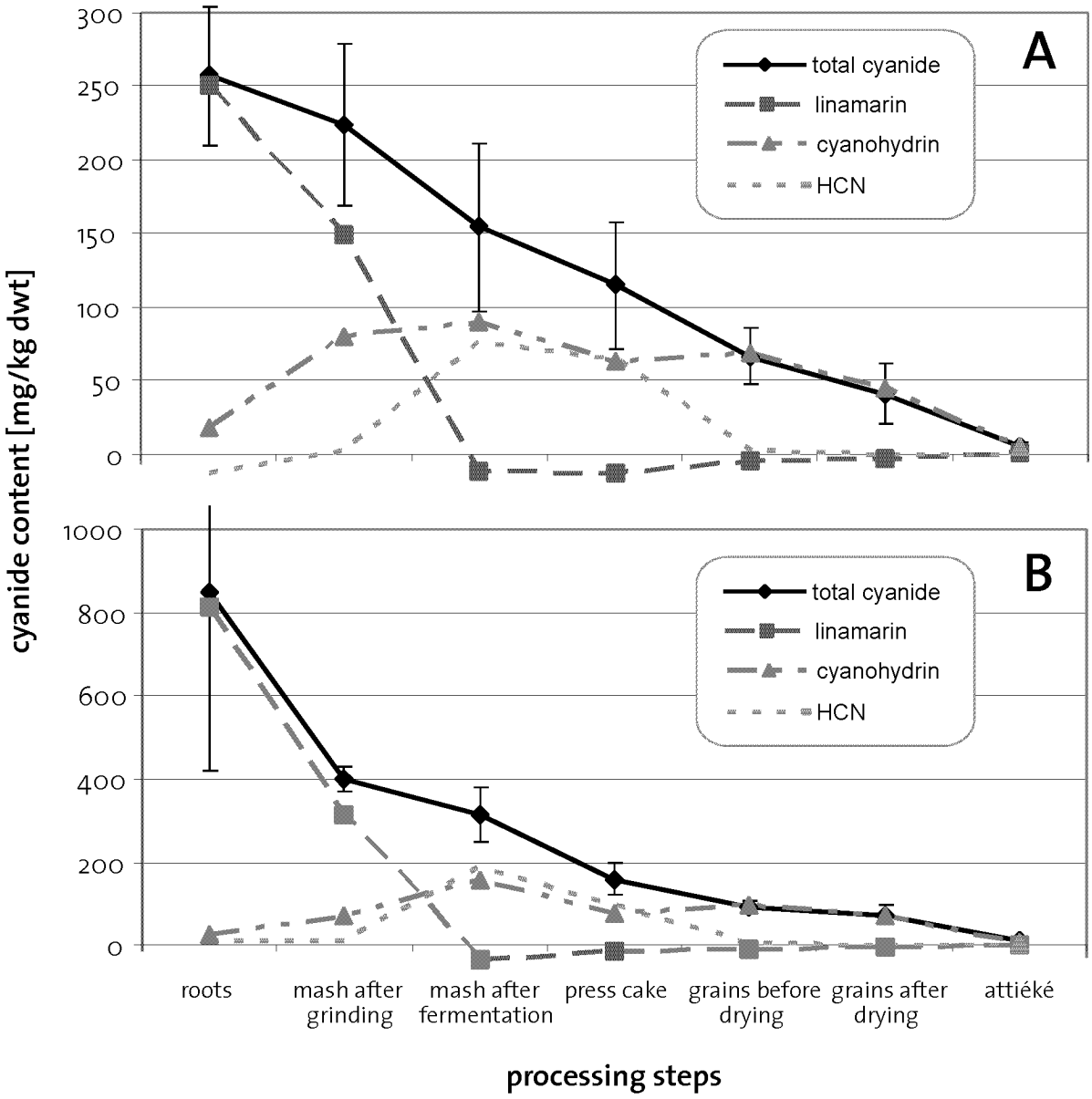


Fig. 7: Development of cyanogenic compounds during attiéké preparation with low (A) and high (B) initial cyanide content

4.4.4 Cyanohydrin

In the roots, only little cyanohydrin was found, which may result from the cutting into pieces (Table 11 and Fig. 7). Cell walls are damaged in this process and degradation of linamarin to cyanohydrin and HCN begins. In the mash after grinding and especially in the mash after fermentation, the cyanohydrin content sharply increased to high levels in both the high and the low cyanide group, and reached a maximum value in the mash after fermentation. At this point, about 54% of total cyanide in the low cyanide group was cyanohydrin, the rest was HCN. In the high cyanide group, 46% was cyanohydrin. As shown for total cyanide, the decrease in cyanohydrin was more pronounced in the high cyanide group than in the low cyanide group. The faster breakdown of linamarin to cyanohydrin led to higher cyanohydrin concentrations earlier during fermentation. The decomposition of cyanohydrin to HCN was further advanced in the high cyanide group compared to the low cyanide group and a higher amount of cyanogenic compounds was already present as HCN.

The pressing step reduced cyanide content for the low cyanide group by 30% and by 50% for the high cyanide group through leaching into the press water. Cyanohydrin could be recovered in the press water. Values between 58 to 131 mg/kg fwt (422 to 1676 mg/kg dwt) were found in the press water with a mean value of 77 mg/kg fwt (926 mg/kg dwt). There was a slight increase in cyanohydrin in the grains before drying. This increase was observed in the traditional attiéké preparation as well as in the model to produce attiéké [see chapter 4.12 (p. 80)]. Drying slightly reduced cyanohydrin content, most likely through evaporation. The final steaming led to a sharp decrease in cyanohydrin content for both groups. The water vapour favoured the evaporation of cyanohydrin (boiling point 86°C). Nevertheless, the main cyanogenic compound in the end product attiéké was cyanohydrin.

4.4.5 HCN

A pattern similar to cyanohydrin was observed for HCN (Table 11 and Fig. 7). In the roots almost no HCN was found. Small amounts may result from the cutting of the roots into pieces, where some cyanohydrin was released from linamarin and decomposed to HCN.

In the mash after grinding, HCN content was only slightly higher than in the roots, but sharply increased in the mash after fermentation. The degradation of linamarin to cyanohydrin and glucose triggered a further decomposition of cyanohydrin to HCN. Cyanohydrin degrades non-enzymatically to HCN at a pH above 4 (McMahon *et al.*, 1995). The maximum HCN content was reached in the mash after fermentation. In the low cyanide group, about 45% of the cyanogenic compounds were present as HCN, whereas it corresponded to about 54% in the high cyanide group. Pressing of the mash reduced

HCN through leaching but did not change the ratio of cyanohydrin to HCN. The sharpest decrease in HCN content occured during granulation. Expansion of the surface facilitated evaporation of HCN (boiling point 27.5 °C; Nweke and Bokanga, 1994) during this step. Drying and steaming only slightly reduced the HCN content.

4.5 Cyanide reduction during traditional attiéké production using new varieties

Samples from attiéké preparations were not only collected in the village of Adiopodoumé. Additionally, two producers from this village were invited to prepare the traditional attiéké at the Centre Suisse. This enabled to follow the process more closely and facilitated sample collection with direct cyanide extraction. In these experiments, we also produced attiéké with new varieties, introduced from the International Institute of Tropical Agriculture (IITA) in Nigeria. The aim was to increase the number of available varieties for farmers and possibly replace the local variety IAC in a few years. The following varieties were grown and harvested at the age of 18 months after planting: TMS 92/00057, 92/00061, 92/00067, 92/00325, 92/00326, 92/00398, 92/00427, 92/01425, 92/02327, Anader1, Anader2, Bonoua, IAC, TME1, Okolyawo (TME7) and Olekanga (TME9). The harvested roots were packed in plastic bags, transported to the CSRS in Abidjan and stored at ambient temperature in the shade. Experiments were carried out within five days after harvest.

Attiéké produced from new varieties

The total cyanide content in the roots and the attiéké end products varied in a wide range (Table 12). Based on these results the cassava varieties were divided into two groups. Group 1 comprised varieties, which contained <15 mg/kg dwt total cyanide in attiéké. The following eleven varieties were included in group 1: TMS 92/00061, 92/00067, 92/00325, 92/00326, 92/00398, 92/01425, Anader1, Bonoua, IAC, TME1 and Okolyawo. Group 2 comprised the five varieties TMS 92/00057, 92/00427, 92/02327, Anader2 and Olekanga, which all contained >15 mg/kg dwt total cyanide in the end product. Although the division into the two groups was not based on the cyanide content of the roots, it was interesting to note that in group 1 varieties with a mean value for total cyanide in the roots of 276 mg/kg dwt (min. 52, max. 747) were present, whereas in group 2 the mean value was 491 mg/kg dwt (min. 143, max. 931) (Table 12).

Tab. 12: Total cyanide content [mg/kg dwt] in roots and attiéké end products. Varieties are grouped based on the mean total cyanide content in attiéké

variety	total cyanide in roots	total cyanide in attiéké
group 1 (<15 mg/kg dwt in the end product)		
92/00061	746.5	13.5
92/00067	461.2	2.3
92/00325	52.1	3.3
92/00326	313.3	0.0
92/00398	139.2	0.1
92/01425	257.5	0.7
Anader1	137.1	5.6
Bonoua	82.1	0.0
IAC	529.3	16.4[1]
TME1	90.5	5.5
Okolyawo	231.0	12.7
group 2 (>15 mg/kg dwt in the end product)		
92/00057	406.4	40.5
92/00427	254.5	18.1
92/02327	723.3	137.4
Anader2	930.5	102.0
Olekanga	142.5	22.9

[1]Although the mean cyanide content for IAC in attiéké was >15 mg/kg dwt in this experiment, IAC was classified in group 1, since cyanide content for attiéké productions with IAC normally were <15 mg/kg dwt [see chapter 4.4 (p. 51)]

4.5.1 Group 1: low cyanide content in the end product

Total cyanide: Results for total cyanide, linamarin, cyanohydrin and HCN contents at the different steps of preparation for group 1 are shown in Fig. 8 and in Table A4 (p. 106) in the Appendix. Results are presented in percentage of the inital total cyanide content of the roots. The total cyanide content in attiéké corresponded to 2.2% when compared to the root (starting material). Fermentation, pressing and steaming can be regarded as the important steps for cyanide removal, as these were the steps where most of the cyanogenic compounds were reduced with regard to total cyanide. These steps were also identified to be crucial in the traditional attiéké preparation with the variety IAC.

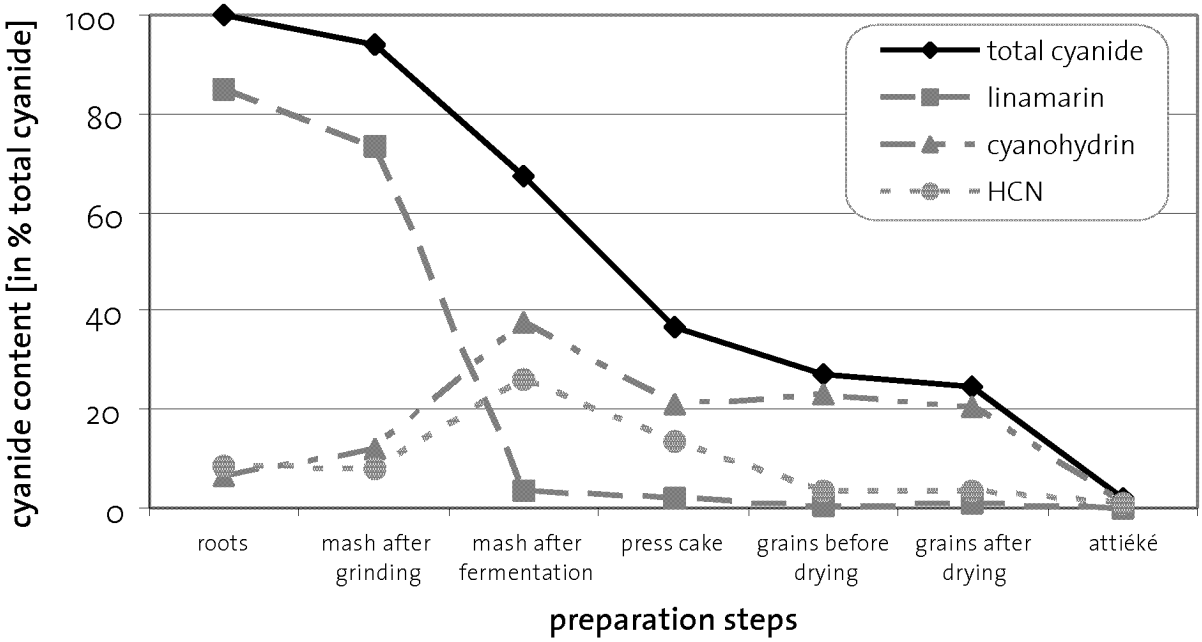


Fig. 8: Cyanide contents of group 1 varieties during attiéké production

Linamarin: Linamarin represented 85% of total cyanide in the roots. In the mash after fermentation, almost all linamarin was degraded to cyanohydrin and glucose, only about 5.1% of total cyanide after fermentation was present as linamarin. This portion remained constant for all preparation steps and the end product. Of the remaining 2.2% of total cyanide in the end product, only 5.2% were linamarin.

Cyanohydrin: The initial cyanohydrin content in the roots (6.8%) increased to 56% in the mash after fermentation. This result can be explained by the degradation of linamarin by the endogeneous linamarase. The impact of the bacteria in the inoculum will be discussed in chapter 4.4 (p. 51). Cyanohydrin proportion reached a maximum in the grains before drying, accounting for 85% of total cyanide. This high ratio of cyanohydrin can be explained by the fact that at this stage, almost no linamarin was left and HCN content decreased fast, as granulation expanded the surface. The ratio of cyanohydrin in the end product was 41% of total cyanide.

HCN: HCN content increased from 9.6% in the roots to a maximum of 11% in the mash after fermentation. Volatility kept the ratio of HCN at a low level for all attiéké preparation steps. Remarkably, the HCN ratio in the end product was high, accounting for 54% of total cyanide. This indicates steaming did not to remove all HCN from the final product. The gelatinisation of the grains may prevented the HCN evaporation.

4.5.2 Group 2: high cyanide content in the end product

Total cyanide: Results for total cyanide, linamarin, cyanohydrin and HCN contents at the different steps of preparation for group 2 are shown in Fig. 9 and in Table A5 (p. 107) in the Appendix. Results are presented in percentage of the inital total cyanide content of the roots. Total cyanide content during preparation declined to 13% for attiéké compared to the initial cyanide content of the roots. As already seen for group 1, the important steps for cyanide reduction were fermentation, pressing and steaming, leading to a considerable reduction in total cyanide content.

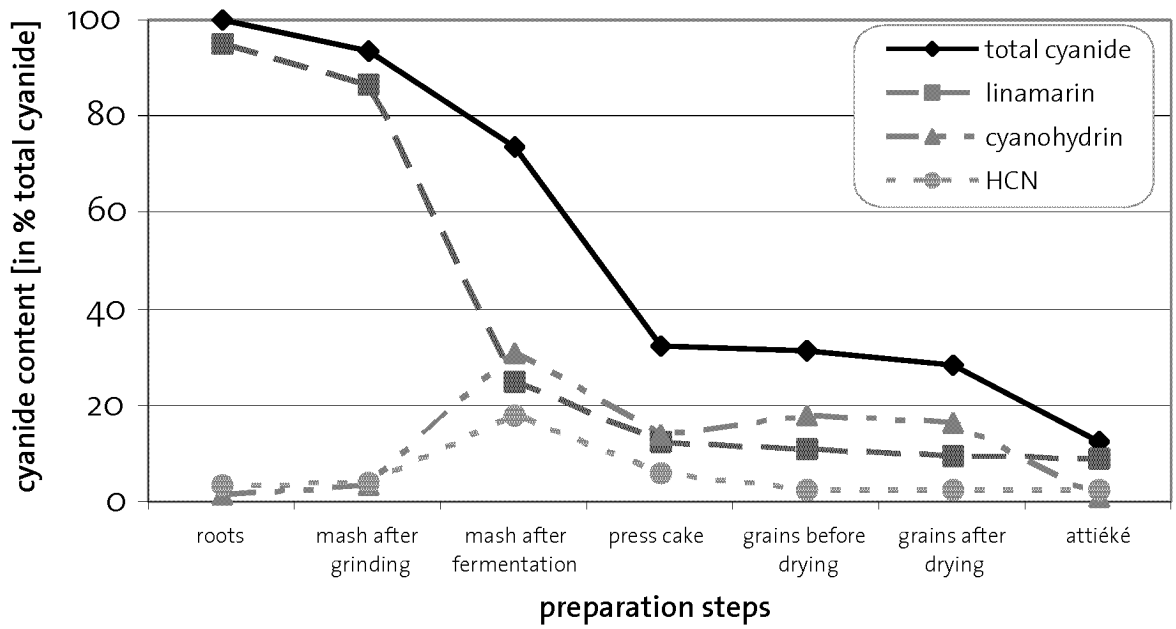


Fig. 9: Cyanide contents of group 2 varieties during attiéké production

Linamarin: Linamarin (95% in the roots) degraded to cyanohydrin and glucose during fermentation. However, the degradation was only partial, leaving 34% linamarin in the mash after fermentation. In comparison, the ratio for group 1 was only 5.2% after fermentation. The linamarin ratio remained constant at about 34% in the grains before drying. Out of the 9.1% of total cyanide in the end product, 72.5% was linamarin. This result indicates a low linamarase activity in roots from group 2. The degradation of linamarin was by far not complete after the fermentation. These results are supported by the fermentation experiments discussed below (p. 62).

Cyanohydrin: The cyanohydrin fraction steadily increased during attiéké preparation from 1.5% in the roots to 58% in the grains after drying. Since not all linamarin was converted to cyanohydrin and glucose during fermentation, some of it was still degraded to cyanohydrin in the grains after drying and the ratio remained constant at about 10%. The final steaming almost completely removed cyanohydrin through degradation and evaporation, leaving only 7% of the inital total cyanide.

HCN: The changes in the HCN fraction during attiéké preparation in the high cyanide group were similar to those in the low cyanide group. The HCN fraction increased from 2.9% in the roots to a maximum of 14% in the mash after fermentation. In the steps after fermentation the portion of HCN was low due to the high volatility of HCN. In the end product, the HCN fraction corresponded to 21% of total cyanide. The gelatinisation probably prevented the HCN evaporation, as we have postulated for group 1.

Comparing group 1 with group 2, a different distribution of cyanogenic compounds in the end product can be observed. For group 1, the ratio of linamarin, cyanohydrin and HCN in the end product of group 1 was 5, 41 and 54%, respectively. In group 2, linamarin accounted for 72%, cyanohydrin for 7% and HCN for 21%. The faster and complete degradation of linamarin to cyanohydrin and glucose during fermentation led to high amounts of cyanohydrin and HCN early during the preparation of attiéké. In group 2, linamarin was not completely degraded during fermentation, cyanohydrin and subsequently HCN were still formed by degradation of linamarin during pressing, grain forming and steaming. Therefore, the absolute content of cyanohydrin and HCN was higher in group 2 than in group 1, although the portion of cyanohydrin and HCN were lower in this group since high amounts of linamarin were left in the end product.

4.5.3 Fermentation experiments carried out with new varieties

The above discussed experiments clearly indicated an only partial degradation of linamarin during fermentation with group 2 varieties. Approximately 34% of total cyanide was present as linamarin after fermentation (Fig. 9) and in the end product as much as 72% of total cyanide accounted for linamarin. The fermentation period was therefore thought to play an important role in attiéké preparation and to considerably influence the cyanide content of the final product. To study the fermentation in detail, experiments were carried out with the same varieties (groups 1 and 2) as described in chapter 3.7 (p. 42). The results are presented in Table 13 and in Fig. 10.

Tab. 13: Mean cyanide content during fermentation in percent of initial value during fermentation of group 1 varieties (n=11) and group 2 varieties (n=5)

time	total cyanide		linamarin		cyanohydrin		HCN	
[min]	[%]	s.d.	[of total cyanide]	s.d.	[of total cyanide]	s.d.	[of total cyanide]	s.d.
group 1 (<15 mg/kg dwt cyanide in the end product)								
0	100.0	0.0	85.6	5.5	9.6	6.7	4.7	4.0
30	91.9	10.9	38.9	18.5	34.4	22.5	18.6	11.3
60	89.8	9.6	16.9	17.2	43.5	25.3	29.4	16.0
90	83.2	9.6	7.7	9.4	39.0	19.5	36.5	13.8
120	88.5	20.1	10.7	10.5	33.3	17.6	44.6	17.4
180	91.2	20.2	11.1	13.7	27.1	17.9	53.1	14.8
300	81.2	24.8	6.6	5.9	19.7	14.0	55.0	12.1
600	82.0	16.6	1.9	2.0	28.1	13.1	52.0	11.9
group 2 (>15 mg/kg dwt cyanide in the end product)								
0	100.0	0.0	92.0	4.4	4.4	3.4	3.6	4.9
30	92.7	4.3	71.8	8.4	14.1	7.9	6.8	3.0
60	96.3	4.3	65.9	12.9	18.0	8.3	12.3	5.4
90	85.7	6.0	48.8	16.1	19.6	7.9	17.3	9.9
120	85.1	12.8	43.9	17.3	19.2	6.7	22.0	11.8
180	98.6	14.6	49.4	14.6	22.6	7.9	26.6	7.2
300	82.7	15.0	26.4	15.5	20.4	5.6	35.9	14.3
600	93.6	19.1	9.1	9.8	28.0	18.9	37.7	25.1

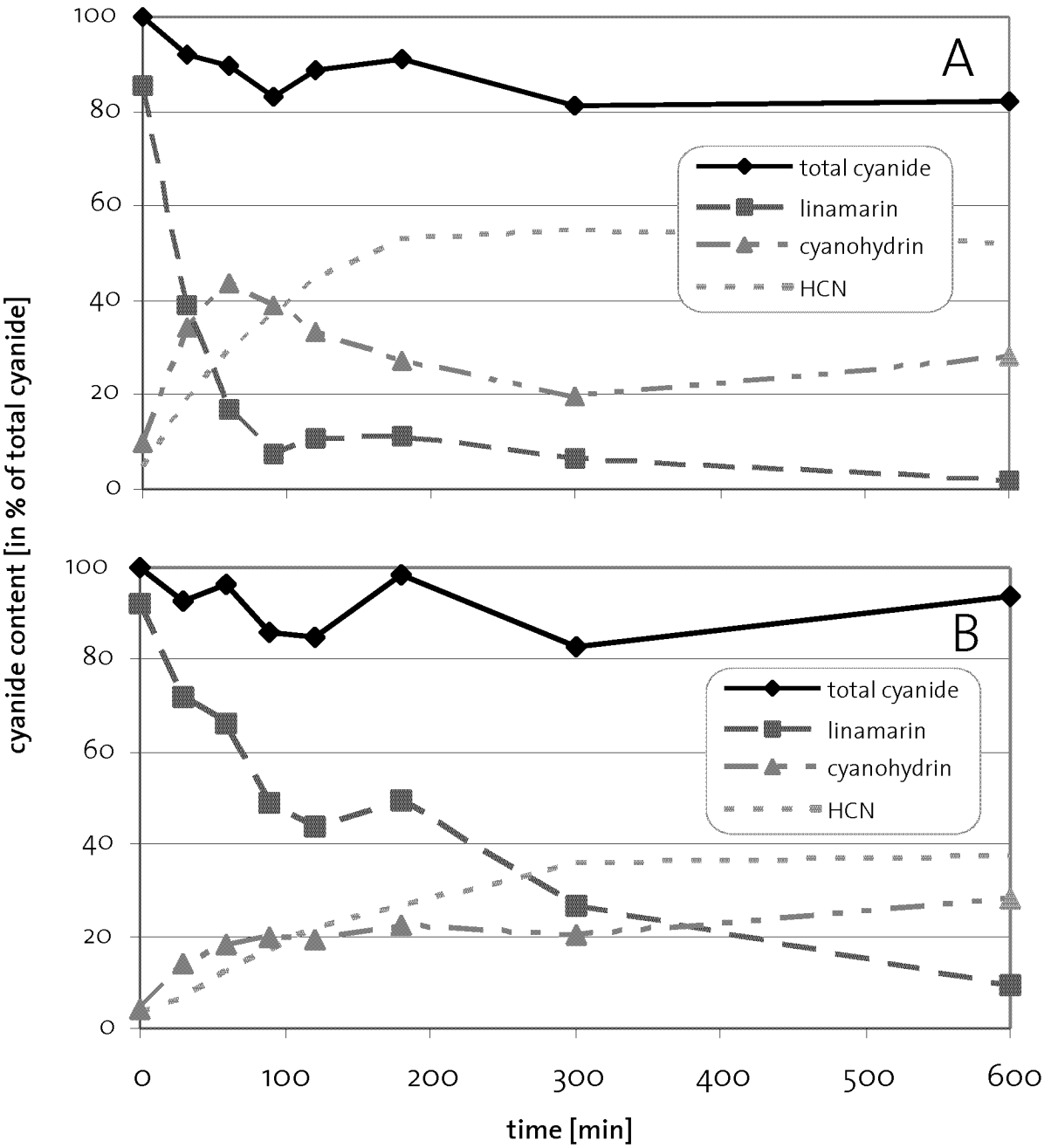


Fig. 10: Mean cyanide content during fermentation in percentage of group 1 (A) and group 2 (B) varieties

Total cyanide: The degradation of total cyanide was about the same for both groups, although the cyanohydrin and HCN contents were higher in group 1 than in group 2 at the start of fermentation. At this step, 86% of cyanogenic compounds were present as linamarin, 9.6% as cyanohydrin and 4.7% as HCN in group 1 (Table 13 and Fig. 10A). In group 2, linamarin accounted for 92% of total cyanide content, cyanohydrin for 4.4% and HCN for 3.6% (Table 13 and Fig. 10B). The fast degradation of linamarin to cyanohydrin and glucose and subsequently to HCN (see below) might explain this result as sampling was not always possible at the first minute of fermentation. After 90 min, total cyanide content dropped from 100% to 83% of the initial value in group 1 and further decreased to 82% after 10 h. The loss in total cyanide may be due to the volatility of cyanohydrin and HCN. In group 2, total cyanide content dropped only to 94% after 10 h.

Linamarin: For group 1, most of the linamarin was degraded in the first phase of the fermentation process. After 90 min of fermentation, 9.3% of the cyanide fraction accounted for linamarin. Thereafter, the degradation rate was slowed down considerably, at the end of fermentation 2.3% ot the total cyanide content were still present as linamarin. In group 2, the degradation rate of linamarin to cyanohydrin and glucose was slower. At the start of fermentation, the ratio of linamarin was higher in group 2 (92.0%) than in group 1 (86%). After a fermentation time of 90 min, 57% of the cyanide fraction accouted for linamarin, at the end of fermentation 12% of the total cyanide content was still present as linamarin in group 2.

Comparing the results obtained for both groups, a faster degradation of linamarin in group 1 was observed. After less than 30 min, half of the linamarin content was already degraded to cyanohydrin and HCN, whereas in the second group it took 180 min to reach the same linamarin level. This result clearly indicates that the endogeneous linamarase is mainly responsible for the degradation of linamarin. It has been shown by Coulin *et al.* (2006) that the growth of bacteria present in the inoculum was not advanced after 90 min of fermentation. The contribution of bacterial β-glucosidases can therefore be considered as minimal at this stage of the fermentation.

Cyanohydrin and HCN: The cyanohydrin fraction reached a maximum of 48% after 60 min of fermentation in group 1, then slowly decreased to 34% at the end of fermentation. The fast production of cyanohydrin also led to a fast conversion of cyanohydrin to HCN. The HCN fraction steadily increased from the start of fermentation, reaching a plateau after 180 min and staying high until the end of fermentation. In group 2, cyanohydrin was formed at a lower rate and the cyanohydrin concentration was still increasing, reaching 28% at the end of fermentation. The HCN content also stayed at lower levels in group 2 (about 35%) at the end of fermentation than in group 1 (52%). Cyanohydrin as well as HCN ratio were still increasing in group 2 after a fermentation time of 10 hours, indicating the degradation of linamarin to cyanohydrin and glucose not to be complete.

4.6 Determination of linamarase activity in new varieties

The results obtained so far strongly suggest fermentation time to play an important role in allowing the complete degradation of linamarin to cyanohydrin and glucose. However, in some varieties (group 2) the breakdown was not complete after fermentation. The residual linamarin did not decompose during steaming and was recovered in the end product attiéké. To verify the assumption that a low endogeneous linamarase activity could be the reason for the residual linamarin found in attiéké, the activity of this enzyme was assessed in 6 varieties of group 1 and in all varieties of group 2. Experiments were carried out using the procedure described in chapter 3.6 (p. 41). The results are presented in Table 14. The more HCN was liberated in 4 h, the higher the linamarase activity was for this variety.

Tab. 14: Total cyanide content of cassava roots and HCN liberated in 4 h by extracts from root parenchyma

variety	number of samples	total cyanide content mg/kg dwt	standard deviation mg/kg dwt	number of samples	HCN liberated in 4 h mg/kg dwt
group 1 varieties (<15 mg/kg dwt cyanide in the end product)					
92/00061	8	261.8	125.6	1	3.7
92/00067	17	215.7	106.4	8	5.3 ± 3.0
92/00325	2	85.1	46.7	1	13.7
92/00398	2	124.8	20.4	1	6.2
IAC	20	453.6	323.3	1	5.2
Okolyawo	2	187.5	61.5	1	11.7
group 1 varieties (>15 mg/kg dwt cyanide in the end product)					
92/00057	2	329.2	109.2	2	3.7 ± 1.9
92/00427	7	172.7	65.7	5	0.7 ± 0.1
92/002327	2	617.1	150.2	1	2.6
Anader2	9	458.6	195.4	1	1.0
Olekanga	4	96.3	21.3	3	0.9 ± 0.1

In group 1 with nearly complete degradation of linamarin to cyanohydrin and glucose during fermentation, linamarase activity in the root parenchyma was higher than in group 2, with the exception of the variety 92/0067. This variety showed the same activity as the variety with the highest activity in group 2, 92/00057.

These results suggest, that linamarase activity of a variety is indeed an important factor with regard to cyanide content in the end product. It should be considered, however, that these results are preliminary, since the method used gives only an estimate of the linamarase activity, and only a small number of samples were analysed. Results need to be confirmed in additional studies.

4.7 Importance of fermentation for the final cyanide content of attiéké

Several authors have stressed the importance of fermentation for the final cyanide content of products (Amoa-Awua *et al.*, 1996; Westby and Choo, 1994; Ikediobi and Onyike, 1982). Two types of fermentation are distinguished: fermentation of grated roots like attiéké and fermentation of soaked roots like gari (Westby and Choo, 1994). The influence of bacteria on cyanogenic compounds seems to be different for the two types. In fermentation of soaked roots, bacteria are involved in cell wall degradation to liberate linamarase. They also contribute to cyanide degradation by producing β-glucosidases, which may degrade linamarin to cyanohydrin and glucose.

In studies with grated roots, only a minimal contribution of bacteria on the cyanide content of the final products has been shown (Westby and Choo, 1994; Giraud *et al.*, 1993; Maduagwu, 1983), although some studies disagree (Amoa-Awua *et al.*, 1996; Idediobi and Onyike, 1982). The results of this study suggest the contribution of bacteria on the cyanide content of the end product attiéké to be minimal. Several points support this view:

- Grinding of the roots leads to an intimate contact of the endogeneous linamarase with linamarin. In the variety IAC, the breakdown of linamarin to cyanohydrin took place in the first hour of fermentation [Fig. 10 (p. 64)], where bacterial growth was not advanced (Coulin *et al.*, 2006).
- Moreover, enzymes present in the inoculum such as cellulases, would need to be highly active to break down cell walls in such a short time. However, Coulin (2004) showed that enzymes such as polygalacturonases in the traditional inoculum break down the cell structure of the mash after grinding, leading to a smooth mash after fermentation. He showed that after a fermentation time of 7 h, the relative hardness of the mash was lowered from 90% directly after milling to 15%. In the first two hours, relative hardness decreased from 90% to 80% only. Breakdown of cell walls therefore needs some time to start. However, as shown in this study, the degradation of linamarin to cyanohydrin and glucose was already completed after 2 h of fermentation.

- In varieties with a low linamarase activity such as Anader2 or Olekanga, the fermentation time of 10 h using a traditional inoculum was not sufficient to completely degrade linamarin to cyanohydrin and glucose. The contribution of bacteria might be studied with such varieties, however, the activity of bacterial β-glucosidases in the traditional inoculum was not sufficient to degrade linamarin completely.
- In attiéké garba preparation, a less-fermented inoculum is used. As shown by Coulin (2004) the activity of polygalacturonases was lower in this inoculum, leading to a coarser grained mash after fermentation. The degradation of linamarin to cyanohydrin was, however, not affected by using a less fermented inoculum. The degradation was as fast as for attiéké preparation.

In conclusion, the influence of the traditional inoculum on the quality of the end product is undisputed (Coulin, 2004), however the impact on cyanide content of attiéké and attiéké garba can be regarded as minimal.

4.8 Preparation of attiéké garba

A few years ago, the ethnic group Ebrié began to market a lower quality attiéké, named attiéké garba. This product is about five times cheaper as the higher quality product and is offered as a complete meal together with fish or meat. This product is not consumed at home but is sold, primarily to children, young people, and generally to poorer groups of the population.

The production of attiéké garba is simpler than the one leading to attiéké, some steps being shortened or omitted. The fermentation of attiéké garba is initiated by an inoculum, which is left to ferment for only one day compared to attiéké, where always a two-days inoculum is used. Additionally, less inoculum is added to the milling process, only about 3% of inoculum is used, instead of 10% for attiéké production. Compared with attiéké preparation, the pore size in the milling disc is wider for attiéké garba yielding coarser grains in the resulting mash. The producers of attiéké garba state that using a younger inoculum will influence the texture of the fermented mash. Since the younger inoculum is less overgrown by fungi and possibly contains less cell wall degrading enzymes (Coulin *et al.*, 2006), the resulting mash is more coarse grained after fermentation.

After a fermentation time of about 12 h, the mash is pressed, sieved and steamed directly. The granulation and the drying step of the traditional attiéké preparation are omitted. Steaming time is about 10 min shorter than for the attiéké production and is finished, when grains turn glassy due to water absorption. In the traditional attiéké preparation, when grains turn glassy, they are stirred, and steaming will continue for about 5-10 min. The resulting attiéké garba is a sticky and mealy product with a lot of unwanted fibres and provides a high energy source at a low cost. The product is filled in large plastic bags and sold to market sellers. For producers, attiéké garba is more attractive than attiéké because of the shorter production time and less laborious work.

Quality characteristics of attiéké garba

Attiéké garba differs from the traditional attiéké in quality, appearance and taste. More palm oil is added during production to prevent a sticky end product. Hence, attiéké garba is of dark yellow colour with an oily taste. The granulation and the drying steps are omitted and the resulting grains are of different and irregular size. Additionally, no fibres are removed and the end product contains high amounts of undesired fibres. Attiéké garba is more sour in taste than attiéké, although the pH of attiéké garba with 4.10 (n=10) is only slightly, statistically not significantly (t-test, $p<0.05$), lower than of attiéké (pH 4.17; n=9). In attiéké garba preparation, the steaming time is shorter, which may result in reduced removal of volatile acids (Firmin, 1998), thus contributing to the sourer taste of attiéké garba. A summary of the differences in the processing steps between attiéké and attiéké garba is shown in Table 15.

Tab. 15: Differences between attiéké and attiéké garba production and quality characteristics

	Attiéké	Attiéké garba
Production differences:		
Milling	fine-grained	coarse-grained
Inoculum	about 10% of inoculum (2 d old)	about 3% of inoculum (normally 1 d but varying)
Palm oil addition	about 0.1% (v/w)	about 1% (v/w)
Grain forming	yes	no
Drying	yes	no
Fibre removing	yes	no
Steaming	about 20 to 30 min	15 to 20 min
Packaging	in plastic bags of 0.3 to 1 kg	wrapped in leaves and put in large plastic bags of about 10 kg
Consistency	slightly sticky	sticky
Price	about 100 CFA per portion	about 20 CFA
Consumption	consumed at home and sold	not consumed at home, only sold
Quality characteristics:		
Colour	white or light yellow	dark yellow
Structure	round and regular grains, few fibres	irregular grains of different size, high amounts of undesired fibres
Consistence	slightly sticky	sticky and mealy
Taste	slightly sour	sour

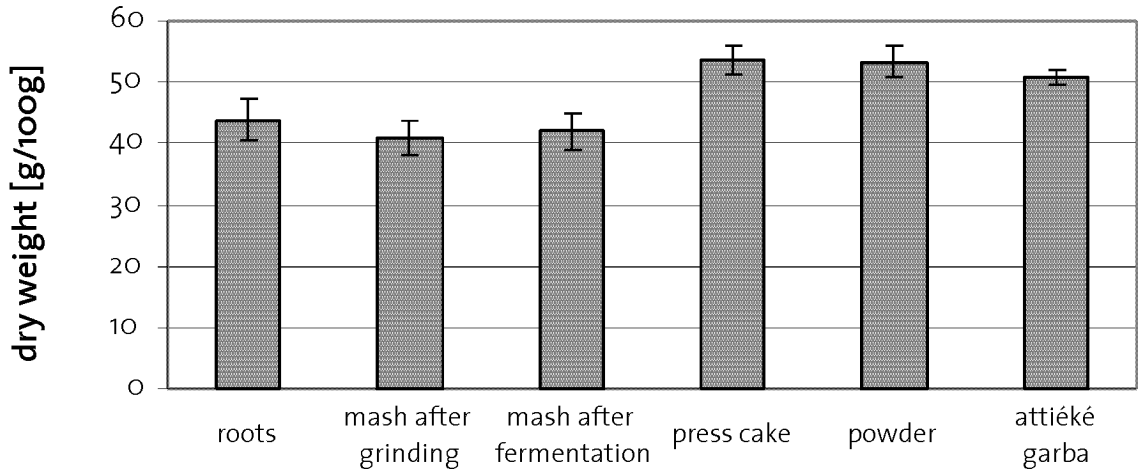
CFA: 100 CFA = 0.25 sFr.

Dry weight and pH of attiéké garba

Since the preparation of attiéké garba is a simplification of the preparation of attiéké, dry weight was similar. Samples were taken during the following preparation steps of attiéké garba: roots, mash after grinding, mash after fermentation, press cake, powder after sieving and attiéké garba. The results are summarised in Fig. 11 and details are given in Table A6 (p. 107) in the Appendix.

The dry weight for the peeled roots, mash and press cake was comparable to the dry weight in attiéké preparations (Fig. 5, p. 51), the differences were statistically not significant (t-test, $p>0.05$). In the powder after sieving, dry matter was statistically significantly lower (53.3 g/100g) than for the corresponding attiéké preparation step (granulation) (56.2 g/100g), since no grains were formed and no drying step was performed in attiéké garba production. This also led to a lower dry weight content in the final product of 50.9 g/100g instead of 53.6 g/100g for attiéké, which was also

statistically significant (t-test, $p<0.05$). Remarkably, variation in dry weight content of attiéké garba was not higher than for attiéké, despite the less strict preparation process parameters. This has also been verified for further attiéké garba samples purchased on the market. Overall, 33 samples were analysed, the dry weight contents for all attiéké garba samples was 50.3 g/100g ± 1.6.



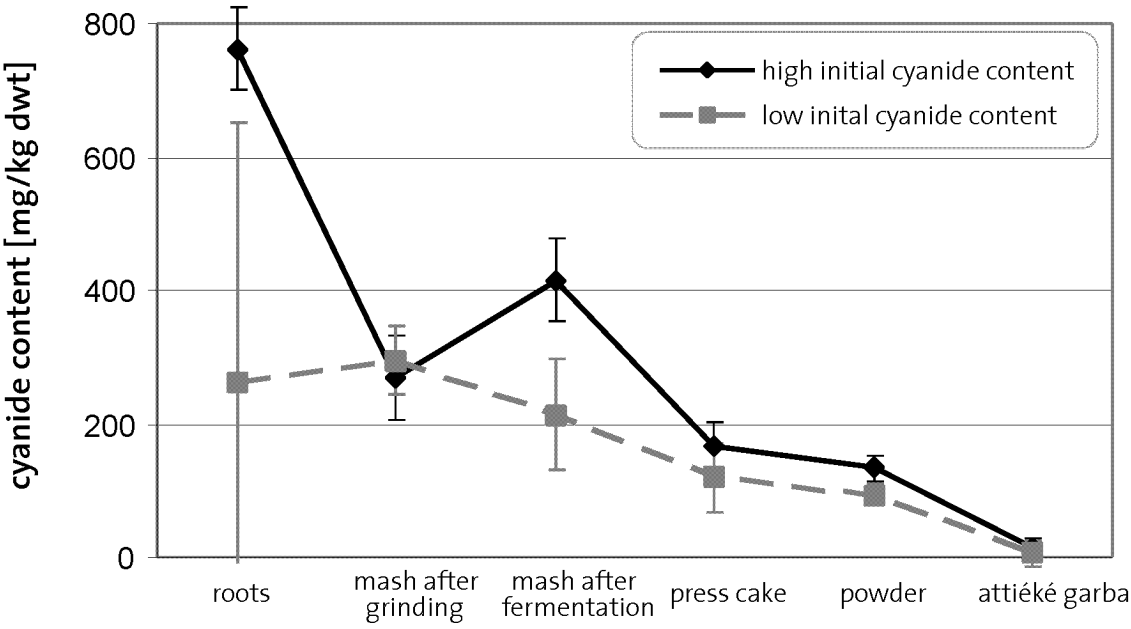
dry weight [g/100g]	43.9	40.9	42.0	53.6	53.3	50.9
standard deviation	3.5	2.8	3.0	2.5	2.6	1.3
number of samples	13	5	10	13	13	13

Fig. 11: Dry weight (g/100g) of attiéké garba preparations (n=13) at different stages of production

4.9 Cyanide reduction during traditional production of attiéké garba

Total cyanide content

Total cyanide of roots, intermediate products and the end product attiéké garba was determined for 13 preparations as described in chapter 3.4 (p. 35). For all attiéké garba preparations the bitter variety IAC was used, the cyanide content of roots varied greatly between 159 and 1612 mg/kg dwt. As stated for attiéké preparation, decrease in cyanide content was different for roots with a high cyanide content compared to roots with a low cyanide content. As for attiéké, two groups were formed, one group containing the roots with an initial cyanide content of less than 400 mg/kg dwt and the other comprising roots with an initial cyanide content of more than 400 mg/kg dwt. The results for total cyanide content of these two groups are shown in Fig. 12 and details are given in Table A7 (p. 107) in the Appendix.



	roots	mash after grinding	mash after fermentation	press cake	powder	attiéké garba
high initial cyanide content	762	270	416	168	133	14
low inital cyanide content	262	295	214	119	94	6

Fig. 12: Total cyanide content of attiéké garba preparations with low (n=8) and high (n=5) initial cyanide content

For roots with an initial cyanide content of less than 400 mg/kg dwt, a reduction by 19% of the initial cyanide content during fermentation (Table 16) was found from a mean value of 262 mg/kg dwt to 214 mg/kg dwt. This reduction was significantly lower than for the fermentation of attiéké. Linamarin might be less accessible during attiéké garba preparation, since the mash is coarser grained. Pressing reduced the cyanide content in attiéké garba by 34% of the inital cyanide content by leaching cyanide containing liquid. Sieving of the press cake did not significantly reduce total cyanide, but the final steaming led to a sharp decrease in total cyanide content by 36% of the inital cyanide content. The mean total cyanide content of attiéké garba prepared with roots with a low initial cyanide content was 6.0 mg/kg dwt, corresponding to 2.9% of initial cyanide content.

Tab. 16: Cyanide reduction in % of the initial cyanide content of each step of attiéké garba preparation

	low initial cyanide content (n=8) reduction in %	high initial cyanide content (n=5) reduction in %
fermentation	18.6	49.4
pressing	33.7	23.7
sieving	8.8	6.1
steaming	36.0	18.9
attiéké garba (remaining cyanides)	2.9 (6.0 mg/kg dwt)	1.9 (14 mg/kg dwt)

For the group with high initial cyanide content, fermentation significantly reduced the cyanide content by 49% (Table 16). This reduction was in the same range as for the traditional attiéké preparation. Steaming reduced cyanide content by 19%. This was lower than for the low cyanide group, mainly because cyanide content was already significantly reduced during fermentation. In the end product, 1.9% of the initial total cyanide was left, corresponding to a mean value of 14 mg/kg dwt. This was slightly above the upper limit of the recommendations of 10 mg/kg dwt for cassava flour by Codex Alimentarius (FAO/WHO, 1991).

Linamarin

Cyanide reduction during attiéké garba preparation was further studied in 10 productions by analysing the cyanogenic compounds linamarin, cyanohydrin and HCN as described in chapter 3.4 (p. 35). The division into two groups with low (n=6) and high (n=4) initial cyanide content was retained. Results are summarised in Fig. 13 and details are given in Table A8 (p. 108) in the Appendix.

As for attiéké preparation, linamarin was by far predominant in the roots, where only minimal amounts of cyanohydrin were present. Fermentation led to a complete degradation of linamarin to cyanohydrin. The linamarin content in the mash after fermentation and for all following steps could be regarded as zero. Negative values resulted from the indirect determination of linamarin.

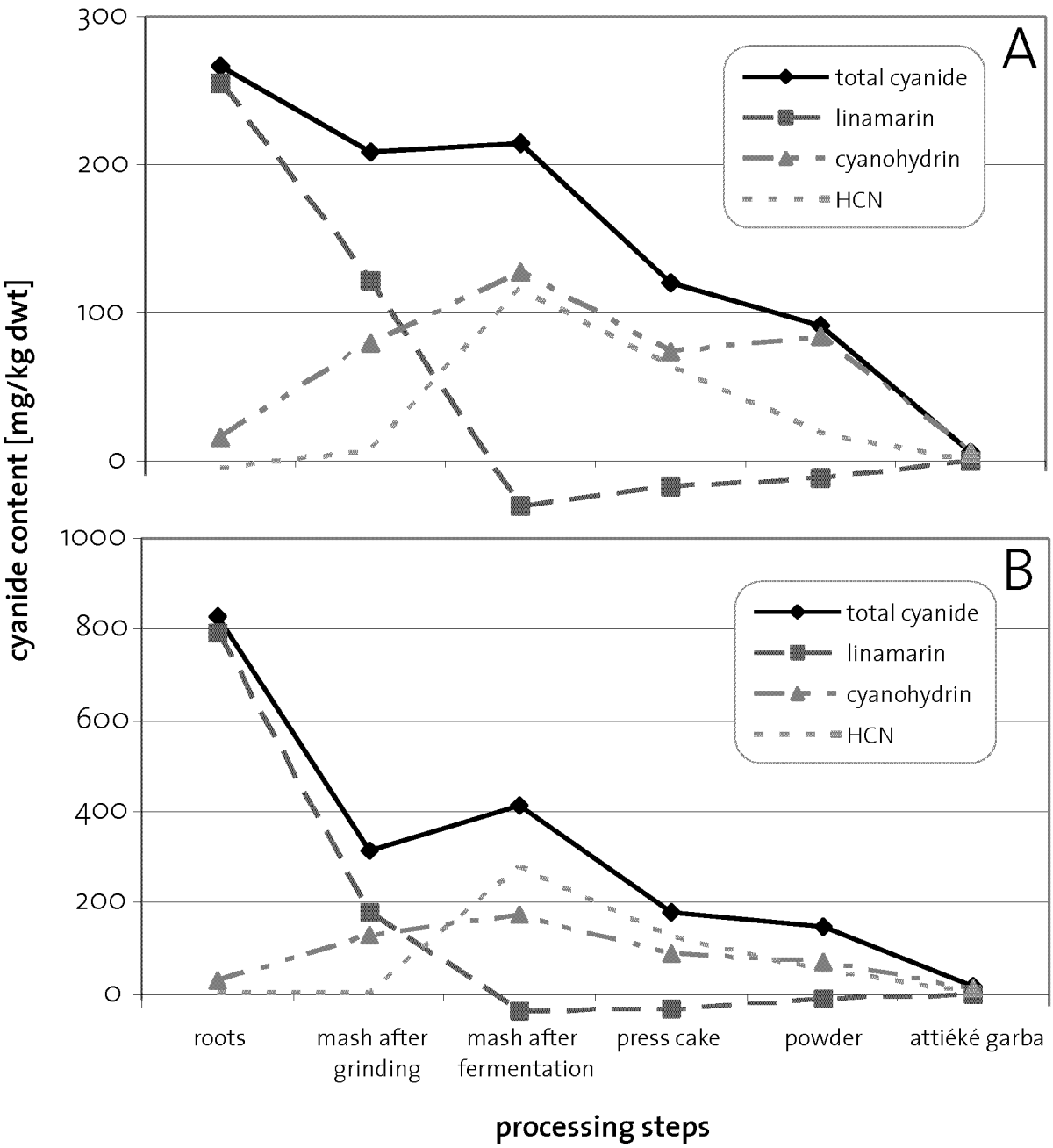


Fig. 13: Cyanogenic compounds during attiéké garba preparations with low initial cyanide content (A) and high initial cyanide content (B)

Cyanohydrin and HCN

Cyanohydrin and HCN were low in the roots, but sharply increased during fermentation and reached a maximum in the mash after fermentation for both the high and low cyanide group. Cyanohydrin accounted for 52% of total cyanide in the low cyanide group after fermentation, HCN accounted for 48%. In the high cyanide group, cyanohydrin and HCN represented 39% and 61%, respectively. As observed for attiéké preparation, the faster degradation of linamarin to cyanohydrin and glucose in the high cyanide group led to higher concentrations in cyanohydrin earlier during fermentation for the high cyanide group. Degradation of cyanohydrin to HCN was further advanced in the mash after fermentation. Pressing reduced the cyanohydrin and HCN concentration by a factor two by leaching of these compounds into the press water. Powder preparation by sieving did not significantly reduce cyanohydrin concentration for both groups, but HCN content was significantly decreased, since expansion of the surface through sieving facilitated evaporation of HCN. Steaming finally led to a significant decrease in cyanohydrin and HCN. As for the attiéké preparation [Table 11 (p. 55)], cyanohydrin was the most abundant cyanogenic compound in the end product attiéké garba, accounting for 92% in the low cyanide group and for 79% in the high cyanide group, respectively.

4.10 Comparison of attiéké and attiéké garba

As described earlier the two products attiéké and attiéké garba differ considerably. In this study, we tried to find out whether the shortening of the attiéké preparation to obtain attiéké garba leads to an increase in cyanogenic compounds in the end product. Depending on the levels of the cyanogenic compounds in the roots, a high and a low cyanide group of attiéké and attiéké garba were formed. For both groups, total cyanide in attiéké garba was higher than for attiéké. In the low cyanide group, total cyanide reached 6.0 mg/kg dwt compared to 5.1 mg/kg dwt for attiéké. In the high cyanide group, total cyanide for attiéké garba was 16 mg/kg dwt versus of 11 mg/kg dwt for attiéké. This is slightly higher than the recommendations by Codex Alimentarius. Hence, attiéké garba can be regarded as safe with respect to cyanide content as can attiéké.

However, the development of the cyanide content during attiéké garba preparation considerably differs from attiéké preparation. In the low cyanide group, total cyanide was considerably higher for attiéké garba after the fermentation step. Only about 19% of cyanide were removed whereas in attiéké preparation, about 41% of total cyanide were removed at the same processing step. Pressing then removed cyanide to about the same level for both preparations. However, since no grains were formed and no drying step

was carried out during attiéké garba preparation, cyanohydrin and HCN content was high before steaming. This step then decreased cyanogenic compounds to low levels in both end products.

In the high cyanide group, the differences in cyanogenic compounds of attiéké and attiéké garba preparations were not as pronounced as for the low cyanide group. Nevertheless, we observed the same trends as for the low cyanide group. After fermentation, total cyanide decreased by about 58% for attiéké and by about 49% for attiéké garba. The HCN content in attiéké garba was significantly higher than in attiéké after fermentation. Total cyanide content before steaming was about twice as high for attiéké garba than for attiéké. Steaming then decreased cyanohydrin and HCN to low levels for both groups.

4.11 Cyanide content of various cassava products

In Côte d'Ivoire, various cassava products apart from attiéké and attiéké garba are produced and consumed by the population. A variety of products was purchased on the market in Adiopodoumé or freshly prepared by two women from the village Adiopodoumé. Table 17 summarises products, their way of production and the cassava variety normally used for the product. For some products, bitter and sweet varieties may be used depending on market supply. However, due to the lower price, the variety IAC was generally used.

Tab. 17: Preparation of various cassava products

Product name	Varieties used	Preparation
Bonoua cooked	sweet varieties	Roots are peeled, cut to pieces and cooked in salted water.
Cassava flour	bitter and sweet varieties	Peeled roots are sun-dried for a period of one week up to one month and then ground and used to prepare products such as boules de manioc, beignets de manioc, placali and claclo.
Placali	bitter and sweet varieties	Roots are peeled, cut to pieces and ground. The mash is left to ferment overnight and then sieved to remove fibres. The mash is cooked in water until a sticky dough results.
Placali from flour (pressed fermented mash)	bitter and sweet varieties	A fermented dry mash is mixed with water and sieved to remove fibres. The mash is cooked in water until a sticky dough results.
Foutou manioc	sweet varieties	Peeled roots are cooked in water and ground to a sticky dough in a mortar.
Foutou banane	sweet varieties	Peeled roots and plantain pieces are cooked in water and ground to a sticky dough in a mortar.
Boules de manioc	bitter and sweet varieties	Cassava flour, water and salt are mixed, formed to small balls and fried in peanut oil.
Beignets de manioc	bitter and sweet varieties	Cassava flour, water, sugar and yeast are mixed, formed to small balls and fried in peanut oil.
Claclo	bitter and sweet varieties	Plantain pieces and cassava flour are mixed, formed to small balls and fried in peanut oil.

In the following paragraphs, results for these products will be discussed. The dry weight and contents of cyanogenic compounds were determined as described in chapters 3.3 (p. 34) and 3.4 (p. 35), respectively. In Table 18 dry weight and cyanogenic compounds for various products are summarised. Cyanide content for attiéké and attiéké garba are given for comparison.

Tab. 18: Dry weight and cyanide content of various products prepared from bitter and sweet cassava varieties

product (n=x)	dwt [g/100g]	Total cyanide [mg/kg dwt]	Linamarin [mg/kg dwt]	Cyanohydrin [mg/kg dwt]	HCN [mg/kg dwt]
Bonoua raw (n=8)	44.7 ± 4.3	229.6 ± 92.2	213.9 ± 84.8	9.6 ± 7.7	6.1 ± 2.5
Bonoua cooked (n=9)	38.8 ± 6.7	190.8 ± 70.2	187.1 ± 67.5	1.7 ± 2.6	2.0 ± 2.7
Cassava flour (n=4)	88.6 ± 2.2	15.4 ± 15.4	10.3 ± 12.0	2.2 ± 4.6	2.9 ± 6.7
Placali (n=4)	25.7 ± 4.0	49.4 ± 12.7	0	27.6 ± 8.6	21.8 ± 5.7
Placali flour (n=3)	51.9 ± 6.5	89.8 ± 45.2	0	70.0 ± 34.5	19.8 ± 11.1
Foutou manioc (n=1)	40.9	52.3	53.0	-3.6	2.9
Foutou banane (n=2)	35.8	49.8	42.8	1.2	6.0
Boules de manioc (n=2)	77.4	3.1	-0.58	-0.1	3.7
Beignets de manioc (n=1)	67.8	1.6	-4.4	1.2	4.8
Claclo (n=3)	71.6 ± 5.8	4.0 ± 2.8	0.5 ± 1.4	0.3 ± 1.1	3.3 ± 1.3
Attiéké (n=11)	54.3 ± 1.1	7.2 ± 4.4	0.3 ± 1.1	6.6 ± 3.9	0.3 ± 1.5
Attiéké garba (n=28)	50.4 ± 1.5	10.9 ± 6.5	1.9 ± 6.7	8.3 ± 6.5	0.7 ± 2.9

Bonoua raw and cooked

A common method of preparation used in Côte d'Ivoire is cooking of cassava pieces in water. The preparation of cooked cassava pieces is described in chapter 3.9 (p. 44). Total cyanide content of fresh cassava pieces (n=8) was high (230 ± 92 mg/kg dwt), taking into account that Bonoua is considered to be a sweet variety, which was confirmed by Mosso *et al.* (2000), who found 89 mg/kg dwt of total cyanide for the variety Bonoua. In our study most of the cyanogenic compounds were in the form of linamarin (93%). Cooking reduced total cyanide only by about 20% to 191 ± 70 mg/kg dwt. Nambisan and Sundaresan (1985) reported that cyanide reduction after cooking in pieces of 50 g was about 50%. This piece size was also used in this study, the difference in reduction remains to be elucidated.

Free cyanides were reduced from 15.7 ± 8.8 mg/kg dwt to about 3.7 ± 4.2 mg/kg dwt, a reduction by about 80%. Padmaja (1995) reported a reduction in free cyanide of 90% after 15 min cooking time, which is comparable to our results.

Cassava flour

Cassava flour (n=4) was bought on the market in Adiopodoumé. The total cyanide of the flour ranged from 3.2 to 36 mg/kg dwt, with a mean value of 15 mg/kg dwt, linamarin content representing two thirds of the total cyanide content.

Different results of total cyanide content in cassava flour are reported. Mosso *et al.* (2000) reported a cyanide content of 0.9 mg/kg dwt for cassava flour from Côte d'Ivoire prepared from the bitter variety IAC. Higher amounts of cyanide have been found by Mlingi *et al.* (1992), who found an elimination of cyanide of 71% after 8 d and 73% after 17 d of treatment [details are given in Table 5 (p. 31)]. This resulted in a flour containing 145 mg/kg dwt, mainly in the form of linamarin (96%). The differences in the results for cassava flour may be related to the different processing techniques.

Placali and placali flour

Placali and placali flour were purchased on the market in Adiopodoumé, the preparation of these products is described in chapter 3.9 (p. 44). Total cyanide content for placali (n=4) and for placali flour (n=3) was 49 ± 13 mg/kg dwt and 90 ± 45 mg/kg dwt, respectively (Table 18). No linamarin was found in placali and in placali flour, cyanohydrin represented the main part of cyanogenic compounds in both products, accounting for 28 and 70 mg/kg dwt for placali and placali flour, respectively. This may be explained by the low pH in the mash after fermentation, stabilizing cyanohydrin in the samples. However, considerable amounts of HCN could also be found. Placali flour contained 20 ± 11 mg/kg dwt HCN, whereas in placali 22 ± 6 mg/kg dwt HCN were present. The astonishingly high HCN content of the end products could be due to the highly viscous structure of placali, hindering the escape of HCN.

Mosso *et al.* (2000) reported total cyanide values of 2.3 mg/kg dwt for placali prepared with Bonoua and 8.4 mg/kg dwt for placali prepared with the variety IAC. The high cyanide content of placali analysed in this study indicates the use of a bitter variety.

Foutou manioc and foutou banane

Foutou manioc (n=1) and foutou banane (n=2) were freshly prepared as described in chapter 3.9 (p. 44). In contrast to all other products (except for bonoua cooked), linamarin was almost exclusively present. Total cyanide content for foutou manioc was 52 mg/kg dwt and 50 mg/kg dwt for foutou banane. Since linamarase is inactivated during cooking, the linamarin could not be degraded to cyanohydrin and HCN, thus remaining in high quantities in the end product.

Boules de manioc, beignets de manioc and claclo

Boules de manioc (n=2), beignets de manioc (n=1) and claclo (n=3) were freshly prepared by two women from the villige Adiopodoumé. Cyanide content was low, 3.1 mg/kg dwt for boules de manioc, 1.6 mg/kg for beignets de manioc and 4.0 mg/kg dwt for claclo, respectively. The cyanide content of the flour used to prepare these products seem to have the highest impact on the cyanide content of the final product, since frying in oil does not significantly reduce the cyanide content (Padmaja, 1995).

4.12 Cyanide reduction in attiéké and attiéké garba prepared on a laboratory scale

Several steps of attiéké and attiéké garba preparation were studied in a model to produce attiéké. This model mimicks the steps of the traditional attiéké preparation. It was developed by Coulin (2004) and allows to produce attiéké in small quantities. Proceedings on how to prepare attiéké and attiéké garba on laboratory scale are described in chapter 3.8 (p. 42). The model allows to study important steps related to cyanide removal in depth. Moreover, the preparation procedures can be altered to determine the influence on cyanide content of the final product. Several experiments were carried out to determine the importance of fermentation, influence of the inoculum and steaming time on the cyanide content of the end product attiéké.

Attiéké preparation

About 1.5 kg of cassava roots were peeled and attiéké was prepared according to chapter 3.8 (p. 42). Samples for the analysis of dry weight and cyanogenic compounds were taken during the following steps: roots, mash after grinding, mash after fermentation, press cake, grains before and after drying and attiéké. Cyanide determinations were carried out as described in chapter 3.4 (p. 35). In Fig. 14, cyanide contents at various stages of preparation are shown. Results obtained for the model system were similar to those for the traditional attiéké preparation. The model proved to be suitable to study different attiéké preparation steps in detail.

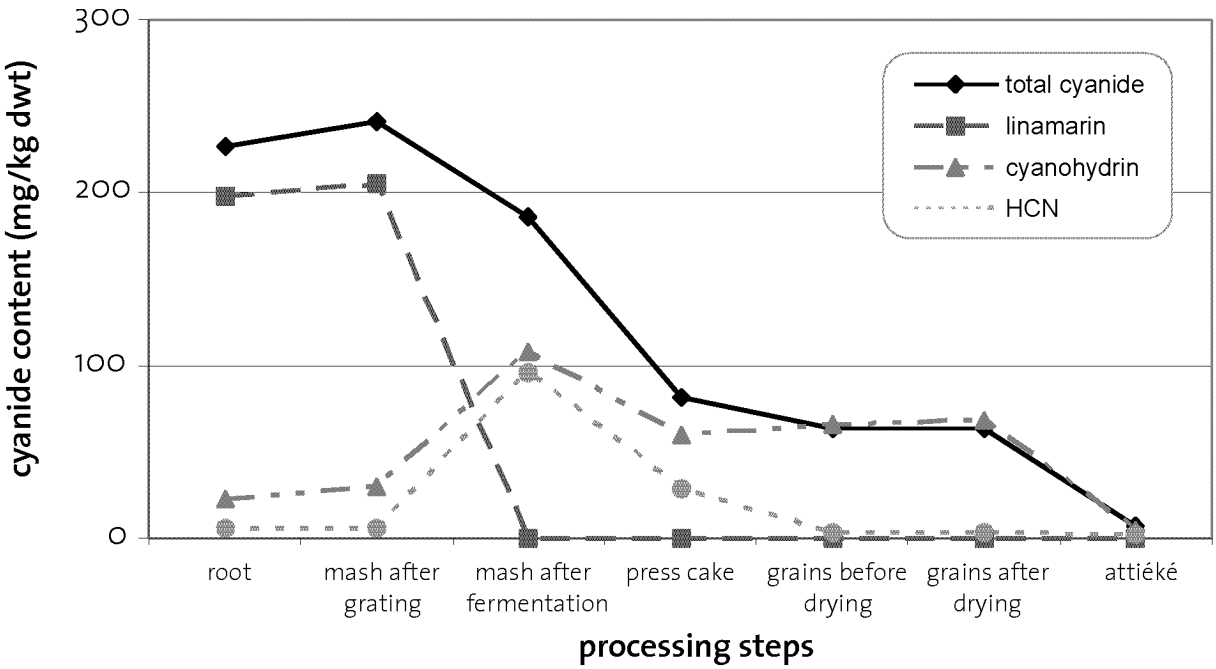


Fig. 14: Cyanide content at various stages of attiéké production in a model system

Fermentation experiments with and without inoculum

Fermentation plays an important role in the attiéké production, as in this step, linamarin is degraded completely to cyanohydrin and glucose. To investigate the contribution of the inoculum on the degradation of linamarin, fermentation experiments with and without the addition of inoculum were carried out as described in chapter 3.8 (p. 42). Samples were taken at an interval of 30 min and analysed for cyanogenic compounds as outlined in chapter 3.4 (p. 35). The results of this set of experiments are summarised in Fig. 15.

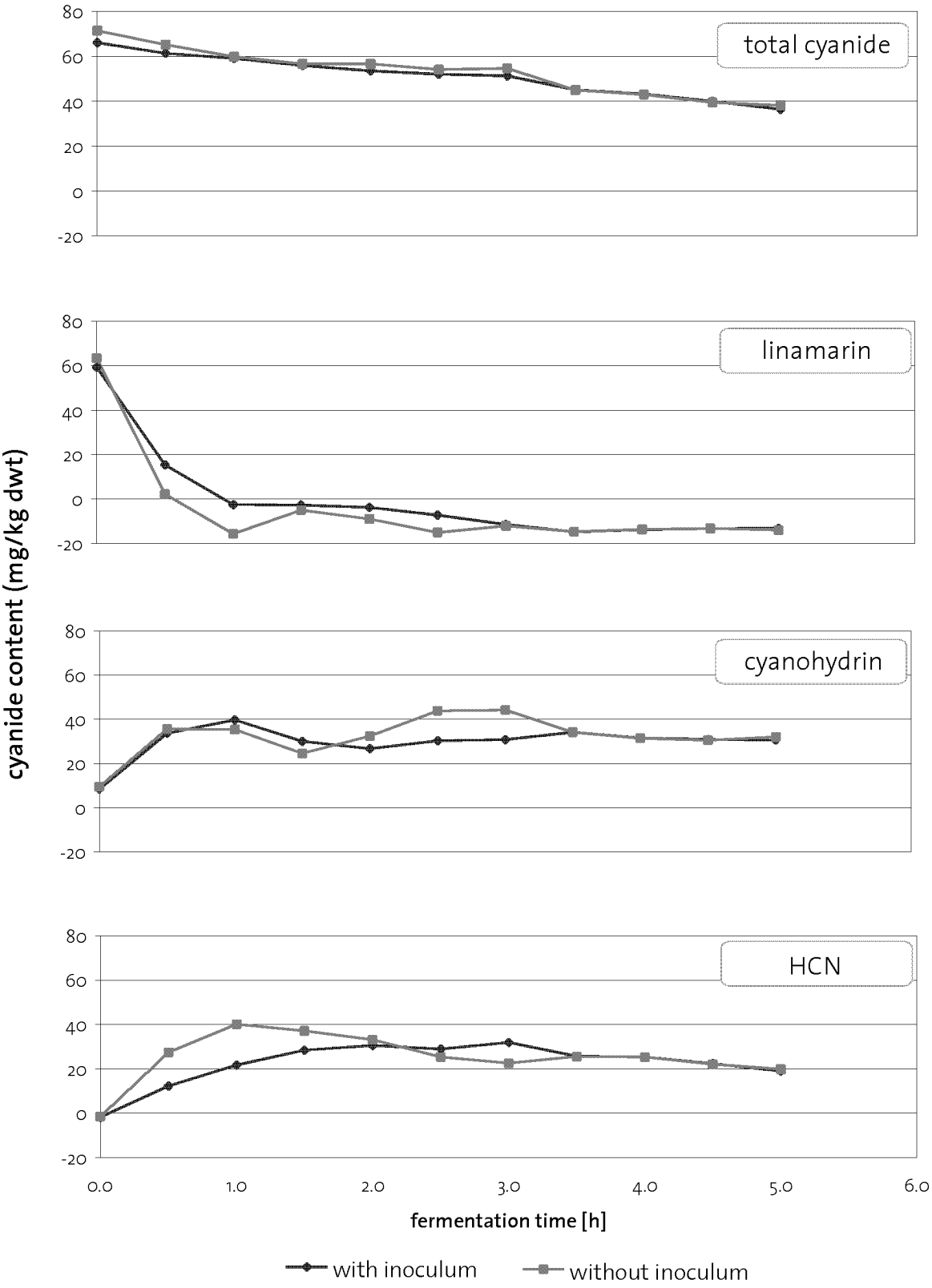


Fig. 15: Cyanogenic compounds during fermentation with and without inoculum

Fermentation with inoculum led to similar results as for the traditional procedure [see chapter 4.4 (p. 51)] carried out with the variety IAC or other varieties with high linamarase activity. Total cyanide only slightly differed during the 5 h of fermentation in the mash with and without inoculum. In the mash with inoculum, total cyanide content was slightly lower, since the addition of inoculum, containing less cyanide than the roots, decreased cyanide content of the mash.

Degradation of linamarin to cyanohydrin was completed after 30 min of fermentation in the mash without inoculum, which was slightly faster than in the mash with inoculum. From this result it may be concluded that the addition of inoculum was not essential for the degradation of linamarin. The activity of the endogeneous root linamarase was sufficient for the complete degradation of linamarin. As pointed out by several authors (Amoa-Awua *et al.*, 1996; Westby and Choo, 1994; Ikediobi and Onyike, 1982) bacterial β-glucosidases seem to be of little importance for the linamarin degradation in attiéké preparation. Nevertheless, the inoculum plays an important role in attiéké preparation. As shown by Coulin (2004), the inoculum is the main factor in determining the overall quality of attiéké.

The development of cyanohydrin was comparable in both experiments. The increase in the sample without inoculum after 2.5 and 3 h remains to be elucidated and was not observed in other experiments.

On the other hand, the HCN content seemed to be influenced by the addition of an inoculum. Liberation of HCN from cyanohydrin in the mash without inoculum was faster than from the mash with inoculum. This observation could be explained by the lower pH in the mash with inoculum. Since cyanohydrin is stabilised by lower pH, the liberation of HCN from cyanohydrin might be slower in the mash with inoculum.

Steaming

Steaming is an important step with regard to cyanide removal in the preparation of attiéké. It liberates volatile cyanohydrin and HCN and reduces cyanogenic compounds to low levels. The influence of steaming time on the development of cyanogenic compounds was studied in the model system by steaming 50 g of grains after drying for a defined period of time. Samples were analysed for dry weight, total cyanide, free cyanide and HCN as described in chapter 3.3 (p. 34) and 3.4 (p. 35). Results for steaming times ranging from 0 to 45 min are shown in Fig. 16.

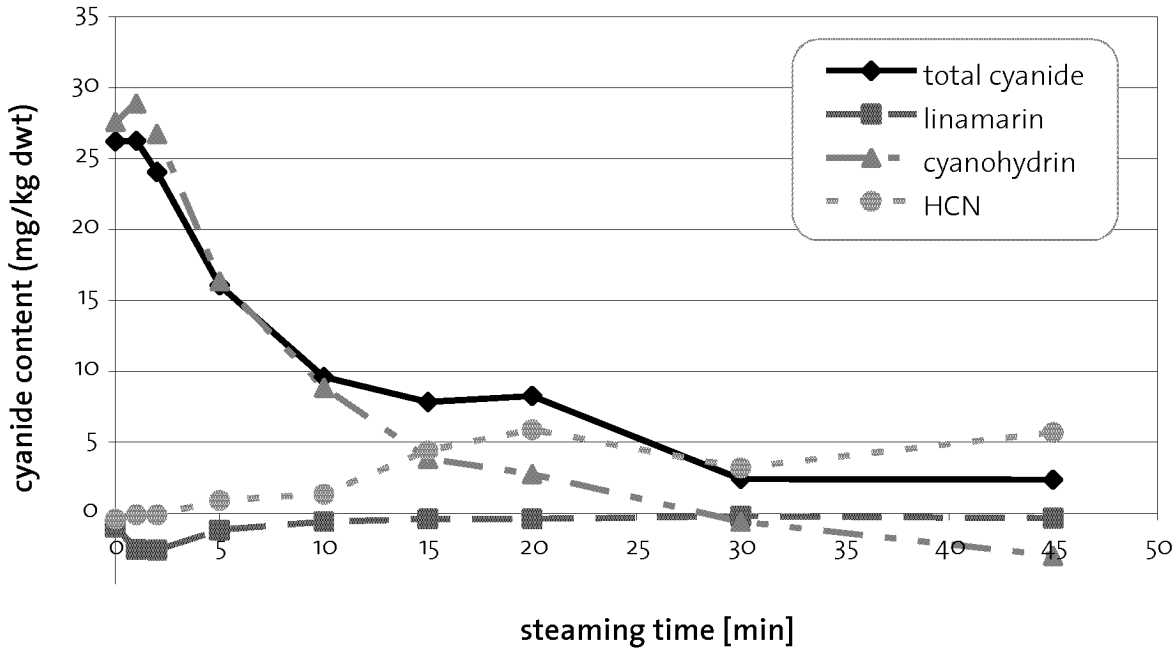


Fig. 16: Development of cyanogenic compounds during steaming

Total cyanide was reduced during steaming to a minimum value after about 30 min. Steaming for a longer time had little influence and cyanogenic compounds were not further reduced. Linamarin had been completely degraded during the fermentation step and therfore played no role during the steaming process. Cyanohydrin steadily decreased during steaming and was completely degraded after about 30 min. HCN content increased in the first 20 min due to conversion of cyanohydrin to HCN. Further steaming did not change the HCN content. As a consequence of these results a steaming time of 25-30 min can be recommended with regard to cyanide removal. This corresponds well to the steaming time used in the traditional attiéké production.

Influence of the duration of fermentation on cyanide content of attiéké garba

To determine the influence of the duration of fermentation on the cyanide content of attiéké garba, experiments with a duration of fermentation of 0, 1, 3 and 5 h were carried out. About 1.5 kg of cassava roots were peeled and attiéké garba was prepared following the procedure described in chapter 3.8 (p. 42) and analysed according to chapter 3.4 (p. 35). Table 19 lists cyanogenic compounds in the end product obtained after fermenting for 0, 1, 3 and 5 h.

Tab. 19: Cyanogenic compounds (mg/kg dwt) in the end product attiéké garba after different durations of fermentation times

fermentation (h)	total cyanide	linamarin	cyanohydrin	HCN
0	17.7	12.6	-0.8	5.9
1	10.2	4.2	-0.8	6.8
3	5.9	0.4	-0.7	6.2
5	0.1	0.4	-0.9	0.6

Total cyanide content in the end product decreased with increasing duration of fementation, linamarin could be recovered in attiéké garba after fermenting for 0 and 1 h. The results support our findings that fermentation is important for the cyanide content of the end product. Fermentation time should be at least 3 h to allow the complete conversion of linamarin to cyanohydrin, although a duration of 5 h was needed to remove almost all cyanogenic compounds in the end product.

In Fig. 17, cyanide content at various stages of preparation are shown. Even without fermentation total cyanide decreased, since degradation of linamarin also took place during pressing and powder preparation (Fig. 17). Linamarin degradation was not complete after a fermentation time of 0 and 1 h. Some linamarin was still left in the mash after fermentation and a small amount was recovered in the end product. Cyanohydrin as well as HCN increased only slightly in the non-fermented mash. Apparently, cyanohydrin and HCN produced during pressing and powder preparation evaporated continuously and cyanohydrin and HCN stayed at low levels.

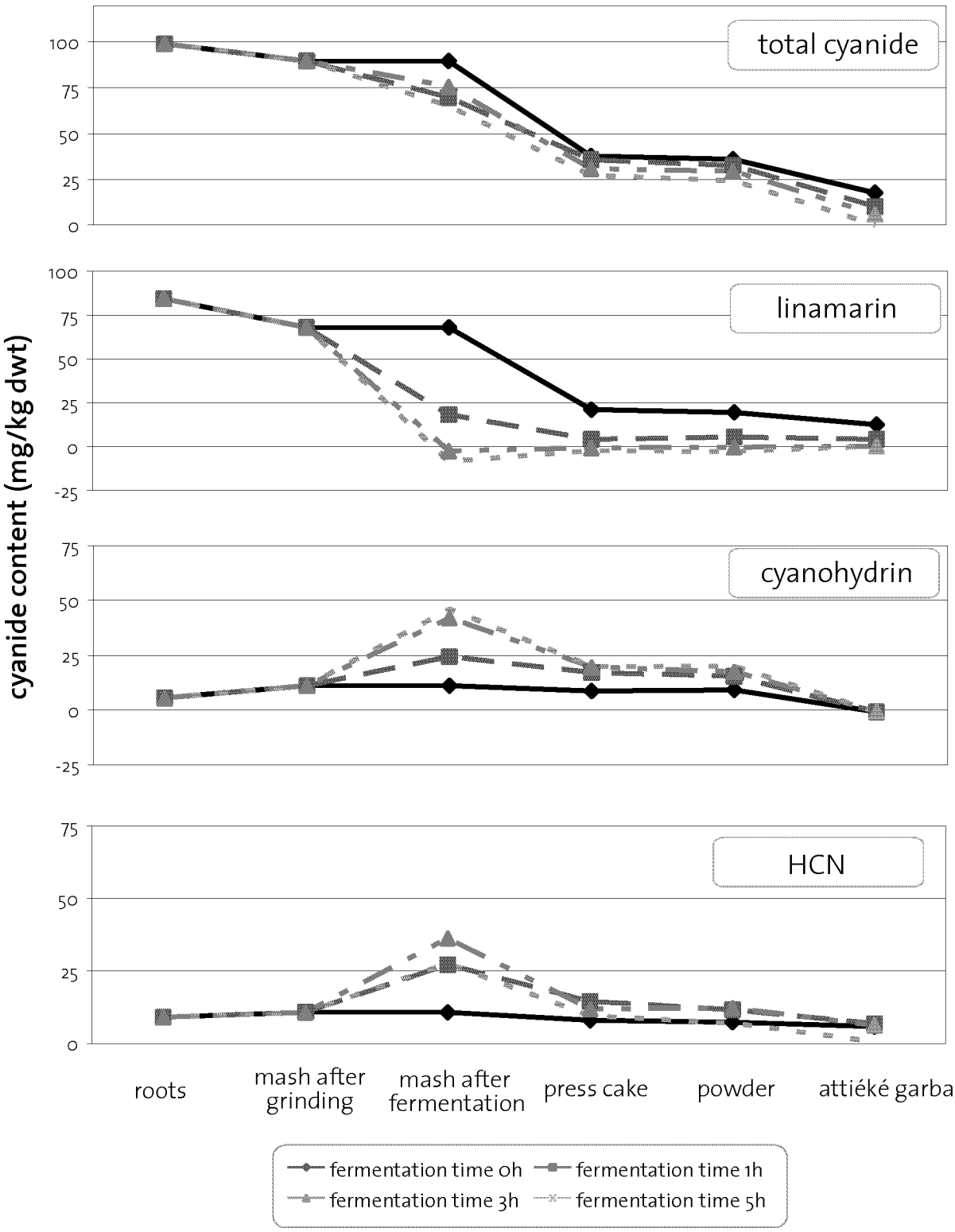


Fig. 17: Cyanogenic compounds during attiéké garba productions with different duration of fermentation

Linamarin was completely degraded to cyanohydrin and HCN after 3 and 5 h of fermentation time (Table 19). The latter reach a maximum in the mash after fermentation, as for traditional attiéké and attiéké garba preparation. During pressing and steaming cyanohydrin and HCN were reduced to low levels.

These findings support the hypothesis that fermentation should proceed until linamarin is completely degraded to cyanohydrin. This enables to reach low levels of cyanogenic compounds in the end product and leads to a safe product.

These experiments were carried out with a variety containing low amounts of cyanogenic compounds. It is to be expected that in varieties with a high amount of cyanide such as IAC, effects are more significant, although this remains to be elucidated.

5 Conclusions and Outlook

The present study showed the importance of extensive preparation to obtain a safe product for varieties with high initial cyanide content, such as IAC. An optimised processing has to ensure that cyanogenic compounds are reduced to low levels in the end products attiéké and attiéké garba, even if they are prepared from high cyanogenic roots. The traditional preparation of attiéké can be regarded as safe concerning cyanide content. A mean value of 5.9 mg/kg dwt in the end product of 19 attiéké preparations was measured, which is below the tolerated 10 mg/kg dwt for flour (Codex Alimentarius). A shortening of the processing, as seen in the attiéké garba preparation, does not lead to a significant increase in cyanide in the end product. In 20 attiéké garba samples, a mean cyanide content of 9.2 mg/kg dwt was measured in the end product.

In the present study, three important steps in the production of attiéké and attiéké garba with regard to cyanide reduction were identified: fermentation, pressing and steaming. During fermentation, cyanogenic glycosides were degraded by the endogeneous linamarase to cyanohydrin, which subsequently disintegrated spontaneously to HCN. The fermentation process led to a decrease in total cyanide of approximately 50% in attiéké preparation and of approximately 30% in attiéké garba preparation. Differences might be explained by the fact that a coarser grained mash and a less developed inoculum was used for attiéké garba compared to attiéké preparation. Coulin (2004) showed that in the inoculum used for attiéké garba preparation the concentration of cell wall degrading enzymes is lower which helps to maintain a coarser grained mash. The degradation of linamarin to cyanohydrin might be slower during attiéké garba production, since linamarin was not released from the cells as fast as in attiéké preparation. The complete degradation of linamarin to cyanohydrin and glucose was attained later in the fermentation step. Pressing removed about 20% of total cyanide in the attiéké preparation and about 30% in the attiéké garba preparation. During steaming, approximately 15% of total cyanide evaporated in attiéké preparation. In attiéké garba preparation, the steaming step removed about 30% of total cyanide, since more cyanogenic compounds were still left in the product before steaming. The total cyanide content of the end product was higher in attiéké garba (3% of initial cyanide content) than in attiéké (2% of initial cyanide content).

Fermentation was shown to be the most important step for cyanide reduction. The fermentation process in attiéké and attiéké garba preparation fulfills several purposes:

- A prerequisite for an optimal fermentation is milling. This process brings the enzyme linamarase into contact with linamarin, starting its degradation to cyanohydrin and glucose.
- During fermentation, all cyanogenic glycosides are degraded to cyanohydrin and, subsequently, to HCN. The activity of the endogeneous linamarase is normally sufficient for the complete degradation of linamarin.
- The long fermentation time of 15 h for attiéké and attiéké garba production allows the HCN to evaporate during fermentation. Total cyanide content of the mash is reduced by about 50% for attiéké and by about 30% for attiéké garba during fermentation.

The contribution of the inoculum used in the production of attiéké and attiéké garba on cyanide degradation was shown to be minimal. Moreover, β-glucosidases from bacteria did not play an important role with regard to cyanide reduction. Several hypothesis support this view:

- Milling prior to fermentation led to an intimate contact of linamarase and linamarin. The complete degradation of linamarin to cyanohydrin took place in the first hour of fermentation for varieties with high linamarase activity such as IAC, TMS 92/00061, 92/00067, 92/00325, 92/00326, 92/00398, 92/01425, Anader1, Bonoua, TME1 and Okolyawo.
- In varieties with a low activity of endogeneous linamarase such as TMS 92/00057, 92/00427, 92/02327, Anader2, Olekanga, β-glucosidases from bacteria did not sufficiently contribute to the degradation of linamarin.
- Cell wall degrading enzymes in the inoculum, would need to be highly active to free linamarin and linamarase from the cells. Coulin (2004) showed that the relative firmness of the mash after grinding decreases only by about 10% during the first two hours of fermentation, while linamarin was already completely degraded to cyanohydrin and glucose.
- Moreover, in attiéké garba preparation, a less-fermented inoculum was used and the fermentation time was shorter. The activity of cell wall degrading enzymes was shown to be lower in this inoculum (Coulin, 2004), leading to a desired coarser grained mash after fermentation. The decomposition of linamarin was only slightly affected by the shorter fermentation time and the less-fermented inoculum. The degradation of linamarin was almost as fast as for attiéké preparation.

In varieties with a high linamarase activity, the remaining cyanides in the end product were below the tolerated 10 mg/kg dwt for flour (Codex Alimentarius). However, in varieties with a low linamarase activity, the decomposition was not completed after fermentation and cyanide contents between 18 and 140 mg/kg dwt with a mean value of 65 mg/kg dwt were found. The results in this study showed that in these varieties, linamarase activity was lower than for varieties with low cyanide content in the end product.

Several studies have tried to correlate total cyanide content to linamarase activity but failed (Bradbury and Egan, 1992; Nambisian and Sundaresan, 1994; Iglesis *et al.*, 2002). In this study, the preliminary results on linamarase activity also showed no correlation. As a consequence, varieties with a low linamarase activity such as TMS 92/00057, 92/00427, 92/02327, Anader2 and Olekanga, should not be used for attiéké preparation. The low linamarase activity leads to an incomplete degradation of linamarin and an elevated cyanide content in the end product.

Other varieties such as IAC, TMS 92/00061, 92/00067, 92/00325, 92/00326, 92/00398, 92/01425, Anader1, Bonoua, TME1 or Okolyawo possess a high endogeneous linamarase activity. Furthermore, some among these varieties contain lower levels of cyanide in the roots as IAC. This leads to an even faster break down of linamarin after milling.

In the present study, some questions remained unanswered and should be adressed in a future work:

- Linamarase activity might be an important factor in choosing new varieties, which possibly replace IAC as the preferred variety for attiéké and attiéké garba preparation in Côte d'Ivoire. The selection of a appropriate variety should include studies on its linamarase activity and the velocity of cyanide degradation. The absolute cyanide content of a selected variety might not be as important as a fast degradation of linamarin, since this leads to low cyanide content in the end product, regardless of the absolute cyanide content in the roots.
- For attiéké garba preparation, quality parameters other than cyanide content, have not been studied in this thesis. Shelf life of attiéké garba is shorter than attiéké, which might be influenced by the less-fermented inoculum or by shorter steaming of attiéké garba. Other hitherto unknown factors may be responsible for spoilage of attiéké garba and should be studied as well.

- The differences in cyanide content of roots from the same plant and roots of the same variety but different plants is still not sufficiently known. Further studies need to be carried out to decide how many roots need to be sampled to determine a representative mean cyanide content of a variety. Other factors such as age of the plant or maturity of the roots may influence the cyanide content of the root and should be investigated as well.
- Cyanide content for some products such as placali and foutou manioc is four to five times higher than the tolerated 10 mg/kg dwt for flour (Codex Alimentarius). These products are not consumed on a daily basis, nevertheless might cause health problems. The degradation of cyanogenic compounds in these products should be studied in detail and some recommendations on the variety to be used for the preparation of these products should be established.

In depth knowledge of cyanogenic compounds of cassava and their degradation should help to select new varieties which exhibit desired attributes such as yield, resistance against pests and diseases, starch content, cyanide content and quality of the edible product. This knowledge would also improve the safety of the end products derived from cassava such as attiéké and attiéké garba.

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7 APPENDIX

Tab. A1: Calibration curves for the determination of cyanides (absorbance at 600 nm)

concentration	acetone cyanohydrin calibration curve (n = 16)		KCN calibration curve (n=4)		linamarin calibration curve (n=2)			
[mg HCN/l]	mean value	s.d.[1]	mean value	s.d.	[mg linamarin/l]	[mg HCN/l]	mean value	s.d.
0	0.069	0.076	0.077	0.004	0	0	0.079	0.001
1	0.124	0.077	0.155	0.008	1	0.11	0.091	0.002
3	0.302	0.075	0.312	0.025	5	0.55	0.121	0.005
5	0.473	0.070	0.479	0.023	10	1.09	0.162	0.006
8	0.738	0.067	0.732	0.072	30	3.28	0.318	0.009
10	0.909	0.065	0.891	0.058	50	5.47	0.461	0.018
15	1.359	0.063	1.276	0.106	100	10.93	0.921	0.029
20	1.763	0.065	1.758	0.117				
slope	0.0860		0.0831				0.0759	
y-intersect.	0.0506		0.0661				0.0752	
correlation coefficient	0.9996		0.9990				0.9978	

[1] standard deviation

Tab. A2: Dry weight (g/100g) of samples taken during attiéké preparation

	number of samples	dry weight	standard deviation
peeled roots	16	42.4	3.8
mash after grinding	14	37.9	3.6
mash after fermentation	19	40.9	2.9
press cake	21	53.1	1.4
grains before drying	21	51.4	1.3
grains after drying	21	56.2	1.5
attiéké	20	53.6	1.6

Tab. A3: Cyanide content of samples taken at different steps during attiéké preparation (mg/kg dwt)

	initial cyanide content of roots <400 mg/kg dwt			initial cyanide content of roots >400 mg/kg dwt		
	number of samples	mean value	s.d.	number of samples	mean value	s.d.
roots	12	263.7	61.3	8	750.7	390.2
mash after grinding	10	206.6	64.4	4	382.2	50.8
mash after fermentation	10	145.9	61.2	8	283.6	83.5
press cake	12	95.1	34.3	8	140.3	51.3
grains before drying	12	61.2	19.2	8	92.26	14.5
grains after drying	12	41.3	15.2	8	74.3	19.0
attiéké	12	3.9	2.4	7	9.3	5.7

Tab. A4: Relative cyanide content in percent during attiéké preparations with low cyanide contents (< 15 mg/kg dwt) in the end product (n=11)

steps	total cyanide		linamarin		cyanohydrin		HCN	
	[%]	s.d.	[%]	s.d.	[%]	s.d.	[%]	s.d.
roots	100.0	0.0	84.8	13.2	6.8	6.3	8.4	9.6
mash after grinding	93.7	37.3	73.4	11.2	12.2	7.2	8.1	6.4
mash after fermentation	67.2	28.3	3.4	7.2	37.6	5.2	26.1	7.5
press cake	36.8	23.1	2.0	3.5	21.0	3.3	13.8	3.9
grains before drying	27.3	15.3	0.6	1.3	23.1	1.7	3.6	2.2
grains after drying	24.6	14.3	0.9	2.2	20.4	2.1	3.3	1.9
attiéké	2.2	3.0	0.1	0.2	0.9	0.6	1.2	0.5

Tab. A5: Relative cyanide content in percent during attiéké preparations with high cyanide contents (> 15 mg/kg dwt) in the end product (n=5)

step	total cyanide		linamarin		cyanohydrin		HCN	
	[%]	s.d.	[%]	s.d.	[%]	s.d.	[%]	s.d.
roots	100.0	0.0	94.9	3.2	1.5	0.6	3.6	2.9
mash after grinding	93.7	20.8	86.5	4.1	3.4	1.6	3.8	2.7
mash after fermentation	73.5	15.4	24.7	20.9	30.6	11.7	18.1	10.1
press cake	32.4	10.6	12.3	8.5	14.1	4.5	6.0	4.0
grains before drying	31.2	10.1	10.8	8.8	17.8	7.4	2.6	2.0
grains after drying	28.5	8.9	9.7	8.4	16.5	7.1	2.3	1.9
attiéké	12.6	4.8	9.1	3.2	0.9	0.7	2.6	2.6

Tab. A6: Dry weight (g/100g) of samples taken during attiéké garba preparation

	number of samples	dry weight	standard deviation
peeled roots	13	43.9	3.5
mash after grinding	5	40.9	2.8
mash after fermentation	10	42.0	3.0
press cake	13	53.6	2.5
powder after sieving	13	53.3	2.6
attiéké garba	13	50.9	1.3

Tab. A7: Cyanide content of samples taken at different steps during attiéké garba preparation (mg/kg dwt)

	initial cyanide content of roots lower than 400 mg/kg dwt			initial cyanide content of roots higher than 400 mg/kg dwt		
	number of samples	mean value	s.d.	number of samples	mean value	s.d.
roots	8	262.5	90.3	5	762.0	479.4
mash after grinding[1]	3	(294.9)	(108.9)	2	(270.1)	(35.4)
mash after fermentation	6	214.0	77.0	4	415.6	54.5
press cake	8	118.8	40.0	5	167.5	22.0
powder	8	93.7	21.7	5	132.8	17.4
attiéké garba	8	6.0	5.4	5	14.3	9.7

[1] n=1

Tab. A8: Cyanogenic compounds (mg/kg dwt) during attiéké garba preparation with low initial cyanide content (A) (n=6) and high initial cyanide content (B) (n=4) in the roots

	total cyanide	linamarin	cyanohydrin	HCN
A: roots (< 400 mg/kg dwt)	266.9	255.7	16.1	-4.9
mash after grinding[1]	(208.2)	(121.5)	(80.1)	(6.6)
mash after fermentation	214.0	-30.4	128.2	116.2
press cake	120.7	-17.6	74.3	64.1
powder	91.1	-11.1	83.7	18.5
attiéké garba	6.0	-0.2	5.7	0.5
B: roots (>400 mg/kg dwt)	826.6	791.9	30.3	4.5
mash after grinding[1]	(316.1)	(180.7)	(130.3)	(5.2)
mash after fermentation	415.6	-37.2	175.1	278.0
press cake	181.1	-32.7	88.8	125.0
powder	146.6	-8.5	72.4	53.7
attiéké garba	16.4	-0.8	11.4	3.1

[1] n=1

Tab. A9: Cyanogenic compounds (mg/kg dwt) in a model to prepare attiéké (n=1)

	total cyanide	linamarin	cyanohydrin	HCN
roots	226.3	197.5	23.1	5.7
mash after grinding	240.9	205.1	29.7	6.1
mash after fermentation	186.1	-16.8	107.5	95.4
press cake	81.4	-8.1	60.3	29.2
grains before drying	63.8	-6.5	66.4	3.9
grains after drying	64.1	-7.3	67.9	3.5
attiéké	6.9	-0.1	4.4	2.6

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